THE ROLE OF A BIOFILM AND ITS CHARACTERISTICS IN MICROBIOLOGICALLY INFLUENCED CORROSION OF STEEL

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DEDICATION

To

Mukesh and Varsha Jhobalia (my parents)

and

Vidhi Jhobalia (my sister)

for their continued support through out this work

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Chapter 1: Introduction

Corrosion is the deterioration of a material due to interaction with its environment (Jones, 1996). It is the process in which metallic atoms leave the metal or form compounds in the presence of water and gases. Chemical corrosion, such as hydrolysis and biological corrosion and biofouling are naturally occurring processes, which have been both a benefit and a problem for centuries (Videla, 2002). Micro-organisms help in decomposition of waste by breaking down organic matter. Biodeterioration, however, may also decompose our natural treasures and monuments (Gaylarde et al., 1995). In aquatic environments microbes may attack and breakdown substrates and inorganic materials using them as source of food and necessary critical nutrients. Biofouling occurs when creatures excrete enzymes which can for example digest such things as bridges and concrete waterways (Gaylarde et al., 1995). Sometimes this may occur in concert with or independent from simple chemical corrosion from sea water or other environmental effects, such as hydrolysis. At the same time, biofouling is also characterized by attachment of organisms, both plant and animal, to structures and hulls and building biomass which may block flow in pipes, (such as the organisms which blocked water cooling pipes in the Chernobyl nuclear power plant disaster) or create tremendous weight imbalances on deep water structures (Cloete, 2003).

For 4000 years, man has tried various means to manage and reduce the degradation effects of organism attachment (fouling), corrosion and consumption of man-made structures and vessels in marine, freshwater, and aquatic environments. Fouling can increase drag on ships and raise operating costs (Floerl et al., 2003). In the energy

shortage days of the 1970's, extremely effective toxins were used to eliminate any fouling.

Micro-organisms are also associated with deleterious effect in oilfield operations including corrosion of piping and equipment, plugging of injection or disposal wells and souring of fluids and reservoirs. Even though the first publications on biocorrosion were made near the end of the 19th century, a mechanistic interpretation of the field only began to be rigorously made in the early 1960s (Videla, 1996). The only exception has been the pioneering work of von Wolzogen Kuhr and Van der Vlugt published in 1934 (Kuhr, et al., 1934), which can be considered the first attempt to interpret biocorrosion in electrochemical terms. In 1960s and 1970s most research was aimed at proving or disproving the theory that corrosion of iron by sulfate reducing bacteria (SRB) occurred according to the cathodic depolarization theory (CDT) (Videla, 2001). Since then microbiologically influenced corrosion (MIC) has been considered a significant problem in oil and gas fields.

Since the early 1980s, biocorrosion has been demanding more attention from researchers working in different disciplines due to an increasing exchange of intellectual and technical knowledge among specialists of different disciplines and the development of new and more sophisticated techniques for the study of metal/solution interface.

> *"The present day definition of biocorrosion, microbial corrosion, or microbiologically influenced corrosion (MIC) describes it as an electrochemical process where the participation of the micro-organisms is able to initiate,*

facilitate, or accelerate the corrosion reaction without changing its electrochemical nature." (Videla, 1996)

This is a very broad definition in itself and demands some prior understanding of biocorrosion. Microbe related corrosion problems are seldom straightforward and require a considerable amount of investigation often involving sophisticated methodology and equipment (Videla, 2001). It has been shown that not only do bacterial cells have the ability to accelerate biocorrosion, but that extra-cellular products in the absence of micro-organisms are also aggressive (Gaylarde, 1997; Beech, 1998). The mixed bacterial consortia and the numerous organic and inorganic species present in a micro-habitat make the process of biocorrosion complex.

Mild steel and stainless steel are the most frequently used materials of construction in the oil and gas industry and elsewhere. Many investigators have tried to study the corrosion of iron and steel in the presence of SRB (Starosvetsky et al., 2000). SRB are non-fermentative anaerobes that obtain energy for growth from the oxidation of organic substrates using inorganic sulfur oxy-acids or nitrate as the terminal electron acceptors (Feio et al., 2000). The way in which SRB influence the anaerobic corrosion of iron is, however, still a subject of some controversy (Rainha et al., 1997).

To study the influence of sulfate reducing bacteria on anaerobic corrosion of iron a detailed investigation of the role of the bacterial biofilm is mandatory. The effect of vital factors including flow and dissolved iron concentration on bacterial biofilm and hence on corrosion has not been investigated in much depth.

The current project aims to study sulfate reducing bacterial biofilm and its effect on the corrosion of mild steel.

CHAPTER 2: LITERATURE REVIEW

2.1 Corrosion

Corrosion mitigation came complimentary to human interest along with the use of metal objects. Most metals are unstable in aqueous surroundings. Usually a considerable amount of energy is used to extract metals from their ores. In certain environments metals combine chemically with other elements or compounds to form different compounds returning to their lower energy levels (Jones, 1996).

Metals deteriorate through corrosion when in contact with water (moisture), acids, bases, salts, organic compounds, and other solid and liquid chemicals. Gaseous materials including acid vapors, ammonia gas, and sulfur containing gases can also corrode metals.

Any process involving deterioration and degradation of metals can be identified as corrosion. Rusting of steel is the most common example. Corrosion is an electrochemical process similar to an electrical battery. Metal atoms exposed to a humid environment can give up electrons forming positively charged ions which pass into the solution. Localized corrosion coupled with corrosive environments like seawater may cause greatly enhanced growth of fatigue cracks. Pitting corrosion is accelerative on micro-structural changes induced by welding operation (Jones, 1996).

Corrosion is provoked through unintentional chemical or electrochemical action taking place at the surface. Metals have a tendency to get oxidized, some rather easily compared to other. The relative strength of this tendency is tabulated to give galvanic series, useful in determining potential usefulness for structural and other application of metals through their placements.

The two processes, anodic and cathodic reactions, one involving metal dissolution which generates electrons consumed by the other respectively (Nesic et al., 1995), have to balance their charges. The sites hosting these processes can be placed close or far on the metal surface. This seemingly simple observation has a huge impact on corrosion control to avoid the most sinister or confined deterioration.

A cathodic reaction in the immediate vicinity is needed to consume the electrons produced by corrosion. Hydrogen ions first react with these electrons to form atomic hydrogen, and finally molecular hydrogen gas, in which if the acidity level is high, i.e. low pH, and then this molecular hydrogen will readily become gas. The "polarizing" film (thin gaseous film at the surface of the metal), formed by hydrogen inhibiting further corrosion, is beneficial in improving water and metal clearance to reduce corrosion (Yu et al., 2002). Dissolved oxygen will react with the hydrogen, which will convert it into water and destroy the film, and eventually increase the rate of corrosion. Also some bacteria are known to destroy this film according to the cathodic depolarization mechanism well known in biocorrosion literature (Borenstein, 1994).

