Investigation of Microbiologically Influenced Corrosion (MIC) by Sulfate Reducing Bacteria (SRB) Biofilms and Its Mitigation Using Enhanced Biocides

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ABSTRACT

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Investigation of Microbiologically Influenced Corrosion (MIC) by Sulfate Reducing Bacteria (SRB) Biofilms and Its Mitigation Using Enhanced Biocides

Director of Dissertation: Tingyue Gu

Microbiologically influenced corrosion (MIC) has been a challenge in the oil and gas industry as well as other industries such as water treatment facilities, nuclear power plants and pulp and paper plants. Sulfate-reducing bacteria (SRB) are considered a major factor because sulfate is a widely available oxidant in anaerobic environments.

This work studied the influence of flow effects on SRB biofilm growth in a glass cell bioreactor. Correlation between the cylindrical coupon rotation rate in the glass cell and the average flow velocity in the pipeline was theoretically derived with rough surface taken into account. Deadlegs attached to pipelines often are more prone to MIC. Because there is often no flow or low flow near the bottom of a deadleg, an open flow loop for MIC investigation in deadlegs using small flow rates to achieve similar mass transfer effects to fast flow pipeline was proposed. Mass transfer simulation using the computational fluid dynamics (CFD) software FLUENT was employed. Unlike regular recirculating flow loops used in most MIC studies, this setup was better at emulating real MIC in pipelines, avoiding the recycling of metabolic byproducts and damage of cells due to the high shear stress in the recycling pump.

MIC treatment is usually about biocide treatment. Because of increasing environmental concerns and tightening regulations, a more environmentally benign
treatment to mitigate MIC was evaluated in this work, utilizing biodegradable chelators such as ethylenediaminedisuccinate (EDDS) and N-(2-Hydroxyethyl)iminodiacetic acid disodium salts (HEIDA) to enhance biocide performance against planktonic and sessile SRB. It showed that biocide dosage to control biofilm growth could be reduced considerably when a chelator was used with the biocide. Furthermore, when 10% to 15% (v/v) methanol was added to the binary combination of biocide and EDDS treatment, mitigation of planktonic SRB growth was improved from an inhibiting effect to a kill effect. D-amino acids as signaling molecules to disassemble biofilms were also investigated as biocide enhancers. It was found that 10 µM of a mixture of D-amino acids combined with 500 ppm EDDS successfully enhanced 30 ppm THPS to remove an established SRB biofilm. They showed great promise to enhance biocides for the mitigation of SRB biofilms.

Lab investigation of MIC after simulated hydrotesting revealed that severe pitting, as high as 40 µm pit depth in 30 days, occurred with greater pit depth when it was spiked with SRB. CO₂ and H₂S/CO₂ combined with MIC were also found to accelerate corrosion.

A mechanistic model for MIC in the presence of CO₂ was presented based on the electrochemical model for general CO₂ corrosion. A localized MIC pitting rate can be predicted with the help of biofilm porosity indicators.
DEDICATION

To

Xianwen Hu (my mother)

May she rest in peace

and

Xiangling Gao (my wife)
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CHAPTER 1 INTRODUCTION

On March 02, 2006 a serious crude oil leak was discovered on an Alaska Prudhoe Bay oil field pipeline which forced the operation to shut down, aggravating turmoil in the global oil market (Barringer, 2006). Microbiologically influenced corrosion (MIC) was highly suspected as the reason for this failure (Jacobson, 2007).

MIC, as its name indicates, is a corrosion process with the activity of microorganisms which may initiate, facilitate or accelerate corrosion reaction (Videla, 1996). It has become increasingly important in the oil and gas industry due to aging pipelines and massive use of water flooding in enhanced oil recovery. It is also a major problem in many other industries such as nuclear power and water utilities. Billions of dollars worldwide are lost every year due to MIC (Flemming, 1996).

MIC attack has been reported on many common engineering metals and alloys such as carbon steel, stainless steel, aluminum, copper and its alloy (Javaherdashti, 1999). Nonmetallic materials like polymers, concretes, wood and stone were reported to suffer MIC as well (Jack, 2002; Ding et al., 2017).

An open-to-air system can include aerobic condition on the top where oxygen is abundant and anaerobic condition in the bottom where oxygen is depleted by microbial respiration of the top layer aerobes. Hardy & Brown (1984) provided evidences that the alternation between anaerobic and aerobic exposure can cause severe corrosion. The complexity, however, obscures the interpretation of MIC mechanisms. Systems in the oil and gas industry are usually strictly anaerobic. Oxygen scavengers are used to remove the
trace oxygen content. To simplify the difficulties of studying MIC mechanisms, this work has focused on anaerobic MIC research.

It is believed that sessile bacteria within biofilms, rather than planktonic bacteria, are directly responsible for MIC attack. Biofilm, a collection of microorganisms attached to a solid surface, forms when planktonic bacteria adhere to the metal surface (O'Toole et al., 2000). By secreting extracellular polymeric substances (EPS), which trap all sorts of particulates in the surroundings, SRB show a strong tendency to adhere to available surfaces (Characklis, 1990). In response to environmental variations such as nutrient availability and flow conditions, biofilms adhere, grow, maintain and detach. Thus, in this work flow rate influence on biofilm growth and, sequentially, on metal corrosion has been of interest.

In the oil and gas industry, deadlegs attached to pipelines are prone to corrosion (Ifezue & Tobins, 2014) or MIC due to the accumulated water (in oil/water two-phase flows) and nutrients (Habib et al., 2005). The failure in Alaska draws attention to MIC and associated underdeposit corrosion in deadlegs. One challenge among researchers has been that lab experiments do not simulate field application well, one reason being the difficulties to reproduce field conditions in a lab. In real pipe flows the linear velocity usually exceeds 1 m/s, which makes the cost of culture medium unaffordable in a regular MIC open loop even with a diameter of one inch unless recycling or partial recycling is used. Meanwhile, a recycling pump at a fast flow rate will damage microbes in the fluid. Recycling also makes the microbial growth conditions deviate from field conditions since the metabolic by-products are also recycled during this process. In view of MIC in
deadlegs, it is possible to design an open flow loop for MIC investigation using smaller flow rates since the region near the bottom of a deadleg is often a no flow or low flow region. A commercial computational fluid dynamics (CFD) software program FLUENT was explored to simulate fast flow field conditions in a low velocity MIC lab by mass transfer similarity.

When biofilm control is needed, mechanical or chemical methods are applied. In the oil and gas industry, smart pigs are regularly utilized to clean pipe interior, but their application is limited because of cost and down time. Chemicals like biocides are widely used to control biofilm. Biofilm protects bacteria from environmental attack. Meanwhile, sessile bacteria have different physiologies from planktonic cells that respond to limited nutrition by decreasing metabolic activity (Davies, 2003). All these help sessile bacteria resist biocide attack and make it more difficult to remove biofilm than to kill planktonic cells (Davies, 2003; Meyer, 2003). It is costly and often causes environmental concerns to use large scale applications of biocides in industry. Because environmental policies are becoming more and more stringent, any method to reduce the dosage of biocide is desirable. In this work, green biocide enhancers to improve biocide performance on biofilms were investigated.

Hydrotest is a routine procedure for pipeline to test the integrity before its service. MIC is a concern during and after hydrotesting because bacteria introduced by hydrotest fluids may form biofilms and, subsequently, biofilms may flourish during pipeline operations. In this work, lab investigation of MIC during the hydrotesting and subsequent exposure to pipeline fluids was carried out. A mechanic MIC model combined with a
general CO$_2$ model was proposed to predict localized corrosion due to MIC in the presence of CO$_2$. 
CHAPTER 2 LITERATURE REVIEW

The concept of MIC or biocorrosion, an electrochemical process, was proposed as early as the 1930s (von Wolzogen Kuhr and van der Vlugt, 1934). Due to its complexity and lack of understanding, 70 years later, some corrosion engineers still are unconvinced about MIC and called it a “myth” (Little & Wagner, 1997). Recent catastrophes related to MIC (Jacobson, 2007; van Derbecen, 2010) have revealed the importance of understanding MIC mechanisms and, more importantly, preventing MIC.

2.1 Microorganisms involved in MIC

The activities of microorganisms can interact either directly or indirectly with metals such as iron, copper and some alloys as well as in different environments, changing (accelerating or inhibiting) the electrochemical process and causing MIC when the process is accelerated. A variety of phylogenically and physiologically different microorganisms causing MIC have been reported (Beech & Gaylarde, 1999) including metal-reducing bacteria (MRB), metal-depositing bacteria (MDB), slime-producing bacteria, acid-producing bacteria (APB), fungi, and, most importantly, SRB.

SRB reduces sulfate to sulfide, coupled with oxidation of low-chain organic carbons such as lactate, pyruvate, acetate and propionate. The reaction can be expressed as:

\[ SO_4^{2-} + 2CH_3CHOHCOO^- \rightarrow 2CH_3COO^- + 2CO_2 + HS^- + OH^- + H_2O \] (2-1)

Other than organic carbon, H₂ can also be utilized as the sole energy source for SRB (Thauer et al., 2007). SRB were considered to be obligate anaerobes until the late 1970s.
New findings have revealed that some of SRB strains can tolerate oxygen and revive when switched to anaerobic environments, which has explained the phenomena that severe corrosion was observed when alternations between aerobic and anaerobic conditions occurred (Hardy & Brown, 1984; Sass & Cypionka, 2007).

SRB growth behavior is complicated. Other than limiting factors such as nutrients of ferrous and sulfate ions and carbon sources, environmental conditions such as flow rate, temperature, and pH of medium can influence on the microbial growth and metabolic activities (Marchal et al., 2001).

### 2.2 Mechanism of MIC due to SRB

As early as in the turn of the 20th century, bacteria activity in the corrosion process was discovered (Videla & Herrera, 2005). The first report of MIC on iron in the presence of sulfur was not until 1923 (Fitzgerald, 1993). Sulfate-reducing bacteria are among the most notorious bacteria causing MIC.

Since the first MIC theory was proposed in 1934, mechanisms of MIC have continued to be developed based on new findings on microbial activities, metabolism, biofilms and corrosion theory. A number of reviews on MIC have been published (Hamilton, 1985; Little et al., 1992; Beech & Gaylarde, 1999; Javaherdashti, 1999; Videla & Herrera, 2005; Skovhus et al., 2017).

#### 2.2.1 Classical mechanism for MIC due to SRB

von Wolzogen Kuhr and van der Vlugt (1934) pioneered MIC research and interpreted the MIC mechanism due to SRB using cathodic depolarization theory (CDT):

\[
\text{Anodic reaction: } 4Fe \rightarrow 4Fe^{2+} + 8e^{-}
\]
Water dissociation: \[ 8H_2O \rightarrow 8H^+ + 8OH^- \] (2-3)

Cathodic reaction: \[ 8H^+ + 8e^- \rightarrow 8H(ads) \] (2-4)

Cathodic depolarization: \[ SO_4^{2-} + 8H(ads) \xrightarrow{\text{hydrogenase}} S^{2-} + 4H_2O \] (2-5)

Corrosion product: \[ Fe^{2+} + S^- \rightarrow FeS \] (2-6)

Overall reaction: \[ 4Fe + SO_4^{2-} + 4H_2O \xrightarrow{\text{SRB}} FeS + 3Fe(OH)_2 + 2OH^- \] (2-7)

At the cathode, protons act as electron acceptor, where the sulfate is reduced to sulfide by SRB. By the hydrogenase of the bacteria, hydrogen adsorbing on the metal surface is removed. The corrosion rate is thus accelerated since the required high activation energy step of combining hydrogen atoms is bypassed.

CDT shows MIC to be an electrochemical process, which has become the base concept in this field. The bioenergetics of coupling of the oxidation of lactate to acetate and CO\(_2\) with the reduction of sulfate to sulfide is elucidated on Figure 2-1 (Pereira et al., 2007). Electron transfer and sulfate transfer occur through adenosine diphosphate (ADP) and adenosine triphosphate (ATP) system via hydrogenase and enzymes. Dinh et al. (2004) proposed a pathway of electron flow for iron corrosion:

\[ Fe \rightarrow \text{electron transport system} \rightarrow \text{sulfate reduction enzymes} \]

However, how the extracellular electron transfer occurs between metal-biofilm and within biofilms remains unclear. Hernandez and Newman (2001) reviewed and explained possible excreted shuttles to play the role. They stated that at different layers of biofilms, the mechanisms for energy generation are different. At the top of biofilms, outer-
membrane can interact with ions directly; while within biofilms and at the metal surface, it is another story. Recent discoveries on microbial nanowires (Reguera et al., 2005) demonstrated that conductive pili can transfer electrons to extracellular electron acceptors.

Figure 2-1. Schematic showing Desulfovibrio vulgaris Hildenborough derives energy for growth by coupling the oxidation of lactate to acetate and CO₂ with the reduction of sulfate to sulfide. (Pereira et al., 2007).

However, the lack of information on ferrous sulfide film and, more importantly, biofilm makes it vulnerable to contradictory practices where low corrosion rates were observed in anaerobic field conditions while large amounts of SRB were found nearby or
vice versa (Starkey, 1985; Tiller, 1985). With considerable focus on the MIC in the last two decades, the nature and mechanisms of MIC have been developed. SRB attack on metal was found to be caused by colonies of SRB under patchy biofilms, forming localized corrosion (Rosnes et al., 1991; Little et al., 1992; Videla & Herrera, 2005).

The “cathodic depolarization,” however, has been questioned by many researchers (Hamilton, 1985; Little et al., 1992; Heitz, 1996; Videla & Herrera, 2005). They argued that the “depolarization” is not a strictly electrochemical term in this theory, and only indicates a process occurring at the cathode. Moreover, CDT did not explain the role of sulfide in the anodic reaction and, more importantly, the interaction with films (e.g. oxides, sulfides and biofilm), making it difficult to accept. Little et al. (1992) also challenged that H\(^+\) concentration in Equation (2-4) is very small in neutral environments and the removal of H\(^+\) in reaction Equations (2-4) and (2-5) by SRB can hardly increase the corrosion rate.

2.2.2 Development of mechanisms of MIC due to SRB

A number of controversial experiments either confirmed or disproved the CDT since the 1960s while the awareness of MIC has been increased in industry (Hamilton, 1985). Iverson (1966) used benzyl viologen as an oxidant in place of sulfate in the experiment to avoid the complication of induced sulfide film and observed that cathodic hydrogen was generated by evidence of reduction of the redox indicator, where the cathode was physically separated from the anode and only connected electrochemically by a salt bridge. Hardy (1983) experimentally demonstrated that the oxidization of
cathodic hydrogen depolarized the anode mild steel; however, he also found that this depolarization was transient.

Arguing the hydrogenase’s role on the atomic hydrogen removal, Costello (1974) proposed that the cathodic depolarization at neutral pH contributes to the dissolved hydrogen sulfide generated by SRB shown as below:

Cathodic reaction: \[ H_2S + e^- \rightarrow HS^- + \frac{1}{2} H_2 \] (2-9)

Hydrogenase, not like Equation (2-5) suggested, may just play a secondary role of removing molecular hydrogen. However, this downplayed the important role of hydrogenase biocatalysis.

King and Miller (1971) in their classical paper hypothesized that ferrous sulfide as a cathode adsorbed the atom hydrogen, where the critical electrochemical reaction was, minimizing the direct role of SRB on the corrosion process. In addition to the focus on the cathodic reaction as mentioned above, some researchers proposed other mechanisms summarized by Beech and Gaylarde (1999) (Table 2-1). While the direct role of SRB in the MIC continues to be contested by some researchers (King and Miller, 1971; Crolet, 2005), the development in other fields like microbial fuel cell (MFC) has illustrated the capabilities of bacteria to oxidize organic matters and transfer electrons to the anode, generating current (Logan et al., 2006). Sherar et al. (2011) obtained a high resolution image showing that SRB are attached on the surface via organic filaments and possible microbial nanowires (Figure 2-2). Bacteria may obtain energy from metal via nanowires. This finding provided new proof of SRB involvement in the electron transfer process during corrosion (Gu & Xu, 2010; Xu & Gu, 2011).
Table 2-1. Suggested mechanisms of metal corrosion by SRB (Beech & Gaylarde, 1999)

<table>
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<tbody>
<tr>
<td>Cathodic depolarization by hydrogenase</td>
<td>von Wolzogen Kuhr and van der Vlugt, 1934; Bryant et al., 1991.</td>
</tr>
<tr>
<td>Anodic depolarization</td>
<td>Salvarezza and Videla, 1986; Daumas et al., 1988; Crolet, 1992.</td>
</tr>
<tr>
<td>Sulfide</td>
<td>Little et al., 1998.</td>
</tr>
<tr>
<td>A volatile phosphorus compound</td>
<td>Iverson and Ohlson, 1983.</td>
</tr>
<tr>
<td>Sulfide-induced stress</td>
<td>Edyvean et al., 1998.</td>
</tr>
<tr>
<td>Corrosion cracking</td>
<td></td>
</tr>
<tr>
<td>Hydrogen-induced cracking or blistering</td>
<td>Edyvean et al., 1998.</td>
</tr>
</tbody>
</table>

Gu et al. (2009) thus proposed a so-called BCSR theory (biocatalytic cathodic sulfate reduction) stating that only the sessile SRB in contact with the metal surface have the direct impact on corrosion. In this theory, the cathodic reaction is shown as below:

Cathodic reaction: \[ SO_{4}^{2-} + 9H^{+} + 8e^{-} \rightarrow HS^{-} + 4H_{2}O \]  \hspace{1cm} (2-10)

Anodic reaction (2-2) occurs on the metal surface, while reaction (2-10) happens in the cytoplasm with the help of biocatalysis from SRB. This can refer to the bioenergetics model as shown on Figure 2-1. For the thermodynamics perspective, the standard redox potential of Fe/Fe^{2+} is -447 mv; on the other hand, the standard potential of SO_{4}^{2-}/HS^{-} is -217 mv (Thauer et al., 2007). This coupled reaction is thermodynamically as favorable as
the lactate oxidation/sulfate reduction reaction ($E^0$ of CO$_2$+acetate/lactate: -430 mv). It is reasonable that at the metal surface, when in lack of organic carbon, SRB may derive energy from iron oxidation for survival (Xu & Gu, 2011). The subsequent experimental observation of increased pitting corrosion due to SRB starvation validated this theory.

Figure 2-2. SEM image of two SRB cells with “filaments” anchored on carbon steel coupon from a culture medium without organic carbon. (The filaments did not appear when for a culture medium with organic carbon.) (Sherar et al., 2011)

The BCSR theory points out the electron origin and fate and leaves out the details for the extracellular electron transport (EET) process to accommodate various EET mechanisms. There are two methods for EET, namely, direct electron transport (DET) and mediated electron transport (MET) (Zhang et al., 2015; Jia et al., 2017c). When cell walls are in direct contact with metal surfaces, extracellular electrons are transferred to the cytoplasm via cell wall membrane bound proteins such as c-type cytochromes or nanowires (pili, as shown in Figure 2-2), the process of which is called DET. For cells to
utilize redox-active electron mediators such as $\text{H}^+$/H$_2$ (assisted by hydrogenases), riboflavin and flavin adenine dinucleotide (FAD) for facilitating electron transferring, this process is named MET. Gu (2012b) and Gu et al. (2015) pointed out that the classical CDT is correct for hydrogenase-positive SRB MIC when $\text{H}^+$/H$_2$ is viewed as an electron shuttle. Indeed, this shuttle is actually quite common in microbes that have hydrogenase enzymes (Bergel, 2007).

Overall, MIC due to SRB is an electrochemical process which is unanimously accepted. However, there cannot be a sole mechanism governing the MIC process in such a large variety of conditions (e.g., materials, pH and consortium). More likely, they may co-exist and play a role to some extent.

2.2.3 Quantification of anaerobic MIC model

From an engineering perspective, the ultimate goal of study on corrosion is to predict and prevent corrosion. Unfortunately, with the lack of a singular mechanism governing MIC, risk factor models based on experience were often the only models available (Sooknah et al., 2007). When corrosion factors such as temperature, pH, operation factors and biocide usage were graded and incorporated into this model, MIC susceptibility was then obtained. A mechanistic model, however, derived from physical phenomena, can provide meaningful and more accurate results if the intrinsic mechanism is well understood.

2.2.3.1 Background of corrosion thermodynamics and kinetics

According to Faraday’s law (Landolt, 2002), the corrosion rate can be obtained by measuring the corrosion current density:
$$CR = \frac{iM_w}{nF\rho} \quad (2-11)$$

Where \(F\) is the Faraday constant, 96485 C/mol; \(M_w\) is the molecular weight, g/mol; \(\rho\) is the material density, kg/m\(^3\); \(n\) is the number of electrons involved in the electrochemical reaction and the unit of CR is mm/y.

When the reaction is in charge transfer control, the so-called Butler-Volmer equation is used to describe the relationship between the current density and potential:

$$i = i_0 \exp \left[ \frac{\alpha nF}{RT} \eta \right] - i_0 \exp \left[ -\frac{(1 - \alpha) nF}{RT} \eta \right] \quad (2-12)$$

where

- \(i_0\): exchange current density, A/m\(^2\);
- \(\alpha\): a proportionality factor called the charge transfer coefficient, dimensionless;
- \(R\): universal gas constant;
- \(T\): temperature, K;
- \(\eta\): over-potential, V (\(\eta = E - E_{eq}\));
- \(E\) is the electrode potential, V;
- \(E_{eq}\) is the equilibrium potential, V.

The induced empirical Tafel coefficients \(\beta_a\) and \(\beta_c\) are easily measurable, which are defined as:

$$\beta_a = \frac{dE}{d\ln i_a} = \frac{RT}{\alpha nF} \quad (2-13)$$

$$\beta_c = \frac{dE}{d\ln i_c} = \frac{RT}{(1 - \alpha)nF} \quad (2-14)$$

Thus Equation (2-12) can be expressed as:
\[ i = i_0 \exp \left( \frac{\eta}{\beta_a} \right) - i_0 \exp \left( -\frac{\eta}{\beta_c} \right) \]  

Butler-Volmer Equation (2-12) is valid when the reaction is in charge transfer control (when the charge transfer rate is much smaller than the mass transfer rate), which means the surface concentrations of species are equal to those in the bulk solution. When mass transport including mass diffusion and convection contributes to the corrosion rate, there is a concentration profile of reactants and products from the bulk solution and at the electrode surface. The overall current density \( i \) can be expressed as:

\[ \frac{1}{i} = \frac{1}{i_c} + \frac{1}{i_{\text{lim}}} \]  

where

\( i_c \): current density in charge transfer control, A/m\(^2\);

\( i_{\text{lim}} \): current density in mass transfer control, A/m\(^2\).

