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Microbiologically Influenced Corrosion and Its Impact on Metals and Other Materials

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15.1 Introduction

Microbiologically influenced corrosion (MIC), or biocorrosion, is blamed for causing serious problems in many fields, such as the oil and gas industry, water treatment systems and sewer systems. The integrity, safety and reliability of the pipeline and equipment are severely affected by MIC. In 2006, MIC was a primary suspect in the leakage of Alaska oil pipelines. The leak caused a major production shutdown that resulted in an oil price spike (Jacobson 2007). MIC may account for 20% of all corrosion losses (Flemming 1996).

Sulphate-reducing bacteria (SRB) are the more common microbes involved in MIC. They may be introduced in a system via multiple ways, including secondary oil recovery and hydrotest. SRB oxidise organic carbons to harvest electrons. The electrons are used for sulphate reduction that causes biogenic...
hydrogen sulphide gas is not only toxic to living organisms but is also a cause in reservoir souring and a corrosion threat. SRB tend to live in a biofilm community, which provides protection against harmful environmental factors such as pH swings and biocides. Biofilm is a mass transfer barrier, which may lead to a local shortage of organic carbon at the bottom of the biofilm. Electrogenic microbes such as SRB living as “bottom feeders” on a steel surface may switch from organic carbon to elemental iron as the electron donor for the reduction of an oxidant such as sulphate under biocatalysis, leading to pitting corrosion (Xu and Gu 2011). Besides SRB, electrogenic nitrate-reducing bacteria (NRB) and methanogens may also cause MIC (Uchiyama et al. 2010; Xu et al. 2013) because they are capable of utilising electrons from steel for nitrate and CO$_2$ reduction, respectively. Gu and Xu (2013) lumped these electrogenic microbes as XRB, that is, X-reducing bugs in which X represents oxidants (electron acceptors), including sulphate, nitrate/nitrite, CO$_2$ and so on.

Numerous theories have been proposed to demonstrate the mechanisms of MIC (Videla and Herrera 2005). The cathodic depolarisation theory (CDT) was first introduced to explain MIC by von Wolzogen Kühr and van der Vlugt (1934). CDT interprets how MIC occurs from the electrochemical aspect. However, CDT cannot explain MIC caused by hydrogenase-negative SRB and other non-sulphate-reducing bacteria. Gu et al. (2009) presented a new MIC theory called the biocatalytic cathodic sulphate reduction (BCSR) theory. It provides a bioenergetics explanation of MIC. In BCSR, iron dissolution (oxidation) and sulphate reduction in the cytoplasm of cells are two key steps of MIC. The cell potential of the resultant redox reaction is +230 mV, which yields a negative Gibbs free energy change. It means this process is thermodynamically favourable. SRB harvest energy to support their metabolism from this process. SRB biofilms serve as biocatalysts. They lower the high activation energy of sulphate reduction (Gu et al. 2009). Analogously, the theory that explains MIC caused by NRB is called biocatalytic cathodic nitrate reduction (BCNR) (Gu and Xu 2013; Xu et al. 2013). NRB reduce nitrate to N$_2$ or NH$_4^+$, with a cell potential of +1196 mV and +805 mV, respectively, when combined with iron oxidation.

MIC has been classified into three categories to distinguish the various mechanisms (Gu 2012a; Gu and Xu 2013). Type I MIC is caused by electrogenic bacteria. Electrogenic bacteria, which can actively form pili for electron transfer and energy distribution, perform respiration metabolism (Chang et al. 2006). They use carbon steel or other non-noble metals as electron donors intentionally because the reduction potentials of the ions in these metallic materials are sufficiently negative to form thermodynamically favourable redox reactions when coupled with the reduction of an oxidant such as sulphate and nitrate. Bacteria utilise electrons released from elemental metal oxidation and reduce the oxidant intracellularly.

Type II MIC is defined as the corrosion caused by the metabolites secreted by microbes. These metabolites are oxidants such as volatile fatty acids. The
MIC caused by acid-producing bacteria (APB) belongs to this category (Gu and Xu 2013). Copper MIC caused by SRB is also Type II MIC. Type III MIC is caused by microbial species that secrete enzymes or other corrosive chemicals, which degrade non-metallic materials containing organic carbon as one of the components.

MIC detection and mitigation are essential for pipeline security. Planktonic cells can be easily detected. However, biofilms are the main culprit in MIC and they are more elusive. The detection and enumeration of sessile cells are more critical. Microbiology tests using culture media may be used for detection. Molecular biology methods such as quantitative polymerase chain reaction (qPCR), confocal laser scanning microscopy (CLSM) and denaturing gradient gel electrophoresis (DGGE) are also applied to identify sessile cells. The main methods used to mitigate MIC in the field are pigging and biocide application (Videla 2002). Pigging can remove most of the established biofilm by physical scratching. Using biocide is a chemical way to reduce the bacteria population. Tetrakis hydroxymethyl phosphonium sulphate (THPS) and glutaraldehyde are two widely used biodegradable biocides in the oil and gas industry. Biocide can be applied during the pigging process. Some pipelines, especially older pipelines, are unpiggable due to their designs. They include pipes with small or multiple diameters, bends and connections, and so on (Tiratsoo 2013). Because of the various defense mechanisms adopted by biofilms, sessile cells in biofilms require 10 times the dosage of biocide for planktonic cells (Mah and O'Toole 2001). Owing to the fact that a large amount of biocide is needed, the cost of biocide treatment is high. Environmental hazards are another concern for the increasing biocide usage in the field. Raad et al. (2003, 2007) introduced ethylenediaminetetraacetic acid (EDTA) as a biocide enhancer to strengthen the effect of biocides while lowering their concentrations. Recently, Kolodkin-Gal et al. (2010) reported that \(\text{d-tyrosine}, \text{d-methionine}, \text{d-tryptophan}\) and \(\text{d-leucine}\) mixed with equal concentration as low as 10 nM (overall) could trigger the disassembly of \textit{Bacillus subtilis} biofilm. Xu et al. (2012b) demonstrated that an equimolar mixture of these four \(\text{d-}\)amino acids could promote the biocidal effect of THPS. A synergistic THPS and \(\text{d-tyrosine}\) or \(\text{d-methionine}\) combination was found to effectively prevent the \textit{Desulfovibrio vulgaris} biofilm formation and remove the established biofilm (Xu et al. 2012a, 2013).