Swift moving water and solid particles contained in the water will eradicate the film from the metal surface. There are other forces which quicken the corrosion including free hydrogen ion (indicated by low pH) which speeds up the release of electrons and, high water temperatures which increase all reaction rates. Thus the huge domain of natural and environmental agents affects the corrosion rate even though there are no special conditions involved.

Modern corrosion science has descended through two subjects. The first being electrochemistry, which helps in deriving the mechanism that is fundamental to corrosion of all metallic objects and the second is metallurgy, which deals with the characteristics of metals and their alloys combined with the methods of amalgamation of various metals and casting of alloys into desired shapes.

The losses due to corrosion are of principal concern worldwide. Corrosion has been even more detrimental then just degradation. Plant shutdowns, waste of valuable resources, loss or deterioration of products, reduced efficiency, high maintenance, environmental pollution and classy overdressing are just a few examples of the problems caused by corrosion.

Biocorrosion or microbiologically influenced corrosion as described earlier is an electrochemical process in which the participation of micro-organisms is able to initiate, facilitate, or accelerate the corrosion reaction without changing its electrochemical nature. Different species of bacteria have been found to affect the corrosion process in different ways. Some of the species of organisms commonly related with corrosion of metal and other materials have been described below.

2.2 Bacterial corrosion

Certain fungi are capable of producing organic acids and have been blamed for corrosion of steel and aluminum (Little, 2001), as in the highly publicized corrosion failures of aluminum aircraft fuel tanks. In addition, fungi may produce anaerobic sites for SRB and can produce metabolic byproducts that are useful to various bacteria.

2.2.1 Aerobic Slime Formers

Aerobic slime formers are a diverse group of aerobic bacteria. They are important to corrosion mainly because they produce exo-poly saccharides (EPS) that make up what is commonly referred to as "slime." This polymer is actually a sophisticated network of sticky strands that bind the cells to the surface and control what permeates through the deposit (Blanco et al., 1996).

The stickiness traps all sorts of particulates that might be floating by, which, in dirty water, can result in the impression that the deposit or mound is an inorganic collection of mud and debris. The slime formers and the sticky polymers that they produce make up the bulk of the distributed slime film or primary film that forms on all materials immersed in soil.

Slime formers can be efficient "scrubbers" of oxygen, thus preventing oxygen from reaching the underlying surface (Videla, 1996). This creates an ideal site for SRB growth. Various types of enzymes are often found within the polymer mass, but outside the bacterial cells. Some of these enzymes are capable of intercepting and breaking down toxic substances (such as biocides) and converting them to nutrients for the cells.

2.2.2 Iron/Manganese Oxidizers

Bacteria that derive energy from the oxidation of Fe^{2+} to Fe^{3+} are commonly reported in deposits associated with MIC (Starosvetsky et al., 2001). They are almost always observed in tubercles (discrete hemispherical mounds) over pits on steel surfaces. The most common iron oxidizers are found in the environment in long protein sheaths or filaments. While the cells themselves are rather indistinctive in appearance, these long filaments are readily seen under the microscope and are not likely to be confused with other life forms.

The observation that filamentous iron bacteria are "omnipresent" in tubercles might be, therefore, more a matter of their easy detection than of their relative abundance. An intriguing type of iron oxidizers is the *Gallionella* bacterium, which has been blamed for numerous cases of corrosion of stainless steels (Muthukumar et al., 2003).

Besides the iron/manganese oxidizers, there are organisms that simply accumulate iron or manganese. Such organisms are believed to be responsible for the manganese nodules found on the ocean floor (Murdoch et al., 1999). The accumulation of manganese in biofilms is blamed for several cases of corrosion of stainless steels and other ferrous alloys in water systems treated with chlorine or chlorine/bromine compounds.

2.2.3 Methane Producers

Only in recent years have methane producing bacteria (methanogens) been added to the list of organisms believed responsible for corrosion. Like many SRB, methanogens consume hydrogen and thus are capable of performing cathodic depolarization (Zhang et al., 2003). While they normally consume hydrogen and carbon dioxide to produce

methane, in low nutrient situations these strict anaerobes will become fermenters and consume acetate instead.

In natural environments, methanogens and SRB frequently coexist in a symbiotic relationship: SRB producing hydrogen, CO_2 and acetate by fermentation, and methanogens consuming these compounds, a necessary step for fermentation to proceed (Zhang et al., 2003). The case for facilitation of corrosion by methanogens still needs to be strengthened, but methanogens are as common in the environment as SRB and are just as likely to be a problem. The reason they have not been implicated before now is most likely because they do not produce distinctive, solid byproducts.

2.2.4 Organic Acid Producers

Various anaerobic bacteria such as *Clostridium* are capable of producing organic acids. Unlike SRB, these bacteria are not usually found in aerated macroenvironments such as open, recirculating water systems (Lutey et al., 1996). However, they are a problem in gas transmission lines and could be a problem in closed water systems that become anaerobic.

2.2.5 Sulfur/Sulfide Oxidizers

This broad family of aerobic bacteria derives energy from the oxidation of sulfide or elemental sulfur to sulfate. Some types of aerobes can oxidize the sulfur to sulfuric acid, with pH values below 2.0 (Yamanaka et al., 2002). These *Thiobacillus* strains are most commonly found in mineral deposits, and are largely responsible for acid mine drainage (Sasaki et al., 1998) which has become an environmental concern. They

proliferate inside sewer lines and can cause rapid deterioration of concrete mains and the reinforcing steel therein (Nica et al., 2000).

They are also found on surfaces of stone buildings and statues and probably account for much of the accelerated damage commonly attributed to acid rain. Wherever *Thiobacillus* bacteria are associated with corrosion, they are almost always accompanied by SRB. Thus, both types of organisms are able to draw energy from a synergistic sulfur cycle (Overmann et al., 2000). The fact that two such different organisms, one a strict anaerobe that prefers neutral pH, and the other an aerobe that produces and thrives in an acid environment, can coexist, demonstrates that individual organisms are able to form their own microenvironment within an otherwise hostile larger world.