The mass flux is given as:

\[ N_B = -D_B \frac{dC_B}{dy} \bigg|_{y=0} \]  

where

\( N_B \): flux of species B;

\( D_B \): diffusion coefficient of species B;

\( C_B \): concentration of species B in the diffusion layer;

\( y \): distance from the electrode surface in the diffusion layer.

By integration of Equation (2-17) and according to Faraday’s law, the maximum current density called the limiting current density becomes:
\[ i_{lim,B} = -nFD_B \frac{C_{B,b}}{\delta} \]  \hspace{1cm} (2-18)

where the subscript b means bulk; \( \delta \) is the diffusion layer thickness.

2.2.3.2 Modeling the pitting growth of MIC due to SRB

Based on the CDT theory, Peng et al. (1994) predicted the pitting growth in a low sulfate system where sulfate was the growth-limiting substrate by numerically solving the mass-conservation Equation (2-19) considering the time evolution of the moving bio-corroded boundary. It should be pointed out that the authors mistakenly missed sulfate consumption by bulk SRB cells in the biofilm in their corrosion rate calculation.

\[ \frac{\partial C_s}{\partial t} + u \cdot \nabla C_s = D \nabla^2 C_s \]  \hspace{1cm} (2-19)

where

- \( u \): average velocity, m/s;
- \( D \): sulfate diffusion coefficient, m\(^2\)/s;
- \( t \): time, s;
- \( C_s \): sulfate concentration on the biofilm surface, mol/m\(^3\).

With the boundary condition:

\[ -D \frac{\partial C}{\partial n} = \frac{\mu_{max} C X_f}{K_m + C} \]  \hspace{1cm} (2-20)

where

- \( C \): sulfate concentration within the biofilm, mol/m\(^3\);
- \( \mu_{max} \): maximum specific rate of substrate uptake, mol SO\(_4^{2-}\)/(kg·s);
- \( K_m \): apparent half-saturation constant, mol/m\(^3\);
- \( X_f \): biomass, kg/m\(^2\);
n: unit outward normal to surface (reaction zone).

Gu et al. (2009) based on the BCSR theory proposed a model predicting the pitting growth of MIC. “Biofilm aggressiveness” was used to indicate the ability of SRB to facilitate cathodic sulfate reduction, which is dependent on bacteria species, metabolic environment and sessile SRB density within biofilm on the cathode. By computer simulation, it was demonstrated that when biofilm thickness is small, the charge transfer resistance is the dominant factor; when there is a deep pit, mass transfer distance increases and the mass transfer resistance becomes dominant. Further, this model was extended to describing MIC due to acid producing bacteria (APB) as well (Xu et al., 2016). However, both models focused on the cathodic reduction. Further development should be incorporated into models by taking into account of H₂S and FeS film, but their effects on MIC are still poorly understood.

2.3 Biofilm and its characteristics

Biofilm is a collection of microorganisms attached to a solid surface in a matrix formed by extracellular polymeric substances (EPS), or “sticky slime polymers,” trapping all sorts of particulates in the surroundings (Characklis, 1990; Beyenal & Lewandowski, 2002).

2.3.1 Role of biofilms on corrosion

Biofilms are in direct contact with metal surface and generally believed to be the main reason contributing to corrosion instead of planktonic cells. Heterogeneous biofilms formed on the surface are normally patchy, resulting in pitting corrosion underneath. The role of biofilms on the corrosion can be summarized as below (Videla & Herrera, 2005):
(a) act as a mass transfer barrier for the chemical species; (b) remove the protective film when detached or cause it to dissolve by such metabolic products as organic acids; (c) form concentration cells due to patchy biofilms; (d) alter the electrochemical properties of metal-biofilm interface.

2.3.2 Mass transfer and shear effects

Biofilms behave like a barrier of mass transfer for bacteria to resist the toxic substances (such as biocides) and shear damage (Denyer, 1995; Morton et al., 1998). It was reported that cells in biofilms can have up to 500 times more resistance than planktonic cells against biocides (Ismail et al., 2002). In MIC, mass transfer was linked to nutrient distribution in the medium and the transport of corrosive species and corrosion products. Thus, the demands for the study of mass transfer and shear effects on MIC are of significance for understanding how the biofilms attach, grow, and detach.

A case of MIC on carbon steel was reported at linear velocities of about 0.35 m/s (Lee & Characklis, 1993). The flow effects on MIC were discussed. Two key factors influencing the biofilm growth are hydrodynamics and nutrients. Hydrodynamic conditions have two interlinked effects: mass transfer and shear. The latter causes the detachment of biofilms. A good evidence of shear stress on the biofilm detachment was provided by P. Stoodley in an online video (https://www.youtube.com/watch?v=vckVw_rQC48). Biofilm accumulation is a net effect of cell attachment, detachment, and growth, and hydrodynamics influences all these processes. Bouwer (1987) pointed out that increased surface irregularity due to biofilm formation can influence particle transport rate and biofilm attachment rate by (1)
increasing convective mass transport near the surface (not valid for very thin and dense biofilms such as some SRB biofilms that are less than 100 micron thick); (2) providing shelter from shear forces; and (3) increasing surface area for attachment. It was reported that biofilm thickness increased with nutrient concentration when shear was held constant (Characklis, 1990) but decreased with shear when the substrate-loading rate was constant (Characklis, 1981). Further research on the effects of shear stress on the biofilms (Ramasamy & Zhang, 2005) showed that secretion of EPS corresponded to the sudden increase of shear stress but would reach a quasi-steady state, and the biofilm would become less porous but denser.

There are two boundary layers above the biofilm surface (Stewart, 1998). One is hydrodynamic boundary layer and the other is mass transfer (diffusion) boundary layer. As the controller parameter for microbial activity in biofilms, the distribution of nutrient can be described by a continuity Equation (2-19) with the boundary Equation (2-20). In case of no substrate consumption in the bulk solution, the external mass transfer will be equal to internal mass transfer.

\[
N_s = k_1(C_b - C_i) = D_f \left. \frac{dC}{dn} \right|_{n=L_f}
\]  

(2-21)

where:

- \( k_1 \): external mass transfer coefficient;
- \( L_f \): average biofilm thickness;
- \( D_f \): effective diffusivity of the growth-limiting nutrient in the biofilm.

To determine the value of \( k_1 \), empirical Equation (2-22) was proposed to calculate the Sherwood number (Sh) for biofilms (Stewart, 1998):
\[ Sh = 2 \text{Re}^{0.5} \text{Sc}^{0.5} \left( \frac{d_h}{L} \right)^{0.5} (1 + 0.0021 \text{Re}) \] (2-22)

Where:

- \( \text{Sc} \): Schmidt number (\( \text{Sc} = \mu/(\rho D) \));
- \( \text{Re} \): Reynolds number (\( \text{Re} = \nu d_h \rho/\mu \));
- \( \text{Sh} \): Sherwood number (\( \text{Sh} = k_1 d_h/D_w \));
- \( \mu \): fluid viscosity (g/cm/s);
- \( \nu \): fluid velocity;
- \( \rho \): fluid density (g/cm\(^3\));
- \( d_h \): hydraulic radius;
- \( D_w \): molecular diffusivity in culture medium.

Fan et al. (1990) proposed an empirical equation correlating the effective diffusivity \( D_f \) in the biofilm with \( D_w \) and biomass density \( X \) (kg/m\(^3\)):

\[
\frac{D_f}{D_w} = 1 - \frac{0.43X^{0.92}}{11.19 + 0.27X^{0.99}}
\] (2-23)

The effective diffusivity in biofilms varied from one location to another, as the result of heterogeneous biofilms. Biofilm behavior is complicated because it consists of living organisms, which may have a physiological response under higher flow rate conditions, causing denser biofilms than those grown in lower flow rate conditions and, thus, rearranging the internal biofilm structure. Thus, the effective diffusivity in biofilms may change with flow rate. To measure the local effective diffusivity, Beyenal et al. (2002) used a limiting-current density measurement. By measuring local consumption of ferricyanide, local mass transport coefficient was calculated.
2.4 Prevention, control and mitigation of MIC

With the aging pipelines, especially when water wetting of pipe wall occurs, the risk of MIC has drastically increased. More and more MIC cases have been reported. The desire to prevent, control and mitigate MIC is of significance in industries such as food, water treatment and the oil and gas.

In the oil and gas industry brush scraping or pigging has been routinely used to clean the pipelines; however, its cost and the interruption to production limit its use. Cathodic protection (CP) is popular in industry to protect pipelines, bridges and instruments from external corrosion attacks. Videla (1996) reported that 0.950 v (vs. Cu/CuSO₄ reference electrode) is necessary to prevent corrosion in the presence of SRB. A review by de Romero et al. (2009) indicated that 0.950 v is insufficient to protect the MIC due to SRB and stated that once the biofilm attached, CP may not be effective in preventing corrosion and, specifically, localized corrosion. Some reports (de Romero et al., 2009; Gu & Xu, 2010) mentioned that impressed current actually promotes SRB growth.

Beneficial bacterial biofilms (BBBs) have been proposed as a means to inhibit MIC. Zuo (2007) summarized the possible mechanisms for such inhibition: (1) removal of corrosive substances by bacterial activities; (2) antimicrobials generated by certain bacteria to suppress corrosive bacteria such as SRB growth; (3) formation of protective biofilm. This strategy provides an environmentally friendly corrosion control approach; however, it is far from mature and abundant research is needed to develop this technology before field practice. It must be noted that there is some evidence showing
BBB may become corrosive when environmental conditions change. Thus, BBB is only useful for strictly controlled environments or special settings.

Biocides are widely used to control and mitigate MIC. They can be divided into two groups: oxidizing chemicals such as ozone and non-oxidizing chemicals. Non-oxidizing biocides are normally preferred in industry such as the oil and gas industry where there are concerns of unintended chemical corrosion caused by biocides (Laopaiboon et al., 2006). Among the popular biocides used in oil fields are quaternary ammonium compounds (QAC), bromo-nitropropanediol (BNPD), glutaraldehyde and tetrakis hydroxymethyl phosphonium sulfate (THPS). Glutaraldehyde and THPS are the most widely used due to their broad-spectrum and biodegradable properties.

Figure 2-3 illustrates their structures. Their mechanisms of action as a biocide are different: glutaraldehyde mainly blocks cell walls by cross-linking the amino groups (Denyer, 1995; Russell, 2002; Greene et al., 2006; Laopaiboon et al., 2006) while THPS with its biologically active phosphine disrupts the disulphide bonds in cell proteins and enzymes (Ballantyne & Jordan, 2004).

![Glutaraldehyde and THPS structures](image-url)

Figure 2-3. Glutaraldehyde and THPS structures
Biofilms are known to protect sessile bacteria from biocide attacks acting as a mass transfer barrier (Denyer, 1995; Morton et al., 1998). Stoodley et al. (1999) reported that mass transfer resistance was increased by denser biofilms. Furthermore, bacterial metabolic activity decreased due to the limited nutrition supply and shorter exposure to biocides, increasing its resistance to biocides overall. Meanwhile, physiology of sessile bacteria may also be changed due to bacterial adaptation to biocides (Morton et al., 1998; Fux et al., 2005). It is believed that 10x or higher dosage of a biocide is required to kill the sessile bacteria than what is required for treating planktonic bacteria (Davies, 2003; Meyer, 2003). With increasing concern over the environment and tightened regulations in recent years, a more effective and environmentally benign biocide treatment is desired. Methods to enhance the biocide applications include: (1) utilization of agents to enhance the cell permeability; (2) modification of the chemical structure of a biocide for better delivery; (3) combination of biocides with complementary mechanisms of action (Denyer, 1995).

One of the novel techniques to enhance the biocide efficacy is to utilize chelators with biocides. The real mechanism for chelator enhanced biocide treatment is not certain yet. It is believed that by chelating ions such as manganese, zinc, iron, bacterial metabolism and biofilm growth will be affected (Dunne & Burd, 1992). Calcium is essential for bacteria to bond polymer molecules in biofilms (Carpentier & Cert, 1993). Hence, bacterial adhesion is sensitive to chelators. Iron chelators were found to suppress biofilm formation (Weinberg, 2004). Taweechaisupapong & Doyle (2000), Banin et al.
(2006) and Chudzik et al. (2007) pioneered the utilization of chelators combined with other antivirus agents to sanitize medical instruments.

Raad and Sherertz (2001) patented the use of a lock solution consisting of ethylenediaminetetraacetic acid (EDTA) that is a chelator and an antibiotic to eradicate biofilms on catheters for cancer patients. Raad et al. (2003) found that EDTA combined with minocycline is very effective in eradicating biofilms on catheter surfaces and the cocktail treatment is further improved by adding 25% (v/v) ethanol (Raad et al., 2007). The new triple combination achieved efficacy in removing biofilms on catheters after 15 min of exposure.

EDTA has long been used as a chelator in many industries. It biodegrades slowly but may accumulate in the environment after it is discharged (European Commission, 2004). Alternative chelators have been developed including ethylenediaminedisuccinate (EDDS) and N-(2-Hydroxyethyl)iminodiacetic acid disodium salts (HEIDA). EDDS was claimed to be a readily biodegradable chelator (Schowank et al. 1997). Jaworska et al. (1999) reported that the degradation or mineralization (the process of conversion of organic substance to carbon dioxide) half-life of [S,S]-EDDS in soil is two-three days. Based on a die-away test, it is, indeed, 6.3 days in unacclimated river water. HEIDA has an OECD (Organization for Economic Co-operation and Development) 306 value of 89% in 28 days. It reaches passing level (60%) within 20 days (Dow Chemical Co. technical report XUS40855.01).

D-amino acid mixtures at nanomolar concentrations (~10 nM) were recently found to be able to prevent biofilm formation and trigger biofilm disassembly (Kolodkin-
Gal et al., 2010). Although exogenous D-amino acid was found to inhibit bacterial growth in the 1940s, before Cava et al. (2011) it did not receive much attention because of the high concentration dosage. The authors reviewed the D-amino acids’ role in regulating stationary phase cell wall remodelling and biofilm disassembling as signaling molecules. Its application on mitigating membrane biofouling and promoting biofilm detachment using D-tyrosine was soon confirmed (Xu & Liu, 2011). They suggested it may be caused by the release of amyloid fibers which function as clue to link cells in the biofilm together. Sanchez et al. (2014) found that D-amino acids were not effective in enhancing biocide mitigation on planktonic bacteria, which indicates that sessile bacteria might not develop resistance to such D-amino acids. Since D-amino acid is naturally produced by many bacteria, it shows promise in field applications with a good environmental profile.

2.5 Iron sulfide film

Ferrous sulfide is the main corrosion product in MIC of steel due to SRB. Shoesmith et al. (1980) investigated the formation of iron sulfides on the steel in aqueous H$_2$S. The different formations of ferrous sulfide, varied from mackinawite, greigite, smythite and pyrrhotite, determine if the film is either protective or non-protective. Videla et al. (2005) reviewed that with increased ferrous ion concentration, the film transformed from adherent and continuous film to looser film, and, thus, higher corrosion rates were observed.

Lee and Characklis (1993) compared the results in the presence and absence of biofilms using a chemostat and reported that the metal surface maintained its scratch line
when the formation of ferrous sulfide film was intentionally prevented before biofilms accumulated, while visible localized corrosion was observed when the formation of FeS was formed before the biofilm. Liu et al. (2000) compared three kinds of films, SRB biofilm, FeS film using chemosynthesis and a semi-permeable membrane separating SRB in the media from coupon surfaces. They found the corrosion rate on coupons with SRB biofilm was larger than with the other two films.

Herein the role of ferrous iron is emphasized. Lee and Characklis (1993) observed that no corrosion was detected in an iron-free medium. Meanwhile, Marchal et al. (2001) found that SRB grew well at pH of 6.0, but not at pH of 7.4 in Fe$^{2+}$-free medium containing mild steel coupons, which confirmed the importance of ferrous iron for the SRB growth. Hence, they stated that 0.5 μM of ferrous ion is required for SRB growth.

As previously mentioned in Section 2.2.2, King and Miller (1971) addressed the role of FeS in MIC. Lee and Characklis (1993) concluded that the product of ferrous sulfide “caused” MIC. However, this is questionable. Sulfide is the ultimate reduction product and it cannot accept any electrons and be reduced furthermore (Gu, 2012a). There must be some other chemicals like oxygen or sulfate involved in this reaction serving as terminal electron acceptor (Gu, 2012a). In strictly anaerobic SRB corrosion systems, a porous FeS film allows more sessile SRB cells to attach. Because the film is semi-conductive, it provides a large cathodic surface area where SRB sessile cells can harvest electrons. In a strictly anaerobic condition without the presence of an utilizable oxidant, FeS corrosion is questionable in the absence of an electron acceptor. It was
likely that oxygen was not completely eliminated in many SRB experiments, especially in experiments involving flow.

### 2.6 Methodology for MIC study

MIC is a complex process requiring multidisciplinary collaborations including microbiology, chemistry, biochemical engineering. While a number of questions require answers in this area, new analytical, microbiological, electrochemical and microscopic techniques have been developed in lab research and field application.

#### 2.6.1 Flow loop

In lab research, batch experiments are easily operated and controlled. However, metabolic products will accumulate and inhibit cell growth, which differs from field practice such as pipeline flow. To simulate MIC in pipeline, continuous flow loops are normally used. Lee and Characklis (1993) used a flow loop studying corrosion of mild steel under anaerobic biofilm. Nitrogen was continuously purged during experiments to minimize air permeation. Flow velocity was around 0.3 m/s with a pump recycling the effluent. Electrochemical analysis was carried out via a potentiostat. Two points must be addressed for the flow loop with recirculation: (1) microbes in the fluid may be damaged during recycling at a fast flow rate; (2) the metabolic by-products are also recycled during this process, which is a significant deviation from field conditions. Hence, while a flow loop with open end is preferred, the high cost hinders its application in a long run in a lab at a high flow velocity, e.g. 1m/s, which is typical for the oil and gas industry.
2.6.2 Glass cell

A glass cell equipped with a rotating cylinder is a widely used device in electrochemical study in corrosion and MIC (Nesic et al., 1996; Mansfeld, 2003). Coupled with a potentiostat, electrochemical analysis such as open-circuit or corrosion potential $E_{\text{corr}}$, polarization resistance and EIS (Electrochemical impedance spectroscopy) can be carried out. The flow effects on the corrosion mechanism can be studied by varying the rotation speed.

Electrochemical noise (EN) measurement, a method to indicate the localized corrosion, has been reported as promising to investigate pitting corrosion (Kearns et al., 1996). Mansfield (2003) reviewed the applications of EN in studies of MIC and stated that the statistical parameters, like Localization Index (LI), should be used with caution to determine the prevailing corrosion mechanism, where LI is defined as the standard deviation of current divided by the root mean square of the current fluctuation.

More importantly, the rotation speed can be related to pipe flow velocity based on mass and momentum simulation between glass cell and pipe flow, which makes the laboratory glass cell experiments meaningful for field applications (Silverman, 1984, 1988, 2004 & 2005).

The shear stress $\tau$ on the surface of rotating cylindrical coupons can be calculated using the following equation (Silverman, 1984):

$$\tau_{\text{cyl}} = 0.079 \, \text{Re}^{-0.30} \, \rho (\omega r)^2$$

where

$\omega$: rotation speed (radians/s);
r: cylindrical coupon diameter (cm);

ρ: density (g/cm³);

Re: Reynolds number.

By assuming the surface to be hydraulically smooth for both pipe and rotating cylinders, Silverman (1988) also proposed an equation to correlate the rotation speed of cylindrical coupons in a glass cell with the average linear velocity in pipe flow:

\[ u_{cyl} = 0.1185 \left( \frac{\mu}{\rho} \right)^{-0.25} \left( \frac{d_{cyl}^{3/7}}{d_{pipe}^{5/28}} \right) Sc^{-0.0857} u_{pipe}^{5/4} \]  (2-25)

where:

cyl: rotating cylinder electrode;

pipe: pipe;

\( \mu \): fluid viscosity (g/cm/s);

\( u_{cyl} \): peripheral linear cylinder velocity (cm/s);

\( u_{pipe} \): the mean pipe flow velocity (cm/s);

Sc: Schmidt number (\( \mu/(\rho D) \)).

Figure 2-4 is the correlation between cylinder rotation rate and pipe velocity. The viscosity, density, Schmidt number and diameter of cylindrical coupon used in calculation are 0.01 poise, 1 g/cm³, 1000, and 1.2 cm, respectively.
While $u_{cyl}$ is set equal to $u_{pipe}$ to achieve the wall stress similarity, Equation (2-25) can be rearranged to the following equation (Silverman, 2005) to satisfy the relationship between the pipe and the rotating cylinder electrode (RCE):

$$d_{cyl} = \left[ 8.442d_{pipe}^{0.1786}Sc^{0.0857}\left(\frac{\mu}{\rho}\right)^{0.25}u_{pipe}^{-0.25}\right]^{2.333} \tag{2-26}$$

However, the above correlations are built based on an assumption of hydraulic smoothness. For rough surface like metal surfaces with thick biofilms, modification to the above equations should be made. The details for this will be discussed in Chapter 3.

### 2.6.3 Cell count

Cell count is used to determine the concentration of cells in suspension, which is important for characterizing the activity of microorganisms. Generally it can be divided
into: direct count or estimations of cell numbers. The latter, which includes determinations of turbidity, metabolic activity and dry mass (Tortora et al., 2002), will not be discussed in this work.