15.2 MIC Mechanisms and Classifications

The essence of anaerobic MIC of elemental iron (\(\text{Fe}^0\)) is the following \(\text{Fe}^0\) oxidation reaction:

\[
\text{Anodic: } \text{Fe} \rightarrow \text{Fe}^{2+} + 2e^- \quad \text{(iron oxidation)} \quad -E^\circ = +447 \text{ mV} \quad (15.1)
\]
To keep the oxidation reaction going and to maintain electroneutrality, the electrons released by Reaction 15.1 must be absorbed by an electron acceptor. The reduction potential for Fe\(^{2+}/Fe^0\) is \(-447\) mV, in which \(E^{o'}\) stands for the reduction potential (also known as redox potential) at 25°C, pH 7, 1 M solutes (1 bar gases) except H\(^+\) with the standard hydrogen electrode (SHE) as the reference. Because Reaction 15.1 is written as an oxidation reaction, \(E^{o'} = -447\) mV is expressed as \(-E^{o'} = +447\) mV to indicate the reaction direction. The prime in \(E^{o'}\) and other related symbols in bioelectrochemistry denotes pH 7. Table 15.1 shows some \(E^{o'}\) values used in this work.

### 15.2.1 BCSR Theory

Sulphate reduction occurs in the cytoplasm of an SRB cell via the adenosine phosphosulphate (adenylylsulphate) (APS) pathway requiring ATP sulphurylase, APS reductase and bisulphite reductase enzymes (Thauer et al. 2007):

\[
\text{SO}_4^{2-} \rightarrow \text{APS} \rightarrow \text{HSO}_3^- (\text{bisulphite}) \rightarrow \text{HS}^- (\text{bisulphide})
\]  

(15.2)
The overall sulphate reduction reaction can be expressed as

\[
\text{SO}_4^{2-} + 9\text{H}^+ + 8\text{e}^- \rightarrow \text{HS}^- + 4\text{H}_2\text{O} \quad E^o' = -217\text{mV}
\]  

(15.3)

When sulphate reduction is coupled with Fe\textsuperscript{0} oxidation, the cell potential for the redox reaction is \(\Delta E^o' = +230\text{ mV}\) (calculated from \(447\text{ mV} - 217\text{ mV}\)) under the conditions defined for \(E^o'\). This positive cell potential corresponds to \(\Delta G^o' = -178\text{ kJ/mol sulphate}\) based on the following equation:

\[
\Delta G^o' = -nF\Delta E^o'
\]

(15.4)

where \(n\) is the number of electrons involved in the redox reaction (8 in this case) and \(F\) is the Faraday constant (96,485 C/mol). The negative Gibbs free energy change (\(\Delta G^o'\)) value indicates that the redox reaction coupling Fe\textsuperscript{0} oxidation with sulphate reduction is thermodynamically favoured (i.e. energy is produced) under the conditions defined for \(E^o'\). If actual temperature, concentration and pressure values deviate from these conditions, \(E^o'\) values will shift based on the Nernst equation. Thus, \(\Delta E^o'\) and \(\Delta G^o'\) will change. However, \(\Delta G^o'\) in this case will still be negative because \(\Delta G^o' = -178\text{ kJ/mol}\) is far from borderline negative.

Fe\textsuperscript{0} is a fuel molecule. It has been used as the sole energy source in bioenergetics studies not intended for MIC investigations in the past (Biswas and Bose 2005; Ghafari et al. 2008; Ginner et al. 2004). The “burning” of Fe\textsuperscript{0} with sulphate as the oxidant produces energy that benefits SRB metabolism. In fact, Fe\textsuperscript{0} is slightly more energetic than lactate under the conditions defined for \(E^o'\) because Fe\textsuperscript{2+}/Fe\textsuperscript{0} has a slightly more negative \(E^o'\) value of \(-447\text{ mV}\) (Table 15.1) compared with \(-430\text{ mV}\) for CO\textsubscript{2} + acetate/lactate. Favourable thermodynamics does not mean that corrosion will happen at an appreciable speed if kinetics is retarded. Sulphate reduction has a high activation energy. It will proceed only with SRB biocatalysis involving multiple enzymes in the SRB cytoplasm. Thus, Gu et al. (2009) proposed the BCSR theory by treating Fe\textsuperscript{0} oxidation as the anodic reaction and biocatalysed sulphate reduction as the cathodic reaction. The word “cathodic” is used in BCSR merely to indicate that the reaction is a reduction reaction for the purpose of mechanistic modelling (Xu and Gu 2011). There is no physical cathode at the site where sulphate reduction occurs, that is, the SRB cytoplasm.

### 15.2.2 Electrogenesis in MIC

Organic carbon molecules such as lactate and acetate are soluble. They can diffuse into the SRB cytoplasm to be oxidised. They donate electrons in the oxidation process in the cytoplasm where they are used for sulphate reduction. This is not the case for Fe\textsuperscript{0} as an electron donor. The iron (or steel) matrix is insoluble and thus Fe\textsuperscript{0} cannot enter the cytoplasm. Fe\textsuperscript{0} oxidation
occurs extracellularly without catalysis. In SRB MIC that uses sulphate as the terminal electron acceptor, the electrons released by Fe$^0$ oxidation must be transported across the SRB cell wall to the cytoplasm. This kind of electron transfer is impossible from an iron surface to a planktonic cell because electrons do not “swim” in water. Only sessile cells in a biofilm can do it. Microbes that are capable of cross-cell wall electron transfer are known as electrogens.