2.3 Sulfate reducing bacteria (SRB)

Sulfate reducing bacteria are among the micro-organisms most frequently implicated in microbially influenced corrosion of iron, copper and ferrous alloys (Fonseca et al., 1998). Sulfate-reducing bacteria include all unicellular bacteria capable of reducing sulfate to sulfide (Mohanty et al., 2000; Nielsen, 1987). The most common of the genera is *Desulfovibrio*. They can grow in a pH range 5 to 10 and the temperature range of 5 ^{o}C to 50 ^{o}C (Javaherdashti, 1999). The most commonly found species of this genus in anaerobic regions of mud, soils, marine and estuarine sediments is *Desulfovibrio desulfuricans* (Dzierzewicz et al., 2003). Analysis of the 16S rRNA gene sequences has helped to detect and distinguish *Desulfovibrio* species from other micro-organisms (Shukla et al., 2000; Devereux et al., 1990; Leu et al., 1998).

Sulfate reducing bacteria are obligate anaerobes which obtain energy for growth by oxidation of organic substrates and use sulfate as the external electron acceptor; as a result sulfate is reduced to sulfide (Costello, 1974). The sulfate reducing bacteria form a physiologically distinctive group of anaerobic bacteria, their oxidative metabolism being based, not on fermentation, but on the reduction of sulfate or certain other inorganic sulfur compounds. Their physiology has broad analogies to that of nitrate reducing bacteria (denitrifying bacteria), but they are all exacting anaerobes and no examples of facultative aerobes are known.

SRB are usually lumped into two nutrient categories, those that can use lactate and those that cannot (Hilton et al., 1988). The latter generally use acetate and are difficult to grow in the laboratory on any medium. Lactate, acetate, and other short chain

fatty acids usable by SRB are produced by other organisms in the environment. Therefore, these organisms depend on other organisms for their nutritional needs.

SRB reduce sulfate to sulfide, which usually shows up as hydrogen sulfide or, if iron is available, as black ferrous sulfide. In the absence of sulfate, some strains can function as fermenters and use organic compounds such as pyruvate to produce acetate, hydrogen, and carbon dioxide. Many SRB strains also contain hydrogenase enzymes, which allow them to consume hydrogen. Most common strains of SRB grow best at temperatures from 25 °C to 35 °C. A few thermophilic (living in high temperature) strains capable of functioning efficiently at more than 60°C have been reported (Javaherdashti, 1999).

Tests for the presence of SRB have traditionally involved growing the organisms on laboratory media, quite unlike the natural environment in which they were found. These laboratory media will allow growth of only certain strains of SRB, and even then some samples require a long lag time before the organisms will adapt to the new growth conditions. As a result, misleading information has been obtained regarding the presence or absence of SRB in field samples.

2.4 Microbiologically influenced corrosion

2.4.1 Introduction

Microbiologically influenced corrosion has been defined as an electrochemical process in which the presence of micro-organisms is able to initiate, facilitate or accelerate the corrosion reaction without changing its electrochemical nature (Videla, 1996). Micro-organisms are associated with deleterious effects in several oil field operations including corrosion of pipes and equipment, plugging of injection or disposal wells and souring of fluids and reservoirs (Galbraith et al., 1987). Nearly 20% of all corrosion damages seem to be microbial in nature. Costerton and Boivin (1990) estimated that the MIC damage in production, transport, and storage of oil could amount to some hundred million US dollars in the United States every year due to SRB alone, not including the costs for lost oil and clean-up of environmental pollution.

SRB have been implicated in the corrosion of cast iron and steel, ferritic stainless steels, 300 series stainless steels (also very highly alloyed stainless steels), copper nickel alloys, and high nickel molybdenum alloys (Videla, 1989). They are almost always present at corrosion sites because they are in soils, surface water streams and waterside deposits in general. Their mere presence, however, does not mean they are causing corrosion. The key symptom that usually indicates their involvement in the corrosion process of ferrous alloys is localized corrosion filled with black sulfide corrosion products.

An excellent case of microbially influenced corrosion was the one in Lost Hills Oilfield operated by Chevron USA, Oil Production Company. The new oil and water

gathering system experienced pinhole leaks in various segments of the gathering system just 18 months after start up. Internal inspection of the leaking piping showed severe microbial attack occurring under deposits of fracture sand and/or iron sulfide (Strickland et al., 1996).

Another example of MIC is the McElroy water injection system. The average thirty day corrosion rate of McElroy injection water system operated by Petrolite Corporation was observed to be about 40 mpy (mils per year). At sixty days the rate exceeded 100 mpy on several sections. The tests were conducted on five 2-inch chuck type bio-probes installed on a section of two inch line upstream of the injection well head. The bio-probes were flush mounted to the inner pipe surface (Agostini et al., 1989) which means the coupons were definitely exposed to flow conditions.

In another laboratory research conducted by BP Corporation's steel specimens were introduced in two systems. One was inoculated with bacteria and the other was kept in a sterile condition. The corrosion rate in the inoculated water increased with increasing exposure time, while a relatively low and constant corrosion rate appeared in the sterile control (Lou et al., 1994).

Another example of biocorrosion would be the Brazilian offshore water injection system (Videla et al., 1989). The Brazilian offshore seawater injection systems had been studied for two types of steel, N-80 and carbon steel, in a detailed analysis of biofouling and corrosion under different seawater conditions. The monitoring device included an on-line Bioprobe™ installed under pressure. Both steels used in the injection water systems showed a poor corrosion resistance in seawater. The corrosiveness of the injection

seawater based on chemical considerations alone was not enough to cause the degree and type of attack observed on metal surfaces. The SEM observations also revealed that micro-organisms were involved in the corrosion process.

All these cases strongly suggest that a detailed understanding of microbially influenced corrosion is very important. Studying the effect of various physical parameters such as temperature, flow rate and substrate concentration on biofilm growth is very important.

The study of microbial corrosion process demands a more in-depth understanding of the bacterial biofilm. The bacterial biofilm is necessarily composed of EPS and bacterial cells in a gel-like matrix which is filled with water. The Figure 1 below shows the bacterial biofilm on metal surface.