Direct microscopic count can determine the concentrations of cells in suspension under microscope using a hemocytometer (Figure 2-5). To better visualize cells or distinguish live cells from dead cells, stains such as crystal violet and Nile blue are usually applied. By counting the cell numbers in the four corners and the middle squares, within the big square (containing 5 × 5 squares) dimension of 1 mm × 1 mm and 0.1 mm sample depth, the concentration of suspension is then equal to the cell numbers × 5 × 10⁴ cells/ml. The count is easily obtained, but only appropriate for pure strains. When a consortium or sessile bacteria are present, other methods must be employed. The Most Probable Number (MPN) method is a statistical estimating technique. By serial dilutions until no bacteria remains in the tubes, the cell number can be calculated from an MPN table based on the numbers of positive tubes in the set (Tortora et al., 2002). Each set can have 3, 5, or more replicates. MPN results in a probable estimate, detecting only viable organisms. This labor-intensive procedure and cost may limit its application. This method may also underestimate the bacteria population because of the difficulty of culturing for wild bacteria. Vester and Ingvorsen (1998) presented an improved MPN method to detect SRB by using natural media and a radio-labeled tracer of ³⁵SO₄²⁻. The results showed a 100-fold to 1000-fold higher than the normal MPN value.
With recent advances in molecular biology, novel methods have been applied to identify and quantify environmental bacteria, the large part of which cannot be cultivated. PCR (polymerase chain reaction), discovered by Kary Mullis in 1983, has introduced novel innovations in molecular biology, genetics and biochemistry (Walker, 2002). It is a powerful, sensitive and automatic tool. It was reported that by “real time” quantitative PCR (qPCR), a single *Bacillus anthracis* spore was detected in 100 liters of air (Makino & Cheun, 2003). For SRB, dissimilatory sulfite reductase (DSR) is the primary enzyme in dissimilatory sulfate reduction. By determining the copy numbers of the dsrA gene coding for the α-subunit, qPCR was used to quantify the bacteria (Kondo *et al.*, 2008). This method has been applied to the oil and gas industry (*Zhu et al.*, 2005; Larsen *et al.*, 2006).
In the OU biocorrosion Lab, Zhao (2008) used qPCR to detect and quantify SRB in natural seawater.

### 2.6.4 Surface visualization and analysis

In MIC research, the visualization of biofilm structure and spatial information is of great concern as well as the corrosion pitting area, depth and distribution. Scanning electron microscopy (SEM) is widely used to analyze the coupon surfaces and compositions with high resolution when coupled with EDAX or EDS (energy-dispersive analysis of X-rays) (Beech, 2004; Videla & Herrera, 2005). Transmission electron microscopy (TEM) and SEM require sample dehydration, which may cause the loss of information of the structure. Environmental scanning electron microscopy (ESEM) and atomic force microscopy (AFM) require partly dehydrated samples, which provide information that is closer to real hydration. Confocal laser scanning microscopy (CLSM) is a novel procedure used for visualizing biofilm without biofilm damage (Neu, 2000).

To determine the chemical compositions on the surface, biofilm and abiotic films included, EDAX, XRD (X-ray diffraction) and XPS (X-ray photoelectron spectroscope) are generally used. Elemental distribution over the surface and composition information can be obtained (Beech, 2004; Videla & Herrera, 2005; Li et al., 2016).

For detailed pitting corrosion observation, information with high resolution can be obtained via SEM or AFM; however, the depth and density of pitting cannot be easily quantified through such methods. Laser profilometry can measure, visualize, and analyze the topography associated with the specimens, and give the pit depths, pit shapes and the volume damage rate (Patella et al., 2000; Koul, 2003).
CHAPTER 3 INVESTIGATION OF SRB BIOFILM

3.1 Introduction

As pointed out in Chapter 1, sessile bacteria in biofilms, not planktonic cells, contribute directly to MIC. It is often found that in practice MIC occurs with stagnant fluids remaining in the pipeline as well as at flow conditions. At high shear stress, biofilms may be detached and then MIC is inhibited. A study between flow rate and MIC was conducted in this work.

An electrochemical apparatus called a rotation cylinder glass cell is widely used in the study of corrosion in flow conditions. Based on the similarities of wall shear stress and mass transfer coefficient between rotation coupon and pipeline, the rotation speed of coupons in the glass cell can be correlated to linear velocity in pipeline flow, which is a major focus for field engineers (Silverman, 1984, 1988, 2004 & 2005). A surface with biofilms, usually 20-200 µm or more thickness, may not be suitable for the assumption of hydraulically smooth surfaces for the Silverman correlation and a modification was made to account for rough surface.

The synergistic effects of underdeposit attack and MIC recently drew attentions due to the Alaskan pipeline leak in March 2006 (Jacobson, 2007). Old transit lines with a decreased flow rate can accelerate sand deposition and also possibly biofilm buildup in low flow regions or stagnant deadlegs. While the penetration of corrosion inhibitors will be hindered by sand deposits, the stagnant condition in deadlegs will trap water because the water will separate from oil if the flow rates are too low during multiphase flows. Deadlegs also trap nutrients and provide surfaces that allow a synergistic microbial
consortium to flourish. The bacteria in the consortium produce metabolites such as organic acids, inorganic acids, ammonia, and sulfides. These by-products cause corrosion when their local concentrations become sufficiently high. However, MIC researchers have long been troubled in lab experiments due to the inability to reproduce field conditions in pipe flows that have linear velocities often exceeding 1 m/s. This kind of high velocity in an open MIC flow loop makes the cost of culture medium prohibitive unless it is recycled or partially recycled. Meanwhile, a recycling pump at a fast flow rate will damage the microbes in the fluid. Recycle also makes the microbial growth conditions deviate from field conditions due to metabolite buildup. Since the region near the bottom of a deadleg is often a no-flow or low flow region, it is possible to design an open flow loop for MIC investigation in deadlegs using small flow rates. In this work, a mass transfer similarity computer simulation was employed to expand low velocity MIC lab results for fast flow field conditions using a computational fluid dynamics software FLUENT.

3.2 Objectives

- To investigate the effect of flow rates on SRB biofilm growth and corresponding MIC;
- To correlate the rotating coupon glass cell results to pipeline flow on the rough surface;
- To expand low velocity MIC lab results for fast field conditions using computer simulation.
3.3 Experimental setup

The investigation of flow rates on SRB biofilm growth was carried out in a 2-liter glass cell with a rotating shaft in the center (Figure 3-1). A C1018 cylindrical coupon was mounted on the shaft and served as a working electrode. The coupon had an outer diameter of 1.20 cm and an exposed surface area of 5.40 cm$^2$. Electrochemical measurements were conducted using a Gamry potentiostat PC4. Ag/AgCl electrode served as the reference electrode which was connected to the glass cell via a Luggin capillary. Before each run, glass cell parts were autoclaved at 121ºC for 40 min; heat-sensitive parts such as pH probe and Luggin capillary were sterilized using 70% isopropyl alcohol. The coupon was polished using 200, 400, 600 grit abrasive paper, successively. It was then sterilized using pure isopropyl alcohol and UV light for 20 min before use. It should be noted that this glass cell system was very difficult to keep a strictly anaerobic condition without continuous N$_2$ sparging. The sparging tended to remove H$_2$S from the culture medium and thus increased the SRB broth pH.
Figure 3-1. Schematic of an electrochemical glass cell bioreactor: (1) reference electrode, (2) temperature probe, (3) Luggin capillary, (4) working electrode, (5) hot plate, (6) gas output, (7) bubbler for gas, (8) pH electrode, and (9) counter electrode. (Figure courtesy of Daniel Mossier at Ohio University, 2004.)

*D. vulgaris* ATCC®7757 strain (previously known as *D. desulfuricans* subsp. *Desulfuricans*) was used in this research. ATCC 1249 medium was used for culturing SRB. In the test, modified ATCC 1249 medium (Table 3-1) was used by reducing the amount of Fe(NH₄)₂(SO₄)₂ from 200 ppm to 25 ppm to diminish the interference by over-precipitated FeS. 1.8 to 2.0 liters of sterilized medium was poured into the glass cell with an additional 0.5 g/l of cysteine as the oxygen scavenger. It was then sparged with
filtered N₂ for at least 45 min. During experiments, small amounts of N₂ were continuously fed into the glass cell to maintain a positive overhead pressure. The medium temperature was maintained at 37°C using a hot plate. Linear polarization resistance (LPR) and open potential were monitored by a Gamry software in addition to coupon weight loss measurement. Planktonic SRB numbers were counted under an optical microscopy using a hemocytometer. After the experiment was completed, coupon cross sections and biofilm morphology were checked under SEM. Before SEM observations for biofilm morphology, the wet coupons were prepared by critical point drying as follows: Firstly the coupons were exposed to 4 wt% of glutaraldehyde for one hour and then washed with ethanol using a series of 30%, 50%, 70% and 100% v/v successively to dehydrate the cells gradually. Then the coupons were critical point dried. To increase the conductivity for better observation under SEM, the coupons were gold-coated before the SEM observation (Wen et al., 2009). Coupon surfaces were then cleaned using the Clark’s solution according to the ASTM G1-03 standard and, again, checked under SEM for pitting.
Table 3-1. Composition of modified ATCC 1249 medium for SRB

<table>
<thead>
<tr>
<th>Component</th>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component I</td>
<td>MgSO₄</td>
<td>2.0 g</td>
</tr>
<tr>
<td></td>
<td>Sodium Citrate</td>
<td>5.0 g</td>
</tr>
<tr>
<td></td>
<td>CaSO₄</td>
<td>1.0 g</td>
</tr>
<tr>
<td></td>
<td>NH₄Cl</td>
<td>1.0 g</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>400 ml</td>
</tr>
<tr>
<td>Component II</td>
<td>K₂HPO₄</td>
<td>0.5 g</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>200 ml</td>
</tr>
<tr>
<td>Component III</td>
<td>Sodium Lactate</td>
<td>3.5 g</td>
</tr>
<tr>
<td></td>
<td>Yeast Extract</td>
<td>1.0 g</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>400 ml</td>
</tr>
<tr>
<td>Component IV</td>
<td>Fe(NH₄)₂(SO₄)₂</td>
<td>0.127 g</td>
</tr>
</tbody>
</table>

3.4 Results and discussion

3.4.1 Modification of correlation between rotation speed in glass cell and average flow velocity in a straight pipe with rough surface

For flow in the horizontal section of a pipe, the wall shear stress ($\tau_{pipe}$) can be expressed as following:

$$
\tau_{pipe} = \frac{\Delta p_l d_{pipe}}{4}
$$

(3-1)

where $\Delta p_l$ is the pressure drop per unit pipe length and $d_{pipe}$ is the pipe inner diameter. $\Delta p_l$ can be calculated from friction factor $f$ and average linear velocity $u_{pipe}$:

---

1 This section has been published as a NACE conference paper, CORROSION/2007, Paper No.07516.
where \( \rho \) is the fluid density. Substituting Equation (3-2) to Equation (3-1), \( \tau_{pipe} \) can be obtained:

\[
\tau_{pipe} = \frac{f}{8} \rho u_{pipe}^2
\]  

The friction factor \( f \) was obtained from literature (Fox & McDonald, 1992; Shames, 2003). Conveniently, in turbulent flow, the explicit equation of \( f \) can be calculated as below (Shames, 2003):

\[
f = \frac{0.25}{\left[ \log \left( \frac{\varepsilon}{3.7d_{pipe}} + \frac{5.74}{\text{Re}^{0.8}} \right) \right]^{2}}
\]  

where \( \text{Re} \) is Reynolds number \( (\text{Re} = \frac{\rho <u>_d}{\mu}) \);

\( \mu \) is the fluid viscosity;

\( <u> \) is average linear velocity of pipe flow;

\( \varepsilon \) is the pipe roughness.

The formula is valid for \( 5 \times 10^3 \leq \text{Re} \leq 10^8 \) and \( 10^{-6} \leq \varepsilon/d_{pipe} \leq 10^{-2} \).

Rather than using the empirical correlation for the shear stress on the surface of a rotating cylindrical coupon in a glass cell \( (\tau_{cyl}) \) proposed by Silverman (1984), when surface roughness is considered, \( \tau_{cyl} \) was obtained using the correlation by Sedahmed et al. (1979):

\[
\tau_{cyl} = 0.714\text{Re}^{-0.39}(\varepsilon/d_{cyl})^{0.2}p(u_{cyl})^2
\]  

where:

\[
\tau_{cyl} = 0.714\text{Re}^{-0.39}(\varepsilon/d_{cyl})^{0.2}p(0.5\omega d_{cyl})^2
\]
where \( Re \) is Reynolds number in glass cell;

\[
d_{\text{cyl}} \text{ is the coupon diameter, cm;}
\]

\[
u_{\text{cyl}} \text{ is the linear velocity on the cylindrical coupon surface, cm/s;}
\]

\( \omega \) is angular velocity, radians/s.

Shaw and Hanratty (1977) suggested that the mass transfer coefficient in fully developed pipe flow can be described as:

\[
k_{\text{pipe}} = 0.0889 \sqrt{\frac{\tau_{\text{pipe}}}{\rho}} S_{\text{c}}^{-0.704}
\]

where \( S_{\text{c}} \) is the dimensionless Schmidt number \( (S_{\text{c}} = \frac{\mu}{\rho D}) \), from 693 to 37,200;

\( D \) is the diffusion coefficient, m\(^2\) s\(^{-1}\);

\( k_{\text{pipe}} \) is mass transfer coefficient, m\( \cdot \)s\(^{-1}\);

\( \tau_{\text{pipe}} \), kg\( \cdot \)m\(^{-1}\)\( \cdot \)s\(^{-2}\);

\( \rho \), kg\( \cdot \)m\(^{-3}\);

\( \sqrt{\frac{\tau_{\text{pipe}}}{\rho}} \), so-called friction velocity, m\( \cdot \)s\(^{-1}\).

Meanwhile, according to Silverman (1988) the mass transfer coefficient for a rotating cylinder in a glass cell follows the equation:

\[
k_{\text{cyl}} = \frac{\tau_{\text{cyl}}}{\rho u_{\text{cyl}}} S_{\text{c}}^{-0.644}
\]

which is valid for \( 200 < Re < 1.5 \times 10^5 \) and \( 3 < S_{\text{c}} < 30,000 \).

By equating Equation (3-6) and Equation (3-7) for mass transfer coefficient similarity in pipe and annular flows, the relationship between the linear rotation velocity of the cylindrical coupon and the average linear velocity in pipe flow for different pipe diameters \( (d_{\text{pipe}}) \) can be expressed as follows (Silverman, 1988 and 2005):
\[ u_{cyl} = 0.1185 \left[ \left( \frac{\mu}{\rho} \right)^{-0.25} \left( \frac{d_{cyl}^{3/7}}{d_{pipe}^{5/28}} \right) Sc^{-0.0857} \right] u_{pipe}^{5/4} \]  

(3-8)

where \( \mu \) is in poise;

\[ \rho, \text{ kg} \cdot \text{m}^{-3}; \]

\( d_{cyl} \) and \( d_{pipe}, \text{ m}, \)

\( u_{cyl} \) and \( u_{pipe}, \text{ m} \cdot \text{s}^{-1}. \)

Since rotation speed in rpm is more often used, the following formula was employed to convert the angular velocity \( \omega \) (radians/s) to tangential linear velocity \( u \) (m·s⁻¹):

\[ u = \omega \cdot r = \frac{2\pi n}{60} \cdot r \]  

(3-9)

where \( n \) is the rotation speed (rpm) and \( r \) is the radius of the cylindrical coupon (m). Figure 3-2 shows the relationship between rotation speed of the cylindrical coupon and average linear velocity of the pipe flow based on Equation (3-8).
Figure 3-2. Correlation between rotational speed in glass cell and average flow velocity in a straight pipe based on Equation (3-8). (Sc=1,000, μ=0.01 poise, ρ=1 g/cm³, d=1.2 cm.) (Wen et al., 2007)

When $u_{cyl}$ is set to be equal to $u_{pipe}$ and Equation (3-8) is rearranged, according to Silverman (2005) it becomes:

$$d_{cyl} = \left[ 8.442d_{pipe}^{0.1786}Sc^{0.0857}\left(\frac{\mu}{\rho}\right)^{0.25}u^{-0.25} \right]^{2.333} \quad (3-10)$$

By plotting Equation (3-10) using typical parameter values, the correlation between the rotating cylindrical coupon diameter in the glass cell and the average flow velocity in a straight pipe is shown on Figure 3-3. By simultaneously taking simulation of shear stress and mass transfer, the required rotating cylindrical coupon diameter can be estimated.
when the pipe diameter and flow velocity are known. This correlation indicates that the lab research results in a glass cell can be applied, in practice, to pipe flow.

Figure 3-3. Correlation between rotating cylindrical coupon diameter in glass cell and average flow velocity in a straight pipe based on Equation (3-10). (Sc=1,000, μ=0.01 poise, ρ=1 g/cm$^3$, d=1.2 cm.) (Wen et al., 2007)

3.4.2 Flow effects on SRB biofilm growth and corrosion rate

Experiments were carried out in the glass cell with varied rotation rates of 0, 1000 and 3000 rpm. Figure 3-4 shows the comparison of potentiodynamic sweeps for SRB growth on a C1018 steel surface in ATCC 1249 medium at 37ºC. $\beta_a$ and $\beta_c$ were obtained as 55 mV/decade and 250 mV/decade respectively. The potentiodynamic sweeps indicate

---

2 Part of this section has been published in a NACE conference paper, CORROSION/2006, Paper No.06666.
that the corrosion process is in charge control and insensitive to velocity. However, Figure 3-5 suggests some effects of flow rates on the corrosion rates obtained by LPR. Their corresponding weight losses were 0.061 g for the static test; 0.27 g for the 1000 rpm test and 0.115 g for the 3000 rpm test, respectively. Mild agitation at 1000 rpm caused the highest corrosion rate as well as the largest pitting diameter (Figure 3-6). When the rotation rate was raised to 3000 rpm, the corrosion rate and pitting diameter decreased, but were still higher than in stagnant conditions. It has to be mentioned that LPR is a method to measure the uniform corrosion. Thus, it can be used to compare with weight loss derived corrosion rate (using Tafel constants calibrated for a specific MIC system), but not pitting rate in corrosion mechanism and biocide efficacy studies (Jia et al., 2017b & 2017c). The spikes in corrosion rate in Fig. 3-5 were unusual. In this work, there was a possibility of Ag/AgCl reference electrode poisoning by sulfides in the glass cell. A sulfide resistant electrode or a good salt bridge is recommended for future work.

In a glass cell with a rotating cylinder coupon (Figure 3-1), it was difficult to prevent oxygen ingress without nitrogen sparging. However, the sparging removed some H$_2$S from the culture medium and this elevated its medium pH as discussed by Jia et al. (2017a) recently. It is recommended that in the future, the entire setup should be placed in an anaerobic chamber without relying on continuous nitrate sparging.

While MIC is typically a localized corrosion, as Figure 3-6 suggests. In this work, weight loss and pitting images were primarily used to characterize MIC due to SRB. LPR was not used as a primary indicator.
Figure 3-4. The comparison of potentiodynamic sweeps for SRB growth on a C1018 steel surface in ATCC 1249 medium at 37°C.

Figure 3-5. LPR measurements for the glass cell experiments at three different rotation rates. Initial cell counts were $1.0 \times 10^5$ cells/ml (for 0 rpm), $1.1 \times 10^5$ cells/ml (for 1000 rpm) and $1.0 \times 10^5$ cells/ml (for 3000 rpm), respectively. (Wen et al., 2006a)
As mentioned in Chapter 1, oxygen is an important factor to cause corrosion. When carrying out glass cell experiments, especially in high speed rotation, oxygen leaking is possible. A continuous bubbling of nitrogen into the system can maintain a small positive pressure, avoiding the oxygen leaking. Duplicate experiments should be employed to validate any pitting attack. Bubbling may strip H₂S out of the medium and thus causes increased pH in the medium.

The planktonic cell count (Figure 3-7) shows that at 1000 rpm rotation rate, SRB grew the fastest and reached the stationary phase 2-3 days earlier, although there was little difference among them on Day 4 and afterward. The planktonic cell growth was obviously affected by coupon rotation. Although the coupon and shaft in this glass cell may not be an efficient impeller for agitation, the medium indeed was well agitated. Checked by BioSan Sani-Check® Product 100 test kits, sessile SRB concentration was found between $1.9 \times 10^5$ and $1.9 \times 10^6$ cells/cm² on the coupon surface at rotation rate of 1000 rpm.
By repeating the experiment running at 0, 1000, 3000 rpm, separately, the morphologies of SEM biofilm on the coupon surfaces were obtained (Figure 3-8 to Figure 3-10). After a five-day test, peanut-shaped sessile SRB were clearly visible on coupon surfaces at a stagnant condition and a rotating rate of 1000 rpm while it was difficult to detect any sessile SRB at a rotating rate of 3000 rpm. The coupon surface was smooth with broken films where bare metal base was exposed (Figure 3-10). Using the Silverman (1988) correlation, the rotation rate of 3000 rpm in the glass cell can be correlated to a pipe velocity of 3.5 m/s for 10” pipeline. However, the high flow rate might prevent SRB adhesion and biofilm growth and cause the detachment of biofilms, resulting in a lesser MIC attack.
Figure 3-8. SEM biofilm images on coupon surface for 0 rpm rotation rate. (Wen et al., 2006a)

Figure 3-9. SEM biofilm images on coupon surface for 1000 rpm rotation rate. (Wen et al., 2006a)

Figure 3-10. SEM biofilm images on coupon surface for 3000 rpm rotation rate. Sessile SRB were hard to find. (Wen et al., 2006a)
Figure 3-11. The comparison of cross section of film on C1018 at different rotation rates: (a) 1,000 rpm; (b) 3,000 rpm. (Wen et al., 2006a)

Figure 3-11 shows a cross section comparison of coupon surfaces between 1000 rpm and 3000 rpm. The metal surface was much more rugged at 1000 rpm with thicker film coverage than the one at 3,000 rpm, which indicates more severe corrosion.

As mentioned in Chapter 2, biofilm thickness decreased with increasing shear stress when the substrates were continuously loaded, and increased with nutrient concentration when shear was held constant (Characklis, 1981 & 1990). Because this work was a batch test, the substrates were consumed and their concentrations decreased with time. Therefore there was a maximum biofilm thickness when flow rate increased. High flow rate prevented SRB attachment on the metal surface. Without sessile SRB, the corrosion of metal at high flow rate was more likely due to H₂S corrosion and erosion.

3.4.3 Mass transfer simulation for expansion of low flow MIC results to fast flow pipeline situations

3.4.3.1 Modeling Biofilm Kinetics

The equations describing the biofilm system are listed below:
- Total mass conservation (continuity equation) for an incompressive fluid:

\[ \nabla \cdot u = 0 \tag{3-11} \]

where \( u \) is the velocity, m/s.

- Momentum conservation for the fluid flow over the biofilm:

\[ \frac{\partial u}{\partial t} + u \cdot \nabla u = -\frac{1}{\rho} \nabla p + \nu \nabla^2 u \tag{3-12} \]

where

- \( \rho \): density, kg/m\(^3\);
- \( \nu \): kinematic viscosity, Pa·s;
- \( p \): pressure, Pa.

- Mass conservation for the limiting substrate (sulfate):

\[ \frac{\partial C_s}{\partial t} + u \cdot \nabla C_s = D \nabla^2 C_s \tag{3-13} \]

where

- \( D \): sulfate diffusion coefficient, m\(^2\)/s;
- \( t \): time, s.