The sessile cells in a biofilm can transfer electrons using either direct electron transfer (DET) or mediated electron transfer (MET). In DET, cell membrane-bound proteins such as c-type cytochromes are involved. The cells are either directly in contact with a metal surface or via conductive nanowires (pili) as shown in Figure 15.1. The proteins transfer extracellular electrons from outside the cells to the cytoplasm. This is opposite to the electron transfer direction in the bioanode of a microbial fuel cell (MFC). For cells that are unable to perform DET, redox-active electron mediators (also known as shuttles or carriers) are used by electrogens. These chemicals absorb and discharge electrons when they move between two locations. They are recycled for repeated uses. For hydrogenase-positive SRB, hydrogen (H$_2$) is used as an electron carrier. Other common electron carriers include riboflavin, nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD). Gu and Xu (2013) tested riboflavin and FAD to promote (accelerate) MIC by *D. vulgaris* against carbon steel. They found that both of them accelerated weight loss and pit depth growth without changing planktonic and sessile cell densities. Gu and Xu (2010) called these electron mediators MIC promoters (i.e. MIC accelerators). Gu (2011) filed a provisional patent on the use and detection of electron mediators in MIC tests and MIC forensics in 2011.

The classical CDT first proposed by von Wolzogen Kühr and van der Vlugt (1934) describes the use of hydrogenase in the “depolarising” of a cathode to push the iron oxidation reaction forward by removing the adsorbed hydrogen atoms on a cathode. This theory is applicable to hydrogenase-positive SRB. In fact, it is a case of using H$_2$ as the electron carrier for cross-cell wall electron transfer. In bioelectrochemistry, the use of H$_2$ as the electron carrier has been well documented, albeit in non-corrosion research such as MFCs (Gu 2012a). Recently, Venzlaff et al. (2013) presented electrochemical data showing direct electron transfer from a steel surface to SRB.

**FIGURE 15.1**
Schematic illustration of DET across SRB cell walls.
Most cells are not electrogenic because they do not need to transport electrons across cell walls. Typical electron donors such as organic carbons are usually soluble and can diffuse into the cytoplasm. Some cells can become electrogenic when the need arises. Sherar et al. (2011) discovered that pili were formed on the iron surface only in the absence of organic carbon during the culturing of SRB cells isolated from an oil well. It was likely that the SRB cells formed pili to transfer electrons when they switched from organic carbon to Fe\(^0\) as an electron donor. Some SRB isolated from carbon steel pipelines were initially found to be hydrogenase-positive, but they would change to hydrogenase-negative after incubation in an enriched medium (Bryant et al. 1991). It was likely that soluble organic carbon in the culture medium served as electron donor. They readily diffuse to the cytoplasm of the SRB cells without the need for H\(_2\) as the electron carrier. The formation of pili and the production of hydrogenase enzymes consume resources. The SRB cells produce them only when they are needed for electron transfer.

Without the help of pili, direct cell wall to metal surface contact is required for DET. This means only a monolayer of sessile cells in a biofilm are directly involved in harvesting electrons from Fe\(^0\) oxidation. This limits the severity of MIC. With the help of pili networking among cells and between cells and the steel surface, several layers of sessile cells may be involved directly in harvesting electrons from Fe\(^0\) oxidation. Thus, MIC can become more severe. Similarly, electron mediators can allow more than a monolayer of sessile cells to be active in extracellular electron transfer, leading to accelerated MIC (Gu and Xu 2013). Type I MIC’s key process is about the transport of extracellular electrons for use in the reduction of an oxidant (e.g. sulphate) in the cytoplasm of a cell. Figure 15.2 illustrates electron transfer in MIC by SRB.

The “motive” for SRB attacks on steel was not fully explained until Xu and Gu (2011) designed a starvation experiment based on their understanding of the bioenergetics of MIC. Their theoretical analysis of Type I SRB MIC suggests that SRB cells starved of organic carbon should be more aggressive because they switch from organic carbon to Fe\(^0\) as the electron donor for energy production. To prove this, they first grew *D. vulgaris* biofilms on C1018 carbon steel coupons in ATCC 1249 medium (full-strength medium) to maturity. Then, the coupons were retrieved and dropped into new anaerobic vials containing modified ATCC 1249 media, including full-strength medium (i.e. full-strength ATCC 1249 medium), full-strength medium minus 90% carbon source (mild starvation), full-strength medium minus 99% carbon source (severe starvation) and full-strength medium minus 100% carbon source (extreme starvation). The vials were incubated for another 7 days at 37°C. The experimental data clearly demonstrated that when removing 90% and 99% of the organic carbon in the culture media, the weight losses increased significantly. Severe organic carbon starvation produced the largest normalised weight loss, while mild organic carbon starvation yielded the deepest pit depth. They suggested that deeper pits required advancement of the SRB
biofilm towards the newly created pit bottom. This means that the biofilm needed some external organic carbon to grow a little because scavenging the dead cells and exopolymers in the biofilm was insufficient. These data fully supported the theoretical prediction that SRB switched from organic carbon to Fe$^{0}$ in order to obtain energy. The data also corroborated with the electron transfer theory in Type I MIC, which requires direct biofilm attachment to the pit bottom for the pit to grow (Figure 15.2).

**15.2.3 BCNR Theory**

Nitrate injection has been widely used in reservoir souring mitigation to suppress biogenic H$_{2}$S production downhole by SRB (Gieg et al. 2011). It promotes respiration by NRB to suppress sulphate respiration by SRB. However, not many people realise that nitrate reduction by NRB can also cause MIC. This means that if nitrate is not completely utilised downhole, it may cause MIC in pipelines. Some SRB can utilise nitrate as well. MIC by NRB can be explained by a theory called BCNR theory that is completely analogous to BCSR. NRB can cause Type I MIC, which means that only electrogenic NRB can do this. Figure 15.3 illustrates the role of electron transport in MIC by NRB.