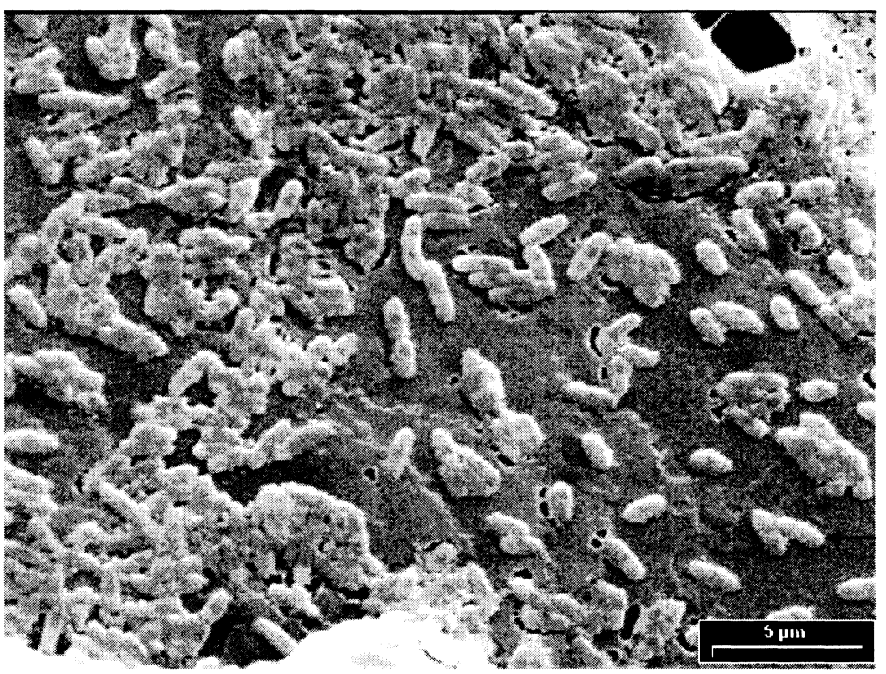


Figure 1. SEM image of a biofilm on metal surface showing the bacterial cells (Videla, 2001).

According to Flemming (1996) a more extensive research is required in the field of biocorrosion. We need to understand the biofilm growth dynamics, physical and chemical characteristics, and nature of biofilm. A more through understanding of the biocorrosion mechanism through the use of simple monitoring methods is needed.

2.4.2 Microbial corrosion mechanism

The fact that bacterial corrosion process occurs at near neutral pH in anaerobic environments raises issues about the cathodic reaction (Hamilton, 1985). There is no doubt that the overall reaction can be described by,

$$4\,Fe + MgSO_4 + 4\,H_2O \longrightarrow FeS + 3\,Fe(OH)_2 + Mg(OH)_2 \qquad (1)$$

King and Miller (1991) proposed that ferrous sulfide acted as absorber of molecular hydrogen. Iron sulfide would be regenerated and maintained as cathode by the action of hydrogenase system.

On the other hand, Costello proposed that hydrogen sulfide, H_2S, rather than the hydrogen ion would act as the cathodic reactant, i.e.

$$2\,H_2S + 2\,e^- \longrightarrow 2\,HS^- + H_2 \qquad (2)$$

A number of more recent studies have shown that sulfate reduction can occur with cathodically formed hydrogen (Hardy et al., 1981). Based on the available literature and the understanding of the chemistry of microbial systems the following reaction scheme has been proposed to explain the reactions that would typically happen in an anaerobic sulfate reducing bacterial media in the presence of elemental iron,

$$2\,MgSO_4 \longrightarrow 2\,Mg^{2+} + 2\,SO_4^{2-}$$

$$8\,Fe \longrightarrow 8\,Fe^{2+} + 16\,e^-$$

$$16\,H_2O \longrightarrow 16\,H^+ + 16\,OH^-$$

$$14\,H^+ + 14\,e^- \longrightarrow 7\,H_2$$

$$2\,SO_4^{2-} + 8\,H_2 \longrightarrow 2\,S^{2-} + 8\,H_2O$$

$$4\,H^+ + 2\,S^{2-} \longrightarrow 2\,H_2S$$

$$2\,H_2S + 2\,e^- \longrightarrow 2\,HS^- + H_2$$

$$2\,HS^- + 2\,Fe^{2+} \longrightarrow 2\,FeS + 2\,H^+$$

$$6\,Fe^{2+} + 12\,OH^- \longrightarrow 6\,Fe(OH)_2$$

$$2\,Mg^{2+} + 4\,OH^- \longrightarrow 2\,Mg(OH)_2$$

$$8\,Fe + 8\,H_2O + 2\,MgSO_4 \longrightarrow 2\,FeS + 6\,Fe(OH)_2 + 2\,Mg(OH)_2$$

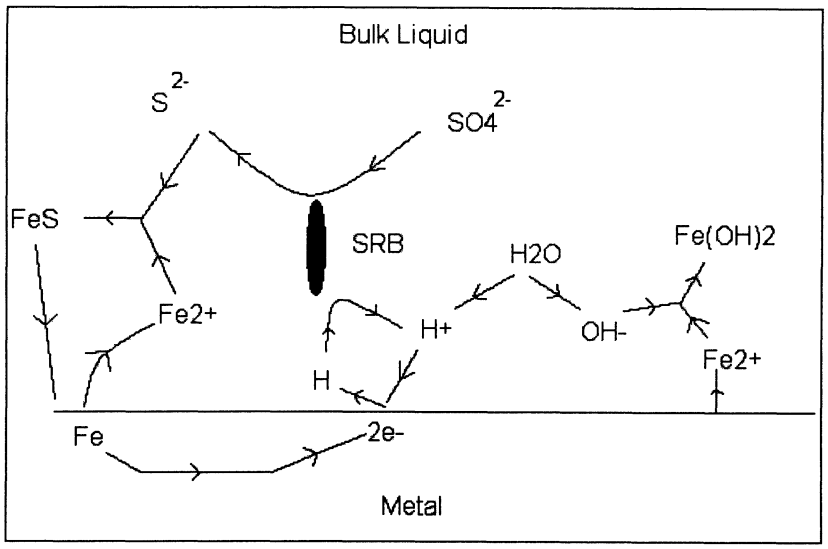


Figure 2. Metal-microbe interaction scheme depicting the reactions

The above reaction scheme (Figure 2) indicates that iron sulfide would be the predominant corrosion product as shown by some authors. The biofilm formation process has been described in great detail by Videla (1996) in his book. The process has been depicted in Figure 3. The major steps in biofilm formation include:

