Investigation of Type II of Microbiologically Influenced Corrosion (MIC) Mechanism and Mitigation of MIC Using Novel Green Biocide Cocktails

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Master of Science

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This thesis titled

Investigation of Type II of Microbiologically Influenced Corrosion (MIC) Mechanism and Mitigation of MIC Using Novel Green Biocide Cocktails

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Microbes play key roles in most Microbiologically Influenced Corrosion (MIC) cases. Sulfate Reducing Bacteria (SRB) attack steel primarily through Type I MIC mechanism. The electrons released by iron oxidation are utilized for sulfate respiration of SRB with energy production. Sulfate reduction occurs in SRB cytoplasm under biocatalysis utilizing electrons transferred from outside the cells. In Type II MIC, microbes secrete corrosive metabolites such as organic acids and hydrogen sulfide. These oxidants can oxidize metals extracellularly without biocatalysis. Since the application of biocide in oil pipelines is under strict environmental regulations, promotion of biocide efficacy by using biocide enhancers is desired. D-Amino Acids (DAAs), may serve as environmental-friendly biocide enhancers, are addressed in this research.

Type I MIC mechanism cannot explain anaerobic copper corrosion by SRB because of unfavorable thermodynamics, while copper corrosion by Nitrate Reducing Bacteria (NRB) in the anaerobic environment can be theoretically explained by Type I MIC. This work examines experimental evidence to support the theories that anaerobic copper corrosion by SRB is Type II and the mechanism of copper MIC by NRB may belong to Type I MIC.
Bin Fu (my father)

and

Pei Zhang (my mother).
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1 INTRODUCTION

Corrosion is a chemical deterioration process on materials (usually metals) that can occur in specific environments leading to changes in the material properties. Corrosion causes metals and alloys to change from an elemental state (e.g., Fe\(^0\)) to an ionized state (e.g., Fe\(^{2+}\)). The rusting of carbon steel is the most common form of corrosion, which is not only detrimental to equipment but also harmful to the environment. Facilities such as underground pipes and offshore oil platforms can be seriously compromised in certain situations, severe environmental impacts at the same time.

The involvement of microbes as a corrosive agent can both facilitate and increase the corrosion reaction of metals. This type of corrosion known as Microbiologically Influenced Corrosion (MIC) can be costly and it is also difficult to predict. Booth (1964) proposed that MIC was responsible for about half of all corrosion failures in pipelines.

Financial losses due to MIC have been reported in numerous countries. Walsh et al. (1993) estimated the annual cost directly associated with MIC to be about 30-50 billion dollars each year in the US. In South Africa, Escom, a national power utility reported that the MIC of carbon steel in their power plants costs millions of dollars annually to repair corroded systems (Bibb, 1986). Corrosion problems are common in nuclear facilities as well as oil and gas industries. For example, in Iranian power and petroleum industries, many serious damages were caused by MIC. One of the famous cases was a fire in reformer heater tubes due to microbial corrosion (Ghassem and Adibi, 1995).
Garrett et al. (1891) published the first report proposing that microorganisms might be the prime reason in the metal corrosion process in some cases. They suggested that microbial metabolic activities enhance the corrosion process of lead cables. At the beginning of the 20th century, many reports showed that corrosion of water pipes possibly were affected by iron and sulfur bacteria (Gaines, 1910). However, most people at the time didn’t understand the mechanisms of biocorrosion, until von Wolzogen Kuehr and van der Vlugt (1934) published their Cathodic Depolarization Theory (CDT), to explain MIC. It was the first theory which attempted to describe MIC mechanisms by using electrochemical knowledge. However, the theory did not address all the factors involved in the corrosion process. Thus, today, the CDT is only accepted as a classic, historical explanation, rather than a completely accurate mechanism (Thierry and Sand, 2002). Given the different phenomena of biocorrosion in various metals, more discussion and further experimental research have taken place for a better understanding of MIC mechanisms.

Cast iron, carbon steel and stainless steel are all vulnerable to MIC, and they often involve SRB (Hamilton, 1985); Acid Producing Bacteria (APB) (Little et al., 1988); Nitrate Reducing Bacteria (NRB) and Methane Producing Bacteria (MPB). When protected by biofilms, bacteria are able to live in the most extreme environments. Desulfovibrio, one SRB species, can grow in situations with pH ranging from 5 to 10 and temperatures from 5 to 50°C (Tatnall, 1993). Dowling et al. (1991) demonstrated that different species of microbes exist in biofilms, developing synergistic communities (consortia) which both enhance bacterial metabolism and provide them with strong
protection against harsh environmental factors such as pH swings. The consortia also increase difficulties of microbial mitigation in practical environments.

Microorganisms interact with the surface environments of metals (Videla, 1997). Biofilms that secrete extracellular polymeric substances (EPS) help to facilitate the attachment of the cells on the metal surfaces. The biofilms formation occurs instantly on the metal surface in the three-component system (metal + solution + microorganisms) (Videla, 2002). Monroe (2007) suggested that the development of biofilms not only protect sessile cells but also aid cells to adapt to different environments. Moreover, when establishing biofilms, microbes change the electrochemical conditions around the metal surface, such as pH values and concentrations of ions, resulting in different corrosion rates of specific metals (Videla, 1989).

SRB biofilms can be very dense in anaerobic situations, and are the major cause of MIC pitting corrosion (Videla and Herrera, 2005). Growing in anaerobic conditions, SRB gain energy from redox reactions, such as combined reactions of sulfide reduction and organic carbon oxidation, for their metabolic activities. Hydrogen sulfide is a corrosive metabolic product generated by the sulfate reduction reaction. SRB and other microbes are suspected to have been responsible for the 2006 Alaska oil pipeline leak (Jacobson, 2007) and pitting corrosion in Ontario Hydro’s nuclear plants (Brennenstuhl and Doherty, 1990).

Frey (1998) showed that the mitigation of MIC relies on mechanical and chemical methods to control microbial biofilms. In the gas and oil industries, physical scrubbing is commonly used to remove biofilms in the pipes, but the applications are limited as a result of complicated shapes of pipes and the high cost. Chemical methods such as
biocides are frequently utilized. However, selecting one of the currently available biocides can be difficult. The highly potent biocides may not be capable of completely stopping the microbial corrosion, given that biofilms protect sessile cells from chemical attacks (Meyer B., 2003). If large-scale use of biocide is applied, it will be costly, and can be harmful to the environment (King, 2007). This suggests that safer options are needed when using biocides, including green biocides and biocide enhancers. D-Amino acids are considered environmentally friendly biocide enhancers because they are biodegradable in the environment and also have highly efficacious in improving biocides (Wen et al., 2012; Xu et al., 2012b; Xu and Liu, 2011).
2 LITERATURE REVIEW

2.1 Corrosion

Corrosion is a naturally occurring deterioration process which is a physicochemical interaction between materials (usually metals and alloys) and their environment, often leading to changes in the material properties (Einar, 1989; Junker, 2009). Rusting iron and steel are the most common forms of corrosion. Due to corrosion, damage and destruction of infrastructures can lead to large financial losses for oil, gas, and nuclear energy industries as well as many other industries. Cases of corroded pipelines are shown in the Fig. 2-1.

*Figure 2-1. Pipeline corrosion (Underground solutions, 2013)*

The amount of money spent on repairing and replacing damaged equipment reaches hundreds of billions of dollars each year. In a U.S. corrosion study, the direct cost
of national metallic corrosion was $276 billion in 2002, which was over 3% of the U.S. Gross Domestic Product (GDP) that year (Koch et al., 2002)

2.2 Carbon Steel and Copper

Iron and Iron alloys are widely used for equipment and facilities in energy industries. Carbon steel is an iron alloy created by using a different range of carbon content along with various metals. The National Association of Corrosion Engineers (NACE) defines carbon steel as an “alloy of carbon and iron containing up to 2 mass percent carbons and up to 1.65 mass percent manganese and residual quantities of other elements -- except for those intentionally added in specific quantities for deoxidation (usually silicon and/or aluminum).” Iron and carbon steels are the most commonly corroded metals. They are selected as fundamental materials not in relation to their corrosion resistance, but for their other good metallic properties and low cost.

Compared to carbon steel, copper and its alloys have better corrosion resistance. Pure copper has a reddish-orange color on the freshly explored surface. It is very soft and malleable, and one of its outstanding physical characteristics is that it is ductile. Because of its good thermal and electric conductivity, copper is usually used as a conductor of heat and electricity. The most common compounds of copper are oxidation status with +1 and +2, named cuprous and cupric, respectively (Holleman and Wiberg, 2001). When copper is in an atmospheric environment, it slowly forms a dark-brown oxidation layer, which protects it from further corrosion. Many copper-made facilities show this kind of oxidization result after about 10 years of oxidization. Copper reacts with sulfides and chloride and produces a layer of light green; a good example of slow oxidization is seen on the Statues of the Library. Copper and its alloys are widely used in various facilities
world-wide. Moreover, brass and bronze (copper alloys) have appeared throughout human history for more than 4000 years, and they are still used in important facilities in modern society.

2.3 Microbiologically Influenced Corrosion

Microbiologically Influenced Corrosion (MIC) was defined by Videla (1997) as a type of corrosion process in which “the microorganism is able to initiate, facilitate, or accelerate the corrosion reaction without changing its electrochemical nature.” Microorganisms play important roles in the cycles of N\textsubscript{2}, S, Si, P and some heavy metals that have brought both benefits and disadvantages to society. In early history, human battled microorganisms to survive because bacteria biodegraded organic materials, like food and wooden tools. Today, it is common knowledge that microorganisms also attack inorganic materials, such as metals and minerals (Flemming, 1996). In some reports, bacteria have been identified as millions of dollars’ worth of damages in industries, one such an example is $55 million to fix stainless steel heat exchangers (Brennenstuhl et al., n.d.), and $20 million to repair and replace the buried lead insulation of power lines (Rehm, 1980). In the early 1970s, MIC was reported to be the reason for destruction of mineral materials and plastics causing over $100 million of repair and restoration annually (Flemming, 1996).

In the oil and gas industries, MIC has been identified as the reason for a large percentage of metal corrosion and a large share of the economic losses. The corrosive effects caused by MIC may range from the destruction of metals to environmental impacts (Sand, 1996). All materials including metals, plastics, organic materials and minerals are vulnerable to attack by microorganisms. Beech and Gaylarde (1999)
estimated that about 34% of the entire corrosion financial payout was for MIC damage to
equipment. Financial costs for protecting facilities and installations from destruction by
both MIC and biofouling are very high.

2.4 Biofilms

There are two different forms of bacteria in a natural situation: planktonic bacteria
and sessile bacteria. Planktonic refers to the bacteria that can freely float and swim in
water and sessile prefers to cells inside biofilms. Since Antonie van Leeuwenhoek first
discovered planktonic bacteria in 1673 (Egerton, 2006, 1968), planktonic bacteria existed
as the recognized form of bacteria for many centuries, and have been researched for a
better understanding for microbiology. The average size of microorganisms is measured
on a micrometer scale. Because the planktonic cell surfaces are hydrophilic, their
nutrition is accessible from their surroundings. Due to the rough surface of metals,
microbes can grow in inaccessible areas such as in deep pits and inner crevices, leading
to an increase in corrosion rate (Videla, 1997). However, because there is no protective
coating surrounding the cells, planktonic bacteria are easily destroyed by antibacterial
agents, like biocides (Lee and Brenda, 2003).

Bacteria grow in communities to protect themselves in some unfriendly situations
and adapt to their environment (Olson et al., 2002). These groups of bacteria, called
sessile bacteria, are encased in an extracellular matrix of carbohydrates forming biofilms
(Costerton et al., 1995; Hall-Stoodley et al., 2004). This is an aggregate of cells and their
self-produced matrix of extracellular polymer substances (EPS), which is the composite
of nucleic acids, proteins and polysaccharides, that adhere to each other and to the surface
of objects (Hall-Stoodley et al., 2004). Biofilms serve as shields for sessile cells.
Moreover, biofilms increase the capability of sessile cells resistance to hostile environments. Costerton et al. (1995) first noted that biofilms are 500 times more resistant to antibacterial agents than planktonic cells. Research showed that biofilms predominately exist in most full-nutritional situations, and cause severe problems in environmental, medical and industrial environments (Mah and O’Toole, 2001).

Biofilms are observable in most natural environments and “pathogenic ecosystems” (Costerton et al., 1999, 1978). The first observation of biofilms was in the growth of a marine bacterial biofilm (Zobell, 1943). Costerton et al. (1999) found that surfaces were covered by biofilms in fresh water and in many others situations. Fig. 2-2 presents the basic steps in biofilm formation.

Figure 2-2. Five steps of a biofilm formation (Stoodley et al., 2002)

The first step is the “initial attachment of cells on the surface.” There are at least three mechanisms to explain how bacteria forms a biofilm (Dalton et al., 1996; Heydorn et al., 2000; Korber et al., 1995; Tolker-Nielsen et al., 2000); however, Stoodley et al. 