### 3.4.3.2 CFD Simulation by FLENT

#### 3.4.3.2.1 Grid and mesh by Gambit

A 2D mesh was generated using software Gambit (Figure 3-12). The deadleg is located at the half size of the main pipe so that the flow is fully developed at the location of the deadleg.
3.4.3.2.2 \textit{Boundary conditions}

To simplify the simulation, the velocity distribution at the inlet is uniform. Pressure drop along the pipe is ignored. Temperature is kept at 37°C. Except for the bottom surface reaction where the SRB biofilms grow, no reaction occurs in the volume. Hence the boundary conditions can be written as below:

Inlet: \( y=0.00236 \)

Wall boundary at bottom of the deadleg:

\[
R_s(C_s, C_x) = \frac{\mu_{\text{max}}CX_f}{K_m + C}
\]  \hspace{1cm} (3-14)

where

\( C_s \): sulfate concentration at the biofilm surface, mol/m\(^3\);

\( C_x \): biomass, mol/m\(^3\);

\( R_s \): consumption rate of sulfate on the biofilm surface, mol/(m\(^2\)·s);

\( \mu_{\text{max}} \): maximum specific rate of substrate uptake, mol SO\(_4^{2-}\)/(kg·s);

\( K_m \): apparent half-saturation constant, mol/m\(^3\);

Figure 3-12. 2D mesh of the pipe and the deadleg: L/D=2, the total length of pipe=50D.
\( X_r \): biomass concentration within the reaction zone times the reaction zone thickness, kg/m².

The constants were calculated from Nielsen (1987): \( \mu_{\text{max}} = 3.89 \times 10^{-10} \text{ mol SO}_4^{2-} / (\text{kg} \cdot \text{s}) \); \( X_f = 4.8 \times 10^{-4} \text{ biomass kg/m}^2 \); \( K_m = 1.4 \times 10^{-3} \text{ mol/m}^3 \); the sulfate diffusion coefficient in water system was \( 8.27 \times 10^{-10} \text{ m}^2/\text{s} \).

A UDF (user defined function) macro using DEFINE_SR_RATE was hooked to FLUENT. The scale was adjusted to certain geometries. Convergence was monitored by the default value of 0.001 for each item such as continuity, x-velocity and y-velocity. The implicit method was used to solve the Navier-Stokes equation.

For laminar flow in lab-scale flow loop, the laminar model was selected; for turbulent flow in pipeline, the standard \( \kappa-\varepsilon \) model was applied. The model constants have the following default values: \( C_{1\varepsilon} = 1.44, C_{2\varepsilon} = 1.92, C_{\mu} = 0.09, \sigma_\kappa = 1.0, \sigma_\varepsilon = 1.3 \).

Figure 3-13 shows the simulation of turbulent flow of velocity of 1 m/s in a deadleg with \( L/D = 6, D = 1.016 \text{ m (40 in)} \). Near the biofilm, the mass fraction of sulfate is 0.0023, about 99% of the inlet fraction. The outlet of sulfate fraction is almost the same as the inlet. Since the sulfate consumption rate is quite slow, the convection term in Equation (3-13) is dominant. Therefore the mass distribution in deadleg changes little from the inlet.
Figure 3-13. Contour of mass fraction of \( \text{SO}_4^{2-} \) in the deadleg for the case of \( v=1 \text{ m/s}, L/D=6, D=1.016 \text{ m (40 in)} \).

The corresponding simulation of laminar flow of velocity of 0.0001 m/s in a deadleg with \( L/D=6, D=0.0254 \text{ m (1 in)} \) is shown in Figure 3-14. The mass fraction of sulfate near the biofilm is 0.00235, 99% of the inlet fraction. As Figure 3-14b illustrates, there is a mass distribution along the bottom of the deadleg, which is affected by the geometry and the flow rate in the main pipe. Comparing Figure 3-13 and Figure 3-14, the analogy between laminar flow in 0.0254 m (1 in) pipe and turbulent flow in 1.016 m (40 in) pipe based on the mass fraction simulation is possible.

Varying the \( L/D \) ratios, the analogy is still similar. For turbulent flow of velocity of 1 m/s in a deadleg with \( L/D=4, 2, 1, D=1.016 \text{ m (40 in)} \), variance of \( L/D \) caused little change in the local mass fraction of sulfate. For laminar flow in a deadleg with \( L/D=4, 2, 1, D=0.0254 \text{ m (1 in)} \), due to the slow sulfate consumption rate by SRB biofilm, there is only a slight difference among those conditions, which confirms that the surface reaction at the bottom of the deadleg is the limiting step.
b. contour of mass fraction of $\text{SO}_4^{2-}$

b. mass fraction of $\text{SO}_4^{2-}$ near the bottom surface of the deadleg

Figure 3-14. Contour of mass fraction of $\text{SO}_4^{2-}$ in the deadleg for the case of $v=0.0001\text{m/s}$, $L/D=6$, $D=0.0254\text{ m (1 in)}$.

Since flow rates also influences the biofilm attachment, growth and detachment, the velocity profile (Figure 3-15) is compared between the laminar flow at $0.0001\text{ m/s}$, $L/D=6$, $D=0.025\text{ m (1 in)}$ and the turbulent flow at $1\text{ m/s}$, $L/D=6$, $D=1.016\text{ m (40 in)}$.

Even for a high flow rate at the main pipe, the velocity near the bottom in the deadleg is small (Figure 3-15a). The shear stress influence on the biofilm is negligible.

a. contour of velocity at $1\text{ m/s}$, $L/D=6$, $D=1.016\text{ m (40 in)}$

b. contour of velocity at $0.0001\text{ m/s}$, $L/D=6$, $D=0.0254\text{ m (1 in)}$

Figure 3-15. Comparison of velocity profile with similar mass fraction near the bottom of deadleg at different flow conditions.
3.4.3.3 Flow Loop Design

Figure 3-16 shows a 1” ID flow loop with identical deadlegs distribution along the pipe that will be constructed and operated inexpensively. The pipe material will be CPVC with a maximum temperature tolerance of 200°F. For a 1” pipe, a 4.4 l/day corresponds to an average linear velocity of 0.1 mm/s. Conditions of blank control, sand only, bacteria only, and sand plus bacteria are produced in four different deadlegs that contain coupons at their bottoms. Each coupon holder resembles a plunger with three small disk coupons on top. The holder can be placed above or below the full-port ball valve. The valve will be closed after the holder is extracted if sampling is needed during a run.
3.5 Summary

- Using wall shear stress and/or mass transfer similarity simultaneously, correlation between the cylindrical coupon rotation rate in the glass cell and the average flow velocity in the pipeline was theoretically derived with rough surface taken into account.

Figure 3-16. Design of an open-ended multi-channel flow loop with 4 deadlegs.
• Mild agitation facilitated initial planktonic cell growth, while sessile SRB was hard to detect at high shear stress.

• The potentiodynamic sweeps indicated that the corrosion process of MIC due to SRB is in charge control and insensitive to flow velocity. However, when the flow rate is high enough to prevent SRB adhesion and biofilm growth and detach the biofilm, less MIC attack may occur.

• The mass transfer simulation for expansion of low flow MIC results to fast flow pipeline situation has been investigated by using FLUENT simulation in 2-D. Due to the low sulfate consumption rate by SRB biofilms, the analogy between laminar flow in a lab-scale pipe and turbulent flow in a field pipe based on the mass fraction simulation is possible. The design of using open-ended flow loop to study MIC is practical. Unlike the regular recirculation flow loop in previous MIC studies, the open-ended flow loop avoiding the recycling of metabolic byproducts and damage to cells due to high shear stress in the recycling pump, has its advantage for studying the real MIC in pipeline.
CHAPTER 4 BIODEGRADABLE CHEMICALS TO ENHANCE BIOCIDE PERFORMANCE

4.1 Introduction

Bacteria entrapped in the matrix glued by EPS are protected against environmental influences such as fluid shear and toxic chemicals. 10x or higher concentrations of biocides are generally required to remove sessile bacteria than to kill planktonic cells. Although there are other methods like smart pig or utilizing “good bugs” to compete with “bad bugs,” the primary mitigation method still relies on biocides. With increasing environmental concern and tightening regulations, a more environmentally benign treatment with relatively low cost is desired.

A mixture of EDTA, ethanol and antibiotics has been successfully applied to eradicate biofilms on catheters (Raad et al., 2003 & 2007). Due to its low biodegradability (European Union Risk Assessment Report, 2004), alternative chelators such as EDDS and HEIDA have been used in industry as replacements. In this chapter, biodegradable chemicals to enhance biocide performance against SRB were explored.

4.2 Experimental conditions

It should be stated that some of the experimental procedures have been included in those published journal papers (Wen et al., 2009, 2010 & 2012). Chemicals and bacteria used in this chapter are listed in Table 4-1.
<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chelators</td>
<td>EDTA</td>
<td>Fisher Scientific, Fair lawn, NJ, USA</td>
</tr>
<tr>
<td></td>
<td>EDDS</td>
<td>Octel Performance Chemicals (now Innospec in Ellesmere Port, Cheshire, UK)</td>
</tr>
<tr>
<td></td>
<td>HEIDA*</td>
<td>Sigma-Aldrich, St. Louis, MO, USA</td>
</tr>
<tr>
<td>Alcohol</td>
<td>methanol</td>
<td>Fisher Scientific, Pittsburgh, PA, USA</td>
</tr>
<tr>
<td></td>
<td>iso-propanol</td>
<td>Fisher Scientific, Pittsburgh, PA, USA</td>
</tr>
<tr>
<td>D-amino acids</td>
<td>D-tyrosine</td>
<td>Fisher Scientific, Fair lawn, NJ, USA</td>
</tr>
<tr>
<td></td>
<td>D-leucine</td>
<td>Fisher Scientific, Fair lawn, NJ, USA</td>
</tr>
<tr>
<td></td>
<td>D-tryptophan</td>
<td>Fisher Scientific, Fair lawn, NJ, USA</td>
</tr>
<tr>
<td></td>
<td>D-methionine</td>
<td>Fisher Scientific, Fair lawn, NJ, USA</td>
</tr>
<tr>
<td>Biocide</td>
<td>glutaraldehyde</td>
<td>Fisher Scientific, Pittsburgh, PA, USA</td>
</tr>
<tr>
<td></td>
<td>THPS</td>
<td>Rhodia, USA</td>
</tr>
<tr>
<td>Bacteria</td>
<td><em>D. vulgaris</em> ATCC 7757</td>
<td>ATCC</td>
</tr>
<tr>
<td></td>
<td>Desulfovibrio alaskensis</td>
<td>ATCC</td>
</tr>
<tr>
<td></td>
<td>ATCC 14563</td>
<td></td>
</tr>
</tbody>
</table>

*It was neutralized with sodium hydroxide to obtain a disodium salt before use.*
4.2.1 Chelators to enhance biocide inhibition of planktonic SRB

Two strains of SRB were used to evaluate the chelators’ performance of enhancement to biocides: one was *D. vulgaris* and the other was *D. alaskensis*, a marine strain. By comparison, the chelators’ effects in different media were evaluated.

To grow cell seed culture, the recommended ATCC 1249 medium and ATCC 1250 medium were used, respectively. In the subsequent tests the ATCC 1249 medium was modified by reducing the ferrous concentration to 25 ppm. Enriched artificial seawater (ASW) was used for ATCC 14563 growth since SRB grows too slowly for laboratory tests without enrichment. The enriched ASW contains synthetic seawater mix (Instant Ocean®, Aquarium Systems, Inc., Mentor, OH, USA) 36 g, Fe(NH$_4$)$_2$(SO$_4$)$_2$ 125 mg, sodium lactate (60 wt% syrup) 4.5 ml and yeast extract 1 g in 1 liter of water. This ASW is popular and Table 4-2 illustrates the comparison between ASW and the typical natural seawater (Atkinson and Bingman, 1996).

<table>
<thead>
<tr>
<th></th>
<th>Salinity (1000 ppm)</th>
<th>Na$^+$</th>
<th>Mg$^{2+}$</th>
<th>Ca$^{2+}$</th>
<th>K$^+$</th>
<th>Sr$^{2+}$</th>
<th>Cl$^-$</th>
<th>SO$_4^{2-}$</th>
<th>BO$_3^{2-}$</th>
<th>CO$_3^{2-}$</th>
<th>HCO$_3^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea-water</td>
<td>35</td>
<td>470</td>
<td>53</td>
<td>10.3</td>
<td>10.2</td>
<td>0.09</td>
<td>550</td>
<td>28</td>
<td>0.42</td>
<td>1.90</td>
<td></td>
</tr>
<tr>
<td>Instant Ocean®</td>
<td>29.65</td>
<td>462</td>
<td>52</td>
<td>9</td>
<td>9.4</td>
<td>0.19</td>
<td>521</td>
<td>23</td>
<td>0.44</td>
<td>1.90</td>
<td></td>
</tr>
</tbody>
</table>

*All measured in millimoles per kilogram. Typical seawater density is 1026 kg/l and pH 8.4.*
Tests were carried out in 100 ml vials. A general procedure is described below for vial tests:

1) Sterilize culture media by autoclaving at 121°C for 20 minutes.
2) Purge the media with filtered nitrogen for at least 45 min to remove dissolved oxygen.
3) During purging, adjust pH of the medium to 7.0.
4) After purging, transfer the media to an anaerobic chamber with a nitrogen environment.
5) Add 50 ml culture medium to each vial.
6) Add a specified amount of a biocide and/or a biocide enhancer.
7) Inoculate the vials with a 2-4 day old SRB seed culture.
8) Seal the vials and remove them from the chamber.
9) Incubate at 37°C for certain duration.

Planktonic SRB cell numbers were monitored by counting the samples withdrawn from vials using sterile syringes under an optical microscope. A hemocytometer counting chamber was used.

4.2.2 Chelators to enhance biocide inhibition of SRB biofilm

The ATCC 14563 strain was used in this section. Two media, enriched ASW and modified ATCC 1250 medium with 25 ppm Fe^{2+}, were used for SRB growth.

4.2.2.1 Substratum for biofilm growth

One to three disk-shaped C1018 coupons serving as the substratum for biofilm growth were seated at the bottom of vials with 100 ml of culture medium. Each coupon
had a top exposed surface area of 1.12 cm². The bottom and side surfaces were coated with Teflon paint to avoid crevice corrosion. Prior to immersion into the vials, each coupon was polished successively by using a series of abrasive papers with 200, 400 and 600 grits.

4.2.2.2 SEM observations

The efficacy of biocide enhancers was evaluated by checking the entire coupon surface to see if sessile SRB existed within biofilms under SEM. At 200X magnification, the figure of an SEM image is representative of about 0.3% of the total exposed coupon surface. The procedure for sample preparation was described previously in Chapter 3.3.

Glutaraldehyde is a common fixative because it can cross-link proteins (Hayat, 2000). It did not misguide the biocidal effects during inhibition tests since the experimental duration was around eight days and the highest concentration used in tests was 500 ppm, which was much lower than the concentration used for fixation.

4.2.2.3 EDDS and glutaraldehyde in inhibiting SRB biofilm formation

Tests were carried out in vials at 37°C containing one to three C1018 coupons with 100 ml of culture medium. The experimental procedure was described previously. Coupons were inserted into vials before adding the medium. The initial pH in the enriched ASW was between 7.8 and 8.1, and in the modified ATCC 1250 medium it was around 6.8. Test duration was about eight days since it takes time to mature for biofilms.

4.2.2.4 EDDS and glutaraldehyde in treating established SRB biofilms

Biofilms were pre-grown in the modified ATCC 1250 medium. The standard procedure described in Chapter 3 was used with the following specifications: to make
sure SRB biofilm was growing, one coupon was retracted for SEM analysis four days after inoculation. In six-eight days after inoculation, coupons covered with pre-grown biofilms were removed and rinsed with sterilized distilled water. They were then transferred into vials with fresh modified ATCC 1250 medium. A specified concentration of EDDS and glutaraldehyde was then added according to the test matrix (Table 4-3). After eight days, the experiments were terminated and SEM observation was conducted to check the SRB biofilms.

Table 4-3. Test matrix for EDDS enhancement of glutaraldehyde treatment of SRB biofilms on carbon steel surfaces in ASW

<table>
<thead>
<tr>
<th>Medium</th>
<th>Inhibiting biofilm formation</th>
<th>Treating established biofilms</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASW</td>
<td>Modified ATCC 1250</td>
<td>Modified ATCC 1250</td>
</tr>
<tr>
<td>Glutaraldehyde (ppm)</td>
<td>25</td>
<td>0, 30</td>
</tr>
<tr>
<td>EDDS (ppm)</td>
<td>0, 2000</td>
<td>0, 2000</td>
</tr>
</tbody>
</table>

4.2.2.5 Treatment of EDDS and THPS in killing established SRB biofilm in flow conditions

A glass cell equipped with a rotating shaft was used as the bioreactor. A C1018 carbon steel cylindrical coupon was used. SRB biofilms were pre-cultured on the C1018 coupon surface in a 100 ml bottle seated in a nitrogen-filled canister. After SRB biofilms developed, the coupons were removed from the bottle, washed with sterilized distilled
water, mounted on the shaft and transferred into the glass cell bioreactor. After 3-6 days’ exposure in the glass cell, the coupon was removed and processed for SEM observation.

4.2.3 Triple combo of a chelator, alcohol and biocide for the mitigation of planktonic SRB and biofilms

*D. vulgaris* ATCC 7757 was used in this section. ATCC 1249 medium was used for cell culture. Because of the interactions between the nutrients in the culture medium and the biocide, the biocide efficacy increases in a less nutritious medium. A 1/4 strength diluted medium containing only four ingredients in the ATCC 1249 medium including MgSO$_4$, lactate, yeast extract, and Fe$^{2+}$ at concentrations 1/4 of those in the full ATCC 1249 medium was used for the test (Table 4-4).

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgSO$_4$ /g</td>
<td>0.5</td>
</tr>
<tr>
<td>Sodium lactate /g</td>
<td>0.875</td>
</tr>
<tr>
<td>Yeast extract /g</td>
<td>0.25</td>
</tr>
<tr>
<td>Fe(NH$_4$SO$_4$)$_2$ /g</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Experiments followed the standard procedures described previously with some specified differences. Methanol was purged with nitrogen for at least 45 min prior to use. Each vial contained 50 ml of the medium for the planktonic cell test and 100 ml of the medium for the coupon test, respectively. There were two coupons in each vial for comparison.
4.3 Results and discussion

4.3.1 Chelators to enhance biocide inhibition of planktonic SRB and biofilms

4.3.1.1 Effects of chelators to enhance biocide inhibition of planktonic SRB growth

The effects were evaluated by comparing the time duration of inhibition of planktonic bacterial growth, the method of which had been used by researchers von Rege and Sand (1998), Gardner and Stewart (2002), and de Savaria and de Mele (2005). The time duration of inhibition is defined as suppression or delay in attaining stationary phase by planktonic bacteria.

4.3.1.1.1 Efficacies of biocide enhancers in modified ATCC 1249 medium

In this section, the ATCC 7757 SRB strain was used. The chelators, such as EDDS and HEIDA, did not inhibit the bacteria growth when it was used alone (Figure 4-1 and Figure 4-2a). The enhancements of THPS and glutaraldehyde by EDDS and HEIDA are shown in Figure 4-1 and Figure 4-3, respectively. It must be noted that biocides such as glutaraldehyde and THPS will interact with culture media, and planktonic bacteria will overcome biocide inhibition and propagate when low biocide dosage is used. As shown, 20 ppm and 30 ppm of THPS failed to inhibit SRB growth completely. There were two and eight day delay of cell number take-offs, respectively. Similarly, SRB growth was not inhibited by glutaraldehyde when a dosage of 20 ppm or 30 ppm was used while 50 ppm of glutaraldehyde suppressed the SRB growth within the test duration up to 12 days (Figure 4-3). When combined with EDDS, the time duration of inhibition increased. Although with 20 ppm of THPS, 500 ppm and 2000 ppm of

3 This section has been published in World Journal of Microbiology and Biotechnology, 28 (2), 431-435, 2010.
EDDS failed to show much difference with respect to the biocidal enhancement, interestingly, SRB numbers gradually increased for the latter and reached the stationary phase four days later after it took off. When the dosage of THPS was increased to 30 ppm, the addition of EDDS enhanced the biocidal effect and the SRB number was kept at low level for 10 days until the test ended.

Figure 4-1. EDDS enhancement of THPS in modified ATCC 1249 medium. (Initial cell concentration was $1.14 \times 10^6$ cells/ml. Final pH range 6.8-7.1.) (Wen et al., 2010)

The chelator HEIDA showed a similar performance on the enhancement of biocide effects on SRB growth (Figure 4-2). With the addition of 1000 ppm of HEIDA, 30 ppm of THPS inhibited SRB growth throughout the test duration of 10 days. However, 500 ppm of HEIDA failed to enhance THPS effectively.
Figure 4-2. HEIDA enhancement of THPS in modified ATCC 1249 medium: (a) initial cell concentration was $1.21 \times 10^6$ cells/ml. Final pH range 7.0-7.1; (b) initial cell concentration was $1.14 \times 10^6$ cells/ml. Final pH range 6.4-7.0. (Wen et al., 2010)

Chelators enhance glutaraldehyde on SRB growth as well (Figure 4-3). The time duration of inhibition was increased when EDDS was added in addition to glutaraldehyde. When comparing treatment of 30 ppm glutaraldehyde alone to treatment of 500 ppm EDDS and 30 ppm glutaraldehyde, the time duration of inhibition increased
from three to seven days. The SRB growth was inhibited completely during a test period of 12 days when treated with 1000 ppm of EDDS and 30 ppm of glutaraldehyde. Considering the different action mechanisms between glutaraldehyde and THPS, it is reasonable to believe that EDDS may contribute to the chelating effect. By chelating some ions such as Mg$^{2+}$ and Ca$^{2+}$, the outer cell membrane for Gram-negative cells such as SRB is targeted, the permeability of which is then increased (Vaara, 1992).