1.) Transportation of organic material to metal surface

2.) Transport of microbial cells from bulk to surface

3.) Attachment of microbial cells

4.) Growth within the biofilm

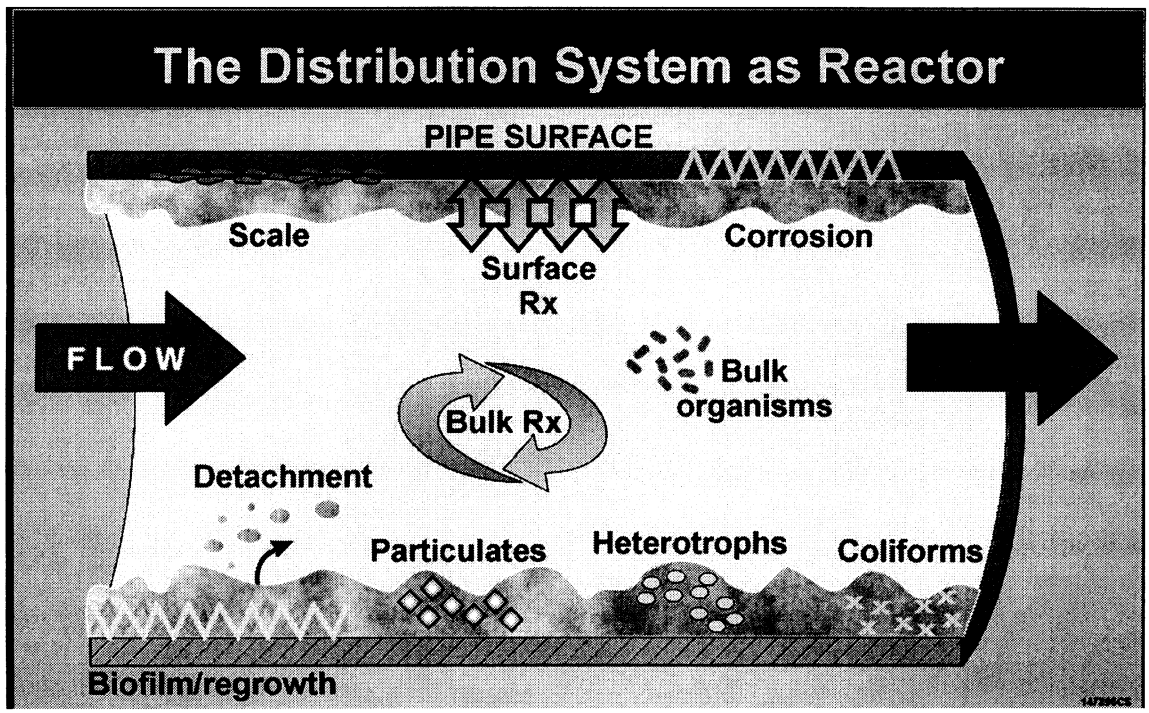


Figure 3. Biofilm formation process inside a pipe showing all stages (Dirckx et al., 1996)

There has been some controversy as to whether MIC can cause pitting corrosion. Some published work in the literature indicates that the biocorrosion process is localized in nature though there are a few oppositions. The theory behind localized corrosion of iron is attributed to the simultaneous biofilm and iron sulfide film growth.

If the metal undergoes corrosion simultaneously along with the biofilm formation there would also be a corrosion product layer formation. The metal surface would then be covered simultaneously with a biofilm and with an iron sulfide film. If there is sufficient iron in the medium the iron sulfide film would cover up the metal before biofilm formation.

At very low iron concentrations a biofilm would be developed before an iron sulfide film could cover up the metal. At intermediate concentrations the iron sulfide film formation will occur simultaneously with biofilm growth. At these intermediate concentrations there could be several occurrences of pitting corrosion. The metal surface would be divided into permanent anodic and cathodic sites. There have been some speculations about the intermediate concentrations but no published data is available co-relating the iron concentration and corrosion rate and its nature. The current research attempts to solve this problem by trying to identify the level of super-saturation at which metal would undergo pitting corrosion. Such research would be of significance when modeling the biocorrosion process.

Flow interferes with the corrosion process by disrupting the protective film on the metal surface. Effect of flow is important in bacterial corrosion process because it not only affects the transfer of species to the metal surface but also influences the overall bacterial adhesion process and the transfer of nutrients to the metal surface. Lee and Characklis (1993) studied the corrosion of mild steel at low flow velocities. They observed cell growth at velocities of up to 0.35 m/s. The current study involved investigation of the corrosion process at higher flow velocities.

2.5 Corrosion mitigation

The best protection against MIC is definitely a "CLEAN SYSTEM" (Edyvean et al., 1991). Most of the time people fail to identify a possible attack from SRB's until the system is actually under heavy attack. Many guidelines have been published to identify the possibilities of an attack due to biocorrosion (Stoecker, 1993). Many times the damage occurs not during actual operations but during hydrotesting, storage or lay-up. Hence care should be taken at all times.

The most common preventive methods for biocorrosion include coatings and cathodic protection. Coating based on polyethylene or polyvinyl chloride, as well as heavy coherent coatings based on bitumen or coal – tar pitch gives good protection against microbial attack (Videla, 1996). Cathodic protection has by far been the most common method of protection against corrosion from external attack due to bacteria in soil. The presence of SRB and/or sulfides in the soil demands a decrease in the cathodic potential level to -0.950V (vs. $Cu/CuSO_4$) for protection (Videla, 1996).

Mitigation of an existing MIC problem involves the use of biocides almost invariably. Biocide can be any chemicals able to kill or inhibit the growth and reproductive cycle of bacteria. Examples of some common biocides are glutaraldehyde, formalaldehyde, acrolein, imidazolines (formaldehyde releasing), quaternary ammonium compounds (QAC), quaternary phosponium compounds (THPS), diamines, coco-diamines, polyamines, isothiazolones, organochlorine compounds, dibromonitrilopropionamide (DNBPA), biguanide, methylene bis-thiocynates and alkyl dithiocarbamates. For a biocide to be effective it should be effective against a wide

variety of bacteria, active in low concentrations, able to penetrate the biofilm and kill sessile population, be less toxic and hazardous to the environment and workers and non-corrosive (Sanders, 2002). It is usually hard to find something which will satisfy all these conditions and still be effective. Also the dosage regime and technique are important. A continuous treatment would usually be the best but at the same time expensive. Hence, a pulse treatment (semi-continuous) with a two or three hour gap is generally practical to keep away the problems.

Over the last 30 years, international concern for the environment has placed all previous toxic release methods of controlling corrosion and fouling in very bad light. Toxins, used to prevent biofouling and corrosion, have parts per trillion toxicity and have eradicated life forms in many internationally navigable ports in industrial countries. Their toxicity has resulted in outright bans of their use.

Attempts have been made to reduce and moderate the toxicity concentration in coatings. Simple anti-corrosive coatings, especially those without toxins, have very short service lives. They are quickly attacked and are often consumed by organisms and natural corrosion. Thus only toxic repellents were developed.

Two methods of delivering toxins in coating systems have prevailed 1) soluble coatings and 2) insoluble coatings. These coatings have lead to irregular and uncontrolled discharge of toxins. Thus, concern for the environment has perceptively stymied all known toxic approaches to provide a safe and cost effective management of man-made surfaces in both aquatic and hydrophytic environments. A need exists for a method which can limit the biomass without being antibiotic, i.e., killing organisms of biomass.