![Biofilm Development](image-url)
(2002) stated that each mechanism, individually, doesn’t clearly describe the whole process. There are many important factors such as the properties of surface, and the physical and chemical situations in the environment that can influence the formation. In the second step, planktonic cells fasten themselves on the surface by secreting EPS. EPS also provides structural support during the biofilm formation. In order to develop a mature biofilm, EPS increases the bonds between cells and surfaces to form permanent contacts which are sometimes irreversible attachments.

The early stages of biofilm formation (early development (step 3) and maturation (step 4)) are the growth of the biofilms architecture. With maturation of biofilms, the more complex architecture is built, providing for a more even distribution of bacteria (Davies et al., 1998). The mature biofilms serve as shelters for the sessile cells, protecting the bacteria from severe environments like extreme acidic pH, starvation. This increases the bacteria’s resistance to antibiotics or biocides. Some mature biofilms contain water channels and pores that evenly spread nutrition between cells (Stoodley et al., 2002). The matrix also assists the communication of the bacteria by passing biochemical signals between cells.

Detachment is the last step in the formation of biofilm. Bacteria are dispersed from the biofilms and become planktonic cells growing in the surrounding environment. Many research studies have been exploring the mechanisms of biofilm detachment, given that this final step is not yet clearly understood and is usually treated as a naturally occurring physiological event O’Toole et al. (2000) and Sauer et al. (2002) have found that absence of nutrition will lead to the biofilm detaching from the surface in order for the bacteria to survive in a new habitat.
When bacteria return to a planktonic state, the biofilm formation accomplishes a closing cycle, shown in Fig. 2-2. It is believed that bacterial properties of the same strain in each step of biofilm formation are distinct from the bacteria in the other steps. Sauer and Camper (2001) evidenced this in their lab experiment with *P. aeruginosa*, in order to protect the inner cells so that they adapt to different conditions (Monroe, 2007). Because planktonic cells are unable to absorb the electrons from metals via an aqueous medium, biofilms in most cases cause MIC.

2.5 Microbes

Cast iron, carbon steel and stainless steel are valuable to MIC, and the main types of bacteria involved are SRB (Hamilton, 1985), Acid Producing Bacteria (APB) (Little et al., 1988), Nitrate Reducing Bacteria (NRB) (Ghafari et al., 2008) and Methane Producing Bacteria (MPB).

2.5.1 Sulfate Reducing Bacteria (SRB)

MIC pitting corrosion is commonly caused by SRB biofilms (Videla and Herrera, 2005) because SRB are more frequently found in anaerobic environments than other bacteria such as NRB. For example, pitting corrosion due to SRB was likely responsible for the 2006 Alaska oil pipeline leak (Jacobson, 2007). Growing in anaerobic conditions, SRB gain energy from redox reactions such as the reduction of sulfate or other oxidants combined with the oxidation of organic carbons for their metabolic activities (Gu, 2011). Hydrogen sulfide, a corrosive metabolic product generated by the sulfate reduction reaction; has been a major concern because when hydrogen sulfide reaches high concentrations that can cause reservoir souring in oil fields. Usually, sulfate or different sulfur compounds, sulfate, thiosulfate and sulfite, are the most common electron
acceptors during SRB metabolism (Lovley and Phillips, 1994). However, many SRB are able to reduce other electron acceptors, such as the element sulfur (Böttcher et al., 2005; Finster et al., 1998), fumarate (Tomei et al., 1995), nitrate (Krekeler and Cypionka, 1995) and dimethylsulfoxide (Jonkers et al., 1996), when sulfate or sulfur compounds are depleted in the local environment.

SRB are considered to be strictly anaerobic bacteria and usually live in sulfate-rich anaerobic environments (Cypionka, 2000; Fareleira et al., 2003; Sass et al., 1992). They can be exposed to oxygen for a period of time but they will not grow in the presence of oxygen. The ocean is a nutritious habitat for SRB because of its large sulfate concentration (typically 2,700 ppm). However, SRB can also be found in fresh water that has a much lower concentration of sulfate.

Bacteria can be divided into two groups, heterotrophic bacteria and mixotrophic bacteria (Little et al., 2000). Heterotrophic bacteria use organic compounds such as sugars, alcohols and organic acids as their energy source (CDC, 2010). They are a part of natural substance cycling, so heterotrophic bacteria are widely found in soil and water. Inorganic substrates (e.g., NH₃, N₂, H₂) are the energy sources for autotrophic bacteria. When bacteria use the metabolic mechanisms from both heterotrophic and autotrophic bacteria, they are called mixotrophic bacteria (Eiler, 2006).

SRB, an example shown the Fig. 2-3, are the most common MIC bacteria isolated from oil pipelines. SRB have also been detected in various other environments including fresh water, seawater, drywalls, processed food and the human mouth. Even in some extreme conditions SRB can grow at a pH level ranging from 4-9.5 (Bade et al., 2000), survive in 90°C, as the highest temperature for certain thermophilic species (Stahl et al.,
2007) and tolerate a pressure of 500 atm (Stott et al., 1988). *Desulfovibrio vulgaris* is a common corrosive SRB used in this work.

![Image](image.png)

*Figure 2-3. D. vulgaris* (the bar in the upper right is 0.5 micrometer long) (Brock and Madigan, 2012)

2.5.2 Nitrate Reducing Bacteria (NRB)

Nitrate reduction as a part of denitrification is a reductive process of the ionic nitrogen oxidations, NO$_2^-$ and NO$_3^-$, by Nitrate Reducing Bacteria (NRB), to gaseous products, NO and N$_2$O (Knowles, 1982) or NH$_3$, in the nitrogen cycle shown in the Fig. 2-4. Knowles (1982) also stated that these gaseous products could possibly be further reduced to N$_2$. When oxygen is absent but a large quantity of nitrate is present, bacteria respire on nitrate in such an environment, using the nitrate or nitrite as electron acceptors during the reductions (Seitzinger et al., 2006). In many anaerobic conditions, such as underground (Buresh and Patrick, 1978; Caskey and Tiedje, 1979) and in the ocean (Koike and Hattori, 1978), the major product of the reduction of NO$_3^-$ and NO$_2^-$ is NH$_4^+$ (ammonium) by NRB.
Figure 2-4. Nitrogen Cycle (http://en.wikipedia.org/wiki/File:Nitrogen_Cycle.svg)

There are many species of NRB existing in natural environments. *Bacillus licheniformis* is the strain used in this laboratory work. *B. licheniformis* is commonly found in the soil and also in bird feathers. It can use nitrate as terminal electron acceptor. It is a thermophilic bacterium with 30°C as the optimal growth temperature in spore form (Rey et al., 2004). It is also found in oil and gas pipelines.

In secondary oil recovery, seawater is injected into reservoirs to increase the pressure for driving out more oil; however, the process also brings diverse bacteria into the system. In order to control the microbial souring problems caused by the SRB, nitrate is injected into the pipeline to suppress the growth of SRB by promoting an increased NRB population (Thorstenson et al., 2002). In the research of Thorstenson et al. (2002), when a small dose of nitrate (0.25 mmol/liter) was continuously added to the system, NRB growth strongly reduced the population of SRB as well as activities over a four-month period. After 32 months, measurements of the metal coupons in the system
showed a decrease in corrosion rate from 0.7 mm/year to 0.2 mm/year. Moreover, Feio et al. (2000) showed in their laboratory experiment, in order to decrease the hydrogen sulfide production, nitrate also can be used by SRB instead of sulfite as an alternative electron acceptor. The use of nitrate rather than a biocide has been shown to have less hazardous environmental impacts.

2.6 Mitigation of Biofilms and MIC

2.6.1 Inhibit Growth of SRB Using Biocides

In order to remove biofilms, physical scrubbing, such as pigging, and chemical methods including biocides are commonly used (Videla, 1996). Some pipelines cannot accommodate the scrubbing method because of their complicated shapes, so biocide treatment is widely used in such situations to mitigate MIC (Zuo, 2007). Non-oxidizing biocides are chosen by industries to avoid unplanned chemical corrosion during the mitigation (Laopaiboon et al., 2006). In their report of Pope et al. (1989), microorganisms are able to develop biocide resistance. Moreover, it takes at least an 10 times higher dosage of biocide to remove sessile cells in biofilms than the dosage of biocides required for killing planktonic cells (Videla, 1996b). Thus, sometimes are necessary to repeated applications for eradicating biofilms (Vance and Thrasher, 2005). Additionally, Javaherdashti (2008) mentioned that biocides present serious problems in regard to toxicity, high cost and environmental impacts as well as other negative side-effects.

Glutaraldehyde and Tetrakis hydroxymethyl phosphonium sulfate (THPS) are popular in the oil industry because of their broad spectrum and low environmental impact (Videla, 2002). Glutaraldehyde has a linear five carbon structure, and terminal aldehyde groups that are very reactive. Glutaraldehyde behaves as a cross-linking agent (Denyer,
1995; Greene et al., 2006; Russell, 2002) to inhibit the growth of amino groups on SRB cell walls (Gardner and Stewart, 2002; von Rège and Sand, 1998). In addition to being environmentally friendly, glutaraldehyde also has many performance advantages including harmless byproducts and reactive characteristics in aerobic and anaerobic situations. Union Carbide Corporation (1999) reported that this biocide degrades quickly in alkaline environments.

THPS is a compound of four active phosphine that interrupts the disulphide bonds on cellular proteins and enzymes to kill the microbes (Ballantyne and Jordan, 2004). THPS is “the best in class” among all biocides, and also are very friendly to the environment since it biodegrades quickly in anaerobic and aerobic situations (Esch et al., 2000). It is less poisonous and easier to handle than traditional biocides, so workers are safer in their work environment (Frey, 1998). THPS was the first patented biocide that won the “Designing Greener Chemical Award” in 1997 (US EPA, 1997). In their review, Jones et al. (2012) stated THPS was developed for utilization in the oil and gas industries about 25 years ago, and its performance has steadily increased in both research and applications. However, in recent years, with the heightened concern over the environmental impact from biocides, the number of restrictions on large scale biocide applications has increased. It must be noticed that this biocide is unstable in many practical situations because of its easily biodegradable characteristics. Its high cost is also an issue for oil and gas companies. Thus, there is a need to develop that more efficient dosing for biocides application. Nontoxic and degradable biocide enhancers are needed to improve efficiency.
2.6.2 Biocide Enhancement

Denyer (1995) reviewed several enhancements for biocide applications. He noticed that the utilization of both cell permeable agents and combination of biocides to help obtain enhanced complementary mechanisms. Ethylenediaminetetraacetic acid (EDTA) can enhance antibiotic eradication of microbial biofilms from catheters (Raad et al., 2003). Moreover, it also demonstrated an increased inhibition of SRB growth by THPS and glutaraldehyde (Zhao et al., 2005). Recently, Wen et al. (2009, 2010) confirmed two readily biodegradable chelators, ethylenediaminedisuccinate (EDDS) and N-(2-hydroxyethyl)iminodiacetic acid (HEIDA) disodium salts, enhanced glutaraldehyde and THPS in their inhibition of SRB planktonic growth. Additionally, they also found that a combination of 1000 ppm EDDS + 15% (v/v) methanol enhanced 50 ppm glutaraldehyde removal of established SRB biofilms on a carbon steel coupon surface (Wen et al., 2012). In this work all ppm values are based on mass, unless indicated otherwise.

D-Amino Acids (DAAs) were found to be one of the key factors in the regulation and dispersion of bacterial biofilms and can also inhibit bacterial growth in high concentrations. This may be explained by the ability of DAAs to disrupt peptidoglycan synthesis in the cell walls (Cava et al., 2011). Amino acids are small organic molecules containing a carboxyl and an amino group. There are two different configurations of amino acids, L-amino acids and DAAs. L-amino acids are important components of proteins, which exist in all living things, while only bacteria naturally produce DAAs. The mechanism of biofilm control is that DAAs break the peptide cross bridge of the peptidoglycan in the cell walls, and then interrupt the development of the biofilm (Cava
et al., 2011). Furthermore, they inhibit cell attachment to the metal surface and then disperse the established biofilm into the liquid. Certain DAAs have been identified that they can break down biofilm formation and disperse the existing biofilm. Lam et al. (2009) also quantified DAAs in bacteria and found the D-methionine (D-Met), D-leucine (D-Leu), D-valine (D-Val) and D-isoleucine (D-Ile) are secreted by bacteria to change characteristics of cell wall for adaption in different environments. Moreover, Kolodkin-Gal et al. (2010) showed that some DAAs (D-tyrosine (D-Tyr), D-Met, D-tryptophan (D-Try), and D-leucine (D-Leu)) lead to the dispersion of the established bacterial biofilm of *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. They reported that the combination of the four DAAs at low concentrations works even better on the dispersal of microbial biofilms than individual DAAs at higher concentrations. Xu et al. (2012b) also proved that 6.6 ppm equimolar D-Tyr, D-Met, D-Try and D-Leu mixed with 30 ppm THPS and 500 ppm EDDS, a biodegradable chelator, was far more effective than using THPS alone against *D. vulgaris* biofilm. Additionally, Xu et al. (2013, 2012a) found that the effects of THPS were significantly enhanced by D-Met and D-Tyr, separately, in the lab experiments.