As shown in the two reactions below, nitrate reduction to either nitrogen or ammonia both have rather large positive E°' values, which means that nitrate...
is a more potent oxidant than sulphate. It leads to far more negative $\Delta G^{\circ}$ values. This means the nitrate attack on Fe$^0$ is thermodynamically favoured strongly.

$$2\text{NO}_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6\text{H}_2\text{O} \quad (E^{\circ'} = +760\text{mV}) \quad (15.5)$$

$$\text{NO}_3^- + 8e^- + 9H^+ \rightarrow \text{NH}_3 + 3\text{H}_2\text{O} \quad (E^{\circ'} = +360\text{mV}) \quad (15.6)$$

With biocatalysis by electrogenic NRB for nitrate reduction, Type I MIC occurs. Xu et al. (2013) found that *Bacillus licheniformis* was more corrosive than typical SRB when it was grown as an NRB. It caused a maximum pit depth of 14.5 $\mu$m in 1 week (Figure 15.4).

**15.2.4 Type II MIC by Microbes That Secrete Corrosive Metabolites**

MIC by SRB and NRB belong to Type I MIC that requires electrogenic microbes at the bottom of the biofilm. Type II MIC does not require electrogenic microbes because it is caused by microbes such as APB that secrete corrosive metabolites such as organic acids. Proton (H$^+$) is an oxidant. At a
sufficiently low pH, the proton causes corrosion because it has a sufficiently high reduction potential that will make the oxidation of Fe\(^0\) coupled with its reduction thermodynamically favourable. The following Nernst equation can be written to calculate the reduction potential of H\(^+\) at non-standard conditions:

\[
E(2H^+/H_2) = -\frac{2.303RT}{F} \cdot \text{pH} - \frac{RT}{2F} \cdot \ln \left( \frac{p_{H_2}}{p_o} \right)
\]  

(15.7)

where R is the universal gas constant, T the temperature (in K) and \(p_{H_2}\) the partial pressure of H\(_2\) (in bar). In Equation 15.7, \(p_o\) equals to 1 bar. Plugging in the conditions defined for \(E^o'\), the Nernst equation yields \(E^o'(2H^+/H_2)\) = −414 mV, which differs from SHE’s 0 mV because \(E^o'\) requires pH 7, while SHE requires pH 0 (i.e. 1 M H\(^+\)).

As shown below, both proton reduction and free (i.e. undissociated) acetic acid reduction produce hydrogen gas.

\[
2H^+ + 2e^- \rightarrow H_2 \text{ (proton reduction)} \tag{15.8}
\]

\[
2\text{HAc} + 2e^- \rightarrow 2\text{Ac}^- + H_2 \text{ (free acetic acid reduction)} \tag{15.9}
\]

More recent electrochemical analysis of acetic acid corrosion at the Ohio University Corrosion Institute suggests that it may be more accurate to treat HAc as a reservoir of H\(^+\) rather than viewing it as capable of direct
oxidation (Gu and Xu 2013). Oxidants such as H^+ can be directly oxidised without biocatalysis unlike sulphate and nitrate. Thus, acid corrosion happens abiotically in conventional chemical corrosion. Biofilms in Type II corrosion merely provide a locally more concentrated source of oxidants, and planktonic cells may also participate by secreting corrosive metabolites as well, albeit at lower concentrations because sessile cells tend to have a volumetric cell number density that is two orders of magnitude higher (Gu and Xu 2013). Gu (2012b) points out that Type I MIC is caused by microbes that perform respiration while Type II MIC is caused by microbes that grow fermentatively. The former needs external electron acceptors such as sulphate and nitrate, while the latter create their own electron acceptor. Fermentative microbes tend to produce organic acids (e.g. fatty acids), alcohols and so on during their metabolism. APB are typical examples.

Although H_2S is corrosive, Gu and Xu (2013) argued that SRB attack on Fe^0 is primarily Type I MIC due to cross-cell wall electron transport, rather than Type II MIC caused by secreted H_2S. Their view is supported by the fact that starved *D. vulgaris* cells are more aggressive. Interestingly, elemental copper (Cu^0) corrosion by SRB falls under Type II MIC (Gu and Xu 2013). The E° values for Cu^+/Cu^0 and Cu^{2+}/Cu^0 are +520 mV and +340 mV, respectively. This means Cu^0 oxidation coupled with sulphate reduction will have a negative cell potential of −737 mV with Cu^+ as the oxidation product, or −557 mV with Cu^{2+} as the oxidation product. These very negative values lead to very positive ΔG° values, suggesting that the redox reaction of copper oxidation coupled with sulphate reduction is thermodynamically unfavourable. Thus, this kind of corrosion cannot happen.

Puigdomenech and Taxén (2000) found that the following reaction is strongly favoured thermodynamically:

\[
2\text{Cu}_{(\text{crystal})} + \text{S}^{2−} + 2\text{H}^+ \rightarrow \text{Cu}_2\text{S}_{(\text{crystal})} + \text{H}_2(\text{g})
\]  

Thus, SRB corrosion of Cu^0 should belong to Type II MIC because it is caused by secreted metabolites. Electron transfer happens outside the cells, rather than in the cytoplasm of SRB cells in this case.

### 15.2.5 Type III MIC by Microbes That Degrade Organic Materials

The MIC caused by secreted enzymes that degrade organic matter such as polyurethane and other polymers, as well as plasticisers, for the objective of obtaining utilisable organic carbons for the microbes is termed Type III MIC by Gu and Xu (2013). This kind of MIC is better known as biodegradation of organic materials. There have been documented cases of Type III MIC attacks on plastic structures and electric insulation (Gu 2003). Degradation of wire insulation by microbes in older airplanes threatens aging military and civilian aircraft.
15.3 Examples and Analyses of MIC against Metals and Other Materials

There are numerous reported cases of MIC against metals in many different industries in many parts of the world. MIC failures have been reported for many fields, including the oil and gas industry, fire protection systems, copper potable water systems and heat exchangers, wastewater treatment facilities, the pulp and paper industry, and nuclear and hydroelectric power plants. MIC can also be found in many other industries not mentioned in this chapter.