![Graph](image)

**Figure 4-3.** EDDS enhancement of glutaraldehyde in modified ATCC 1249 medium. (Initial cell concentration was $9.70 \times 10^5$ cells/ml. Final pH range 6.7-7.3.) (Wen et al., 2010)

### 4.3.1.1.2 Efficacies of biocide enhancers in the enriched ASW

In this section, the ATCC 14563 strain was used. The enhancements of EDTA and EDDS on glutaraldehyde in inhibiting SRB growth in the enriched ASW are shown (Figure 4-4 and Figure 4-5). It should be noted that pH adjustment was necessary after
the addition of EDTA. Otherwise, the decreased pH due to EDTA in the test may be misleading. Similarly, EDTA or EDDS alone could not inhibit SRB growth in the enriched ASW. When glutaraldehyde is combined with chelators, the time duration of inhibition increases. When 30 ppm of glutaraldehyde were combined with 1000 ppm of EDTA (Figure 4-4) or 2000 ppm of EDDS (Figure 4-5), the SRB cell numbers did not take off. Testing was stopped after 13 days. The time duration of inhibition for the treatment of 20 ppm of glutaraldehyde combined with 1000 ppm of EDTA or 2000 ppm of EDDS is similar to the one for the treatment of 30 ppm glutaraldehyde alone. 500 ppm of chelators, EDTA or EDDS, however, did not show enhancement on glutaraldehyde inhibition of SRB growth.

Compared to those results in the modified ATCC 1249 medium, a higher dosage of EDDS was needed to enhance glutaraldehyde inhibition of SRB growth in the enriched ASW. This was probably due to the high concentration of salt ions interacting with the chelator and counteracting its chelating ability.
Figure 4-4. EDTA enhancement of glutaraldehyde in the enriched ASW. (Initial cell concentration after inoculation was $1.42 \times 10^6$ cells/ml. EDTA solution pH adjusted to 7 before being added to the broth. Final pH range 6.95-7.4.) (Wen et al., 2010)

Figure 4-5. EDDS enhancement of glutaraldehyde in the enriched ASW. (Initial cell concentration after inoculation was $1.42 \times 10^6$ cells/ml. Final pH range 6.45-7.1.) (Wen et al., 2010)
4.3.1.2 Effects of chelators to enhance biocide inhibition of SRB biofilm establishment*

As discussed in Chapter 2, sessile bacteria are difficult to eradicate. In this study, even when treated with 500 ppm of glutaraldehyde on pre-grown SRB biofilms, sessile SRB were detected as shown on Figure 4-6. A more effective biocide treatment is required for industrial application.

Figure 4-6. Pre-grown SRB biofilm treated with 500 ppm glutaraldehyde in modified ATCC 1250 medium for 8 days. (Wen et al., 2009)

In this work, the performance of chelators’ enhancement on biocide treatment of SRB biofilm was evaluated. Figure 4-7 shows the morphology of control samples in ASW. 2000 ppm of EDTA cannot inhibit sessile SRB growth completely. The comparison of coupon surfaces with and without biocide enhancer treatment in the enriched ASW is shown on Figure 4-8. Combined with EDDS treatment, sessile SRB is hard to detect (Figure 4-8b), while peanut-shaped SRB are clearly visible for the treatment without EDDS (Figure 4-8a). Figure 4-9 and Figure 4-10 show the surface morphology after cleaning using Clarke’s solution. Initial pitting attacks were observed

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*This section has been published in International Biodeterioration & Biodegradation, 63(8), 1102-1106, 2009.
on all the samples. Longer time duration may be needed to evaluate chelators’ enhancement to biocide treatment in respect to reducing pitting attacks.

In modified ATCC 1250 medium, sessile SRB grew well (Figure 4-11a) and 2000 ppm of EDDS alone did not prevent SRB biofilm establishment (Figure 4-11b). It was observed that sessile SRB grew better in modified ATCC 1250 medium than in the enriched ASW since ATCC 1250 medium is more nutritious. Combined with 30 ppm of glutaraldehyde, the addition of 2000 ppm of EDDS enhanced its inhibition on SRB biofilm establishment with sufficient evidence that sessile SRB were absent (Figure 4-12b). In contrast, sessile SRB were visible in treatment with glutaraldehyde alone in the same concentration (Figure 4-12a).
Figure 4-7. Control sample with 0 ppm of glutaraldehyde in ASW for 8 days: (a) without EDTA; (b) with 2000 ppm of EDTA.
Figure 4-8. Effects of EDDS enhancement of glutaraldehyde on preventing SRB biofilm establishment using 25 ppm of glutaraldehyde in ASW for 8 days: (a) without EDDS; (b) with 2000 ppm of EDDS. (Wen et al., 2009)
Figure 4-9. Surface morphology after cleaning for EDDS enhancement of glutaraldehyde on preventing SRB biofilm establishment using 25 ppm of glutaraldehyde in ASW for 8 days: (a) without EDDS; (b) with 2000 ppm of EDDS.
Figure 4-10. Surface morphology after cleaning for control sample with 0 ppm of glutaraldehyde in ASW for 8 days: (a) without EDTA; (b) with 2000 ppm of EDTA.
Figure 4-11. Control sample with 0 ppm of glutaraldehyde in modified ATCC 1250 medium for 8 days: (a) without EDDS; (b) with 2000 ppm of EDDS. (Wen et al., 2009)
Figure 4-12. Effects of EDDS enhancement of glutaraldehyde on preventing SRB biofilm establishment using 30 ppm of glutaraldehyde in modified ATCC 1250 medium for 8 days: (a) without EDDS; (b) with 2000 ppm of EDDS. (Wen et al., 2009)

4.3.1.3 Effects of chelators enhancing biocide treatment on established SRB biofilms

4.3.1.3.1 Treatment in vials without flow

Once biofilms are established, they are difficult to remove. In pipeline service, the most effective method is pigs combined with biocide treatment. However, the high cost and interruption of production precludes the frequent use of this operation. An effective biocide treatment is imperative.
In this work the enhancement of a glutaraldehyde treatment for established SRB biofilms by EDDS was obtained. Figure 4-13 shows the comparison between samples with and without EDDS in the same concentration of glutaraldehyde in a modified ATCC 1250 medium. Without EDDS enhancement, dense biofilms were found on coupon surfaces and sessile SRB were clearly visible (Figure 4-13a). In contrast, with the help of 2000 ppm of EDDS, 30 ppm of glutaraldehyde successfully removed the established SRB biofilm and sessile SRB was undetectable (Figure 4-13b). This combination treatment achieved better biofilm control than the treatment with 500 ppm of glutaraldehyde alone as shown in Figure 4-6. When the dosage of glutaraldehyde was increased to 100 ppm and even as high as 500 ppm, sessile SRB were still detected (Figure 4-14 and Figure 4-6). MPN results also illustrated that 100 ppm of glutaraldehyde did not remove SRB biofilm (Table 4-5). As discussed previously, chelators can remove the divalent cations Mg$^{2+}$, Zn$^{2+}$ and Ca$^{2+}$ from lipopolysaccharide (LPS) by chelating, increasing the permeability of outer membrane for Gram-negative cells (Hancock, 1984; Vaara, 1992). Moreover, it also may weaken the bonding of EPS by chelating Ca$^{2+}$ (Carpentier and Cert, 1993), making the biofilms less dense and easier for glutaraldehyde to penetrate through the biofilms. Thus, a higher efficacy of biocide on inhibition of SRB biofilm growth is achieved by the addition of chelators.
Figure 4-13. Effects of EDDS enhancement of glutaraldehyde on treating established SRB biofilm using 30 ppm of glutaraldehyde in modified ATCC 1250 medium for 8 days: (a) without EDDS; (b) with 2000 ppm of EDDS. (Wen et al., 2009)

Table 4-5. MPN counting for sessile SRB 7 days after biofilm treatment in modified ATCC 1250 medium

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>MPN (cells/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glut 30 ppm, EDDS 0 ppm</td>
<td>$3.6 \times 10^4$</td>
</tr>
<tr>
<td>Glut 100 ppm, EDDS 0 ppm</td>
<td>$2.0 \times 10^4$</td>
</tr>
</tbody>
</table>
Figure 4-14. Pre-grown SRB biofilm treated with 100 ppm glutaraldehyde in modified ATCC 1250 medium for 8 days.

4.3.1.3.2 Treatment of EDDS and THPS in killing established SRB biofilm in flow conditions

After 10 days culturing in a 100 ml bottle without flow, one coupon was removed and prepared for SEM observation (Figure 4-15a). Another duplicate coupon was transferred to a freshly modified ATCC 1250 medium for 5 more days’ exposure without fluid flow before being removed for SEM analysis (Figure 4-15b). Sessile SRB are clearly visible in Figure 4-15. This indicates that the biofilm on the coupon surface remained healthy after the coupon was transferred to the fresh medium.

Figure 4-16 shows the comparison of biofilm growth at a 1,000 rpm rotation rate with and without biocide and biocide enhancer treatment. Without treatment, sessile SRB cells were clearly visible in the biofilms (Figure 4-16a). It was hard to detect sessile SRB after treatment (Figure 4-16b).

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5 This section has been published in AIChE Annual Meeting 2006, pp. 471d/1-471d/6.
Figure 4-15. Coupon surface images for the control sample without biocide treatment: (a) SRB were grown in a bottle for 10 days in modified ATCC 1250 medium; (b) coupon was transferred to a fresh modified ATCC 1250 medium for 5-day exposure without biocide and biocide enhancer treatment. (Wen et al., 2006b)
Figure 4-16. Coupon surface images for 5-day exposure in a glass cell with modified ATCC 1250 medium at rotating rate of 1,000 rpm: (a) without biocide and biocide enhancer treatment; (b) treated with 30 ppm of THPS and 1000 ppm of EDDS. (Wen et al., 2006b)

### 4.3.2 Triple combo of a chelator, alcohol and biocide for the mitigation of planktonic SRB and biofilms

In this work, methanol was used in place of ethanol because of its lower cost (James et al. 1996). Methanol has been used as an anti-freeze to prevent hydrate formation in the oil and gas industry. This problem causes plugs in pipelines and drilling (Ng & Robinson, 1985; Kelland, 2006). Because methanol is a natural metabolic product

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6 Part of this section has been published in World Journal of Microbiology & Biotechnology, 28(2), 431-435.
in various biological processes, it is unlikely that it will accumulate in the environment given its quick biodegradability (Malcolm Pirnie, 1999). It may be argued that some bacteria including SRB can utilize methanol directly or indirectly (Liamleam & Annachhatre, 2007). However, it is also known that a higher concentration of methanol can inhibit their growth (Snedecor and Cooney, 1974). In this work, D. vulgaris ATCC 7757, which does not utilize methanol according to Braun and Stolp (1985), was employed.

**4.3.2.1 Inhibition and killing planktonic SRB**

Methanol alone up to 10% (v/v) did not inhibit SRB growth (Figure 4-17). The SRB reached their stationary phase after one day of growth. When methanol concentration was increased to 25% and 50%, SRB growth was inhibited. The cell number remained at a low level.

Most of the work related to using the triple combo of glutaraldehyde, EDDS and methanol to inhibit and kill planktonic SRB was done by Dr. Dake Xu in the OU biocorrosion lab and published in the journal paper (Wen et al., 2012). The followings are excerpts from the paper:

The combination of EDDS (up to 1000 ppm) and methanol (up to 15%) without a biocide did not prevent SRB growth. Compared to control samples without treatment, however, inhibition of SRB growth was observed as the planktonic SRB numbers were about 5x to 10x lower.
With the addition of 10% to 15% of methanol, mitigation of planktonic SRB growth by 30 ppm of glutaraldehyde and 1000 ppm of EDDS changed from an inhibition effect to a kill effect in the 1/4 diluted medium.

![Graph showing cell counts of SRB growth with different concentrations of methanol](image)

Figure 4-17. Methanol (alone) effect on planktonic SRB growth. SRB grew in low concentration of methanol (up to 10% v/v). SRB cell counts decreased and then stabilized in 25% and 50% methanol.

### 4.3.2.2 Prevention of SRB biofilm establishment

In previous work it was found that 30 ppm glutaraldehyde with 2000 ppm EDDS successfully suppressed the SRB biofilm establishment in modified ATCC 1250 medium. In this work, the triple combo treatment showed promising effects in preventing the establishment (Figure 4-18 and Figure 4-19) by reducing the dosage of EDDS to 500 ppm. It was difficult to observe sessile SRB on the coupon surface after triple compo treatment (Figure 4-19).
4.3.2.3 Treatment for established SRB biofilms

In this study, the new triple biocide combination was evaluated for its efficacy in removing established SRB biofilm in modified ATCC 1249 medium. Figure 4-20 shows that sessile SRB were still visible when treated with 50 ppm glutaraldehyde, 15% methanol and 0 ppm EDDS. However, when a combination of 15% methanol + 50 ppm glutaraldehyde + 1000 ppm EDDS was used, no visible SRB were detected even nine days after the biocide treatment which indicates the established biofilm was successfully
removed (Figure 4-21). Compared to the dual treatment of glutaraldehyde and EDDS described previously, the EDDS dosage was reduced to 1000 ppm when combined with 15% methanol and 50 ppm glutaraldehyde.

Figure 4-20. Effect of 15% (v/v) methanol + 50 ppm glutaraldehyde + 0 ppm EDDS on treating established SRB biofilm in ATCC 1249 medium with Fe$^{2+}$ 25 ppm after 9 days of exposure. Sessile SRB are visible.

Figure 4-21. Effect of 15% (v/v) methanol + 50 ppm glutaraldehyde + 1000 ppm EDDS on treating established SRB biofilm in ATCC 1249 medium with 25 ppm Fe$^{2+}$. After 9 days of exposure, sessile SRB are hard to detect. (Wen et al., 2012)
In addition to SEM inspection, MPN and an SRB test kit were used to verify the effects of methanol, chelators and biocide for controlling biofilms (Table 4-6). The binary combination of 50 ppm glutaraldehyde and 1000 ppm EDDS treatment showed 2-3 log reduction on the sessile SRB concentration compared to the control sample. However, when methanol was combined with biocide, it was difficult to observe any color change by using MPN or the Sani-Check® Product 100 test kit. Even for the pre-grown biofilm treatment of 10% methanol alone, no black color was observed, which indicates no SRB was detected. On the contrary, SEM images clearly showed sessile SRB. The reason is probably an iron (II)-EDDS binding complex in methanol, which caused the loss of the black color as the reaction indicator. The methodology needs to be explored to verify the biocide effects when combined with methanol.

It is not clear how methanol enhances the biocide and/or chelators on inhibiting or killing biofilms. As aforementioned, its acting as a denaturant may contribute to this triple combo capability.
Table 4-6. Results of sessile cell counts (cells/cm²) and SRB test kit for methanol + EDDS + glutaraldehyde on removal of established SRB biofilm after 9-day exposure

<table>
<thead>
<tr>
<th>Methanol concentration</th>
<th>BioSan Sani-Check® test kit</th>
<th>MPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control: methanol 0% (v/v), glutaraldehyde 0 ppm, EDDS 0 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol 0% (v/v), glutaraldehyde 50 ppm, EDDS 1000 ppm</td>
<td>≥4.5 × 10⁵</td>
<td>1.6 × 10⁴</td>
</tr>
<tr>
<td>Methanol ≥10% (v/v), glutaraldehyde 50 ppm, EDDS 1000 ppm</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

4.3.3 D-amino acid enhancement for the mitigation of SRB biofilms

In this work, effects of mixture of D-amino acids (D-tyrosine, D-leucine, D-tryptophan, D-methionine) in preventing SRB biofilm establishment and treating established SRB biofilm were evaluated. D-amino acids alone did not prevent the SRB biofilm build-up with 100 µM dosage (Figure 4-22).

As mentioned in Section 4.3.1.3.1, it was found that 30 ppm of glutaraldehyde worked with 2000 ppm EDDS to remove an established biofilm in ATCC 1250 medium. THPS has an efficacy similar to glutaraldehyde. Herein, the concentration of THPS was

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7 Part of this section has been included in World Journal of Microbiology & Biotechnology, 28, 1641-1646, 2012.
fixed at 30 ppm to evaluate their performance. Results showed that adding one mM of the
D-amino acids mixture, 30 ppm THPS did not remove the established biofilm completely
and sessile SRB were easily found on the coupon surface (Figure 4-23). When 30 ppm
THPS was combined with 10 µM D-amino acids and 500 ppm EDDS, sessile SRB were
hard to detect on the coupon surface (Figure 4-24).

Figure 4-22. Effect of 100 µM D-amino acids on preventing SRB biofilm establishment
after 7-day exposure in ATCC 1249 with 25 ppm Fe²⁺ (final pH 7.05). Sessile SRB are
visible. (Xu et al., 2012)

Figure 4-23. Effect of 1 mM D-amino acids and 30 ppm of THPS with 0 ppm EDDS on
treating established SRB biofilm after 8-day exposure in ATCC 1249 medium with Fe²⁺
25 ppm. Sessile SRB are visible.
D-amino acids showed their potential for removing biofilms when combined with biocide and chelators. With higher concentrations of D-amino acids employed, e.g. 1 ppm D-tyrosine or 100 ppm D-methionine, further work by the OU biocorrosion lab (Xu et al., 2012a; Xu et al., 2016) demonstrated that it enhances biocidal effects of THPS on SRB biofilm significantly. Jia et al. (2017c) extended the application of D-amino acids in combination with other popular biocides such as alkyldimethylbenzylammonium chloride (ADBAC) to inhibit SRB biofilms. However, the role of D-amino acids in this treatment is not clear. It is believed they triggered biofilm disassembly when biofilm aged (Cava et al., 2011). Xu & Liu (2011) observed that the amount of EPS decreased when the concentration of D-tyrosine increased. It may be that they destabilize the biofilm and increase its permeability; therefore, enhancing the biocide efficacy.
4.4 Summary

- Chelators, including EDTA, EDDS and HEIDA, demonstrated their capabilities to enhance biocides (glutaraldehyde and THPS) on inhibiting planktonic SRB growth.
- EDDS, a biodegradable chelator, demonstrated its efficacy in enhancing biocides on inhibiting SRB biofilm establishment as well as treatment of established SRB biofilm. Biocide dosage to control biofilm growth can be reduced considerably when EDDS was used with the biocide.
- The triple combo containing a biocide (glutaraldehyde or THPS), EDDS and methanol demonstrated efficacies in SRB treatment. Mitigation of planktonic SRB growth was improved from an inhibition effect to a kill effect when 10% to 15% of methanol was added into the binary combination of biocide and EDDS treatment. Removal of an established biofilm was achieved with a treatment of 15% methanol, 1000 ppm EDDS and 50 ppm glutaraldehyde in the modified ATCC 1250 medium.
- D-amino acids show promise for enhancing biocides to mitigate SRB biofilms. 10 µM of a mixture of D-amino combined with 500 ppm EDDS successfully enhanced 30 ppm THPS to remove an established SRB biofilm.
CHAPTER 5 LAB EVALUATION OF MIC THREAT FOLLOWING HYDROTESTING\textsuperscript{8}

5.1 Introduction

Hydrotesting is a routine procedure for testing integrity of a pipeline before its service. The time duration varies from a short period of no more than eight hours when pressured to 125\% of its Maximum Allowable Operating Pressure (MAOP), to several months and up to a year (Darwin et al., 2010). Depending on the location of the pipelines, hydrotest fluids can be seawater, freshwater or water from a river or lake.

Although after hydrotesting the pipeline will be dewatered and dried, there may still be water remaining in the down point with potential MIC risk of introducing bacteria and forming biofilm during the hydrotest. During subsequent long-term operations, where organic carbons are available, biofilm may flourish and MIC can occur.

A stainless steel (304L and 316L) storage tank was found leaking at butt welds in piping one month after a hydrotest (Garverick, 1994). Iron bacteria (Gallionella) and iron/manganese bacteria (Siderocapsa) were believed to cause this failure while no SRB or sulfur oxidizing bacteria (SOB) was found near the pits. Darwin et al. (2010) presented two pipeline failures due to MIC during or after hydrotesting using untreated water, one of which was caused by SRB. While a number of hydrotest failures were reported, water treatment, corrosion inhibitors, as well as oxygen scavengers may be added during and after the hydrotest. Balancing the cost of treatments with the risk of corrosion must be considered in determining whether or not using biocide and other chemicals.

\textsuperscript{8} Part of this section has been included in a NACE conference paper, CORROSION/2012, Paper No. C2012- 0001226 and Xu et al. (2013).
Previous simulated hydrotest data from the OU biocorrosion lab using natural clean seawater from the Gulf of Mexico and salty aquifer water showed no pitting during six months of tests (Zhao, 2008). No SRB were detected in the seawater while as few as 13.3 cells/ml of total bacteria were detected by quantitative PCR measurement. Off-shore seawater tends to be relatively clean, meaning that it may lack a sufficient amount of organic carbons and a viable native microbial population for lab experiments.

The hydrotest process, itself, may not incur significant MIC pitting due to the lack of significant amount of organic carbons and microbes. However, after a pipeline is commissioned for service, it may be exposed to a fluid that contains organic carbons from oil, CO₂, H₂S in addition to water. These chemicals are capable of supporting microbial growth as some microbes can utilize CO₂ autotrophically (Rosnes et al., 1991; Thauer et al., 2007). In this work, MIC during hydrotesting and following hydrotesting in the presence of CO₂ and H₂S/CO₂ will be evaluated using untreated and biocide-treated coupons.

5.2 Objectives

- To investigate MIC in hydrotesting using enriched artificial seawater spiked with SRB;
- To investigate MIC following hydrotesting on untreated steel surfaces in the presence of oil, water, CO₂, H₂S, chloride and sulfate;
- To investigate MIC following hydrotesting on biocide-treated steel surfaces in the presence of oil, water, CO₂, H₂S, chloride and sulfate.
5.3 Experimental setup

5.3.1 Bacteria and culture conditions during hydrotreating

To simulate the contaminated water in hydrotests, *D. alaskensis* ATCC 14563 was used as an inoculum. ASW was enriched with Fe(NH$_4$)$_2$(SO$_4$)$_2$ 125 mg, sodium lactate (60 wt% syrup) 4.5 ml and yeast extract 1 g with 36 g of Instant Ocean® salt mix in 1 L of water. To avoid oxygen contamination, 100 ppm of cysteine was added except in biocide of THPS treatment tests.

5.3.2 Test fluids after hydrotreating

Simulated formation water equilibrated with H$_2$S (50 ppm) and CO$_2$ (1 bar) headspace, LVT 200 oil/water ratio 1:10, 65000 ppm NaCl and 22 mM SO$_4^{2-}$, pH of which was adjusted to 7 prior to use by adding sodium hydroxide or hydrogen chloride solution. For biocide-untreated coupon tests, 100 ppm of cysteine was added as oxygen scavenger.