D-Val was produced by some bacteria according to (Lam et al., 2009). D-Val has been used to the culture medium to inhibit fibroblast cell propagation while allowing the growth of normal epithelial cells, leading to important research on the tissue in the human body (Gilbert and Migeon, 1975). Takahashi et al. (1997) investigated microbial asymmetric degradation, which can produce pure D-Val from a DL-Valine racemic mixture. Thus, D-Val appears to be a good choice as a biocide enhancer (if it is efficacious) because of its harmless environmental impact and economical availability.
L-Methionine (L-Met) is one of the essential amino acids in human body, and it is also widely available in nature. Currently, L-Met is produced by chemical synthesis to form a mixture of D-Met and L-Met, followed by the enzymatic conversion of all D-Met from L-Met (Leuchtenberger et al., 2005). D-Met is biodegradable (Cooper, 1966) and can also be produced in high purity by microbial asymmetric degradation. D-Met is not harmful to the environment and a relatively cheap bio-enhancer.
3 BIOCORROSION MECHANISMS AND CLASSIFICATIONS

3.1 MIC Classifications

Gu (2012) stated that most anaerobic MIC attacks can be divided into two different types depending on the two anaerobic metabolisms. In his analysis, Type I MIC is caused by the respiration of microbes using elemental iron (or other active metal) as electron donor. In order to reduce the oxidants, microbes extract electrons from elemental iron and transport them across cell wall into the cytoplasm. For example, sulfate reduction occurs inside the cytoplasm under enzyme catalysis while the oxidation of the elemental iron occurs extracellularly. Thus, electron transport is required. The redox process supplies energy to the cells. SRB and NRB that attack carbon steel belong to Type I MIC metabolism.

Fungi and fermentative bacteria such as Acid Producing Bacteria (APB) secrete organic acids. Protons and undissociated organic acids are extracellularly reduced on the metal surfaces without catalysis using the electrons released by the oxidation of elemental metals (e.g., Fe\textsuperscript{0}). Fermentative microorganisms cause Type II MIC (Gu, 2012). They secrete organic acid (such as HAc) and other fatty acids. The free (i.e., undissociated) organic acids are corrosive. Corrosion by these oxidants also occurs abiotically like the same way as conventional acid corrosion. An additional Type III MIC was also defined by Gu (2012). In order to have the energy and carbon sources, bacteria attack organic polymers. They secrete enzymes that degrade the extracellular organic substances and plasticizers in the polymers as nutrients. It is a non-electrochemical corrosion, also known as the “biodegradation” (Gu, 2013). Anaerobic and aerobic microbes are able to
cause Type III MIC. These classifications of MIC are helpful to explore and understand biocorrosion. The mechanisms for both Type I MIC and Type II MIC are electrochemical. This work does not deal with Type III MIC.

3.2 MIC Mechanisms

Oxygen is usually removed from pipelines and reservoirs for corrosion prevention, but anaerobic corrosion still occurs in many cases. Elemental iron in carbon steel loses electrons and releases the soluble ferrous ion (Fe$^{2+}$) as shown in Reaction (1). It needs electron acceptors such as sulfate or nitrate to take up the electrons in Reaction (2) in order to maintain electroneutrality.

**Oxidation Reaction:**  
Fe $\rightarrow$ Fe$^{2+}$ + 2e$^-$(1)

**Reduction Reaction:** Oxidant + e$^-$ $\rightarrow$ Reducer (2)

Corrosion related to bacteria was discovered as early as 1910 (Gaines, 1910) and then the first explanation for the mechanism of biocorrosion was postulated by von Wolzogen Kuehr and van der Vlugt (1934) with the so-called Cathodic Depolarization Theory (CDT). Iron loses the electrons becoming ferrous ions in an aqueous environment (Reaction 3). The electrons react with water-derived protons to produce hydrogen. Then, the hydrogenase enzyme of SRB catalyzes the conversion from H$_2$ to H$^+$ (Reaction 6). Meanwhile, SO$_4^{2-}$ receives electrons to become HS$^-$ (Reaction 7) in cells, which provides energy for SRB metabolism. FeS and Fe(OH)$_2$ are predicated as the corrosion products. (Ljungdahl et al., 2003).

**Anodic reaction:**  
4Fe $\rightarrow$ 4Fe$^{2+}$ + 8e$^-$(3)
Water dissociation: $8\text{H}_2\text{O} \rightarrow 8\text{H}^+ + 8\text{OH}^-$  \hspace{1cm} (4)

Cathodic reaction: $8\text{H}^+ + 8\text{e}^- \rightarrow 8\text{H}_{\text{ads}}$  \hspace{1cm} (5)

Cathodic depolarization by hydrogenase: $8\text{H}_{\text{ads}} (\rightarrow 4\text{H}_2) \rightarrow 8\text{H}^+ + 8\text{e}^-$  \hspace{1cm} (6)

Sulfate reduction by SRB: $\text{SO}_4^{2-} + 9\text{H}^+ + 8\text{e}^- \rightarrow \text{HS}^- + 4\text{H}_2\text{O}$  \hspace{1cm} (7)

Precipitation: $\text{Fe}^{2+} + \text{H}_2\text{S} \rightarrow \text{FeS} + 2\text{H}^+$  \hspace{1cm} (8)

Precipitation: $3\text{Fe}^{2+} + 6\text{OH}^- \rightarrow 3\text{Fe(OH)}_2$  \hspace{1cm} (9)

Total reaction: $4\text{Fe} + \text{SO}_4^{2-} + 4\text{H}_2\text{O} \rightarrow \text{FeS} + 3\text{Fe(OH)}_2 + 2\text{OH}^-$  \hspace{1cm} (10)

Many publications released after that time adopted this theory. The effective H$_2$ utilization of the *Desulfovibrio* species of SRB has been reported to support the CDT (Pankhania et al., 1986); and in the Cypionka and Dilling's (1986) experiment, hydrogenase-positive *Desulfovibrio vulgaris* cells can depolarize the cathode as described in the CDT.

However, CDT did not provide the complete explanation for corrosion caused by bacteria in the field, because it merely covered the corrosion mechanism for reactions between *Desulfovibrio* species of SRB and metals (Wawer and Muyzer, 1995). Voordouw et al. (1990) demonstrated that even with a small number of cells in the pipelines, intense corrosion can still happen when SRB biofilms have high hydrogenase activity. Thus, the corrosion rate of pipelines is dependent on the total hydrogenase activity of biofilms, rather than relying on the bacteria population (Bryant et al., 1991). Moreover, the effect of sulfide compounds (H$_2$S, sulfide, bisulfide) secreted by SRB in cathodes were overlooked in the CDT; the environmental changes between aerobic conditions and anaerobic conditions were not considered in CDT; the effect of other
corrosive products from microbial metabolites was also ignored in the CDT (Thierry and Sand, 2002). Above all, CDT cannot explain MIC by hydrogenase negative bacteria. Gu et al. (2013) pointed out that CDT is just one MIC mechanism that falls under Type I mechanism for hydrogenase-positive SRB with H₂ as electron carrier enabled by hydrogenase enzymes.

### 3.2.1 Biocatalytic Cathodic Sulfate Reduction Theory

A new mechanism was reported by Gu et al. (2009), that is termed the Biocatalytic Cathodic Sulfate Reduction (BCSR) theory. The BCSR theory clearly analyzed MIC by SRB on carbon steel by introducing bioenergetics and electrochemical kinetics in the process. It assumes the SRB biofilms play a critical corrosive role during MIC on the surface of carbon steel in electron transfer. In an anaerobic condition, microorganisms built their biofilms on the surfaces of iron. At the beginning of their growth, the bacteria consumed organic carbons such as lactate in the medium for their metabolism, and used sulfate as the terminal electron acceptor (Gu, 2009). The Nernst equation is used to calculate the reduction potentials under non-standard situations as follows.

**Anodic:** \( \text{CH}_3\text{CHOHCOO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{CO}_2 + 4\text{H}^+ + 4\text{e}^- \) (11)

\[
E'_{(\text{CO}_2/\text{lactate})} = -0.43V + \frac{RT}{4F} \ln \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{CHOHCOO}^-]}
\]

**Cathodic:** \( \text{SO}_4^{2-} + 9\text{H}^+ + 8\text{e}^- \rightarrow \text{HS}^- + 4\text{H}_2\text{O} \) (12)

\[
E_{(\text{SO}_4^{2-}/\text{HS}^-)} = 0.252V - \frac{2.591RT}{F} \ln \frac{[\text{SO}_4^{2-}]}{[\text{HS}^-]} + \frac{RT}{8F} \ln \frac{[\text{SO}_4^{2-}]}{[\text{HS}^-]}
\]
where R is the universal gas constant and F the Faraday constant. Reactions (11) and (12) are coupled together, releasing energy for respiration of SRB. The reduction potential at pH 7, 25°C, 1 M solutes and 1 bar gases ($E^{o'}$) of lactate/CO$_2$ is $-430$ mV vs. the Standard Hydrogen Electrode (SHE). The apostrophe sign in potentials and Gibbs free energy indicates pH 7 condition (Thauer et al., 2007). Reaction (12) gives $E^{o'} = -213$ mV for SO$_4^{2-}$/HS$, which is very close to the value of $-217$ mV adopted by Thauer et al (2007). The cell potential ($E_{\text{cell}}$) for the redox reaction coupling lactate oxidation and sulfate reduction is $217$ mV, subtracting $-430$ mV from $-213$ mV, under the conditions defined for $E^{o'}$. This corresponds to a Gibbs free energy change of reaction of $\Delta G^{o'} = -168$ kJ/mol sulfate based on the equation $\Delta G = -nFE_{\text{cell}}$, where n is the number of electrons. This means that the redox reaction is thermodynamically favorable and will spontaneously occur. Lactate respiration with sulfate as terminal electron donor provides energy and carbon building blocks for SRB growth. When there is sufficient lactate everywhere in the biofilm and below the biofilm, SRB has no need for electrons from iron oxidation because lactate oxidation provides electrons. The redox reaction is written as Reaction (13).

$$\text{SO}_4^{2-} + 2\text{CH}_3\text{CHOHCOO}^- \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{CO}_2 + \text{HS}^- + \text{OH}^- + \text{H}_2\text{O} \quad (13)$$

Although the redox reaction is thermodynamically favorable, it is kinetically retarded. The hydrogenase enzymes and other enzymes in SRB catalyze sulfate reduction and lactate oxidation and drive the reaction forward at a noticeable rate. It is reported that SRB are able to grow if the change of the Gibbs free energy of reaction is more than $-20$ kJ/mole (Thauer et al., 2007). When SRB cells are in the stationary phase without growth, they also need energy (maintenance energy) to maintain their health (Chen and
The planktonic cells use lactate and sulfate for their metabolism. When the dense biofilm of SRB restricts diffusions of organic carbon to the bottom of the SRB biofilm, the bottom layer of bacteria (especially the monolayer of cells at the very bottom) suffers from carbon starvation. In order to obtain their maintenance energy, these bacteria extract electrons from elemental iron instead of organic carbon. The organic carbon oxidation is replaced by the elemental iron oxidation with the following reactions (Valensi et al., 1966):

\[
\text{Anodic: } 4\text{Fe}^{2+} + 8e^- \rightarrow 4\text{Fe} \\
E_{(\text{Fe}^{3+}/\text{Fe})} = -0.447V + \frac{RT}{2F} \ln[\text{Fe}^{2+}] \\
\text{Cathodic: } \text{SO}_4^{2-} + 9\text{H}^+ + 8e^- \rightarrow \text{HS}^- + 4\text{H}_2\text{O} \quad \text{(BCSR)}
\]

\[
E_{(\text{SO}_4^{2-}/\text{HS}^-)} = 0.252V - \frac{2.591RT}{F} \text{pH} + \frac{RT}{8F} \ln\left[\frac{[\text{SO}_4^{2-}]}{[\text{HS}^-]}\right]
\]

The \(E^0\) for \(\text{Fe}^{2+}/\text{Fe}^0\) is –447 mV (Thauer et al., 2007). It is very close to that for lactate/CO\(_2\). \(\text{SO}_4^{2-}/\text{HS}^-\) has \(E^0 = –213\) mV calculated from Reaction (15). The cell potential \(E_{\text{cell}}\) is +234 mV for the redox reaction of iron oxidation coupled with sulfate reduction under the conditions defined for \(E^0\). This corresponding to Gibbs free energy change of reaction \(\Delta G^0 = –180.8\) kJ/mol sulfate.

\[
\text{SO}_4^{2-} + 9\text{H}^+ + 4\text{Fe} \rightarrow 4\text{H}_2\text{O} + \text{HS}^- + 4\text{Fe}^{2+}
\]

Xu and Gu (2011) proved the BCSR theory through a laboratory experiment using SRB starvation. \textit{D. vulgaris} biofilms were first cultured on C1018 carbon steel coupons in full medium for seven days. After that the coupons covered with mature SRB biofilms were taken out and put into anaerobic vials with the same media except with different
amounts of carbon sources (full amount and reduced amount). The SRB cells under the biofilms were in a starvation condition, and were forced to switch to the elemental iron as their source of energy. After one week of incubation, weight loss of iron increased as expected and pits on the metal iron were enlarged under starvation with 10% carbon source in the medium compared with the data for coupons in the full medium. BCSR successfully explained the mechanisms of MIC of carbon steel by SRB (Gu and Xu, 2010) and also demonstrated that SRB cells under the starvation conditions would directly promote the corrosion (Xu and Gu, 2011). This bioenergetics-based MIC theory needs to be further investigated by using different microbes other than SRB.