One particular area where there are a large number of reported cases of MIC of metals is in the oil and gas industry. In the oil and gas industry, MIC has been reported more frequently in recent years. One of the main reasons for this is the use of water (often seawater) injection in enhanced oil recovery operations to increase reservoir pressure. The practice brings in nutrients and microbes. One serious example of this is the 2006 oil pipeline failure at the Prudhoe Bay oil field in Alaska (Jacobson 2007). It was discovered that a 0.25″ by 0.5″ hole had formed in the bottom of an oil transit pipeline causing 200,000 gallons of crude oil to leak from the pipeline before it was discovered. This led to the shutdown of the production in order to replace both the leaking pipeline and another parallel pipeline. Upon further investigation, it was believed that MIC was very likely to blame for the failure that had huge financial repercussions. Another serious example of pipeline failure largely attributed to MIC was the rupture of a 30″ natural gas pipeline near Carlsbad, New Mexico, on August 19, 2000 (NTSB 2003). The natural gas that was released as a result of the rupture caught fire and burned for nearly an hour. The gas killed 12 people who happened to be camping under the bridge that supported the pipeline; it also caused damage to two steel suspension bridges that were nearby. The cost was almost $1 billion in damages to the El Paso Natural Gas Company, which operated the pipeline. An additional example of early pipeline failure attributed partly to MIC was first observed in 1997 in a well fluid pipeline off the coast of India that transported well fluid from the well platform to the process platform (Samant et al. 1999). This pipeline failed due to the formation of three pinhole leaks at the bottom of the pipeline only 2.5 years after the pipeline was commissioned. While attempting repairs to the pipeline, several additional leaks were found. It was believed that the infrequent use of biocides and pigging were to blame for the failure. In 1991, MIC was blamed for initial pinhole leaks observed in a new oil and water gathering system in the Lost Hills Field in the San Joaquin Valley, California, after being in commission for only 18 months (DuBose, Fortnum, and Strickland 1996). Additional pinhole leaks were discovered in 1993. Eventually, various pieces of the pipeline had to be replaced costing nearly $800,000.
Another area with many reported cases of MIC is in fire protection systems. Most fire protection systems utilise untreated water that is left stagnant for long periods of time. This creates an environment for bacterial growth and biofilm formation. These microbes cause MIC, which results in pinhole leaks especially at the weld seams and along the bottom of the pipeline. They can also cause biofouling, which can hinder the water from flowing through the pipe in an emergency. These pinhole leaks could easily cause water damage of very expensive equipment or documents. There are also many instances in which the leaking pipelines were replaced by installation companies without any research into the cause of the failure or any suggestions for future prevention. Modifications to a fire protection system such as water recirculation and treatment can help limit and regulate MIC (McReynolds 1998). According to Pope and Pope (2000), MIC can be found in both steel and copper piping of fire protection systems. MIC can sometimes cause failures of these systems within months of initial installation. The buildup of biofilm and other corrosion products in the sprinkler heads can also cause them to fail. One specific example of MIC in fire protection systems was reported at the Nevada Test Site’s Device Assembly Facility (DAF) (Edgemon et al. 2010). This facility was initially built in the mid-1980s for underground nuclear weapons testing, but is now used for other purposes. The facility is mostly underground and was built with a fire protection system. The fire protection system was installed with primarily carbon steel coal tar enamel-coated (CTE) pipes, which were welded together. Welding the coated pipes destroyed the integrity of the coating in those areas. This made for an easy place for corrosion, including MIC, to take place. The addition of these defects to the CTE coating also made further delamination of the coating much easier, which only increased the areas susceptible to corrosion. Wall thickness losses ranging from 20% to 80% and tubercles were observed at the welded joints.

Copper is a widely used material in potable water systems and heat exchangers. Copper as a material is relatively corrosion resistant and considered toxic to many microbes in nature (Kikuchi et al. 1999). Despite this, MIC of copper, especially in the form of localised corrosion, has been reported in many parts of the world, especially in low flow and stagnant conditions. One such example of this was the failure of a domestic hot water system installed above the ceiling of a retirement home that failed after only 12 years (Labuda 2003). A portion of the copper pipe had to be removed due to several leaks. Upon examination of the interior of the pipe, a uniform alternating corrosion was observed with average of wall thickness losses alternating between about 28% and 80%. Several through-wall perforations were also found. Another reported case was the repeated failure of the copper heat exchanger coils that networked throughout the campus of a southwestern industrial park built in the early 1980s in the United States (Robinette 2011). The replacement of the leaky coils failed to alleviate the problem, as many of the new replacements failed in as little as 60 days. The situation worsened after one company
threatened to leave the industrial park after they observed a coil leak above their critical data processing computers. Further investigation into the matter revealed that MIC was primarily responsible for the pitting corrosion observed in the leaking coils. The coils had to be coated with lepidocrocite (FeOOH) in order to alleviate the problem.

Another area with MIC problems is in wastewater treatment facilities. The MIC problems can occur in many areas of wastewater treatment facilities, especially areas with stagnant conditions and lots of bacteria present, including stainless steels. One example of MIC in this area was the formation a pinhole leak in the rotating disc in a sedimentation tank, which stopped the shaft from rotating (Sreekumari et al. 2004).

MIC has also caused problems in the pulp and paper industry. While problems due to MIC can occur in various areas of the paper machine if the conditions are right, many of the failures are observed in the splash zone at the wet end of the paper machine (Carpén et al. 2001). These components are made of primarily stainless steel. The white waters used in the papermaking process contain many nutrients at perfect temperatures for microbial growth, which create an excellent environment for MIC to occur.