5.3.3 Coupons and test conditions

Experiments were carried out in 100 ml anaerobic vials. X65 carbon steel coupons were used. One side of the coupon surfaces was polished by 200, 400 and 600 grit abrasive paper, successively, while all other sides were coated with inert Teflon paint. The exposure surface area was 4.4 cm$^2$, the ratio of which to 100 ml liquid matched that in a 36” ID pipe to simulate liquid exposure to pipe wall surfaces. Coupons were sterilized by using pure iso-propanol and UV light. All other parts and liquids were autoclaved at 121°C before use.
5.3.4 Cell count

Planktonic cell count was performed by using a hemocytometer under an optical microscope. Sessile SRB were counted by using a SRB Test Kit (Sani-Check® Product 100) and verified by MPN methods if needed. Before quantification, sessile cells were removed from coupon surfaces by subsequently using a scraper and sonication (up to 30 seconds).

5.3.5 Measurement of sulfate concentration and SEM observation

Sulfate concentrations were measured following the turbidimetric method described by Kolmert et al. (2000). Biofilm morphology was checked under SEM following the procedure described previously on Chapter 3.3. After biofilm removal, surface morphology was checked under SEM as well.

5.3.6 Weight loss and pit depth

Coupon surfaces were cleaned using the Clark’s solution according to the ASTM G1-03 standard. Surface overview and pit depth profile were examined using an Infinite Focus Microscope (IFM) (a surface profilometer with a maximum 200X magnification). Localized corrosion rates were calculated based on pit depth profile data.

5.3.7 Hydrotesting using enriched ASW spiked with SRB

Experiments were carried out in 100 ml anaerobic vials (Table 5-1). After coupons were immersed into 100 ml enriched ASW, SRB were added with an initial concentration of 10 cells/ml and 10⁶ cells/ml, separately and vials were placed at 37°C and 25°C. After 15 days and 30 days, some coupons were removed for analysis, leaving the rest for further tests.
### Table 5-1. Test matrix for enriched artificial seawater spiked with SRB

<table>
<thead>
<tr>
<th>Conditions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>22°C, 37°C</td>
</tr>
<tr>
<td>Bacterium for spiking</td>
<td>D. alaskensis ATCC 14563 (marine SRB)</td>
</tr>
<tr>
<td>Time duration</td>
<td>15 and 30 days</td>
</tr>
<tr>
<td>Enriched ASW</td>
<td>Instant Ocean® salt mix (see Table 4-2) intended for marine aquarium 36 g with Fe(NH₄)₂(SO₄)₂ 125 mg, sodium lactate (60 wt% syrup) 4.5 ml and yeast extract 1 g in 1 L of water</td>
</tr>
<tr>
<td>Coupon</td>
<td>X65 carbon steel</td>
</tr>
</tbody>
</table>

#### 5.3.8 Tests in the presence of oil, water, CO₂, H₂S, chloride and sulfate following hydrotesting using untreated steel surfaces in ASW

Coupons were removed from vials after simulated hydrotesting for 15 days and 30 days in ASW. They were washed with deoxygenized water to remove planktonic cells on the coupon surfaces. Coupons were then put into vials containing 100 ml of LVT-200 oil and simulated formation water.

In the presence of CO₂, the simulated formation water and LVT oil were purged with CO₂ for more than one hour before use. Coupon washing was carried out in a chamber full of CO₂ at 1 bar. Vials were sealed using rubber septa and aluminum caps in the chamber. The test matrix is shown on Table 5-2.
<table>
<thead>
<tr>
<th>Temperature</th>
<th>25, 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time duration</td>
<td>15 days</td>
</tr>
<tr>
<td>Coupon source</td>
<td>Simulated hydrotesting in ASW spiked with 10 and 10^6 cells/ml SRB with exposure time of 15 days</td>
</tr>
<tr>
<td>Test fluid</td>
<td>Simulated formation water with CO_2 (1 bar head space equilibrium pressure), LVT oil/water ratio 1:10, NaCl 65000 ppm, SO_4^{2-} 22mM</td>
</tr>
<tr>
<td>Coupon</td>
<td>X65 carbon steel</td>
</tr>
<tr>
<td>Observation</td>
<td>Planktonic and sessile SRB cell counts, surface morphology before and after film removal</td>
</tr>
</tbody>
</table>

In the presence of CO_2/H_2S, the simulated formation water and LVT oil were purged with N_2 for more than one hour before use. This was conducted in a chamber filled with N_2. Vials were sealed using rubber septa and aluminum caps in the chamber. After the vials were removed from the N_2 chamber, CO_2 was introduced into the medium at the same time as H_2S in a H_2S/CO_2 mixture using the following method: A long-stem needle was inserted deep into the medium as a gas inlet and a short-stem needle was inserted into the headspace as a gas outlet. The H_2S/CO_2 mixture was sparged through the medium for approximately 40 min. After sparging, vials were sealed using wax to cover the cap and bottleneck. The test matrix is shown on Table 5-3. After 15 days and 30 days, coupons were removed for analysis. Liquid pH and sulfate concentration were measured.
Table 5-3. Test matrix for coupons exposed to simulated formation water with CO₂/H₂S

<table>
<thead>
<tr>
<th>Temperature</th>
<th>25, 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time duration</td>
<td>15 days</td>
</tr>
<tr>
<td>Coupon source</td>
<td>Simulated hydrotesting in ASW spiked with 10 and 10⁶ cells/ml SRB with exposure time of 15 days</td>
</tr>
<tr>
<td>Test fluid</td>
<td>Simulated formation water with CO₂ (1 bar head space equilibrium pressure), 50 ppm H₂S, LVT oil/water ratio 1:10, NaCl 65000 ppm, SO₄²⁻ 22mM</td>
</tr>
<tr>
<td>Coupon</td>
<td>X65 carbon steel</td>
</tr>
<tr>
<td>Observation</td>
<td>Planktonic and sessile SRB cell counts, surface morphology before and after film removal</td>
</tr>
</tbody>
</table>

5.3.9 Tests in the presence of oil, water, CO₂, H₂S, chloride and sulfate following hydrotesting using biocide-treated steel surfaces in ASW

Coupons were removed from vials after a simulated hydrotest for 30 days in ASW. They were washed with deoxygenized water and followed by treatment with THPS of 300 ppm for 30 min. All operations were carried out in an anaerobic chamber. Coupons were then put into vials containing 100 ml of LVT-200 oil and simulated formation water. The remainder of the procedure was the same as those previously described.

5.4 Results and discussion

Lab evaluation of hydrotesting has been criticized for the lack of pressure tests to simulate field practice such as in subsea pipelines. Microbes may be inactivated at a high
pressure; however, evidence elucidated that microbes such as SRB can grow at 1 bar as well as 300 bar (Rosnes et al., 1991). Many microbes evolved from the deep-sea environment are genetically equipped to adapt to different pressures. Thus, it is reasonable to believe that lab tests at 1 bar provide useful data on microbial growth for field operations.

5.4.1 Hydrotesting using enriched ASW spiked with SRB

Zhao et al. (2010) demonstrated that enriched ASW with limited nutrients can support SRB growth in no less than 15 days. In this work, it was shown that after a 28-day test, SRB maintained their growth while planktonic SRB numbers did not drop significantly (Figure 5-1). At 37ºC, the medium spiked with 10⁶ cells/ml of SRB started to turn black in half a day (Figure 5-2). At 25ºC, the medium spiked with 10 cells/ml started to turn black after 10 days while the spiked medium with 10⁶ cells/ml turned black on Day 4 (Figure 5-3).

Figure 5-4 and Figure 5-5 show the corrosion rate and corresponding pH and sulfate concentration after exposure of 15 days and 30 days, respectively. In both cases, pH decreased from 8.16 to 6.8 – 6.9 after 15 days and to 7.0 – 7.1 after 30 days. No clear trend could be obtained among spiked SRB concentrations, sulfate consumption, temperature and weight loss. The corrosion rates, however, were small and only initiating pits were visible (Figure 5-6). Sessile SRB were clearly visible after exposure of 15 days and 30 days at ambient temperature and 37ºC.
Figure 5-1. Planktonic SRB (ATCC 14563) growth in the enriched ASW.
Figure 5-2. Comparison of media in vials on Day 2 for tests of X65 in the enriched ASW spiked with and without SRB. With inoculation of $10^6$ cells/ml SRB, media started to turn black at 37°C.
Figure 5-3. Comparison of media in vials on Day 11 for tests of X65 in the enriched ASW spiked with and without SRB. With inoculation of 10 cells/ml SRB, cells started to grow exponentially at 25°C after 10 days.
Figure 5-4. X65 coupon corrosion rates (by weight loss) after 15 days in the enriched ASW*. (Coupon surface area was 1 cm².)

*The corrosion rate of coupon spiked with $1 \times 10^6$ cells/ml SRB was not obtained due to the damage of Teflon coating during cleaning process.

Figure 5-5. X65 coupon corrosion rate (by weight loss) after 30 days in the enriched ASW. (Coupon surface area was 1 cm².)
5.4.2 Tests in the presence of oil, water, CO₂, chloride and sulfate following hydrotests using untreated steel surfaces in ASW

CO₂ and H₂S/CO₂ corrosion of mild steel are of great concern in the oil and gas industry. General CO₂ corrosion is well studied and a reliable model has been developed (Sun and Nesic, 2008). Meanwhile, MIC is characterized as a localized corrosion where pits occur underneath the patchy biofilm (Rosnes et al., 1991; Little et al., 1992; Videla & Herrera, 2005). The biofilm growth, therefore, interferes with CO₂ corrosion and shows MIC characteristics.

The simulated formation water with LVT-200 oil (oil/water vol. ratio 1:10) remained clear after 15 days (Figure 5-7). Planktonic cells at the end of tests were undetectable at 25°C under a microscope (detection limit $5 \times 10^4$ cells/ml). The planktonic cell count at 37°C was less than $4 \times 10^5$ cells/ml. Sulfate concentrations were lower than the initial 22 mM with no significant difference among different test conditions (Figure 5-8). pH for medium at 37°C was 0.4 higher than the one at 25°C.
There was no much difference between samples at the same temperature. When exposure duration in the simulated formation water was extended to 47 days, the corrosion rate at 37°C for samples spiked with 10 cells/ml SRB was about 60% higher than the one at 25°C. In contrast, no distinguishing differences were obtained for samples spiked with 10^6 cells/ml SRB (Figure 5-9). More sulfate was consumed at 37°C than at 25°C. Similar to tests with exposure duration of 15 days, the pH of the medium at 37°C was roughly 0.4 higher than the one at 25°C. Compared with hydrotest samples (Figure 5-5), pH was significantly lower in the range of 5.4 – 5.8 due to the presence of CO₂; however, corrosion rates in term of weight loss showed little difference.

Figure 5-10 to Figure 5-13 show the coupon surface morphology and EDS analysis of the coupon surfaces before biofilm removal for samples spiked separately with 10 cells/ml and 10^6 cells/ml SRB at 25°C. Sessile SRB were clearly visible at each test condition. EDS analysis detected elements of carbon, oxygen and sulfur existing on films, indicating the production of ferrous bicarbonate and iron sulfide. There was a jump at the edge of the clump for elements of C and O on the elemental distribution profile (Figure 5-14), illustrating that FeCO₃ was precipitated and entrapped within the biofilms.

When temperature increased to 37°C, a favorite temperature for the test strain, abundance of sessile SRB were observed as shown on Figure 5-15 and Figure 5-17. Similarly, the EDS analysis indicates a mixture of ferrous carbonate and ferrous sulfide precipitates as well (Figure 5-16 and Figure 5-18).
Figure 5-7. Comparison of media in vials on Day 15 for tests of X65 coupons in simulated formation water and LVT 200 oil with oil/water ratio 1:10, saturated with CO₂ and 1 bar headspace pressure. Media stayed clear.
Figure 5-8. Sulfate concentration and pH of samples after 15 days of exposure for X65 coupons in simulated formation water and LVT 200 oil with oil/water ratio 1:10, saturated with CO₂ and 1 bar headspace pressure. (Weight loss data were not obtained due to Teflon painting damage that changed coupon weight.)

*Control samples (no cells) in hydrotest condition, not in simulated formation water and saturated with CO₂.

Figure 5-9. Weight loss, sulfate concentration and pH of samples after 47 days of exposure for X65 coupons in simulated formation water and LVT 200 oil with oil/water ratio 1:10, saturated with CO₂ and 1 bar headspace pressure following 30-day hydrotest. (Coupon surface area was 1 cm².)

*Control samples (no cells) in hydrotest conditions, not in simulated formation water and saturated with CO₂.
Figure 5-10. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ratio 1:10, saturated with CO₂ and 1 bar headspace pressure at 25°C following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB.

Figure 5-11. EDS analysis of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ratio 1:10, saturated with CO₂ and 1 bar headspace pressure at 25°C following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB.
Figure 5-12. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO$_2$ and 1 bar headspace pressure at 25ºC following hydrotreating for 15 days in ASW spiked with 10$^6$ cells/ml SRB. (Huang et al., 2012; Xu et al., 2013)

Figure 5-13. EDS analysis of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO$_2$ and 1 bar headspace pressure at 25ºC following hydrotreating for 15 days in ASW spiked with 10$^6$ cells/ml SRB.
Figure 5-14. EDS analysis-elemental distribution of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO₂ and 1 bar headspace pressure at 25°C following hydrotesting for 15 days in ASW spiked with 10⁶ cells/ml SRB.

Figure 5-15. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO₂ and 1 bar headspace pressure at 37°C following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB.
Figure 5-16. EDS analysis of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO₂ and 1 bar headspace pressure at 37°C following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB.

Figure 5-17. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO₂ and 1 bar headspace pressure at 37°C following hydrotesting for 15 days in ASW spiked with 10⁶ cells/ml SRB. (Huang et al., 2012; Xu et al., 2013)
Figure 5-18. EDS analysis of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO$_2$ and 1 bar headspace pressure at 37°C following hydrotesting for 15 days in ASW spiked with $10^6$ cells/ml SRB.

Figure 5-19 and Figure 5-20 show a typical coupon surface image after film removal. Anomalous round dots had formed on a large corroded area. Other than the pitted area, the metal surface remained un-corroded. The polishing lines were clearly visible (Figure 5-19). Pit clusters were large enough to be visible to the naked eye. 37°C samples gave larger but fewer clusters of pits than 25°C. Samples spiked with $10^6$ cells/ml SRB gave larger but fewer clusters of pits compared to those spiked with 10 cells/ml SRB. The pits appear to be different from those obtained during the hydrotests (Figure 5-6), which are believed to have been caused by the presence of CO$_2$. CO$_2$ corrosion is normally a uniform corrosion. When patched biofilms pre-formed on the metal surfaces, the CO$_2$ distribution was no longer even. The CO$_2$ differential concentration and SRB activity together contribute to the pits-on-the-mesa-like
phenomenon. Although there was no significant difference in weight loss between the hydrotests (Figure 5-5) and the tests in the presence of simulated formation water saturated with CO₂ (Figure 5-9), SEM and IFM images illustrated that severe pitting corrosion occurred when MIC was coupled with CO₂. The pit depths as shown in Figure 5-20, Figure 5-22, Figure 5-24 and Figure 5-26 were around 9.3 µm, 12.5 µm, 32.0 µm and 22.0 µm, respectively, after 15 days of exposure. The higher temperature produced deeper pits, while the spiked SRB concentration showed no distinguishing effects on the pit growth. The mechanism of MIC corrosion in the presence of CO₂ will be discussed in Chapter 6.

Figure 5-19. SEM images of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO₂ and 1 bar headspace pressure at 25°C following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB.
Figure 5-20. Surface analysis of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO$_2$ and 1 bar headspace pressure at 25ºC following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB. (a) Overview IFM image; (b) a spot; (c) depth profile of the spot. Refer to Figure 5-10 and Figure 5-19 for the SEM images.

Figure 5-21. SEM images of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ratio 1:10, saturated with CO$_2$ and 1 bar headspace pressure at 25ºC following hydrotesting for 15 days in ASW spiked with 10$^6$ cells/ml SRB. (Huang el al., 2012; Xu et al., 2013)
Figure 5-22. Surface analysis of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO₂ and 1 bar headspace pressure at 25°C following hydrotesting for 15 days in ASW spiked with 10⁶ cells/ml SRB. (a) Overview IFM image; (b) a spot; (c) depth profile of the spot. Refer to Figure 5-12 and Figure 5-21 for the SEM images. (Huang et al., 2012; Xu et al., 2013)

Figure 5-23. SEM images of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO₂ and 1 bar headspace pressure at 37°C following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB.
Figure 5-24. Surface analysis of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO$_2$ and 1 bar headspace pressure at 37ºC following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB. (a) Overview IFM image; (b) a spot; (c) waviness of this spot. Refer to Figure 5-15 and Figure 5-23 for the SEM images (Xu et al., 2013).

Figure 5-25. SEM images of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO$_2$ and 1 bar headspace pressure at 37ºC following hydrotesting for 15 days in ASW spiked with 10$^6$ cells/ml SRB. (Huang et al., 2012; Xu et al., 2013)
Figure 5-26. Surface analysis of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ratio 1:10, saturated with CO₂ and 1 bar head space pressure at 37°C following hydrotesting for 15 days in ASW with spiked SRB of 10⁶ cells/ml. (a) Overview IFM image; (b) profile of a spot; (c) waviness of this spot. Refer to Figure 5-17 and Figure 5-25 for the SEM images. (Huang et al., 2012; Xu et al., 2013)

5.4.3 Tests in the presence of oil, water, CO₂, H₂S, chloride and sulfate following hydrotesting using untreated steel surfaces in ASW

The simulated formation water with LVT-200 oil (oil/water ratio 1:10 v/v) remained clear after exposure of 15 days and 30 days. Planktonic cell counts at the end of tests were between 3.5 × 10⁴ and 4 × 10⁵ cells/ml under an optical microscope. Sulfate concentrations were around half of the initial 22 mM without significant differences among different test conditions. After 15 days, pH of the medium at 37°C was around 5.2 compared with 4.9 at 25°C. When exposure time was increased from 15 days to 30 days, the pH increased by 0.2 - 0.3.
Figure 5-27 to Figure 5-30 show the corrosion rates and corresponding pH and sulfate concentrations when the coupons were removed at the end of the tests. There was little difference between the corrosion rates of coupons exposed to simulated formation water and H₂S/CO₂ for 15 and 30 days following the 15-day hydrotect (Figure 5-27 and Figure 5-28). In contrast, after the 30-day hydrotect the corrosion rates decreased when the exposure time was increased to 30 days from 15 days (Figure 5-29 and Figure 5-30). In comparing control samples both in hydrotect conditions (Figure 5-28) and in simulated formation water saturated with H₂S/CO₂ without cells (Figure 5-29), samples spiked with SRB during hydrotecting had larger corrosion rates as a result of the co-action of microbial activity and H₂S/CO₂.

![Graph showing weight loss, pH, and sulfate concentration](image)

Figure 5-27. Weight loss, sulfate concentration and pH of samples after 15 days of exposure for X65 coupons in simulated formation water and LVT 200 oil with oil/water ratio 1:10, 50 ppm H₂S and 1 bar CO₂ following 15-day hydrotect. (Coupon surface area was 1 cm².)

*Control samples (no cells) in hydrotect condition, not in simulated formation water and saturated with H₂S/CO₂.
Figure 5-28. Weight loss, sulfate concentration and pH of samples after 30 days of exposure for X65 coupons in simulated formation water and LVT 200 oil with oil/water ratio 1:10, 50 ppm H2S and 1 bar CO2 following 15-day hydrotest. (Coupon surface area was 1 cm².)

*Control samples (no cells) in hydrotest condition, not in simulated formation water and saturated with H2S /CO2.

Figure 5-29. Weight loss, sulfate concentration and pH of samples after 15 days of exposure for X65 coupons in simulated formation water and LVT 200 oil with oil/water ratio 1:10, 50 ppm H2S and 1 bar CO2 following 30-day hydrotest. (Coupon surface area was 1 cm².)

*Control samples (no cells) in simulated formation water and saturated with H2S /CO2.
Figure 5-30. Weight loss, sulfate concentration and pH of samples after 30 days for X65 coupons in simulated formation water and LVT 200 oil with oil/water ratio 1:10, 50 ppm H$_2$S and 1 bar CO$_2$ following 30-day hydrotest. (Coupon surface area was 1 cm$^2$.)

*Control samples (no cells) in simulated formation water and saturated with H$_2$S/CO$_2$.

Figure 5-31 to Figure 5-51 show the coupon surface morphology and EDS analyses of coupon surface before biofilm removal. A large number of sessile SRB were clearly visible at all test conditions. This illustrates that sessile bacteria can maintain their activity even with a limited nutrition supply for as long as 30 days (in this case LVT-200 oil was the carbon source). Moreover, planktonic cells were also detected due to the detachment of biofilms. Interestingly, the sessile SRB density within biofilms at 25 °C was larger than at 37 °C. The initial SRB inoculations of 10 cells/ml and 10$^6$ cells/ml during the hydrotest did not show much difference on the sessile SRB distribution. EDS analysis detected elements of iron, carbon, oxygen and sulfur on the films, indicating the presence of bicarbonate and iron sulfide.
Figure 5-31. SEM images of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 25°C following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB.

Figure 5-32. EDS analysis of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 25°C following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB.
Figure 5-33. SEM images of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 25°C following hydrotesting for 15 days in ASW spiked with 10⁶ cells/ml SRB.

Figure 5-34. EDS analysis of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 25°C following hydrotesting for 15 days in ASW spiked with 10⁶ cells/ml SRB.
Figure 5-35. SEM images of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 37°C following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB.

Figure 5-36. EDS analysis of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 37°C following hydrotesting for 15 days in ASW spiked with 10 cells/ml SRB.
Figure 5-37. SEM images of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 37°C following hydrotesting for 15 days in ASW spiked with 10⁶ cells/ml SRB.

Figure 5-38. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 25°C following hydrotesting for 30 days in ASW spiked with 10 cells/ml SRB.
Figure 5-39. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 25°C following hydrotesting for 30 days in ASW spiked with 10⁶ cells/ml SRB.

Figure 5-40. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 37°C following hydrotesting for 30 days in ASW spiked with 10 cells/ml SRB.
Figure 5-41. EDS analysis of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 37°C following hydrotesting for 30 days in ASW spiked with 10 cells/ml SRB.

Figure 5-42. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 37°C following hydrotesting for 30 days in ASW spiked with $10^6$ cells/ml SRB.
Figure 5-43. EDS analysis of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ratio 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 37°C following hydrotreating for 15 days in ASW spiked with 10⁶ cells/ml SRB.