3.2.2 Investigation of Copper Corrosion under Anaerobic Condition

3.2.2.1 MIC of Copper by SRB

MIC of copper is a well-known problem in the copper piping used in fire sprinkler systems (Cantor et al., 2006). Pitting corrosion on the surface of copper (and its alloys) is also a common form of MIC, usually in the presence of sulfide ions or SRB. In the report of Labuda (2003), the failure of copper piping system occurred in portable water systems with existing SRB. Moreover, microbes may be one of the causes of water leakage from the copper pipes of 76 houses which led to about a $360,000 financial loss in California (Boucly, 2012).

Some drywalls were reported to contain excessive amount of toxic gases including sulfur and sulfur compounds, causing the corrosion of copper used in housing (Environmental pollution centers, 2009). Copper tubing and joints in refrigerators, copper wiring and copper pipes in houses built with drywall were found to be blackened and pitted (Meinhold, 2009), shown in the Fig. 3-1. Because the manufacturing process
probably used a large amount of tainted water, which contained microbes and organic carbons, it was possible that SRB existed and grew on organic nutrients in biofilms in the drywall. Hydrogen sulfide gas considered as one of dominant factor of copper corrosion was possibly secreted by SRB in drywalls. H₂S gas becomes a corrosive acid when it dissolves into the water layer on the copper surface covered with moisture, forming a black loose corrosion product (Cu₂S). This led to a fast deterioration of the copper metals (for example copper pipes behind refrigerators). In this research, drywalls samples were tested for the presence of SRB and its relation to causing copper MIC in houses.

Figure 3-1. Corrosion of copper wires happened in houses (Meinhold, 2009)

3.2.2.2 Investigation of Copper MIC Mechanism by SRB

Copper is a noble metal that is not easily oxidized, so SRB are unable to directly extract its electrons for sulfate reduction. This suggests that the mechanism of copper corrosion by SRB is very different from the mechanism of carbon steel corrosion by SRB. The standard reduction potentials of Cu⁺/Cu⁰ and Cu²⁺/Cu⁰ are smaller than the
standard reduction potential of oxygen, which is 818 mV (Bard et al., 1985). This number states that corrosion reactions between oxygen and copper are thermodynamically favorable. However, copper oxides form a passive layer on the top of copper metal, protecting copper from further corrosion (Feng et al., 1997).

Researchers have attempted to explain the process of copper MIC (Almeida and de França, 1998; Huang et al., 2004; Labuda and Powell Labs Ltd., 2003), but some of them failed to completely removal of oxygen in their experiments, resulting in misleading data. In real anaerobic conditions, microbial respiration requires an electron acceptor, which is usually oxygen in aerobic respiration, and sulfate or nitrate, etc. in anaerobic condition. The $E^{\circ}$ value for $\text{Cu}^+/\text{Cu}^0$ is +520 mV at 25°C and 1 M $\text{Cu}^+$ (Bard et al., 1985). Compared with $-213$ mV for $\text{SO}_4^{2-}/\text{HS}^-$, $E_{\text{cell}}$ is $-733$ mV for the redox reaction combining $\text{Cu}^0$ oxidation to $\text{Cu}^+$ and sulfate reduction under the conditions defined for $E^{\circ}$. This yields a positive Gibbs free energy change of reaction, which means the redox reaction is thermodynamically unfavorable, thus Type I mechanism is not suitable to explain copper MIC by SRB in anaerobic condition.

Puigdomenech and Taxen (2000) collected numerous thermodynamic data regarding copper corrosion. In their technical report, he found a thermodynamically favorable reaction between Cu and H$_2$S shown below,

$$2\text{Cu (crystal)} + \text{H}_2\text{S} \rightarrow \text{Cu}_2\text{S (crystal)} + \text{H}_2 (g)$$

(C17)

$\text{Cu}_2\text{S}$ has a black color. The hydrogen sulfide secreted by SRB was at a high local concentration under SRB biofilms, and it continuously attacked the copper surface to produce the pits. The anodic and assumed cathodic reactions are the following (Bard et al., 1985):
Anodic: \[2\text{Cu}^+ + 2e^- \rightarrow 2\text{Cu}\]  

\[E_{(\text{Cu}^+/\text{Cu})} = 0.52V + \frac{RT}{F}\ln[Cu^+]\] (for Cu\(^+\) reduction)  

Cathodic: \[\text{HS}^- + \text{H}^+ + 2e^- \rightarrow \text{S}^{2-} + \text{H}_2\]  

The net effect is proton reduction  

\[2\text{H}^+ + 2e^- \rightarrow \text{H}_2\]  

\[E_{(\text{H}^+/\text{H}_2)} = -\frac{2.303RT}{F}\ln[pH] + \frac{RT}{2F}\ln\frac{1}{p_{\text{H}_2}}\]  

\[\text{HS}^- \rightarrow \text{S}^{2-} + \text{H}^+\]  

\[\log\left(\frac{[S^{2-}]}{[\text{HS}^-]}\right) = -13.9 + \text{pH}\]  

Reaction (19) is a combination of Reactions (19a) and (19b). As shown in Reaction (19a), protons as the electron acceptor in the solution use electrons from copper oxidation to produce hydrogen. The equilibrium of HS\(^-\) and S\(^{2-}\) in the solution, shown Reaction (19b), shows that their concentration ratio is correlated to the pH. It means as the certain pH values, the concentration ratio of HS\(^-\) and S\(^{2-}\) is fixed. This states the higher concentration of HS\(^-\) will release more protons to take electrons released from the copper oxidation. The equilibrium potentials of Reaction (18) calculated with different Cu\(^+\) concentrations at pH 7 and 25°C is shown in Table 1.
Table 1

*Equilibrium Potentials of Cu\(^+\)/Cu with Different Cu\(^+\) Concentrations*

<table>
<thead>
<tr>
<th>Concentration of Cu(^+) Ion in the Solutions (ppm)</th>
<th>Equilibrium Potential (E') of Cu(^+)/Cu (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>–0.118</td>
</tr>
<tr>
<td>0.1</td>
<td>–0.177</td>
</tr>
<tr>
<td>1</td>
<td>–0.236</td>
</tr>
<tr>
<td>10</td>
<td>–0.295</td>
</tr>
<tr>
<td>100</td>
<td>–0.354</td>
</tr>
<tr>
<td>1000</td>
<td>–0.413</td>
</tr>
<tr>
<td>10000</td>
<td>–0.472</td>
</tr>
<tr>
<td>100000</td>
<td>–0.531</td>
</tr>
</tbody>
</table>

At pH = 7, Reaction (19a) has equilibrium potential \(E^{\circ'} = –414\) mV. In the condition of 1 Pa partial pressure for H\(_2\) at 25°C, the equilibrium potential \(E'\) of Reaction (19a) is –270 mV. In Table 1, all of the potentials of copper oxidation with different Cu\(^+\) concentrations are negative. Adding any one of these potential values with –414 mV will yield a negative \(E_{\text{cell}}\), corresponding to a positive value of \(\Delta G'\). Based on equilibrium potentials of 2H\(^+\)/H\(_2\) under two partial pressures for H\(_2\) and extreme potential values of Cu\(^+\)/Cu calculated in Table 1, the Gibbs free energy changes of the redox reaction obtained from four conditions are listed in Table 2. It shows that the coupled reaction of Reaction (18) and (19) is not thermodynamically favorable in these four conditions.
Table 2

Changes of the Gibbs Free Energy under Different Conditions

<table>
<thead>
<tr>
<th>Cu(^+) concentration (ppm)</th>
<th>Equilibrium Potential of Cu(^{2+})/Cu (V)</th>
<th>Partial Pressure of H(_2) gas (Pa)</th>
<th>Equilibrium Potential of Reaction (19) (V)</th>
<th>(E_{\text{cell}}) (V)</th>
<th>(\Delta G) (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>–0.118</td>
<td>10(^5)</td>
<td>–0.414</td>
<td>–0.532</td>
<td>102.76</td>
</tr>
<tr>
<td>0.01</td>
<td>–0.118</td>
<td>1</td>
<td>–0.270</td>
<td>–0.338</td>
<td>65.29</td>
</tr>
<tr>
<td>100000</td>
<td>–0.531</td>
<td>10(^5)</td>
<td>–0.414</td>
<td>–0.945</td>
<td>182.54</td>
</tr>
<tr>
<td>100000</td>
<td>–0.531</td>
<td>1</td>
<td>–0.270</td>
<td>–0.801</td>
<td>154.72</td>
</tr>
</tbody>
</table>

In the local areas, severe corrosion will occur when the sulfide ion reaches 0.05 ppm (Horn, 2009), and EPRI (2001) stated only 0.01 ppm of the sulfide ion in the environment would cause harmful results on copper. This implies that the concentration of sulfide ions possibly plays a more important role than proton in copper corrosion. Moreover, in real situations, the deviation of pressure, concentrations and temperatures will give different equilibrium potentials for both reactions, making it more difficult to explain and to predict the real electron acceptors and the accurate corrosion rate on the metal surface below SRB biofilms. Thus, the metabolic activities of SRB also have possibilities to influence the copper corrosion. This suggests that the biocorrosion of copper occurring with H\(_2\)S belongs to the mechanism of Type II MIC with H\(_2\)S or S\(^2-\) as the corrosive metabolite. In this research, lab experiments proposed evidence to support this assumption.
3.2.2.3 Investigation of Copper MIC by NRB

In the field biofilm consortia on the surfaces of pipelines, *B. licheniformis* is one of the common NRB found in oil fields (López et al., 2006). Nitrate was injected into pipelines to cultivate NRB as a terminal electron source competitor against sulfate for creating a shortage of organic carbons to SRB, and this helps to suppress the growth of SRB (Gieg et al., 2011). However, based on the experimental from our lab, *B. Licheniformis* was capable of causing biocorrosion on a metal surface. The mechanism of iron MIC by NRB was similar to one used to describe iron MIC by SRB, i.e., the BCSR theory. Other than steel, a few corrosion failure reports have listed the biocorrosion of copper and its alloy produced by NRB. Thomas (2002) showed that the presence of ammonium secreted by NRB was likely responsible for stress corrosion cracking on the brass condenser tubes of cooling systems. Brass is an alloy of copper and zinc.