Nuclear power plants have also experienced many failures due to MIC, with some plants spending upwards of $100 million on the problem (Angell 2002). The two main areas that experience MIC in nuclear power plants are heat exchangers and fire protection systems. These systems are vital to the safety of nuclear power plants, and their failure could force a plant to shut down operations. Failure of nickel alloy heat exchanger tubes were reported in the late 1980s at several Ontario Power Generation CANDU power plants less than a year after installation. These power plants use heavy water (D₂O) on the tube side of the heat exchangers and raw water on the shell side. These conditions created an ideal environment for microbes to thrive, especially in the shell side of the heat exchanger, which in addition to being difficult to clean, also reduced fluid velocity.

MIC can also cause problems at hydroelectric power plants. Multiple cases have been reported of MIC of stainless-steel components in hydroelectric power plants (Linhardt and Nichtawitz 2003). An example of this was the pitting corrosion observed after only 18 months of service on the turbine blades and discharge rings of the hydroelectric power plant on the Maas River in the Netherlands. Similar problems were also observed at the hydroelectric power plant on the Mun River in Thailand less than a year after being commissioned.

Besides the severe influence of MIC in industrial fields, the effect of microbes on the corrosion of medical implants has also been investigated. The metal implant surface is continually exposed to the biological environment of the human body, which contains water, organic compounds, microorganisms, enzymes, chlorides, amino acids and so on (Hansen 2008). Several strains of microbes, such as aerobic iron-oxidising bacteria (IOB) and SRB, have been found in the surrounding physiological environment in
the human body (Boopathy et al. 2002; Manivasagam et al. 2010; Mylonaki et al. 2006). These bacteria form biofilms, which can cause the biocorrosion of implants and associated infections (Gino et al. 2010; Vianna et al. 2008). Chang et al. (2003) has shown the corrosion of dental metallic materials made of titanium alloys and stainless steel in the presence of *Streptococcus* mutants and the metabolic products, such as lactic acid, carbonic acid and glucan-binding proteins. Moreover, another case from Maruthamuthu et al. (2005) indicated that increasing corrosion currents in nickel, titanium and stainless-steel orthodontic wires were observed in the presence of bacteria such as APB and SRB collected from human saliva. These studies on biocorrosion and electrochemical behaviour of titanium alloys and stainless-steel orthodontic wires in the presence of bacteria reveal that the interactions between the metal implant surface with the microbial cells and their metabolic products have important impacts on implant corrosion propagation and damage (Costerton et al. 2005).

Examples of MIC can be found in a wide range of industries in many parts of the world. MIC is responsible for the degradation of many different metals, including carbon steel, stainless-steel, copper and nickel alloys. Many MIC failures have been reported well before any other corrosion problems were expected, causing a great deal of unanticipated financial losses. MIC even threatens non-metallic materials such as polymers such as polyurethane. A notable case was the polyurethane electrical insulation penetration by filamentous fungi at the Zurich airport in the 1960s. The airport electrical system had to be renovated causing many flight cancellations (Flemming 1998; Pommer and Lorenz 1985). This case belongs to Type III MIC, also known as biodegradation.

### 15.4 MIC Detection and Mitigation

MIC is mitigated primarily using the old-fashioned “spray and scrub” strategy. Biocides and scrubbing are used to remove microbes. In pipelines, pigs can be deployed to scrub the internal pipeline surface and to spray biocides. In the old days, these robotic devices tended to make a noise that resembled the oink noise made by pigs. Some older pipelines were not designed for pigging due to their tight turns and other pig-unfriendly structures. Because a field system is not sterile, microbes always bounce back after a treatment. Repeated treatments are needed. In the oil and gas industry, the decision for treatment is a big one because downtime, chemicals and labour are costly. Reliable detection is critical to the decision making of whether treatment is required or not. Although various electrical resistance (ER) probes and linear polarisation resistance (LPR) probes have been tried for online biofilm detection, their reliability needs improvement. Recently, Ohio University...
filed a provisional patent on the utilisation of biofilm electrogenicity for the detection of electrogenic biofilms (Gu 2012c).

15.4.1 Detection of Planktonic and Sessile Microbes in MIC Forensics

It is relatively easy to assay planktonic cells by analysing the microbes in a liquid sample. However, biofilms are the major culprits in MIC. Planktonic cells indicate the possible presence of biofilms in a particular system. Planktonic cell counts are not directly correlated with sessile cell counts. In a pipeline with fluid flow, planktonic cells are relatively evenly distributed while biofilms may be opportunistic and sporadically distributed. This makes the precise detection of biofilms very difficult.

Anaerobic microbes involved in MIC tend to be rather small. For example, *D. vulgaris* is a rod-shaped microbe with a length of roughly 2 µm. They are barely visible under a light microscope at 400× magnification. Fortunately, living *D. vulgaris* cells are motile and this makes counting them under a microscope much easier because artefacts such as small particles are stationary. Using a haemocytometer, cell counts as low as 50,000 cells/mL can be counted. If cell counts are below this, it would be inaccurate because there are not enough cells to be seen on a haemocytometer. To count the cells, one must grow them to increase the number and then back calculate. The so-called most probable number (MPN) enumeration method can be used. It typically uses a liquid culture medium to culture a sample for a period of time. When the cell population increases, they can be enumerated by comparing with a set of standards. For example, SRB cells are cultured with Fe²⁺-containing medium. The medium will turn black when a sufficient amount of HS⁻ is secreted. It makes S²⁻ available to form FeS that has a black colour. The time for an inoculated medium to turn black corresponds to the original cell count in the sample used in inoculation. Sessile cells are enumerated the same way, except that they have to be dislodged from a biofilm first. Sometimes, an ultrasound burst lasting 15–30 s can be used to remove sessile cells from a coupon surface used in MIC testing. The brief exposure to ultrasound is not sufficient to kill the sessile cells. Harvested sessile cells are suspended in a liquid and then used as the inoculum for MPN enumeration. The original cell count in the harvested sessile cell sample is used to calculate the sessile cell density (cells/cm²) based on the coupon surface. For cells that are easy to grow on a solid medium in a Petri dish, CFU (colony-forming units) can be used to quantify the cell counts. SRB and other anaerobic cells grow very slowly on a solid medium and they require an anaerobic incubator. Thus, a liquid medium is often used in an anaerobic vial for enumeration. Instead of using the ATCC 1249 liquid medium for MPN, Xu et al. (2012a,b) found that their *D. vulgaris* is more easily enumerated using the Sani-Check SRB test kit (Sani-Check® Product #100, Biosan Laboratories, Warren, MI) that comes with a brush-like dipstick in a solid SRB medium (Figure 15.5). The dipstick is used to dip into a sessile cell suspension before being inserted into the vial.
for anaerobic incubation at 37°C. No anaerobic chamber is needed for incubation because the solid medium in the sealed vial keeps the oxygen out. The Fe\(^{2+}\)-containing solid medium turns black (the FeS film colour) during SRB growth. The time required for the black colour to appear correlates with SRB cell counts (cells/mL) based on the vendor’s calibration.