CHAPTER 3: RESEARCH OBJECTIVES AND TEST MATRIX

3.1 Research objectives

Based on previous research and published literature, there is a need to study the effects of a biofilm on microbiologically influenced corrosion of steel in pure sulfate reducing bacteria cultures. The key questions that were addressed in this work included:

1.) What is the effect of physical factors such as temperature and nutrient concentration on the corrosion due to SRB?

2.) What is the nature of a biofilm growing on a metal surface? Does the dissolved iron affect the nature of this film? How does the simultaneous presence of iron sulfide and biofilm affect the corrosion process?

3.) What is the effect of flow on the nature of the biofilm and corrosion?

4.) What is the minimum inhibitory concentration of glutaraldehyde (biocide) effective against SRB?

3.2 Test matrix

The following test matrix (Table 1) was defined to answer the questions.

Table1. Test Matrix for research

Steel Type	C1018
Media	Modified Baar's media
Temperature	5 ~ 37 °C
Culture	*Desulfovibrio desulfuricans (ATCC 7757)*
Pressure	1 bar
Rotational velocity (rpm)	0, 1000, 2000
Fe^{2+} concentration (ppm)	0 ~ 140
Biocide concentration (ppm)	0, 10, 25, 50, 100, 250

The experiments were designed to study the interaction of iron sulfide with the biofilm. The dissolved ferrous ion concentration was varied from 0 to 140 ppm to study the effect of dissolved iron.

The velocity effect was studied in glass cells. The velocity was varied by changing the rotational speed of the coupon. Appropriate correlations were used to transfer this rotational speed to linear velocity.

The experiments were performed on mild steel coupons AISI C1018. The chemical composition of these coupons is given in Table 2. Modified Baar's media was used for the cultivation of the SRB. Composition of the media is as per shown in Table 3.

Table 2. Composition of the mild steel material used in experimentation.

Element	Wt %	Element	Wt %
Al	0.066	Ni	0.03
As	0.01	P	0.016
B	0.0009	Pb	0.036
C	0.2	S	0.009
Ca	0.0004	Sb	0.009
Co	0.007	Si	0.036
Cr	0.052	Sn	0.005
Cu	0.02	Ta	0.005
Mn	0.84	Ti	0.002
Mo	0.028	V	0.002
Nb	0.012	Zr	0.006

Balance Fe

Table 3. Composition of the Modified Baar's media used for cultivation of SRB

Component I	$MgSO_4$	2.0 g
	Sodium Citrate	5.0 g
	$CaSO_4$	1.0 g
	NH_4Cl	1.0 g
	Distilled water	400.0 ml
Component II	K_2HPO_4	0.5 g
	Distilled water	200.0 ml
Component III	Sodium Lactate	3.5 g
	Yeast Extract	1.0 g
	Distilled water	400.0 ml
Component IV	$FeSO_4$	2.1g
	$(NH_4)_2SO_4$	1.0g
	Distilled water	30ml

CHAPTER 4: EXPERIMENTAL SETUP AND PROCEDURE

The experiments were performed in sealed anaerobic bottles and a special glass cell with rotating electrode. Necessary care was taken at all times to avoid contamination. All experiments were carried out under anaerobic conditions.

4.1 Anaerobic bottle experiments

The first step in all experiments involved sterilization of all equipment to be used for the experiment. This included flasks, anaerobic bottles, needles, syringes, de-oxygenation bottle and pipette. The media components were then weighed according to the formulation. The desired quantity of water was taken into a separate flask to prepare different media components. The chemicals were added to the water as per the component requirement. The media formulation has been discussed in the test matrix. After the mixing was complete the flasks were covered with a sponge and aluminum foil. They were then sterilized at 121 ^{o}C for 15 minutes followed by an exhaust cycle of 20 minutes. The media components were then taken from the autoclave and allowed to cool down in a laminar flow hood which was pre-sterilized using UV light. The media when cooled down to a temperature of approximately 45 ^{o}C was transferred to a de-oxygenation bottle and purged with filtered nitrogen to remove oxygen. This procedure was carried out for approximately 45 minutes. Prior tests were done to make sure that the de-oxygenation time was sufficient. The bottle was then sealed and transferred to the glove box immediately. The anaerobic bottles and seals had already been placed into the

glove box. Metal coupons were also polished, cleaned and de-greased and kept ready prior to beginning of this procedure.

Metal coupons were polished with 400 grit silicon-carbide paper, rinsed with alcohol and coated with Teflon. Only one surface of the coupons was left exposed. The Teflon was then allowed to dry overnight. They were then baked in oven at 200 °F to drive out all moisture and volatile substances. The Teflon coated coupons were then polished again using 250, 400 and 600 grit papers, rinsed with alcohol and cleaned using ultrasonication. The coupons were subjected to three ultrasonic bursts of 15 seconds each to remove all forms of dirt and grease on the coupon surface. They were then stored in special packets to avoid exposure to oxygen and were transferred to vacuum desiccators as soon as possible and stored there for two or three hours as necessary.

The anaerobic bottles were filled with sterilized, de-oxygenated medium. Coupons were introduced into the bottles at this time. Filter sterilized $Fe(NH_4)_2(SO_4)_2$ was also added at this point if needed. The bottles were then inoculated with the culture as per the need. Sealed inoculated bottles were incubated at 37 °C. Samples were taken out at regular time intervals using sterilized needles to perform cell counts and measure iron concentrations. Cell counts were done using an improved hemocytometer (Neubauer chamber, Hausser Scientific, Horsham, Pa., USA).

At the end of the experiment the aluminum seals were broken and the coupons were taken out and cleaned with alcohol and bursts of ultrasound in acetone solution. They were further cleaned with Clarke's solution (6 N HCl, with inhibitor) as needed. The coupons were re-weighed to determine the loss in weight.

4.2 Glass cell experiments

For glass cell experiments a setup similar to the one shown in Figure 4 was used. A saturated Ag/AgCl reference electrode used externally was connected to the cell via a luggin capillary and a porous wooden plug. A concentric platinum ring was used as a counter electrode.