NRB consume organic carbons such as sucrose to obtain energy and reduce nitrate as the electron acceptor in order to complete anaerobic respiration, and in the process, nitrates can be reduced to ammonium by following Dissimilatory Nitrate Reduction to Ammonium (Su et al., 2012). In addition, nitrogen is also one of the common reduction products by the NRB during the denitrification. The BCNR (Biocatalytic Cathodic Nitrate Reduction) theory, which is analogous to the BCSR theory, can explain the MIC of steel by NRB as well as copper MIC by NRB. In the NRB culture medium, the reduction reactions forming nitrogen and NH$_4^+$ are shown in the following (Maloy, 1985):

\[
2\text{NO}_3^- + 10\text{e}^- + 12\text{H}^+ \rightarrow \text{N}_2 + 6\text{H}_2\text{O}
\]  (20)
\[ E_{(\text{NO}_3/\text{N}_2)} = 1.246V - \frac{2.764RT}{F} \text{pH} + \frac{RT}{10F} \ln \left( \frac{[\text{NO}_3^-]^2}{P_{\text{N}_2}} \right) \]

\[ \text{NO}_3^- + 8\text{e}^- + 10\text{H}^+ \rightarrow \text{NH}_4^+ + 3\text{H}_2\text{O} \tag{21} \]

\[ E_{(\text{NO}_3/\text{NH}_4^+)} = 0.875V - \frac{2.879RT}{F} \text{pH} + \frac{RT}{8F} \cdot \ln \left( \frac{[\text{NO}_3^-]}{[\text{NH}_4^+]} \right) \]

The \( E^0' \) value of \( \text{NO}_3^-/\text{NH}_4^+ \) is +358 mV and for \( 2\text{NO}_3^-/\text{N}_2 \) is +750 mV. +750 mV is sufficiently high for the oxidation of elemental copper because the \( E^0' \) value for \( \text{Cu}^+/\text{Cu} \) is +530 mV. Some equilibrium potential values for conditions deviating from those defined for \( E^0' \) are listed in Table 1. The oxidation reaction of copper is in the following (Puigdomenech and Taxen, 2000):

\[ \text{Cu} \rightarrow \text{Cu}^+ + \text{e}^- \tag{22} \]

If, assuming the \( \text{Cu}^+ \) concentration is about 1 ppm under an NRB biofilm, the combination of Reactions (20) and (22) gives the cell potential +514 mV, corresponding to \( \Delta G' = -496 \text{ kJ/mole nitrate} \). Combining Reactions (21) and (22), the coupled cell potential is +122 mV and \( \Delta G' \) is -94 kJ/mole nitrate. These negative changes in the Gibbs free energy mean that copper MIC due to nitrate reduction by NRB are thermodynamically favorable. In this research, lab experiments supported the mechanism assumption.
4 RESEARCH OBJECTIVES AND TEST MATERIALS

4.1 Research Objectives

4.1.1 Biocide Enhancing DAAs

The DAA mixtures (D-Try, D-Met, D-Tyr, and D-Leu) that investigated by Xu and Jie (2011) in their experiment were equimolar. Thus, it is unclear whether all types of DAA can make the same contribution on biocide enhancement. The objective of the present study was to demonstrate the enhanced capability of one DAA not tested by our group before, as well as to investigate if L type of Methionine would adversely influence the efficacy of D-Met on its biocide enhancement.

In order to carry out this research, three preliminary assumptions required examination:

1. Can D-Val be used as an enhancer for the biocide THPS to increase the efficiency of MIC mitigation by SRB?
2. What is the best concentration of D-Val for THPS enhancement?
3. Does L-Met influence or inhibit the capability of D-Met as a biocide enhancer?

4.1.2 Investigation of Copper MIC by Bacteria in Anaerobic Environment

Copper MIC occurred in an anaerobic environment with SRB; however, copper, being a noble metal, is difficult to lose electrons in electro-chemical reactions. Direct oxidation of elemental copper coupled with sulfate reduction is found to be thermodynamically unfavorable for Type I MIC because of the high reduction potentials for Cu^{2+}/Cu and Cu^{+}/Cu. It is very possible that the metabolic products of SRB, including hydrogen sulfide, will react with copper metal because its thermodynamics is unfavorable.
(Puigdomenech and Taxen, 2000). The coupled reaction of nitrate reduction by NRB and copper oxidation is thermodynamically favorable in an anaerobic environment because $\text{NO}_3^-$ has large reduction potentials higher than those for $\text{Cu}^{2+}/\text{Cu}$ and $\text{Cu}^+/\text{Cu}$, making Type I MIC possible under biocatalysis. Thus, in the second part of this research, the objective was to demonstrate the various mechanisms to explain the MIC of copper by SRB and NRB in an anaerobic environment.

Five preliminary assumptions require investigations:

1. Can SRB attack the copper alloy 110 in an anaerobic environment?
2. What type of MIC mechanism can reasonably explain the copper MIC caused by SRB?
3. Can SRB attack copper coupons in an anaerobic environment and can SRB-tainted drywall be corrosive?
4. Is NRB able to cause severe MIC against copper alloy 110 in an anaerobic environment?
5. What type of MIC mechanism is needed to explain the effect of NRB on copper in an anaerobic environment?

4.2 Test Matrices

4.2.1 Test Conditions

Pure strain *D. vulgaris* (ATCC 7757) was cultured in ATCC 1249 medium. The culture medium was sterilized in an autoclave at 121°C for 20 minutes and then sparged with pure nitrogen (filter-sterilized) for about one hour to remove dissolved oxygen. The initial pH value of medium was adjusted about 7.2 before autoclaving. A mixture of 100
ml full strength ATCC 1249 culture medium and 100 ppm L-cysteine, as an oxygen scavenger, (Fisher Scientific, Pittsburgh, PA, USA) was placed in 125 ml anaerobic vials (Catalog No. 223748, Wheaton Industries Inc., Millville, NJ, USA). In the test, 1 ml of SRB seed culture was added into each anaerobic vial with an initial SRB seed concentration of $10^6$ cells ml$^{-1}$.

Table 3

*ATCC 1249 Medium for Sulfate Reducing Bacteria*

<table>
<thead>
<tr>
<th>Component</th>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>MgSO$_4$</td>
<td>2.0 g</td>
</tr>
<tr>
<td></td>
<td>Sodium Citrate</td>
<td>5.0 g</td>
</tr>
<tr>
<td></td>
<td>CaSO$_4$</td>
<td>1.0 g</td>
</tr>
<tr>
<td></td>
<td>NH$_4$Cl</td>
<td>1.0 g</td>
</tr>
<tr>
<td></td>
<td>Distilled Water</td>
<td>400 ml</td>
</tr>
<tr>
<td>II</td>
<td>K$_2$HPO$_4$</td>
<td>0.5 g</td>
</tr>
<tr>
<td></td>
<td>Distilled Water</td>
<td>200 ml</td>
</tr>
<tr>
<td>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>IV</td>
<td>Filter-sterilize 5 wt% ferrous ammonium sulfate. Add 0.1 ml of this solution to 5.0 ml of medium prior to inoculation.</td>
<td></td>
</tr>
</tbody>
</table>

Pure strain *B. licheniformis* (ATCC 14580) was cultured to be an NRB. The components of the medium are listed in Table 4.
Table 4  
*Medium for Nitrate Reducing Bacteria*

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>10 g</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>13.9 g</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>2.7 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>1 g</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>1 g</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>2.5 g</td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

Ten milliliters of a trace element stock solution were added into the culture medium each time. The components of the solution are listed in Table 5.
Table 5

Component of Stock Solution of Trace Elements

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnCl₂·4H₂O</td>
<td>180 mg</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>270 mg</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>50 mg</td>
</tr>
<tr>
<td>CuCl₂·2H₂O</td>
<td>24 mg</td>
</tr>
<tr>
<td>NaMoO₄·2H₂O</td>
<td>23 mg</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>19 mg</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>100 ml</td>
</tr>
</tbody>
</table>
4.2.2 Test Matrices

The research experiments were conducted in the following manner.

Table 6

Test Matrix for Mitigation of SRB MIC Using THPS and DAA

<table>
<thead>
<tr>
<th>Test Strain</th>
<th>\textit{D. vulgaris} (ATCC 7757)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Medium</td>
<td>ATCC 1249 Medium</td>
</tr>
<tr>
<td>Coupon Material</td>
<td>UNS C1018 Mild Steel</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37</td>
</tr>
<tr>
<td>Initial pH</td>
<td>7.0 ± 0.1</td>
</tr>
<tr>
<td>D-Met (ppm)</td>
<td>0, 100</td>
</tr>
<tr>
<td>L-Met (ppm)</td>
<td>0, 100</td>
</tr>
<tr>
<td>D-Val (ppm)</td>
<td>0, 50, 100, 200, 500, 1000</td>
</tr>
<tr>
<td>THPS (ppm)</td>
<td>0, 50</td>
</tr>
<tr>
<td>Experimental Setup</td>
<td>Anaerobic Vials</td>
</tr>
</tbody>
</table>
Table 7

*Test Matrix for Investigation of Copper MIC by SRB*

<table>
<thead>
<tr>
<th>Test Strain</th>
<th><em>D. vulgaris</em> (ATCC 7757)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Medium</td>
<td>ATCC 1249 Medium</td>
</tr>
<tr>
<td>Coupon Material</td>
<td>Copper Alloy 110</td>
</tr>
<tr>
<td>Temperature (ºC)</td>
<td>37</td>
</tr>
<tr>
<td>Initial pH</td>
<td>7.0 ± 0.1</td>
</tr>
<tr>
<td>Total Carbon Source Percentages (v/v) in Medium after 3-day Biofilm Establishment</td>
<td>0, 1, 10, 100</td>
</tr>
<tr>
<td>Total Drywall Samples</td>
<td>Six Pieces of Drywall from: Master Bedroom, Master Bathroom, Middle Bedroom, Front Bedroom, Bathroom, Master Bathroom Closet.</td>
</tr>
<tr>
<td>Experimental Setup</td>
<td>Anaerobic Vials</td>
</tr>
</tbody>
</table>
Table 8

*Test Matrix for Investigation of Copper MIC by NRB*

<table>
<thead>
<tr>
<th>Test Strain</th>
<th>B. licheniformis (ATCC 14580)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Medium</td>
<td>NRB*</td>
</tr>
<tr>
<td>Coupon Material</td>
<td>Copper Alloy 110</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37</td>
</tr>
<tr>
<td>Initial pH</td>
<td>7.2 ± 0.1</td>
</tr>
<tr>
<td>Total Carbon Source Percentages (v/v) in Medium after 3-day Biofilm Establishment</td>
<td>0, 1, 10, 100</td>
</tr>
<tr>
<td>Experimental Setup</td>
<td>Anaerobic Vials</td>
</tr>
</tbody>
</table>

*NRB medium recipe is from report of Folmsbee et al. (2006)
5. EXPERIMENTAL SETUP

5.1 DAAs to Enhance Biocide Experiment

5.1.1 Chemicals and Substratum for Biofilm Growth

D-Met, L-Met and D-Val were purchased from Sigma-Aldrich (St. Louis, MO, USA). To reduce the strong acidity, 10,000 ppm of THPS (Sigma-Aldrich, St. Louis, MO, USA) solution was adjusted using a 5 mol/L NaOH solution to a neutral pH just prior being added to the culture medium. In the tests, disc-shaped C1018 carbon steel coupons with diameters of 1.12 cm were sequentially polished with 180, 400 and 600 grit sandpaper. The sides and bottom of all coupons were painted with inert Teflon and the top surfaces were cleaned using 100% (v/v) isopropanol. Then coupons were sanitized under a UV light in a biosafety hood for 15 minutes. Nitrogen sparging was used to deoxygenate the medium, the THPS, and the amino acid solutions for about one hour. Medium mixing and inoculation were performed in an anaerobic, nitrogen-filled glove-chamber. Vials were sealed in the anaerobic chamber and then incubated in at 37°C. Table 9 lists the chemical compositions of the carbon steel C1018.
Table 9

Composition of C1018 Carbon Steel Coupons

<table>
<thead>
<tr>
<th>Element</th>
<th>wt %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (Fe)</td>
<td>98.81-99.26</td>
</tr>
<tr>
<td>Carbon (C)</td>
<td>0.18</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>0.6 – 0.9</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>0.04</td>
</tr>
<tr>
<td>Sulfur (S)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

5.1.2 Mitigation of Biofilm Establishment Experiment

The mitigation of biofilm establishment on coupon surfaces was conducted by placing three coupons and 100 ml full strength medium in each vial. Different concentrations of THPS and amino acids were added to the medium before sealing them in the chamber. After both three and seven-day incubation periods, coupons were removed and examined for sessile SRB cells.

5.1.3 Biofilm Observation under Scanning Electron Microscope (SEM)

In order to observe the biofilm layer on coupon surfaces, the copper coupons were immersed in 4% (wt.) glutaraldehyde (Cat. No. G151-1 from FisherSci.com) for four hours and then dehydrated by a graded series of ethanol baths (25%, 50%, 75% and 100% v/v). The Critical Point Drying (CPD) method was followed to achieve dehydration. Then, observation of biofilms and corrosion products was conducted using an SEM (Model JSM-6390, JEOL, Tokyo, Japan). Because biofilms are not able to conduct electrons, a thin layer of golden plasma is usually coated on the biofilms to
obtain a better vision under the SEM. SEM images were taken of locations exhibiting the most sessile cells on the coupon because sessile cells were not evenly distributed on a coupon.