CLSM is a widely used technique in biological science research, which can provide a high-resolution optical image at a selected depth. CLSM is becoming a popular tool for the investigation of biofilm structures due to its non-invasive nature (Mueller et al. 2006). Using a suitable stain, CLSM can even distinguish living and dead cells. Cell density can be quantified with the help of computer software. In laboratory investigations, CLSM can be an effective tool to quantify biocide efficacy. However, when the surviving cell density is too low after a biocide treatment, not enough cells may be visible under the optical microscope. An MPN method is required to increase the cell density.

In MIC forensics, anaerobic biofilms are difficult to preserve once the specimen is exposed to air. Some biofilm cells are not culturable in a lab culture medium. This makes biofilm identification difficult. Luckily, molecular biology techniques do not require living cells because only their molecular markers are assayed. DGGE and fluorescence in situ hybridisation (FISH) techniques are used to evaluate the diversity and similarity of the dominant microbes in various microbiological communities collected from the field (Friedrich et al. 1999; Teng et al. 2008). It is helpful to identify these dominant microbial species (Carpén et al. 2012; Teng et al. 2008) when making treatment decisions. The FISH technique employs fluorescent probes that bind to chromosome sections that exhibit a high degree of DNA sequence
complementarity. It reveals the presence of specific DNA sequences coded in the probes for the identification of specific microbial species. The DGGE technique can be used to analyse DNA and RNA samples extracted from biofilms. The genetic fingerprints obtained from DGGE are used with the basic local alignment search tool (BLAST). The phylogenetic diversity of corrosive biofilms has been characterised by some researchers using this approach in MIC research (Zhang and Fang 2001).

For biofilm consortia and cultures that have very low concentrations, real-time qPCR can be used. It uses primers to match species-specific sections in DNA molecules extracted from the microbes to fingerprint specific microbial species (Powell et al. 2006). By using a standard curve, the amount of DNA for a microbial species can be correlated with its cell count. Because of its high sensitivity and accuracy, real-time qPCR is widely used to monitor and enumerate microorganisms (Lutterbach et al. 2011; Mitchell et al. 2012). Another popular method of cell enumeration is adenosine triphosphate (ATP) assay. After ATP is dyed with fluorescence, the light emission, which is proportional to the amount of ATP, is measured by ATP photometer. ATP is found in living cells. Thus, ATP assay could be used to quantify the viable microorganisms (Yu et al. 2010). This method, however, is not sensitive enough for low cell counts. Caution must be exercised when using this kind of molecular biology method in biocide efficacy studies because newly killed cells may still have the biomarker molecules (e.g. DNA fragments) intact.

It should be pointed out that the most abundant microbes in a biofilm may not be the MIC culprits as discussed in Section 15.2 of this chapter. In a mixed-culture field biofilm consortium, only one or at most a few bottom layers of microbes are directly involved in Type I MIC. Thus, sampling of the bottom-layer microbes is critical. The existing common mistake of identifying the dominant species of microbes as MIC culprits shows a disregard of MIC mechanisms. The ultimate proof in MIC forensics is the actual corrosion test using the field microbes in a laboratory. After all, a field system is never sterile and you will always find certain microbes. For example, it is nothing unusual to find evidence of the existence of a sulphate reducer in an anaerobic environment when the liquid contains sulphate. Thus, microbiological, molecular biology assay results must be carefully interpreted together with any corrosion test results. It is unfortunate to miss the evidence of MIC, but it can also be very costly to start treatment unnecessarily.

15.4.2 Biocide Mitigation of Biofilms and MIC

Many biocides and biostats (also known as antimicrobials) are used to mitigate MIC and biofouling. They include glutaraldehyde, THPS, bronopol, 2-bromo-2-nitro-1,3-propanediol, tributyl tetradecyl phosphonium chloride, alkyldimethylbenzylammonium chloride, dimethyl benzyl ammonium chloride, chlorine dioxides, calcium hypochlorite, potassium hypochlorite, sodium hypochlorite, dibromonitrilopropionamide (dibromonitrilopropionamide),
methylened bis thiocyanate (methylene bis thiocyanate) and 2-(thiocyanomethylthio) benzothiazole (thiocyanomethylthio benzothiazole). Among them, glutaraldehyde and THPS are the top two choices in many large-scale applications, especially in the oil and gas industry because of their broad-spectrum efficacy and good environmental profiles. Both are readily biodegradable. THPS is officially a green chemical according to the U.S. Environmental Protection Agency (US EPA 1997). The working mechanism of THPS is less understood than glutaraldehyde, which is a cross-linking agent that reacts with amino and sulphydryl groups in proteins and nucleic acids of microorganisms. Lee et al. (2010) explained that THPS can kill SRB through interfering their energy metabolism. Glutaraldehyde is more effective at higher pH (e.g. pH 8), while THPS prefers slightly acidic pH. Zhao et al. (2009) modelled the degradation of THPS at different temperatures and pH. They found that THPS degrades much faster under alkaline pH. Concentrated THPS is quite acidic. Prolonged exposure causes unacceptable corrosion. A biocide cocktail is often used by mixing a biocide with a surfactant, a corrosion inhibitor and perhaps a scale remover. A surfactant can help a biocide distribute to a surface (e.g. inner pipe wall) where a biofilm is present, rather than to the body of the pipeline fluid.