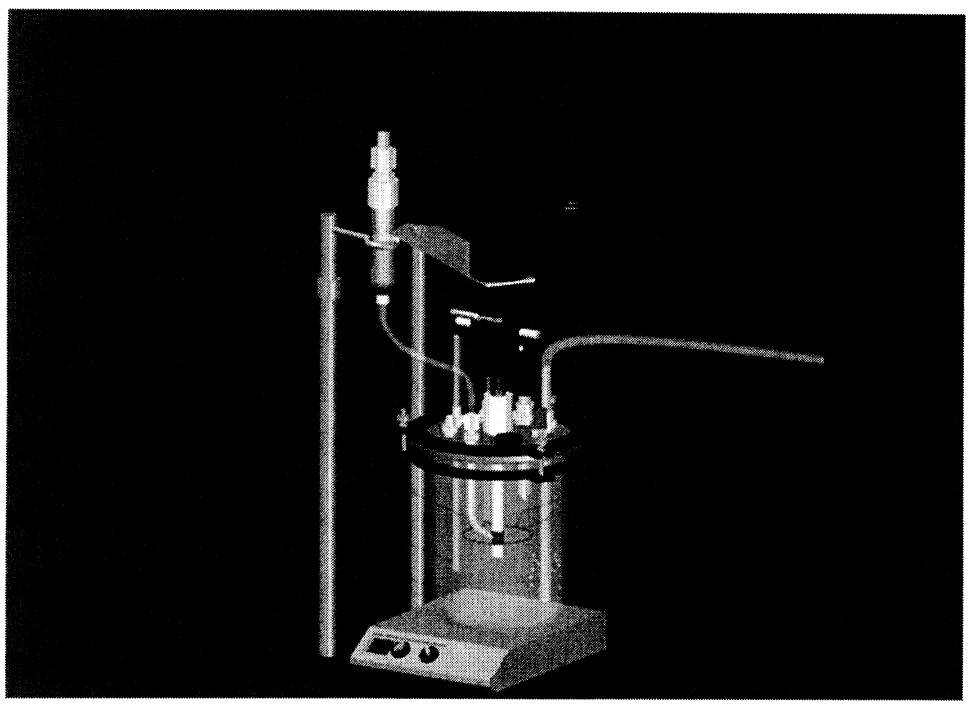


Figure 4. Glass cell setup 1.) Reference electrode, 2.) Rotor, 3.) Lid, 4.) Counter electrode, 5.) Working electrode, 6.) Bubbler, 7.) Hot plate, 8.) Luggin capillary, 9.) Temperature probe, 10.) Glass cell. (Courtesy of Daniel Mosser at Ohio University.)

Prior to the start of the experiment the glass cell and all other accessories including fittings were sterilized in an autoclave. The sterilization was performed at 121 ^{o}C for 15 minutes followed by an exhaust cycle of 20 minutes. The temperature probe, pH probe and counter electrode were cleaned and sterilized using hydrochloric acid and

alcohol. Media was prepared in separate flasks as per the procedure described earlier. After cooling down of the media it was transferred to the glass cell aseptically. All the fittings were connected and the entire setup was made ready in a laminar flow hood to avoid any type of contamination. The glass cell setup was then placed over the hot plate and fastened securely. The temperature of the glass cell was maintained at 37 °C. The media was deoxygenated by purging nitrogen through it. Coupon was not introduced into the glass cell at this point. The coupon was polished and cleaned as per the procedure described earlier. After the purging of media was over the shaft was introduced into the glass cell with the working electrode mounted (mild steel coupon). Also the desired amount of ferrous ammonium sulfate was added to this deoxygenated media. The glass cell was then completely sealed and the shaft was set to the desired rotation if necessary.

Electrochemical corrosion measurements were performed using a potentiostat connected to a PC. Corrosion rates were measured by using the linear polarization resistance (LPR) method and weight loss. The electrochemical method used to monitor corrosion rates are described below.

The linear polarization resistance (LPR) technique is used to monitor the in-situ corrosion rate. In this technique the metal coupon is polarized periodically (half hour interval) to ± 5 mV over the open circuit potential. The equations used to calculate the corrosion rate using LPR are as follows (Jones, 1990):

$$i_{corr} = B \times \frac{1}{R_p} \times \frac{1}{A} \qquad (4.2\text{-}1)$$

where,

B is the 'B' value, R_p is the corrosion resistance in ohm, A is the working electrode surface area in m^2.

The 'B' value can be calculated by using following expression:

$$B = \frac{\beta_a \beta_c}{2.303(\beta_a + \beta_c)} \tag{4.2-2}$$

The β_a and β_c in above equation are the Tafel slopes. They can be expressed as:

$$\beta_a = \frac{2.303RT}{\alpha_a F} \tag{4.2-3}$$

$$\beta_c = \frac{2.303RT}{\alpha_c F} \tag{4.2-4}$$

Where,

T - Absolute temperature in K,

R - Universal gas constant (8.314 J/(mol K)),

α_a and α_c - Symmetry factors for anodic and cathodic reactions.

F - Faraday's constant (96,500 coulombs/equivalent).

The corrosion rate (CR) in mm/yr can then be calculated according to the following equation:

$$CR = \frac{m}{At\rho} = \frac{i_{corr} M_w}{\rho n F} = 1.16\, i_{corr} \qquad (4.2\text{-}3)$$

Where,

m – Metal loss in kg,

t - Time in seconds,

ρ – Density of material in kg/m^3,

M_w – Molecular weight of iron,

F – Faraday's constant and

n – Number of electrons exchanged in the electrochemical reaction.

CHAPTER 5: RESULTS AND DISCUSSION

The experimental results are discussed in the following order:

- Effect of growth medium variation on growth of SRB
- Effect of temperature variation on MIC
- Study of effect of dissolved iron concentration variation
- Study of flow effect on corrosion due to SRB in glass cell
- Determining minimum inhibitory concentration level of biocide

A lot of pre-tests were carried out to check the procedure and setup a standard protocol for the biocorrosion experiments. This included testing of various procedures and equipments for oxygen free sterile environment. The protocol was improved by testing and investigation. The complete protocol has been described in sufficient detail in the experimental procedure chapter.

Interpretation of results and modeling work has been included at the end of each experimental sub-section. Initial tests had shown that the corrosion process terminated almost completely after seven days. The duration of most experiments was thus kept constant at seven days. The samples were taken for cell count at regular time interval. The cell count was done using a light microscope. Figure 5 shows the bacterial cells under the microscope at 400X magnification and Figure 6 shows the cell under an epifluroscence light microscope. The inoculation was generally done using a three day old culture showing a cell count of at least 1 X 10^6 cells/ml.