Figure 5-44. SEM images of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ratio 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 25°C following hydrotreating for 30 days in ASW spiked with 10 cells/ml SRB.
Figure 5-45. EDS analysis of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 25ºC following hydrotesting for 30 days in ASW spiked with 10 cells/ml SRB.

Figure 5-46. SEM images of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 25ºC following hydrotesting for 30 days in ASW spiked with 10$^6$ cells/ml SRB.
Figure 5-47. EDS analysis of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H\textsubscript{2}S, saturated with CO\textsubscript{2} and 1 bar head space pressure at 25°C following hydrotesting for 30 days in ASW spiked with 10\textsuperscript{6} cells/ml SRB.

Figure 5-48. SEM images of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H\textsubscript{2}S, saturated with CO\textsubscript{2} and 1 bar head space pressure at 37°C following hydrotesting for 30 days in ASW spiked with 10 cells/ml SRB.
Figure 5-49. EDS analysis of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 37ºC following hydrotesting for 30 days in ASW spiked with 10 cells/ml SRB.

Figure 5-50. SEM images of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 37ºC following hydrotesting for 30 days in ASW spiked with 10⁶ cells/ml SRB.
Figure 5-1. EDS analysis of X65 coupon surface before acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 25°C following hydrotesting for 30 days in ASW spiked with 10⁶ cells/ml SRB.
Figure 5-52 to Figure 5-59 show coupon surface images after film removal. Pit clusters were visible to the naked eye. At 25°C, severe pitting was evident. At 37°C, the pits were far fewer and smaller compared with 25°C. In regard to weight loss data, a higher temperature caused a larger weight loss. It may indicate that at a higher temperature there was more general corrosion, while at a lower temperature pitting corrosion was more likely. Surprisingly samples spiked with 10^6 cells/ml SRB during hydrotesting consistently produced smaller and fewer pits compared to those spiked with 10 cells/ml SRB. Among tested conditions, coupon at 25°C spiked with 10 cells/ml SRB during hydrotesting exhibited the most serious pitting. When exposure time was increased from 15 days to 30 days, the number of pits and corroded areas also increased.
Figure 5-52. Surface analysis of X65 coupon surface under IFM after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 25ºC following hydrotesting for 30 days in ASW spiked with 10 cells/ml SRB. (a) Overview IFM image; (b) waviness of this spot. The x- and y-axes scales are in microns. Refer to Figure 37 for the SEM images.
Figure 5-53. Surface analysis of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 25°C following hydrotesting for 30 days in ASW spiked with 10⁶ cells/ml SRB. (a) Overview IFM image; (b) waviness of this spot. Refer to Figure 38 for the SEM images.
Figure 5-54. Surface analysis of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ratio 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 37°C following hydrotesting for 30 days in ASW spiked with 10 cells/ml SRB. (a) Overview IFM image; (b) waviness of this spot. Refer to Figure 39 for the SEM image.
a.

Figure 5-55. Surface analysis of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 37ºC following hydrotesting for 30 days in ASW spiked with 10⁶ cells/ml SRB. (a) Overview IFM image; (b) waviness of this spot. Refer to Figure 41 for the SEM image.
Figure 5-56. Surface analysis of X65 coupon surface after acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H\textsubscript{2}S, saturated with CO\textsubscript{2} and 1 bar head space pressure at 25°C following hydrotreating for 30 days in ASW spiked with 10 cells/ml SRB. (a) Overview IFM image; (b) waviness of this spot. Refer to Figure 43 for the SEM image.
Figure 5-57. Surface analysis of X65 coupon surface after acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 25°C following hydrotesting for 30 days in ASW spiked with 10⁶ cells/ml SRB. (a) Overview IFM image; (b) waviness of this spot. Refer to Figure 45 for the SEM image.
Figure 5-58. Surface analysis of X65 coupon surface after acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 37°C following hydrotesting for 30 days in ASW spiked with 10 cells/ml SRB. (a) Overview IFM image; (b) waviness of this spot. Refer to Figure 47 for the SEM image.
Figure 5-59. Surface analysis of X65 coupon surface after acid cleaning: after 30 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 37°C following hydrotesting for 30 days in ASW spiked with $10^6$ cells/ml SRB. (a) Overview IFM image; (b) waviness of this spot. Refer to Figure 49 for the SEM image.
5.4.4 Tests in the presence of oil, water, CO₂, chloride and sulfate following hydrotesting in ASW and biocide treatment

The simulated formation water with LVT-200 oil (oil/water vol. ratio 1:10) remained clear after 15 days (Figure 5-60). Planktonic cells at the end of tests were undetectable at both 25°C and 37°C under an optical microscope (detection limit $5 \times 10^4$ cells/ml). Sulfate concentrations were detected 5 mM lower than the controls (no spiked bacteria during hydrotesting) for both temperatures (Figure 5-61). It indicated that sulfate was consumed by SRB not killed by THPS. This is consistent with the SEM images (Figure 5-62 and Figure 5-64). pH for medium at 37°C was 0.3 higher than the one at 25°C. There was no significant difference between samples at the same temperature. Corrosion rates at 37°C were 2.5 times higher than those at 25°C (Figure 5-61). It must be noted that in this section no cysteine was added into the liquid to avoid oxygen leakage that could possibly interfere with THPS. This may have caused the higher corrosion rate at 37°C.

Figure 5-62 to Figure 5-65 show the coupon surface morphology before biofilm removal and after biofilm removal. Sessile SRB were clearly visible at each test condition (Figure 5-62 and Figure 5-64). Severe pit corrosion was evident (Figure 5-63 and Figure 5-65). Pit depth was about 25 µm for both temperatures. Although weight loss data showed no significant difference between the hydrotests (Figure 5-4 and Figure 5-5) and in the presence of simulated formation water saturated with CO₂, IFM images illustrated that severe pitting corrosion accelerated when MIC was coupled with CO₂. Experimental data herein reveal that even when treated with 300 ppm of biocide for 30 min, biofilm
removal cannot be guaranteed and, thus, severe pitting corrosion may occur on surfaces exposed to a CO₂ environment.

Figure 5-60. Comparison of X65 coupons in simulated formation water and LVT 200 oil with oil/water ratio 1:10 following 30-day hydrotest and biocide treatment. (a) Day 1 for tests equilibrated with 50 ppm H₂S, saturated with CO₂ and 1 bar headspace pressure; (b) Day 1 for tests saturated with CO₂ and 1 bar headspace pressure; (c) Day 15 for both tests at end. Media stayed clear.
Figure 5-61. Weight loss, sulfate concentration and pH of samples after 15 days for X65 coupons in simulated formation water and LVT 200 oil with oil/water ratio 1:10, saturated with 1 bar CO$_2$ following 30-day hydrotest and 30 min treatment of 300 ppm THPS. (Coupon surface area was 1 cm$^2$.)

Figure 5-62. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ratio 1:10, saturated with CO$_2$ and 1 bar head space pressure at 25ºC following hydrotesting for 30 days in ASW spiked with 10 cells/ml SRB and treatment of 300 ppm THPS.
Figure 5-63. Surface analysis of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO$_2$ and 1 bar head space pressure at 25ºC following hydrotesting for 30 days in ASW spiked with SRB of 10 cells/ml and treatment of 300 ppm THPS. (a) Overview IFM image; (b) waviness of a spot.

Figure 5-64. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, saturated with CO2 and 1 bar head space pressure at 25ºC following hydrotesting for 30 days in ASW spiked with 10 cells/ml SRB and treatment of 300 ppm THPS.
5.4.5 Tests in the presence of oil, water, CO₂, H₂S, chloride and sulfate following hydrotesting in ASW and biocide treatment

The simulated formation water with LVT-200 oil (oil/water ratio 1:10 v/v) remained clear after 15 days (Figure 5-60). As well as in the presence of CO₂, planktonic cells at the end of tests were undetectable at both 25°C and 37°C under an optical microscope (detection limit 5 × 10⁴ cells/ml). Sulfate concentrations were around half of the initial 22 mM at 37°C (no data at 25°C) (Figure 5-66). After 15 days, the pH of the liquid at 37°C was 5.4 compared with 5.1 at 25°C. The corrosion rates of control samples showed little difference in the presence of CO₂ and H₂S/CO₂ (Figure 5-61 and Figure 5-66), while higher temperature gives higher corrosion rate. Compared with biocide
treatment (Figure 5-66) and no biocide treatment (Figure 5-30), corrosion rates of biocide-treated coupons were 0.33 mpy at 25ºC and 0.54 mpy at 37ºC lower, respectively, than those of untreated coupons.

Figure 5-66. Weight loss, sulfate concentration and pH of samples after 15 days for X65 coupons in simulated formation water and LVT 200 oil with oil/water ratio 1:10, 50 ppm H2S and 1 bar CO2 following 30-day hydrotest and 30 min treatment of 300 ppm THPS. (Coupon surface area was 1 cm².)

Figure 5-67 to Figure 5-70 show the coupon surface morphologies before biofilm and after biofilm removal. Sessile SRB were clearly visible at each test condition. Pitting corrosion was evident (Figure 5-68 and Figure 5-70). Pit depth at 25ºC was around 21 µm, similar to those of untreated coupons. In contrast, pit depth at 37ºC was 12 µm, half of those of untreated coupons. This reveals that once sessile SRB are formed on metal surface it is difficult to remove, which was discussed in Chapter 4 as well. Sessile SRB may not cause much damage during hydrotesting because of its usually short duration and nutrient limitation. When the pipeline carrying sessile SRB from hydrotesting is
commissioned, it may suffer severe corrosion once the local conditions are favorable to bacteria. When MIC is coupled with CO$_2$ and CO$_2$/H$_2$S corrosion, it is extremely dangerous for the pipeline. This work demonstrates that hydrotesting practices must carefully select test fluids and biocide treatments, while balancing cost and risk control. If possible, draining and drying pipelines and tanks should be performed immediately following hydrotesting and before commissioning to avoid biofilm buildup.

Figure 5-67. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 25ºC following hydrotesting for 30 days in ASW spiked with $10^6$ cells/ml SRB and treatment of 300 ppm THPS.
Figure 5-68. Surface analysis of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ratio 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 25°C following hydrotesting for 30 days in ASW with spiked SRB of 10^6 cells/ml and treatment of 300 ppm THPS. (a) Overview IFM image; (b) waviness of a spot.

Figure 5-69. SEM images of X65 coupon surface before acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H₂S, saturated with CO₂ and 1 bar head space pressure at 37°C following hydrotesting for 30 days in ASW spiked with 10^6 cells/ml SRB and treatment of 300 ppm THPS.
Figure 5-70. Surface analysis of X65 coupon surface after acid cleaning: after 15 days of exposure in simulated formation water and LVT 200 oil with oil/water ration 1:10, 50 ppm H$_2$S, saturated with CO$_2$ and 1 bar head space pressure at 37°C following hydrotesting for 30 days in ASW with spiked SRB of 10$^6$ cells/ml and treatment of 300 ppm THPS. (a) Overview IFM image; (b) waviness of a spot.

5.5 Summary

5.5.1 Hydrotesting using enriched ASW spiked with SRB

- In the enriched ASW, planktonic SRB cell numbers remained steady for the test duration of 28 days at 37°C and 25°C for samples spiked with 10$^6$ cells/ml and 10 cells/ml.

- The weight loss after 15-day and 30-day hydrotests was small, within the range of 0.4 mpy to 3.1 mpy. Only initial tiny pits with diameter of about 2 µm were observed on coupon surfaces after film removal.
5.5.2 Tests in the presence of oil, water, CO₂, chloride and sulfate following hydrotesting using untreated steel surfaces in ASW

- In the presence of oil, water, CO₂, chloride and sulfate following hydrotesting for 15 days, planktonic SRB cell numbers were too low to be detected under a microscope, while sessile SRB were clearly visible after 15 days of exposure at 37°C and 25°C. There was a reduction in sulfate concentration, probably due to consumption by sessile SRB. Precipitates of FeCO₃ and FeS were evident on coupon surfaces.

- In the presence of oil, water, CO₂, chloride and sulfate following hydrotesting for 30 days, planktonic SRB cell numbers were less than 1.5 × 10⁵ cells/ml, while sessile SRB were clearly visible after 40 days of exposure at 37°C and 25°C. Precipitates of FeCO₃ and FeS were evident on coupon surfaces.

- Characteristics of corrosion of X65 in the presence of oil, water, CO₂, chloride and sulfate following hydrotesting for 15 days and 30 days differed from those during hydrotesting. Shallow but larger pits were observed. At 37°C there were larger but fewer clusters of pits than at 25°C. Samples spiked with 10⁶ cells/ml SRB gave larger but fewer clusters of pits compared to those spiked with 10 cells/ml SRB.

- CO₂ corrosion in addition to SRB corrosion was suspected because of a significant increase in C content on the coupon surfaces through EDS analysis compared with samples not exposed to CO₂.
5.5.3 Tests in the presence of oil, water, CO$_2$, H$_2$S, chloride and sulfate following hydrotesting using untreated steel surfaces in ASW

- In the presence of LVT-200 oil, water, CO$_2$, H$_2$S, chloride and sulfate following hydrotesting for 15 days and 30 days, planktonic SRB cell numbers were observed between $3.5 \times 10^4$ and $4 \times 10^5$ cells/ml under a microscope. Sessile SRB were clearly visible after 15 days and 30 days of exposure at 37$^\circ$C and 25$^\circ$C. FeCO$_3$ and FeS were found on coupon surfaces.

- Characteristics of corrosion of X65 in the presence of oil, water, CO$_2$, H$_2$S, chloride and sulfate following hydrotesting for 15 days and 30 days differed from those in hydrotesting and in the presence of oil, water, water, CO$_2$, chloride and sulfate. Severe pitting was observed at 25$^\circ$C. At 37$^\circ$C there were far fewer and smaller pits than at 25$^\circ$C. Samples spiked (during hydrotesting) with $10^6$ cells/ml SRB consistently produced fewer pits compared to those spiked with 10 cells/ml SRB. 30-day coupons had more pits in wider areas than 15-day coupons.

- CO$_2$ corrosion was suspected in addition to SRB corrosion because of the significant increase in C content on the coupon surfaces through EDS analysis compared with samples not exposed to CO$_2$.

5.5.4 Tests in the presence of oil, water, CO$_2$, H$_2$S, chloride and sulfate following hydrotesting in ASW and biocide treatment

- In the presence of LVT-200 oil, water, CO$_2$, chloride and sulfate for 15 days following a 30-day hydrotest, planktonic SRB cell numbers were too low to be detectable under a microscope, while sessile SRB were clearly visible after 15
days of exposure at both 37°C and 25°C. There was a reduction in sulfate concentration probably due to its consumption by sessile SRB.

- Characteristics of corrosion of X65 in the presence of oil, water, CO₂, chloride and sulfate following hydrotecting and biocide treatment for 30 days differed from those in the hydrotest but were similar to those not treated with biocide. Shallow but large pits were observed. 37°C gave larger but fewer clusters of pits than 25°C.

- In the presence of LVT-200 oil, water, CO₂, H₂S, chloride and sulfate following the 30-day hydrotest and biocide treatment, planktonic SRB cell numbers were too low to be detectable under a microscope, while sessile SRB were clearly visible after 15 days of exposure at 37°C and 25°C.

- Characteristics of corrosion of X65 in the presence of oil, water, CO₂, H₂S, chloride and sulfate following the 30-day hydrotest and biocide treatment for 30 days differed from those in the hydrotest but were similar to those not treated with biocide. Pitting Corrosion was observed at 25°C and 37°C. Lower corrosion rates and smaller pitting depth at 37°C were obtained after biocide treatment compared to no biocide treatment after hydrotecting.

- 300 pm of THPS treatment for 30 min failed to remove SRB biofilm completely. When MIC was coupled with CO₂ or H₂S/CO₂, severe pitting corrosion occurred.
CHAPTER 6  MODELING OF MIC IN THE PRESENCE OF CO₂

6.1  Introduction

In Chapter 5, laboratory investigation of MIC following hydrotesting using untreated steel surfaces in the presence of CO₂ or H₂S/CO₂ reveals that severe pitting attacks occurred. The pitting depth, however, was larger than what was predicted by current MIC software MICORP (no CO₂ involved) and general CO₂ and H₂S corrosion software MULTICORP 4.1 (no MIC involved) but much lower than the one predicted in the pure CO₂ environment without passive film formation. This suggests that current models based on BCSR theory (MICORP) and uniform CO₂ and H₂S corrosion (MULTICORP 4.1) are not well suitable for the test conditions. Modifications based on the characteristics of MIC following hydrotesting should be applied.

There is a paucity of literature about mechanistic models of localized CO₂ and H₂S corrosion; nor is there an MIC mathematical model in the presence of CO₂ and H₂S. As discussed in Chapter 5, biofilms may form during and after hydrotesting. It is known that SRB biofilms contain mackinawite which is a conductive component. The coverage of biofilms on steel surfaces is not homogeneous and generally is only a small portion of the whole surface (1% coverage is normal). When exposed to a corrosive environment, the heterogeneous film cannot protect the metal from corrosion. Pitting attacks may occur underneath the biofilms.

As described in Chapter 5, the formation water and oil mixture can provide limited nutrition for maintenance of SRB biofilm growth. A possible reaction can be listed below:
\[ \text{SO}_4^{2-} + 2[CH_2O] + H^+ \rightarrow 2CO_2 + HS^- + 2H_2O \]  \hspace{1cm} (6-1)

where \([CH_2O]\) is a simple notation for organic carbon. The metabolic products lower the local pH, disrupt the film formation, and maintain the continuance of the corrosion process. While the sessile SRB activity may be low and the amount of generated \(CO_2\) and \(HS^-\) may not contribute significantly to the corrosion process, its role to disrupt and detach protective film cannot be ignored. Meanwhile, at low temperatures, there is little ferrous carbonate film formation since the precipitate rate is too low (Nordsveen et al., 2003). It is reasonable to assume that this process is governed by \(CO_2\) corrosion with characteristics of biofilms. In this chapter, an approach for simulating the MIC after hydrotesting in the presence of \(CO_2\) is proposed based on the electrochemical model for \(CO_2\) corrosion (Nesic et al., 1996; Nordsveen et al., 2003) with characteristics of MIC. Experimental results from MIC after hydrotesting in Chapter 5 were used to verify the model.

### 6.2 Modeling development

#### 6.2.1 Assumption for the model

A schematic of mass transfer of species from bulk-fluid phase to pit bottom is illustrated (Figure 6-1). To model the corrosion process of mild steel after hydrotesting in the presence of \(CO_2\), simplification of conditions must be applied due to the complexity of the biofilm system by assuming:
1) Well-developed biofilms have been established during hydrotesting.

2) A protective film covers the metal surface except for the area covered by patchy biofilms. Corrosion process only continues under the biofilms.

3) Due to the limited nutrients, the activity of sessile SRB is low and only maintains the biofilms; there are no planktonic SRB in the bulk fluid.

4) Due to low activity of sessile SRB, the amount of generated HS\(^{-}\) or possible H\(_2\)S is small; thus, its direct contribution to corrosion is omitted.

5) Local pH is assumed to be slightly lower than the bulk pH due to the metabolic products of CO\(_2\) and H\(_2\)S. Its value is calibrated according to the pre-established biofilm thickness: the larger the biofilm thickness, the lower the pH. There is no passive film under the biofilms.
6) Simulation is made in static conditions; the effect of fluid flow is not taken into account. There is only diffusional mass transfer.

7) In the simulated MIC following the hydrotest process, test conditions of temperature (< 50°C), pH (≥ 4.0) with moderate CO₂ pressure are applied.

6.2.2 Electrochemical process in the presence of CO₂

This model describes the MIC process after hydrotesting in the presence of CO₂ which is governed by CO₂ corrosion due to the low activity of sessile SRB. For uniform CO₂ corrosion, the mechanism has been well established and a mechanistic model has been successfully applied to predict the CO₂ corrosion of mild steel corrosion (Nesic et al., 1996; Nordsveen et al., 2003). Although the study of localized CO₂ corrosion is far from mature, the mechanism still applies. Following Nesic et al. (1996) and Nordsveen et al. (2003), the water chemistry and electrochemical reactions for CO₂ corrosion of mild steel are presented below.

6.2.2.1 Water chemistry

As stated previously, the sulfate ion and its corresponding products of a hydrogen sulfide ion and hydrogen sulfide by SRB are not under consideration due to the low activity of sessile SRB. Hence, a simplified water chemistry including dissolution of carbon dioxide from the gas phase to liquid and subsequent hydration and dissociation is reproduced from Nesic et al. (1996) and Nordsveen et al. (2003) in Table 6-1. The values of these equilibrium constants can be calculated from the formulae given in Table 6-1.
Table 6-1. Simplified water chemistry for the CO₂ aqueous environment and formulas for the equilibrium constants*

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Equilibrium constant</th>
<th>Formula for equilibrium constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolution of carbon dioxide</td>
<td>$K_{\text{sol}} = C_{\text{CO}<em>2}/P</em>{\text{CO}_2}$</td>
<td>$K_{\text{sol}} = K_{\text{CO}_2}^d = 0.0454(1.6616 - 5.736 \times 10^{-2} t + 1.031 \times 10^{-3} t^2 - 9.68 \times 10^{-6} t^3 + 4.471 \times 10^{-8} t^4 - 7.912 \times 10^{-11} t^5)$</td>
</tr>
<tr>
<td>Carbon dioxide hydration</td>
<td>$K_{\text{hyd}} = C_{\text{H}_2\text{CO}<em>3}/C</em>{\text{CO}_2}$</td>
<td>$K_{\text{hyd}} = 2.58 \times 10^{-3}$</td>
</tr>
<tr>
<td>Carbonic acid dissociation</td>
<td>$K_{\text{Ca}} = C_{\text{H}^+}C_{\text{HCO}<em>3^-}/C</em>{\text{H}_2\text{CO}_3}$</td>
<td>$K_{\text{Ca}} = 387.6 \times 10^{-4} \left(6.41 - 1.594 \times 10^{-7} T_f + 8.52 \times 10^{-6} T_f^2 + 3.07 \times 10^{-5} P - 0.4772 \times T_k^2 + 0.1180 \times I\right)$ molar</td>
</tr>
<tr>
<td>Bicarbonate anion dissociation</td>
<td>$K_{\text{bi}} = C_{\text{H}^+}C_{\text{CO}<em>3^{2-}}/C</em>{\text{HCO}_3^-}$</td>
<td>$K_{\text{bi}} = 10^{-6.3868 - 0.0737549 T_f + 7.47881 \times 10^{-5} T_k^2}$ molar²</td>
</tr>
<tr>
<td>Water dissociation</td>
<td>$K_{\text{wa}} = C_{\text{H}^+}C_{\text{OH}^-}$</td>
<td>$K_{\text{wa}} = 10^{-29.3868 - 0.0737549 T_f + 7.47881 \times 10^{-5} T_k^2}$ molar²</td>
</tr>
</tbody>
</table>

*Table is taken from Nesic et al. (1996) and Nordsveen et al. (2003).