Microbes develop resistance after prolonged use of the same biocide. The biocide selectively promotes resistant microbes by killing off susceptible microbes. As a consequence, biocide dosages escalate. In the meantime, environmental regulations in various countries, especially the European community, are becoming more restrictive. Xu et al. (2012c) argued that it is very unlikely that a new blockbuster biocide comparable to glutaraldehyde or THPS will be available on the market any time soon. Thus, they advocate the use of biocide enhancers such as chelators and D-amino acids that can reduce biocide dosages while achieving better biofilm and MIC mitigation outcomes.

EDTA has been used to significantly enhance the eradication of biofilms on catheter surfaces when combined with antibiotics (Raad et al. 2003). This technology was patented by Raad et al. (patent publication numbers: US 6165484 A, US 6509319 B1, US 20110201692 A1). The concern for EDTA being applied in industry is its slow biodegradability and potential accumulation in freshwater systems. Ethylenediaminedisuccinic acid (EDDS) replaced EDTA due to its biodegradability. It was reported by Wen et al. (2010) that the combination of EDDS at a concentration of 2000 (w/w) ppm EDDS with either THPS or glutaraldehyde at (an active) concentration of 30 ppm (w/w) showed a strong inhibition effect against planktonic cells of *D. vulgaris* ATCC 7757 and *Desulfovibrio alaskensis* ATCC 14563 compared with biocide treatment alone. EDDS also helped glutaraldehyde in both the prevention of SRB biofilm establishment and the removal of established SRB biofilm test on a C1018 carbon steel surface (Wen et al. 2009).

In oil and gas field applications, methanol has been widely used as a hydrate inhibitor and winterising agent. Wen et al. (2012) and Xu et al. (2012c)
found that with the addition of 10% (v/v) methanol, the efficacy of the binary combination of 30 ppm glutaraldehyde and 1000 ppm EDDS was considerably enhanced in the treatment of planktonic SRB growth, prevention of SRB biofilm establishment and mitigation of souring and MIC caused by SRB.

A most recent development is the use of naturally occurring d-amino acids as biocide enhancers. d-Amino acids are far more prevalent than previously thought. They are found in microbes, plants and even humans, playing various biological functions (Xu et al. 2012a). Experimental data reported by Kolodkin-Gal et al. (2010) demonstrated that a low-concentration mixture of d-amino acids (d-tyrosine, d-leucine, d-tryptophan and d-methionine) triggered the disassembly of bacterial biofilms of B. subtilis, Staphylococcus aureus and Pseudomonas aeruginosa. They suggested that these d-amino acids might have substituted the d-alanine terminus in the peptidoglycan molecules in bacterial cell walls. The d-alanine terminus may be a signal molecule for biofilm dispersal. However, when Xu et al. tested d-tyrosine (2012a) and

![FIGURE 15.6](image_url)

**FIGURE 15.6**
SEM examination of coupons (initially covered with mature SRB biofilms) after 1-h treatment in a Petri dish containing: (a) a solution of MgSO₄ and (NH₄)₂Fe(SO₄)₂ at the same concentration as in the ATCC 1249 medium (control), (b) 50 ppm THPS, (c) 100 ppm d-tyrosine, (d) 50 ppm THPS + 1 ppm d-tyrosine, respectively. Scale bars for the small inserted images are 50 µm. (With kind permission from Springer Science+Business Media, *World Journal of Microbiology & Biotechnology*, A synergistic d-tyrosine and tetrakis hydroxymethyl phosphonium sulfate biocide combination for the mitigation of an SRB biofilm, 28(10), 2012a, 3067–3074, Xu, D., Y. Li, and T. Gu.)
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15.5 Summary and Perspectives

This chapter discussed the impact of MIC on various metals. Type I MIC mechanisms were dissected in detail because the common SRB attacks on Fe⁰ belongs to this category. Bioenergetics and electron transfer theories in bioelectrochemistry were employed to explain the mechanisms. Various MIC examples were given. This chapter also reviewed some common methods to assay planktonic and sessile cell identities and quantities. A new trend in biocide technology using novel biocide enhancers was presented. MIC is becoming more and more important due to reasons such as an aging infrastructure and more frequent practices of water injection in the oil and gas industry. Recent advances in MIC mechanisms and biocide enhancement

d-methionine (2013) individually at much higher concentrations to disperse D. vulgaris biofilms on carbon steel surfaces, the results were disappointing. They argued that the D. vulgaris biofilm is much more recalcitrant than the biofilms tested by Kolodkin-Gal et al. (2010). They added a 50 ppm (w/w) THPS biocide stress and found that it “convinced” D. vulgaris biofilms to disperse. Figure 15.6 shows that 100 ppm d-tyrosine alone did not disperse D. vulgaris biofilm while 1 ppm d-tyrosine enhanced the efficacy of 50 ppm THPS greatly by eradicating the mature D. vulgaris biofilm (initially grown in the full-strength culture medium prior to biocide treatment) successfully after a 1-h treatment. Their sessile cell count data indicated that a 50 ppm THPS + 1 ppm d-tyrosine combination and 100 ppm THPS alone both achieved a 5-log reduction (Table 15.2). They found that d-methionine at 100 ppm was also an excellent biocide enhancer for 50 ppm THPS in biofilm prevention and eradication, as well as mitigation of MIC pitting by D. vulgaris.
technologies will contribute to better MIC mitigation. However, there are still many unresolved issues in MIC. More research investment is required.

References


Wen, J., D. Xu, T. Gu, and I. Raad. 2012. A green triple biocide cocktail consisting of a biocide, EDDS and methanol for the mitigation of planktonic and sessile...