5.1.4 Enumeration of Sessile Cells

Live _D. vulgaris_ cells are motile, thus they can be counted by using a hemocytometer under a light microscope at 400X magnification. However, $5 \times 10^4$ cells ml$^{-1}$ is the lower limitation of observation at 400X magnification. The semi-quantitative most probable number (MPN) method was used as a standard practice when the cell number was too low. The MPN method may use a liquid culture medium or a solid medium. It has a typical accuracy of 1-log in cell numbers; therefore, to ensure the efficacy of a biocide system at least a 2-log reduction needs to be shown. SRB biofilms were scrubbed from a coupon surface with brush-shaped dipsticks that were part of an SRB test kit (Sani-Check® Product #100, BioSan Laboratories, Warren, MI, USA). They were then placed in a 50 milliliter sterilized plastic tube containing ten milliliters sterilized and deoxygenated distilled water. In order to ensure an even distribution of SRB cells in the liquid and on the dipsticks, the tubes were vortexed for about 15 seconds to remove sessile cells still attached to the coupon surfaces. The dipsticks were then inserted into the test kit vials containing the SRB solid medium. These solid medium vials were then placed in a 37°C incubator and the time was recorded when a black (FeS) color began to appear in the medium. This color change was correlated with a specific density of SRB sessile cells base on a chart supplied by the BioSan Laboratories. Each assay was repeated three times.
5.2 Investigation of Copper MIC Experiment

5.2.1 Chemicals and Substratum for SRB Biofilm Growth

Copper Alloy 110 disc-shaped coupons (3/8 inch in diameter) were used in the biofilm growth experiment. Each vial contained three duplicate UV sanitized coupons, one milliliter of SRB seed culture and 100ml full strength ATCC 1249 medium to culture the biofilm for seven days. The observation of established biofilms on copper surface was conducted under the SEM with the same procedures as previous described. Table 10 gives Copper Alloy 110 chemical compositions.

Table 10

<table>
<thead>
<tr>
<th>Chemical Compositions of Copper Alloy 110</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Copper (Cu)</td>
</tr>
<tr>
<td>Oxygen (O)</td>
</tr>
</tbody>
</table>

An X-ray diffraction (XRD) analysis (Rigaku Ultimia IV, Rigaku, Japan) to obtain elemental distribution over the surface was used to determine chemical composition of the copper coupon after MIC by SRB. Elemental distribution over the surface and composition information can be obtained.

5.2.2 SRB Carbon Source Starvation Experiment

Copper coupons (3/8 inch in diameter) were used for the starvation experiment. Each vial contained three duplicate coupons. One milliliter of SRB seed culture and 100ml full strength ATCC 1249 medium were used to culture the biofilm for three days.
After SRB biofilms were well established on the surface, in order to test starvation effects, copper coupons were transferred into new 125 ml vials containing fresh full medium or a medium with a reduced carbon source. In the experiment, full strength medium, 10% of carbon source medium, 1% of carbon source medium, and 0% of carbon source medium were used to simulate various degrees of carbon source starvation. During the SRB metabolic activities, lactate was used as the carbon source. To be safe, citrate was reduced along with lactate for carbon starvation.

5.2.3 Observation of Pits under Infinite Focus Microscope (IFM)

Copper coupon surfaces were cleaned with a 20% (v/v) sulfuric acid solution. The surface overview and pit depth profile were examined under the IFM (Model ALC13, ALICONA, Austria) which has a surface profilometer with a maximum 200X magnification. The pit depth profile can be used to describe the localized corrosion rate.

5.2.4 Drywall Experiment

Six drywall samples from a house in Jacksonville, FL were tested. They were suspected by owner to have caused copper corrosion unlike normal drywalls. The Most Probable Number (MPN) method with a solid medium was used to enumerate SRB cells. Moreover, one drywall sample and two UV sanitized copper coupons were placed into each autoclaved vial that was then sealed in an N₂ filled chamber. All vials were placed in a 37°C incubator for about two months and observed for the appearance of black corrosion products on the copper surfaces. Control groups in this experiment contained two UV sanitized copper coupons in each anaerobic vial without any drywall samples.
5.2.5 Chemicals and Substratum for NRB Biofilm Growth

Copper Alloy 110 disc-shaped coupons (3/8 inch in diameter) were used in the biofilm growth experiment. Each vial contained three duplicate UV-sanitized coupons, one milliliter of NRB culture seed and 100 ml full strength NRB medium with a ten-milliliter trace element solution to culture the biofilm for seven days.

5.2.6 NRB Carbon Source Starvation Experiment

Each vial contained three duplicate UV-sanitized copper coupons, one milliliter of NRB seed culture and 100 ml full strength NRB medium and a ten-milliliter trace elemental solution, which was used to culture the NRB biofilm for three days. In order to test starvation effects, other copper coupons were transferred into new 125 ml vials containing fresh full medium or a medium with a reduced carbon source. In the experiment, full medium, 10% of carbon source medium, 1% of carbon source medium, and 0% of carbon source medium were all used to simulate various degrees of carbon source starvation. During the NRB metabolic activities, sucrose and possible some components in yeast extract were used as the carbon source.
6 RESULTS

Experiments were performed according to the test matrices in the Chapter 4, and followed the test procedures described in the Chapter 5. The accomplishment of experiments and the explanations of results are given in a following order:

1) Experiments for mitigation of SRB biofilm by different concentrations of D-Val with 50 ppm THPS;
2) Experiments for demonstrating whether L-Met poses any adverse effect on the efficacy of D-Met and THPS biocide cocktail in SRB biofilm mitigation;
3) Experiments for copper MIC by SRB;
4) Experiments for carbon starvation effect on copper MIC by SRB;
5) Experiments for copper MIC by SRB living in contaminated drywall;
6) Experiments for copper MIC by NRB;
7) Experiments for carbon starvation effect on copper MIC by NRB.

6.1 D-Val Experiment

As discussed in Chapter 2, D-Val is selected and tested to demonstrate its capability as a biocide enhancer. In this experiment, a gradient of concentrations (50 ppm, 100 ppm, 200 ppm, 500 ppm and 1000 ppm) of D-Val was added to 50 ppm THPS to investigate its effect as a biocide enhancer.
Table 11

*Sessile Cell Counts in ATCC 1249 Medium Three Days after Using Different D-Val Treatments for the Prevention of D. vulgaris Biofilm Establishment*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sessile cell count (cells/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td>≥10⁶</td>
</tr>
<tr>
<td>100 ppm D-Valine</td>
<td>≥10⁶</td>
</tr>
<tr>
<td>50 ppm D-Valine + 50 ppm THPS</td>
<td>≥10⁴</td>
</tr>
<tr>
<td>100 ppm D-Valine + 50 ppm THPS</td>
<td>≥10³</td>
</tr>
<tr>
<td>200 ppm D-Valine + 50 ppm THPS</td>
<td>≥10³</td>
</tr>
<tr>
<td>500 ppm D-Valine + 50 ppm THPS</td>
<td>≥10²</td>
</tr>
<tr>
<td>1000 ppm D-Valine + 50 ppm THPS</td>
<td>≥10³</td>
</tr>
<tr>
<td>50 ppm THPS</td>
<td>≥10⁴</td>
</tr>
</tbody>
</table>

Table 11 displays that 100 ppm D-Val alone had a sessile cell count of 10⁶ cells/cm², same as the cell count from no treatment. When treated with 50 ppm D-Val + 50 ppm THPS, the sessile cell count dropped to 10⁴ cells/cm². However, 50 ppm THPS treatment achieved the same cell count. This indicates that 50 ppm D-Val did not enhance 50 ppm THPS. The treatments of 100 ppm D-Val + 50 ppm THPS, 200 ppm D-Val + 50 ppm THPS both achieved 1 log enhancement, while 500 ppm D-Val + 50 ppm THPS achieved 2-log enhancement. Because 1-log difference in cell count could be due to MPN error, 500 ppm D-Val is recommended. Surprisingly, Table 11 shows that further increasing D-Val concentration to 1000 ppm actually achieved 1-log enhancement.
instead of 2 or more. This could be due to MPN error. Fig. 6-1 demonstrates that no obvious sessile cell inhibition with 100 ppm D-Val alone because the sessile cells are numerous in the SEM image. The results presented the treatments of 50 ppm D-Val + 50 ppm THPS, 100 ppm D-Val + 50 ppm THPS, 200 ppm D-Val + 50 ppm THPS, 500 ppm D-Val + 50 ppm THPS, and 1000 ppm D-Val + 50 ppm THPS in Fig. 6-2, Fig. 6-3, Fig. 6-4, Fig. 6-5 and Fig. 6-6, respectively. In the D-Val + 50 ppm THPS cocktail treatment cases, increased D-Val dosages correspond to fewer sessile cells. They all had fewer cells than in the case with 50 ppm THPS treatment alone. It should be pointed out that SEM images should not be used for sessile counting because the cells were unevenly distributed.

*Figure 6-1.* SEM images for 3-day coupons in *D. vulgaris* culture with ATCC 1249 medium treated with 100 ppm D-Val. (Scale bar for the small inserted image is 50 μm)
**Figure 6-2.** SEM images for 3-day coupons in *D. vulgaris* culture with ATCC 1249 medium treated with 50 ppm D-Val + 50 ppm THPS. (Scale bar for the small inserted image is 50 μm)

**Figure 6-3.** SEM images for 3-day coupons in *D. vulgaris* culture with ATCC 1249 medium treated with 100 ppm D-Val + 50 ppm THPS. (Scale bars for the small inserted images are 50 μm)
Figure 6-4. SEM images for 3-day coupons in *D. vulgaris* culture with ATCC 1249 medium treated with 200 ppm D-Val + 50 ppm THPS. (Scale bars for the small inserted images are 50 μm)

Figure 6-5. SEM images for 3-days coupons in *D. vulgaris* cultures with ATCC 1249 medium with D-Val 500 ppm + THPS 50 ppm mixture. (Scale bar for the small inserted image is 50 μm)
Figure 6-6. SEM images for 3-days coupons in *D. vulgaris* cultures with ATCC 1249 medium with D-Val 1000 ppm + THPS 50 ppm mixture. (Scale bar for the small inserted image is 50 μm)
Figure 6-7. SEM images for 3-day coupons in *D. vulgaris* cultures with ATCC 1249 medium treated with 50 ppm THPS. (Scale bar for the small inserted image is 50 μm)

6.2 D-Met and L-Met Experiment

L-Met can be converted to D-Met. It is cheaper to use a 50:50 L-Met, D-Met mixture if possible. It is desirable to know whether the mixture of L-Met and D-Met will diminish the effect of D-Met in its application as a biocide enhancer.

The data of sessile cell count in Table 12 show there were more than $10^6$ SRB cells/cm² on the coupon surface without any chemical treatment, and the treatments of 100 ppm D-Met, 100 ppm L-Met and 100 ppm D-Met + 100 ppm L-Met had no effect on the sessile cell count. This means Met alone is not biocidal. In Table 12, the sessile cell counts were $10^3$ cells/cm² when the treatments were 100 ppm D-Met +100 ppm L-Met + 50 ppm THPS and 100 ppm L-Met + 50 ppm THPS. There was a 1-log reduction of sessile cells. Table 12 also indicates that 100 ppm D-Met + 50 ppm THPS resulted in a
sessile cell count of $10^2$ cells/cm$^2$, which noticeably enhanced the effect of 50 ppm THPS because it achieved 2-log enhancement compared with the $10^4$ cells/cm$^2$ achieved by 50 ppm THPS alone. This means the treatment containing 100 ppm D-Met and 50 ppm THPS was the most effective cocktail in all treatments. The added L-Met decreased the enhancement effect by 1-log. However, this could be due to MPN error.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sessile cell count (cells/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td>$\geq 10^6$</td>
</tr>
<tr>
<td>100 ppm D-Methionine</td>
<td>$\geq 10^5$</td>
</tr>
<tr>
<td>100 ppm L-Methionine</td>
<td>$\geq 10^5$</td>
</tr>
<tr>
<td>100 ppm D-Methionine + 100 ppm L-Methionine</td>
<td>$\geq 10^5$</td>
</tr>
<tr>
<td>100 ppm D-Methionine + 50 ppm THPS</td>
<td>$\geq 10^2$</td>
</tr>
<tr>
<td>100 ppm L-Methionine + 50 ppm THPS</td>
<td>$\geq 10^3$</td>
</tr>
<tr>
<td>100 ppm D-Methionine + 100 ppm L-Methionine + 50 ppm THPS</td>
<td>$\geq 10^3$</td>
</tr>
<tr>
<td>50 ppm THPS</td>
<td>$\geq 10^4$</td>
</tr>
</tbody>
</table>

The SEM images support the sessile cell count data in Table 12. Fig. 6-8(A) shows the biofilm image on the coupon surface that was treated with 100 ppm D-Met in
the full strength medium for seven days. This image shows that sessile cells of SRB were abundant. However, when the coupon was treated with 100 ppm D-Met + 50 ppm THPS, the sessile cells significantly decreased. It means that 100 ppm D-Met + 50 ppm THPS treatment was effective. When a coupon was treated with L-Met alone (Fig. 6-9(A)), the sessile cells of SRB abundantly covered the coupon’s surface. Fig. 6-9(B) shows a relatively clear coupon surface that was treated with 100 ppm L-Met + 50 ppm THPS. Fig. 6-10(A) shows numerous SRB cells in the treatment using 100 ppm D-Met + 100 ppm L-Met. There was a decrease in the number of cells in the medium to which 50 ppm THPS had been added (Fig. 6-10(B)). It suggests that the combination of 100 ppm D-Met and 100 ppm L-Met with 50 ppm THPS were able to inhibit sessile SRB growth. Compared to the effect of 50 ppm THPS alone (Fig. 6-11), treatments with D-amino acids visibly enhanced the result of THPS biocide mitigation of MIC on carbon steel. A rough conclusion can be made that L-Met and D-Met without THPS did not influence the SRB growth, however, they were effect when combined with 50 ppm THPS. L-Met might have a negative influence on the enhancement of D-Met for 50 ppm THPS, but this requires further investigation.