Note: In the table, $t$ is the temperature in °C; $T$ is absolute temperature in K; $T_f$ is temperature in F; $I$ is ionic strength in molar; and $P$ is the pressure in psi. And $K = k_f/k_b$. 
6.2.2.2 Electrochemical reactions on the metal surface

The electrochemical reactions at the metal surface in the presence of CO₂ have been well documented in literature as described in Chapter 2 (Nesic et al., 1996; Nordsveen et al., 2003). A brief description for these reactions and their parameters is reviewed below.

Derived from the Butler-Volmer equation, a fundamental reaction rate to describe the relationship between the charge transfer current density and potential is expressed as:

\[ i_{ct} = \pm i_0 \times 10^{\pm \frac{E - E_{rev}}{b}} \]  

(6-2)

where the positive sign applies to anodic reaction while the negative sign applies to cathodic reaction; \( i_0 \) is the exchange current density, A/m²; the subscript ct specifies the charge transfer; \( E_{rev} \) is the reversible potential, V; \( E \) is potential, V; and \( b \) is the Tafel slope. The electrochemical reactions and the parameters involved in CO₂ corrosion are listed in Table 6-2.

When mass transfer is taken into account, the overall current density is given by:

\[ \frac{1}{i_j} = \frac{1}{i_{ct,j}} + \frac{1}{i_{lim,j}} \]  

(6-3)

where \( j \) is a specified electrochemical reaction involved in the corrosion process.

Among the electrochemical reactions listed in Table 6-2, water reduction is controlled by charge transfer process since water molecules are supplied in unlimited quantities. The limiting current density of proton reduction and carbon dioxide hydration reduction is obtained using the following equations, respectively:
Table 6-2. Electrochemical reactions and their parameters for CO₂ corrosion

<table>
<thead>
<tr>
<th>Reaction</th>
<th>(i_0, \text{ref})</th>
<th>(C_{H^+, \text{ref}})</th>
<th>(a_1)</th>
<th>(C_{\text{H}_2\text{O}, \text{ref}})</th>
<th>(a_2)</th>
<th>(C_{\text{H}_2\text{CO}_3, \text{ref}})</th>
<th>(a_3)</th>
<th>(\Delta H)</th>
<th>(T_{\text{ref}})</th>
<th>(E_{\text{rev}})</th>
<th>(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2H^+ + 2e^- \rightarrow H_2)</td>
<td>0.05</td>
<td>0.5</td>
<td>10⁴</td>
<td>0</td>
<td>N/A</td>
<td>0</td>
<td>N/A</td>
<td>30</td>
<td>25</td>
<td>(-\frac{2.303RT}{F}\cdot pH)</td>
<td>(\frac{2.303RT}{0.5F})</td>
</tr>
<tr>
<td>(2\text{H}_2\text{CO}_3 + 2e^-)</td>
<td>0.06</td>
<td>-0.5</td>
<td>10⁻⁵</td>
<td>0</td>
<td>N/A</td>
<td>1</td>
<td>10⁴</td>
<td>50</td>
<td>20</td>
<td>(-\frac{2.303RT}{F}\cdot pH)</td>
<td>(\frac{2.303RT}{0.5F})</td>
</tr>
<tr>
<td>(\rightarrow H_2 + 2\text{HCO}_3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{H}_2\text{O} + e^-)</td>
<td>3×10⁻⁵</td>
<td>0</td>
<td>N/A</td>
<td>0</td>
<td>N/A</td>
<td>0</td>
<td>N/A</td>
<td>30</td>
<td>20</td>
<td>(-\frac{2.303RT}{F}\cdot pH)</td>
<td>(\frac{2.303RT}{0.5F})</td>
</tr>
<tr>
<td>(\rightarrow H + \text{OH}^-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{Fe} \rightarrow \text{Fe}^{2+} + 2e^-)</td>
<td>1</td>
<td>0</td>
<td>10⁴</td>
<td>0 for pH&gt;5; 1 for 4&lt;pH&lt;5</td>
<td>0.0366</td>
<td>0</td>
<td>N/A</td>
<td>37.5</td>
<td>25</td>
<td>-0.440</td>
<td>for pH&gt;5; 0.08 for 4&lt;pH&lt;5</td>
</tr>
</tbody>
</table>

*Table is taken from Nesic et al. (1996) and Nordsveen et al. (2003).

Note: the exchange current density \(i_0\) is:

\[
i_0 = i_{0,\text{ref}} \left( \frac{C_{H^+}}{C_{H^+,\text{ref}}} \right)^{a_1} \left( \frac{C_{\text{CO}_2}}{C_{\text{CO}_2,\text{ref}}} \right)^{a_2} \left( \frac{C_{\text{H}_2\text{CO}_3}}{C_{\text{H}_2\text{CO}_3,\text{ref}}} \right)^{a_3} \times e^{- \frac{\Delta H}{RT} \left( \frac{1}{T} - \frac{1}{T_{\text{ref}}} \right)}
\]

where \(\Delta H\) is activation energy; \(T_{\text{ref}}\) is the reference temperature, K; \(T\) is the temperature, K.
6.2.2.2.1 \textit{H}^+ \textit{reduction}

At stagnant conditions, the diffusion limiting current density is calculated by:

\[ i_{\text{lim},H^+} = \left( \frac{D_{H^+} \cdot 2F}{x} \right) \cdot C_{b,H^+} \] (6-4)

where \( D_{H^+} \) is the diffusion coefficient of \( H^+ \) within SRB biofilms, \( m^2/s \); \( x \) is the thickness of the diffusion layer, m; and \( C_{b,H^+} \) is the bulk-fluid concentration of \( H^+ \), \( \text{mol/m}^3 \). The diffusion resistance in the liquid diffusion layer (Figure 6-1) is negligible. Hence, \( C_{b,H^+} \) equals the concentration of \( H^+ \) within biofilms.

The diffusion coefficient in water is a function of temperature and viscosity and can be calculated from the Stokes-Einstein equation:

\[ D_w = D_{\text{ref}} \times \frac{T}{T_{\text{ref}}} \times \frac{\mu_{\text{ref}}}{\mu} \] (6-5)

where the reference temperature \( T_{\text{ref}} \) is 293.15 K; the viscosity of water \( \mu_{\text{ref}} \) is 1.002 kg/(m·s) and the diffusion coefficient for \( H^+ \) ion \( D_{\text{ref}} \) is \( 9.31 \times 10^{-9} \text{ m}^2/\text{s} \). Viscosity \( \mu \) is a function of temperature:

\[ \mu = \mu_{\text{ref}} \times 10^{\frac{1.3272(293.15-T)-0.001053(293.15-T)^2}{T+105}} \] (6-6)

The diffusion coefficient \( D_{H^+} \) within biofilms can be obtained by the following empirical equation (Fan et al., 1990):

\[ \frac{D}{D_w} = 1 - \frac{0.43X^{0.92}}{11.19 + 0.27X^{0.99}} \] (6-7)

where \( X \) is biomass density, kg/m\(^3\). In this model, \( X=60 \text{ kg/m}^3 \).

It must be noted that due to the complexity of the biofilm system, there is a known pH buffer property. To quantity the local pH within the biofilms is beyond the
scope of this research. An assumption for this model was made that within biofilms the local pH is 0.2 lower than the pH in the bulk solution, the value of which is applied in the calculation of $i_0$.

### 6.2.2.2.2 $H_2CO_3$ reduction

The limiting current density $i_{\text{lim}}$ can be calculated from the equation derived by Vetter (1967):

$$i_{\text{lim, } H_2CO_3} = F \times [CO_2]_b \times \left(D_{H_2CO_3}K_{\text{hyd}}k_{\text{hyd}}^f\right)^{0.5}$$

(6-8)

where $[CO_2]_b$ is the maximum concentration of dissolved carbon dioxide, mol/m$^3$. This equals the bulk concentration of dissolved carbon dioxide plus the carbon dioxide generated due to sessile SRB activity as:

$$[CO_2]_b = K_{CO_2}^d \times P_{CO_2} \times 1000$$

(6-9)

where $P_{CO_2}$ is the partial CO$_2$ pressure, bar; the value of $K_{CO_2}^d$ is calculated from the formula listed in Table 6-1. The diffusion coefficient $D_{H2CO3}$ at 298.15 k is $2.0 \times 10^{-9}$ m$^2$/s. The equilibrium constants for the CO$_2$ hydration reaction $K_{\text{hyd}}$ and $k_{\text{hyd}}^f$ are listed in Table 6-1.

Combining the equations of (6-2), (6-3), (6-4) and (6-8), the anodic current density and cathodic current density of each species can be obtained separately. The unknown potential E (corrosion potential or open-circuit potential) can be solved by the charge balance equation:

$$\sum_{1}^{n_a} i_a = \sum_{1}^{n_c} i_c$$

(6-10)
where the subscript a means anodic reaction while c means cathodic reaction; n is the number for anodic or cathodic reactions.

### 6.2.2.3 Transport processes

As sketched in Figure 6-1, transport processes include species such as $\text{Fe}^{2+}$, $\text{Na}^+$, $\text{CO}_2$, $\text{H}_2\text{CO}_3$, $\text{HCO}_3^-$, $\text{CO}_3^{2-}$, $\text{H}^+$, $\text{OH}^-$, $\text{Cl}^-$, $\text{SO}_4^{2-}$, $\text{HS}^-$, $\text{S}^{2-}$, etc, some of which are involved in the electrochemical reactions at the metal surface. The concentration of each species is governed by a general transport equation which describes its spatial distribution within biofilms and the boundaries as shown below:

$$
\frac{\partial \epsilon C_j}{\partial t} = - \frac{\partial (\kappa N_j)}{\partial x} + \epsilon R_j \tag{6-11}
$$

where $C_j$ is the concentration of species $j$, mol/m$^3$; $R_j$ is the source or sink due to chemical reactions, mol/(m$^3$·s); $\epsilon$ is the volumetric porosity and $\kappa$ is the surface permeability of the film, respectively; $N_j$ is the flux of species $j$, which can be described as:

$$
N_j = -D_j \frac{\partial C_j}{\partial x} - z_j u_j F C_j \frac{\partial \Phi}{\partial x} + C_j \nu \tag{6-12}
$$

where $D_j$ is the diffusion coefficient of species $j$, m$^2$/s; $z_j$ is the electrical charge of species $j$; $u_j$ is the mobility of species $j$; $\Phi$ is the electric potential in the solution, V; and $\nu$ is the velocity. The permeability of surface films for transport of species $\kappa$ can be correlated to the volumetric porosity $\epsilon$ as follows:

$$
\kappa = \epsilon^{3/2} \tag{6-13}
$$
The average porosity of biofilm can be estimated from methods described by Lewandowski (2000).

In this model, the second and third term in Equation (6-12) are omitted to simplify the calculation since it is a stagnant closed system. Only transport of H$_2$CO$_3$ within biofilms is considered in this model. The production of H$_2$CO$_3$ by sessile SRB is taken into account. In a small time frame, the concentration of carbon dioxide hydrate is time-independent within biofilms and a pseudo-steady state can be assumed. The transport equation is then solved numerically. After the anodic current density of $i_a$ is obtained, the corrosion rate of mild steel can be calculated by (Sun, 2006):

$$
CR_{Fe} = 1.155i_{a,Fe}
$$

(6-14)

where the unit of $CR_{Fe}$ is mm/y. Since the corrosion occurs locally underneath the biofilms, the corrosion rate of mild steel, herein, is actually pitting rate. Pit depth can be obtained by multiplying CR with time duration.

\textit{a) Initial conditions}

Concentrations of species in the bulk solution are used as the initial conditions. It is assumed that the bulk solution is thoroughly mixed before the mild steel is exposed to the corrosive environment.

\textit{b) Boundary conditions}

As sketched in Figure 6-1, the following are the boundary conditions for Equation (6-12):

at the top of the biofilm,

$$
x = 0, \ C_j = C_{b,j}
$$

(6-15)
at the metal surface,

\[ x = \delta, -D \frac{\partial C_j}{\partial x} = \frac{i_j}{n_j F} \]  \hspace{1cm} (6-16)

where \( n_j \) is the number of electrons involved in the electrochemical reaction for species \( j \).

### 6.2.3 Numerical method

Similar to Gu et al. (2009) the corrosion rate \( CR \) is calculated using a time loop. In each time step of a small time frame, the source term due to sessile SRB activity in Equation (6-11) and corrosion rate \( CR \) are treated as constant. The biofilm thickness \( x \) is the sum of previous SRB biofilm thickness and the pit depth difference obtained by multiplying the corrosion rate \( CR_t \) at time \( t \) with the time step length. Since the biofilm is pre-mature and has low activity due to the limited nutrients, the top surface of biofilms exposed to bulk solution does not move. Pits grow over time as the biofilms fill the void formed by the pits underneath.

### 6.2.4 Results and discussion

The electrochemical parameters and their formulae used in this model have been listed on Table 6-1, Table 6-2 and mentioned in Chapter 2. Other than these, parameters used in this simulation are presented as follows: the initial biofilm thickness (\( x \)) is 30 \( \mu \)m; the biofilm dry density (\( X \)) is 60 kg/m\(^3\) while the source term due to sessile SRB activity in Equation (6-11) is set to a constant based on the experimental data.

This model was developed by using LabWindows/CVI in an ANSI C programming environment. Figure 6-2 illustrates the interface for this model with input variables of temperature, time, \( CO_2 \) partial pressure, the \( pH \) of bulk solution, SRB biofilm thickness and sessile SRB activity. The corrosion rate \( CR \) (pitting rate), pit depth and
their variation with time can be obtained. The figure for corrosion potential vs. time is also presented on the interface. It is advisable to save the raw data for the corrosion potential, corrosion rate and pit depth in separate files which will allow for better graphing using other tools like Excel.

Figure 6-2. Interface for Model for MIC corrosion in the presence of CO₂.

It should be noted that the water chemistry module is not implemented in this model and the pH of bulk solution at a specified condition is either obtained from experiments or calculated from other application software like MULTICORP. The sessile SRB activity is set at 1.00 with respect to the low activity of sessile SRB due to limited nutrients. When rich nutrients are available, sessile SRB increase their activity and factor f is used to calibrate the situation. Once the activity is high enough and the effects of
generated H$_2$S and FeS film cannot be ignored, this model is no longer suitable and must use MIC corrosion in the presence of a CO$_2$ and H$_2$S module. This module has not yet been developed.

Figure 6-3 shows the simulation results of pitting rate and pit depth after a 365-day test for mild steel in formation water at 25ºC and CO$_2$ partial pressure 1 bar. The pitting rate decreases over time while the pit depth has the opposite trend. By assuming biofilms can fill the void created by pits, the biofilm thickness can also increase with time; thus, increasing the mass transfer resistance. Hence, mass transfer limitation gradually takes control as pit depth grows. The corresponding corrosion potential decreased slightly from initial (-0.4646 v) to (-0.4655 v) after 365 days (Figure 6-4).

![Figure 6-3. Simulated pitting rate and pit depth prediction at conditions of temperature 25ºC, CO$_2$ pressure 1 bar and pH 5.0 for mild steel in formation water with pre-matured biofilms thickness 20 µm and ε=0.30.](image-url)
Figure 6-4. Simulated corrosion potential prediction at conditions of temperature 25ºC, CO₂ pressure 1 bar and pH 5.0 for mild steel in formation water with pre-matured biofilms thickness 20 µm and ε=0.30.

Temperature has a positive impact on the pit depth as shown on Figure 6-5 with calibrated pH of 5.0 (25ºC). It shows an approximate linear relationship at low temperature (<20ºC); at higher temperature, the corrosion process is more active, corresponding to the larger slope on the pit depth curve. For temperature over the limit of 50ºC, the precipitation of ferrous carbonate cannot be ignored. This is also out of range for the strain of SRB used in this model. Another model considering precipitation and utilizing the parameters of thermophilic bacteria should be applied.

Figure 6-6 illustrates the impact of initial biofilm thickness on the pit depth. This indicates the accumulation of corrosive species generated by sessile bacteria: the larger the biofilm thickness, the more corrosive the species and, subsequently, the deeper the pits. There is an approximately linear relationship for larger biofilm thickness; while for much thinner biofilm, the depth of pits increases sharply. It must be noted that once rich
nutrients are available, biofilms can be activated and can be corrosive. A new model is required.

Figure 6-5. Simulated temperature effects at conditions of CO$_2$ pressure 1 bar and calibrated pH 5.0 for mild steel in formation water with pre-matured biofilms thickness 20 µm and $\varepsilon=0.30$ after a 365-day test.

The impact of CO$_2$ partial pressure on the mild steel corrosion is shown on Figure 6-7. With increasing CO$_2$ pressure, the pit depth gradually increases. pH corresponding to the CO$_2$ partial pressure is adjusted based on water chemistry using MULTICORP software.

Figure 6-8 shows the potentiodynamic sweep profile for mild steel in the presence of CO$_2$. It clearly shows that the cathodic reaction is controlled by the mass transfer of H$_2$CO$_3$. The contribution of pH is small in comparison to that of H$_2$CO$_3$. Meanwhile, the reduction of H$_2$O is in the charge transfer control.
Figure 6-6. Simulated biofilm thickness effects at conditions of temperature 25°C, CO\textsubscript{2} pressure 1 bar and pH 5.0 for mild steel in formation water with pre-matured biofilms thickness 20 µm and ε=0.30 after a 365-day test.

Porosity of film ε is a sensitive parameter in this model. It varies from 1.0 to a small value, indicating the transience from non-protective to protective biofilm. Its effects on the pitting rate are shown in Figure 6-9. The calculation of porosity of films has been described in literature (Lewandowski, 2000; Nesic & Lee, 2003). However, due to the lack of film information, in this work the porosity is assumed and calibrated using experimental data. The pitting rate differs from 0.81 mm/y to 0.067 mm/y when the porosity varies from 1.0 to 0.1. For protective film, the pitting rate remains constant at a low level. On the other hand, the porosity of biofilms can be used as an indicator of the corrosivity of biofilms.
Figure 6-7. Simulated CO\textsubscript{2} partial pressure effects at conditions of temperature 25\textdegree C and pH 5.0 around for mild steel in formation water with pre-matured biofilms thickness 20 µm after a 365-day test.

Figure 6-8. Simulated potentiodynamic sweep at 25\textdegree C, CO\textsubscript{2} pressure 1 bar and pH 5.0 for mild steel in formation water with pre-matured biofilms 20 µm and ε=0.30, time = 0.
Figure 6-9. Simulated porosity of biofilms effects on pitting rate for mild steel at conditions of temperature 25°C and pH 5.0 in formation water with pre-matured biofilms thickness 20 µm after a 365-day test.

### 6.3 Verification of the model

The simulation results from the model are compared with the experimental data described in Chapter 5 for tests following hydrotesting in the presence of CO₂ as shown on Figure 6-10. The pure CO₂ uniform corrosion model of MULTICORP overestimated the corrosion rate since it does not take into account the biofilm characteristics. With the low nutrient supply, the sessile SRB is at low activity. Hence, the MIC model MICORP based on BCSR theory underestimated the pitting rate without considering the CO₂ effects. In this work, the model implementing the biofilm characteristics into the pure CO₂ model corresponds with the experimental data.
By implementing the biofilm characteristics of a source of corrosive species and protective or non-protective film into the electrochemical model for CO$_2$ corrosion, a mechanistic model for MIC in the presence of CO$_2$ was developed. This model demonstrates that the physical characteristics of biofilms including porosity is important to determine the biofilm corrosivity.
CHAPTER 7 CONCLUSION

Lab investigation of MIC was conducted in a glass cell bioreactor and in anaerobic vials. Improvements of methodology in testing for MIC were made by correlating the cylindrical coupon rotation rate in the glass cell to linear pipe flow with respect to rough surface. In addition, low flow lab testing was modeled using FLUENT software based on mass transfer similarity to predict fast pipe flow in the field using an open-ended flow loop. Because of mass transfer similarity, this flow loop can be used to investigate corrosion of deadlegs in pipelines due to MIC.

Enhancing the mitigation of MIC was achieved by combining biodegradable chelators and biocides. With the help of methanol or D-amino acid, the efficacy of chelator/biocide binary treatment was further enhanced, showing promise in reducing MIC risk in practice.

Lab investigation of MIC during hydrotesting and subsequent exposure to pipeline fluids illustrated that the biofilm build-up during hydrotesting can cause MIC after hydrotest. Biofilms can maintain their activities even with limited nutritional liquids such as oil/water mixture. When exposed to a CO₂ or H₂S/CO₂ environment, severe pitting corrosion due to MIC occurred. Experimental data showed that 300 ppm of THPS treatment for 30 min was not effective enough to eradicate biofilms and reduce MIC.

Based on the uniform CO₂ corrosion model with characteristics of biofilms, a mechanistic MIC model was proposed to predict the MIC pitting rate in the presence of CO₂. Experimental results on MIC following hydrotesting were used to validate this model.
CHAPTER 8 RECOMMENDATIONS AND FUTURE WORK

- MIC is such a complex process that no single mechanism can completely describe this phenomenon. Careful evaluation of corrosion factors, bacterial characteristics and environmental conditions must be considered. This work has focused on SRB biofilms and mitigation of MIC. Further research is mandated using field consortia to confirm the findings used in this study.

- Open-ended flow loop has proven to be closer to field conditions than regular recirculating flow loops. The cost effectiveness of this loop should lead to more MIC research.

- Since biofilms are the key for MIC research, a reliable on-line method to detect and monitor biofilm growth and metabolism should be developed.

- Biodegradable chelators and other chemicals should further be evaluated for their enhancement for biocide efficacy in lab scale and possible field practice. D-amino acid showed promise among these chemicals. A mix of L- and D-amino acids, a naturally produced mix, could reduce the cost drastically. Its performance should be evaluated in future work.

- A preliminary MIC model in the presence of CO$_2$ with some assumptions was proposed. A more accurate local pH profile is needed to incorporate into this model. Flow conditions and other corrosive species should be considered as well. Research could be expanded to predict MIC in the presence of H$_2$S/CO$_2$ in future study.
REFERENCES