Fig. 6-12 shows the BioSan SRB test kit results of the sessile cell counts with various treatments. The extent of the blackening of the solid medium correlates with the amount of sessile SRB scrubbed from a coupon surface. Treatments without THPS had a higher degree of blackening through the medium than treatments with THPS alone. Moreover, 50 ppm THPS alone resulted in a darker color in the medium than treatments with D-amino acids + THPS, indicating that D-Met enhanced 50 ppm THPS.
Figure 6-8. SEM images for 7-day coupons in *D. vulgaris* cultures with ATCC 1249 medium treated with (A) 100 ppm D-Met, (B) 100 ppm D-Met + 50 ppm THPS, respectively. (Scale bars for the small inserted images are 50 μm)
Figure 6-9. SEM images for 7-day coupons in *D. vulgaris* cultures with ATCC 1249 medium treated with (A) 100 ppm L-Met, (B) 100 ppm L-Met + 50 ppm THPS, respectively. (Scale bars for the small inserted images are 50 μm)
Figure 6-10. SEM images for 7-day coupons in *D. vulgaris* cultures with ATCC 1249 medium treated with (A) 100 ppm L-Met + 100 ppm D-Met, (B) 100 ppm L-Met + 100 ppm D-Met + 50 ppm THPS, respectively. (Scale bars for the small inserted images are 50 μm)
Figure 6-11. SEM images for 7-day coupons in *D. vulgaris* cultures with ATCC 1249 medium treated with 50 ppm THPS. (Scale bar for the small inserted images is 50 μm)

Figure 6-12. BioSan picture of *D. vulgaris* growth with different treatments after 7 days
6.3 Experiment on Copper MIC by SRB

When MIC occurs on a copper surface, a layer of corrosion product will cover the copper, like the phenomenon shown in Fig. 6-16. After being soaked in 20% (v/v) sulfuric acid solution, the MIC weight losses of the copper were measured and they were relatively large compared to the weight losses of carbon steel and stainless steel (except the abiotic control). As shown in Fig. 6-13, after a 7-day test the average value of the SRB copper corrosion rate was 0.55 mm/year. The significant weight loss of the copper was detected due to the thick layer of corrosion product that was removed. The corrosion rate of control coupons, which were in the same medium without SRB for the same period of time, was about 0.01 mm/year. Fig. 6-14 shows the final pH values of media that with and without SRB were not significantly different. However, Fig. 6-15 shows that the copper coupons had a different appearance after treatment with and without SRB. The control copper coupons were still shiny and not corroded while the copper samples exposed to the SRB had rough and discolored surfaces.
Fig. 6-13. Corrosion rate of copper coupons with SRB and without SRB (control)

Fig. 6-14. The final pH values of media with and without SRB (control) in the copper MIC experiment

Fig. 6-16 shows the SEM image of one of the copper coupons immersed in the SRB culture and medium for seven days. On all of the coupons, there was a brittle layer of corrosion product on the top of the copper coupons, and severe corrosion underneath.
the corrosion product layers. The SEM image of a copper coupon with a corrosion product layer and biofilms is shown in Fig. 6-17, and a copper coupon without a corrosion product after washing by 20\% (v/v) sulfuric acid solution is displayed in Fig. 6-18. It clearly demonstrates that copper MIC caused by SRB is a general corrosion instead of a pitting corrosion, which occurred in carbon steel MIC caused by SRB.

*Figure 6-15.* Picture of copper coupons exposed to *D. vulgaris* for seven days and then washed by 20\% (v/v) sulfuric acid. Abiotic control coupons (bottom row) immersed in the culture medium without *D. vulgaris* for seven days and washed with 20\% (v/v) sulfuric acid solution
Figure 6-16. SEM picture of copper coupon exposed to *D. vulgaris* for seven days. Coupon surface displays a top layer of corrosion products on copper.

Figure 6-17. SEM image of corrosion products with biofilm on copper coupon exposed to *D. vulgaris* in ATCC 1249 medium for seven days. (Scale bar for the small inserted image is 100 μm)
Copper samples were tested under the Energy-Dispersive X-ray Spectroscopy (EDS) (Model JSM-6390, JEOL, Tokyo, Japan). The EDS image of the copper coupons shows element analysis of the corrosion product in Fig. 6-19, while Fig. 6-20 presents the chemical element analysis of the copper coupon after removing the layer of corrosion product. The corrosion product had very high copper and sulfur components, indicating the brittle layer on the surface should be Cu$_2$S or CuS as discussed in Chapter 3. After the removal of the corrosion product, the EDS analysis provided a chemical element analysis of the copper without the product layer, in which Cu was the dominant element in the situation.
Figure 6-19. EDS analysis of the corrosion product on a copper coupon exposed to *D. vulgaris* with ATCC 1249 medium for seven days.
Figure 6-20. EDS analysis of a copper coupon exposed to *D. vulgaris* for seven days and then washed with 20% (v/v) sulfuric acid.
Fig. 6-21 shows the corrosion product on one of the 7-day copper coupons in SRB culture under the XRD. Cu$_2$S, chalcocite, was found to be the dominant product on the copper surface, as was predicted in Chapter 3. This confirms that copper MIC was caused by hydrogen sulfide secreted by the SRB cultures.

6.4 Copper MIC by SRB in the Starvation Conditions

After a 3-day biofilm establishment period, coupons were transferred into four different media with different carbon source concentrations, (0%, 1%, 10% and 100%), and placed in the incubator for another seven days to see the effects of carbon source starvation on copper MIC by SRB. A control vial with full strength medium (100%
carbon source) but no SRB was used. Fig. 6-22 (A) shows the visual differences for different percentages of carbon sources. There was no SRB in the control bottle, so the copper coupons were not corroded by the medium. No noticeable FeS (black color) precipitated in the 0% carbon source and 1% carbon source media. Black FeS precipitations were observed in the 10% carbon source and in the full media. Fig. 6-22 (B) and Fig. 6-22 (C) show a comparison between the copper coupons appearances before and after cleaning with 20% sulfuric acid solution, respectively.

Figure 6-22. Images of starvation experiment: (A) SRB growth under different percentages of carbon source in the media at 37°C for seven days; (B) image of copper coupons immerged in 0 ppm of carbon source for seven days; (C) image of the same group of copper coupons from (B) washed with 20% (v/v) sulfuric acid solution

From the results of the weight loss measurements shown in Fig. 6-23, there were obvious differences of corrosion rates for coupons from different media. The corrosion of
the copper coupons increased when there was a higher percentage of carbon source in the medium. The reason is that SRB had enough nutrients to grow sessile and planktonic cells, which produced hydrogen sulfide to react with the copper. It also gave evidence that the MIC of copper by SRB belongs to the Type II MIC since copper corrosion correlated with the amount of hydrogen sulfide, the metabolic product of SRB. In Type I MIC, starvation can lead to an increase of SRB attack on carbon steel (Xu and Gu, 2011). Fig. 6-24 shows the final pH values of different media after the starvation experiment.

Figure 6-23. Weight loss corrosion rates of copper coupons in different percentages of carbon source ATCC 1249 media in starvation experiments (Error bars present the differences between the maximum and minimum corrosion rates)
Surface analysis of the copper samples was conducted under IFM after weight loss measurements. A high percentage of carbon sources lead to large deep pits on the copper surfaces, while small pits were found on the metal surfaces placed in the low percentage carbon source medium. The results are opposite those from starvation tests for SRB attack on carbon steel (Xu and Gu, 2011) that is Type I MIC rather than Type II MIC.

Fig. 6-25 is one IFM image of copper under 0% carbon source treatment. Fig. 6-25 (A) gives an overview of the metal surface. Fuzzy polishing lines were showed on the surface, and no obvious large pits could be detected by the naked eyes. However, small pits spread on the surface were detected by the IFM. Fig. 6-25 (B) shows a pit about 12 µm deep and 70 µm wide, which represents the average pit size in this condition.
Figure 6-25. Surface analysis of copper coupon after 3-day biofilm growth, then transferred into fresh medium with 0% carbon source for 7-day incubation: (A) IFM image of copper surface; (B) depth profile of one detected pit with 12 μm depth in this condition

Fig. 6-26 is an IFM image of the copper coupons after the 7-day incubation in 1% carbon source medium. Fig 6-26 (A) shows the copper surface to be generally corroded by SRB, almost covering the polish lines by wide spots. Fig. 6-26 (B) shows that one pit was 7 μm deep and 160 μm wide. This pit represents the average pit size caused by SRB under 1% carbon source condition.
**Figure 6-26.** Surface analysis of the copper coupon after 3-day biofilm growth, then transferred into fresh medium with 1% carbon source for 7-day incubation: (A) IFM image of the copper surface; (B) depth profile of a detected pit with 7 μm depth in this condition

Fig. 6-27 shows the IFM image of the copper surface analysis corroded by SRB in 10% carbon source medium for seven days. Fig 6-27 (A) provides an overview of the copper surface. Compared to the bright color of the copper coupon itself, the corroded
area left a darker color on the surface. Polish lines were not observable. Comparing pits in previous conditions, deeper and wider pits were found in the medium with 10% carbon source. Fig. 6-27 (B) shows a pit that had a 12.5 µm depth and 310 µm widths.

Figure 6-27. Surface analysis of the copper coupon after 3-day biofilm growth, then transferred into fresh medium with 10% carbon source for 7-day incubation: (A) IFM image of copper surface; (B) depth profile of a 12.5 µm deep pit in this condition.

Fig. 6-28 shows the IFM image of the copper surface immerged in an SRB with full medium. Fig. 6-28 (A) presents an overview of the copper surface after the corrosion
product was removed. The SRB culture and metabolic products completely changed the copper surface. Fig. 6-28 (B) presents one pit with a depth of 18 µm and a width of about 200 µm. This shows that copper coupons are subject to damage by SRB with an increased carbon source in the medium. This suggests that the MIC was associated with the amount of metabolic activities by SRB.

Figure 6-28. Surface analysis of the copper coupon after 3-day biofilm growth, then transferred into fresh medium with 100% carbon source for the 7-day incubation: (A) IFM image of the copper surface; (B) depth profile of an 18 µm deep pit
Figure 6-29. Surface analysis of the copper coupon in the control condition: full carbon source medium without SRB for the 10-day incubation. IFM image of the copper surface.

Fig. 6-29 shows a very clear copper surface where polish lines were easily observed in the control medium. No obvious pitting corrosion was detected under the IFM, indicating that medium had no corrosive effect on copper in the experiment.

6.5 Copper and Drywall Experiment

In order to verify the existence of SRB, six contaminated drywall samples, listed in Table 13, from a house in Jacksonville, FL, supplied by Skyetec company in June 2011, were tested using both ATCC 1249 full medium and the Biosan test kit (Sani-Check® Product #100, BioSan Laboratories, Warren, MI, USA) in our MIC lab. After seven days incubation, as Fig. 6-30 (A) shown, black precipitate showed up in 6 vials (#1, #4, #5, #6, #7, and #8), including three of them (#1, #5, and #7) only blackened at the bottom and other three (#4, #6, and #8) were completely black, while no predicate appeared in # 2 and # 3 vials after seven days. This states that bacteria which produce sulfides likely exist in the drywall samples from #1, #4, #5, #6, #7, and #8 vials. The two
vials in the right side (#9 and #10) were control group which had ATCC 1249 medium without drywall samples in this experiment. They didn’t have precipitate after the incubation, which indicating no sulfides involved in the experiment environment. In Fig. 6-30 (A), Drywall samples order is following: #1 and #2: Master Bedroom Closet South Wall; #3: Master Bathroom North Wall; #4 Bathroom West Wall; #5 Front Bedroom North Wall; #6 and #7: Middle Bedroom East Wall; #8: Master Bedroom East Wall; #9 and #10 control group.

The results of the MPN method by using the Biosan kit examinations showed SRB likely exist in the Master Bathroom North Wall sample which in medium kit #4 in Fig. 6-30 (B), and then the #4 solid medium turned to black after seven days incubation. Because the solid medium from the Biosan kit only contains the nutrients for SRB growth, thus, it implies the existence of SRB in this drywall sample. In Fig. 6-30 (B), Drywall samples are under this following order: #1: Master Bedroom East Wall; #2: Middle Bedroom East Wall; #3: Front Bedroom North Wall; #4: Master Bathroom North Wall; #5: Bathroom West Wall; #6 Master Bedroom Closet South Wall. All tests were repeated 3 times, and results are shown in Table 13.
Figure 6-30. Images of ATCC 1249 medium and Biosan results used to test the presence of SRB in drywall samples: (A) black color appearance after incubation in ATCC 1249 medium; (B) black color appearance in the solid medium of the Biosan kit incubation.
Figure 6-31. Images of copper coupons from the drywall experiments: (A) images of copper coupons placed with and without six drywall samples after two month incubation; (B) image of copper coupons placed in the Middle Bedroom East Wall sample for two months; (C) image of copper coupons were placed with the Front Bedroom North Wall sample for two months; (D) image of copper coupons without drywall sample condition.
### Table 13

**Results from Drywall Experiment**

<table>
<thead>
<tr>
<th>Drywall Samples</th>
<th>ATCC 1249 Medium</th>
<th>The Biosan Test</th>
<th>Copper coupons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Master Bedroom East Wall</td>
<td>X</td>
<td>X</td>
<td>Black</td>
</tr>
<tr>
<td>2 Middle Bedroom East Wall</td>
<td>Black</td>
<td>X</td>
<td>Black</td>
</tr>
<tr>
<td>3 Front Bedroom North Wall</td>
<td>X</td>
<td>X</td>
<td>Black</td>
</tr>
<tr>
<td>4 Bathroom West Wall</td>
<td>Black</td>
<td>X</td>
<td>Black</td>
</tr>
<tr>
<td>5 Master Bathroom North Wall</td>
<td>Black</td>
<td>Black</td>
<td>Black</td>
</tr>
<tr>
<td>6 Master Bedroom Closet South Wall</td>
<td>Black</td>
<td>X</td>
<td>Black</td>
</tr>
<tr>
<td>7 No Drywall Samples</td>
<td>X</td>
<td>NA</td>
<td>X</td>
</tr>
</tbody>
</table>

X: No black color  
NA: Not tested

The six drywall samples, listed in Table 13, were individually placed with copper coupons in N₂ filled vials and incubated for about two months. Two copper coupons without any drywall samples were placed in N₂ filled vials designed as control groups in this experiment. After the incubation, all copper coupons which placed with drywall samples had black spots on their surfaces, as shown in Fig. 6-31 (A). In the fig. 6-31 (A), the order of copper coupons placed with drywall sample: #1 Master Bedroom East Wall; #2: Middle Bedroom East Wall; #3: Front Bedroom North Wall; #4: Bathroom West Wall; #5: Master Bathroom North Wall; #6: Master Bedroom Closet South Wall; #7: control group. Images of copper coupons placed with the Middle Bedroom East Wall
sample and Front Bedroom North Wall sample are presented in Fig. 6-31 (B) and Fig. 6-31 (C), respectively. They give a close observation of corroded copper coupons, which had black corrosion products (might be Cu$_2$S) on the surfaces. At the same period of incubation time, coupons in the control condition did not show black color, shown in Fig. 6-31 (D), and they were shiny after removal from the bottles.

Results of the copper coupons with the drywall samples support a possible existence of SRB in the drywalls based on the results of medium examinations; in addition, metabolic products from the bacteria possibly caused the MIC on copper coupons.

6.6 Experiment on Copper MIC by NRB

As described in the Chapter 3, NRB is a possible reason for copper MIC because nitrate reduction coupled with copper oxidation can be thermodynamically favorable. Moreover, reduced nitrate reduction product produced by NRB, ammonium, is able to cause cracking corrosion on copper metal. This research experiment examined whether NRB will cause severe MIC of copper in anaerobic conditions, and then classified the mechanism of copper MIC by NRB.

Fig. 6-32 gives a direct view of the copper coupons inside vials with the NRB strain, B. Licheniformis. In the left two vials, copper surfaces were covered by NRB biofilms, so they were no longer shiny unlike those coupons in the control condition (in the right side) for the same incubation time. NRB biofilms are very fluffy and loose on the coupon surfaces. Fig. 6-33 shows that the established NRB biofilms grew on the copper and was easily removed by physical scrubbing. After using a 20% (v/v) sulfuric
acid solution to remove biofilms, the shiny surfaces of the copper coupons were visible in Fig. 6-34.

![Figure 6-32. Images of the copper coupons immersed in media with NRB and without the NRB (control) for seven days, respectively](image)

![Figure 6-33. (A) Image of copper coupons covered by NRB biofilms in a vial; (B) Image of copper coupons taken out of bottles and NRB biofilms appeared loose](image)
Figure 6-34. Image of copper coupons in a medium with and without the NRB for seven days, following cleansing with a 20% (v/v) H\textsubscript{2}SO\textsubscript{4} solution

The weight loss of the copper coupons in NRB was very small, about 0.5 mg/cm\textsuperscript{2}, which corresponded to 0.02 mm/year of uniform corrosion rate. Fig. 6-35 provides a comparison of the copper corrosion rates in the NRB and the abiotic control conditions. Their final pH values are presented in Fig. 6-36.
Figure 6-35. Corrosion rates of the copper coupons in the 7-day NRB cultures and control conditions (Error bars represent the differences between the maximum and minimum corrosion rate)

Figure 6-36. The final pH values of media with and without NRB in the copper experiments

When placing the corrosion rates of the copper coupons from SRB and NRB together (Fig. 6-37), there is a huge difference in the potential damage that could be
caused by the two different kinds of bacteria. SRB yielded about 0.55 mm/year, while NRB only 0.02 mm/year. It should be noted that in pitting corrosion situations, uniform corrosion rates are not indicative of the likelihood of pipeline leaks. They are listed for those people who may demand to see the numbers due to their habit. We recommend specific weight loss (mg/cm²) and pit depth profile for quantification of pitting corrosion.

![Figure 6-37. Comparison of weight loss based uniform corrosion rates of copper coupons in 7-day SRB and NRB experiments, respectively](image)

A surface analysis of the copper coupons was conducted under the IFM, following cleaning with a 20% (v/v) sulfuric acid solution. Fig. 6-38 shows the coupon surface with clear polish lines from the control condition and pits about two micrometers deep. There were no noticeable changes on the surface of the copper coupon immersed in the NRB culture for seven days as shown in Fig. 6-39. The depth of the pits caused by the NRB was about seven micrometers.
Figure 6-38. Surface analysis of the copper coupon after seven days in a medium without NRB. The one pit found on the copper surface was 1.9 μm in depth.
Figure 6-39. Surface analysis of the copper coupon after seven days in full strength medium with the NRB. A 7 μm deep pit was found on the coupon surface.

6.7 NRB Starvation Experiment

NRB biofilms appeared on the surface of the copper coupons in the full strength medium after three days. Coupons were then transferred into different media with various carbon source concentrations, i.e., 0%, 1%, 10% and 100%. In order to see the effect of carbon source starvation on copper MIC by NRB, bottles were filled with N₂ and sealed in an anaerobic chamber, and then placed in an incubator. After seven days of incubation,
coupons were taken out and cleaned with a 20% (v/v) sulfuric acid solution, followed by weight loss measurements and surface analysis under the IFM.

Figure 6-40. Comparison of weight loss based uniform corrosion rates with different percentages of carbon sources in the media (error bars represent the differences between maximum and minimum corrosion rates)
Fig. 6-41. Final pH values in the culture medium with different percentages of carbon sources after 7 day incubation

Fig. 6-40 indicates that a lower corrosion rate was obtained with a lower percentage of carbon sources in the media, except for the 0% carbon source medium. This suggests that the NRB could cause Type I MIC during carbon starvation (in 0% carbon source medium), while in other medium it caused Type II MIC because the corrosion rate increased with increased carbon source. Increasing carbon source would lead to more corrosive metabolites.

Surface analysis of copper samples was conducted under the IFM after weight loss measurements were taken. Fig. 6-42, Fig. 6-43, Fig. 6-44 and Fig. 6-45 are the IFM images of copper under 0%, 1%, 10% and 100% of carbon source treatments, respectively. Fig. 6-42 (A), Fig. 6-43 (A), Fig. 6-44 (A) and Fig. 6-45 (A) give overviews of the metal surface from all treatments. The images show clear polish lines on the copper
coupons, indicating that the NRB caused no severe MIC on the copper surfaces. Image (B) of the four treatments show depth profiles of detected pits in each condition.

This indicates that the largest and deepest surface pits were found in the 0% carbon source condition, while the smallest pits were from the 1% carbon source medium. This observation supports Gu and Xu (2013) who suggest that Type II MIC tend to lead to more evenly distributed corrosion because corrosive metabolite diffusion is more even compared with localized electron transfer in Type I MIC. As long as a carbon source existed in the medium, the size of the pits increased with the percentages of carbon sources, so the coupons in the full strength medium obtained larger pits than the coupons in the other two media. The sizes of the pits were matched with weight loss measurements of the copper coupons in each condition.
Figure 6-42. Surface analysis of the copper coupon after a 3-day biofilm growth, which was then transferred into a fresh none carbon source medium for 7-day incubation: (A) IFM image of the copper surface; (B) Depth profile of a 8.5 μm deep pit
Figure 6-43. Surface analysis of the copper coupon after a 3-day period of biofilm growth which was transferred into fresh 1% carbon source medium a for 7-day incubation: (A) IFM image of the copper surface; (B) Depth profile of a detected pit with 3.2 μm depth
Figure 6-44. Surface analysis of the copper coupon after acid cleaning: after a 3-day period of biofilm growth, which was then transferred into a fresh 10% carbon source medium for a 7-day incubation: (A) IFM image of the copper surface; (B) Depth profile of a 4.9 μm deep pit
Figure 6-45. Surface analysis of the copper coupon after acid cleaning: after a 3-day period of biofilm growth which then was transferred into a fresh full strength medium for a 7-day incubation: (A) IFM image of the copper surface; (B) Depth profile of a detected pit with 7.2 μm depth
7 DISCUSSION AND CONCLUSION

7.1 Biocide Enhancing DAAs

The results of the present study may be summarized by pointing out, firstly, that the addition of D-Val to 50 ppm THPS enhanced eradication of SRB biofilms on carbon steel surfaces. Increasing D-Val concentration from 50 ppm to 1000 ppm incrementally enhanced the effect of 50 ppm THPS on the mitigation of the carbon steel MIC by SRB. It was found that 500 ppm of D-Val was probably the optimal concentration for 50 ppm THPS against the *D. vulgaris* biofilm. However, this is a relatively high concentration for a biocide enhancer in practical applications. It is suggested that future research should investigate the effect of a mixture of lower concentrations of D-Val as well as higher concentrations of THPS.

Racemically pure D-Met is more expensive than a 50:50 mixture of D- and L-Met. Laboratory research showed that 100 ppm L-Met when added to the D-Met decreased the effectiveness of 100 ppm D-Met as a biocide enhancer for 50 ppm THPS by 1-log. However, it could be due to MPN error. The mixture of L-Met and D-Met (50:50) is cheaper than pure D-Met.

7.2 Investigation of Copper MIC by Bacteria in Anaerobic Environment

The second part of this research showed that copper was vulnerable to SRB attack in an anaerobic environment, resulting in severe corrosion.

In our research, copper MIC caused by SRB was found to follow the Type II MIC mechanism because the tendency of copper corrosion in the SRB strain increased with the SRB metabolic products and also, above all, copper oxidation coupled with sulfate
reduction is thermodynamically unfavorable. In order to confirm that hydrogen sulfide is the cause of the copper MIC by SRB, further investigation of copper MIC belonging to Type II MIC mechanism should be conducted with only hydrogen sulfide in an abiotic anaerobic environment.

NRB had the capability of attacking copper in the anaerobic environment while causing only a relatively small corrosion rate on copper. The mechanism demonstrated in our experimental data suggests that copper MIC by NRB likely belongs to Type I MIC given that the copper was attacked more severely by NRB sessile cells under carbon starvation. This study requires further research, including both biotic and abiotic studies on the mechanism of copper MIC by additional strains of NRB.

To demonstrate the corrosion products from the copper MIC by NRB, it is suggested that the utilization of copper ion assay can be applied in the future work. The commercial MicroMolar Copper Assay Kit measures small concentrations of Cu\(^{2+}\) ion ranging from 0.01 mM to 0.1 mM, using fluorescence binding. This may be used to detect the concentration of Cu\(^{2+}\) ion in medium after 7-day incubation. This will indicate whether elemental copper is oxidized to mainly become Cu\(^+\) or some Cu\(^{2+}\) is also a corrosion product. Doing so will help explain the thermodynamics and kinetics of elemental copper corrosion by NRB.
REFERENCES