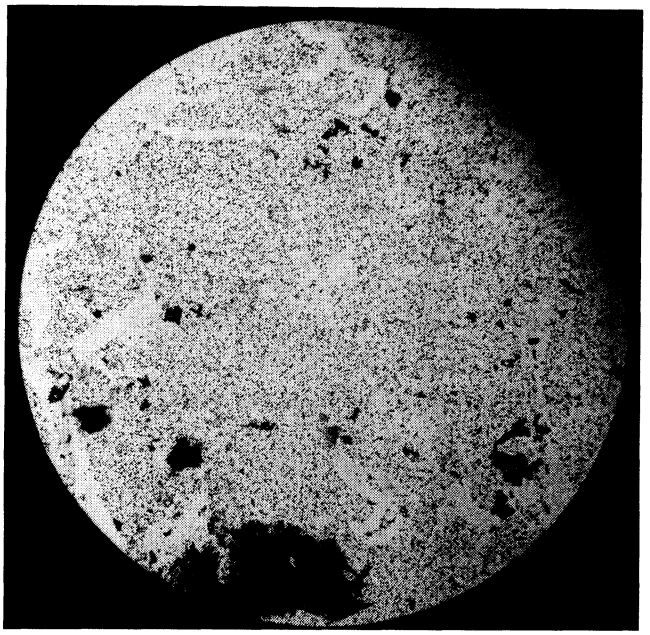


Figure 5. Sulfate reducing bacteria under a light microscope at 400X magnification

The black mass in the above picture is the iron sulfide precipitate. *Desulfovibrio* cells are usually curved rod shaped with flagella. The shape is visible only at high magnifications. The cell count is done at 1000X using a hemacytometer.

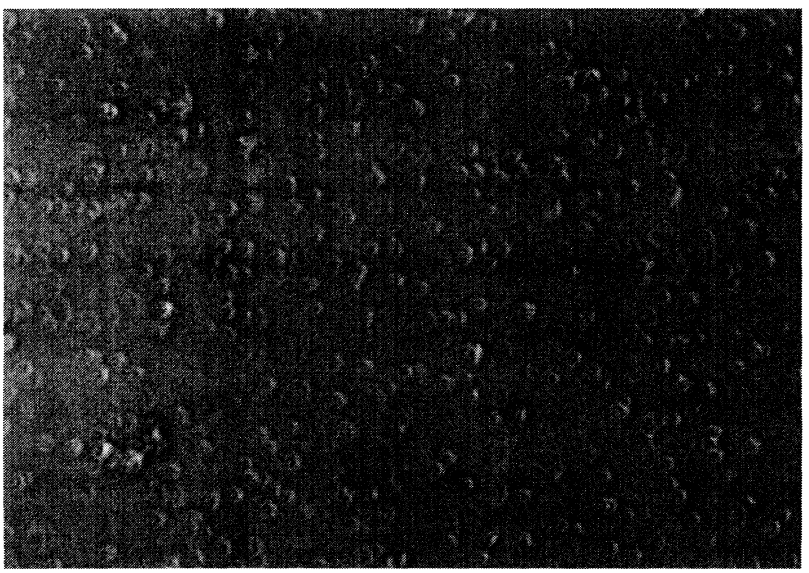
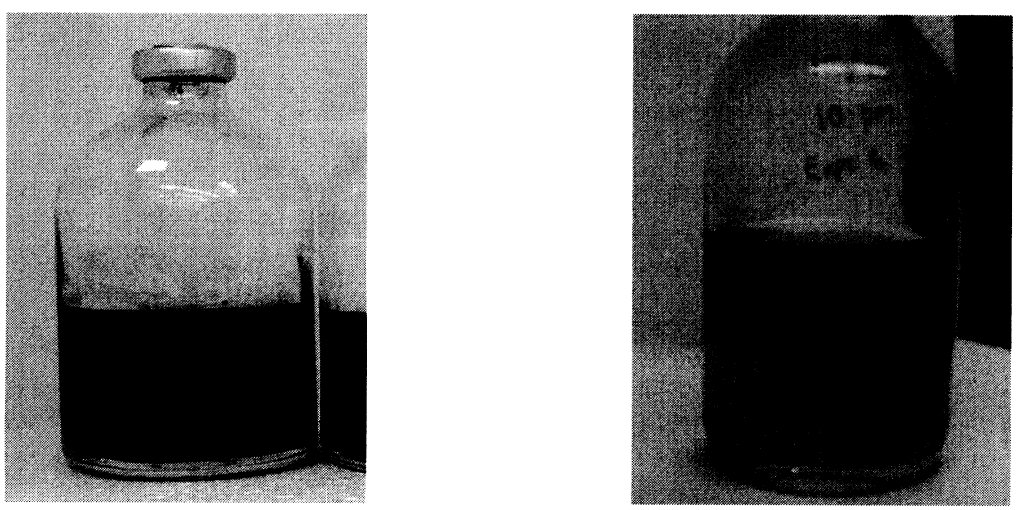


Figure 6. *Desulfovibrio desulfuricans* cells under epifluorescent microscopy at 1600X magnification

5.1 Effect of growth medium variation on growth of SRB

The growth medium composition was varied and some components were substituted by others to improve the experimental procedure. The first set of tests included identifying essential media components. Sulfate reducing bacteria obtain energy by reducing sulfates to sulfides while oxidizing lactate to CO_2 and H_2O (Hilton et al., 1988). Macpherson (1963) observed an increase in the cell population density with the increase in the concentration of media component. The anaerobic pathway depends upon on one or more limiting nutrients including sulfate. The sulfides combine with elemental iron to produce iron sulfide usually resulting in a black iron sulfide precipitate.

Tests were done to identify the limiting component necessary for growth. The culture was grown in media with and without sulfates. All other components were substituted in appropriate amounts to represent the original media. In the culture bottles with no sulfates growth was not observed even after seven days (Figure 7).



(a) (b)

Figure 7. Bottles showing SRB growth in different media (a.) sulfate and (b.) no sulfate

Samples were taken at different time intervals for live cell counts. No live cells were observed in sulfate free media. The iron sulfide precipitation was also absent in the sulfate free media bottle (Figure 7). The tests proved that sulfate is a necessary component for the growth of *Desulfovibrio desulfuricans.* Removal of sulfate can inhibit the growth of these bacteria and thus avoid corrosion problems.

The modified Baar's medium contained $Fe(NH_4)_2(SO_4)_2$ as one of the component. Ferrous ammonium sulfate is a heat sensitive compound. It cannot be heat sterilized as it decomposes. The use of ferrous ammonium sulfate required the use of a filter sterilization technique which made the procedure inconvenient. It was argued that ferrous sulfate and ammonium sulfate in the equivalent amounts would produce the same ionic strength of these ions in the solution. This would avoid the use of ferrous ammonium sulfate complex and thus also avoid the filter sterilization necessary otherwise. Hence, tests were done with ferrous sulfate plus ammonium sulfate and compared with ferrous ammonium sulfate.

The results obtained in terms of corrosion rate (Figure 8) were similar indicating that use of ferrous sulfate and ammonium sulfate had the same effect as the combined use of ferrous ammonium sulfate. Hence, in later experiments the two separate compounds were used instead of the ferrous-ammonium complex. This further simplified the process of controlling iron for different iron concentration experiments.

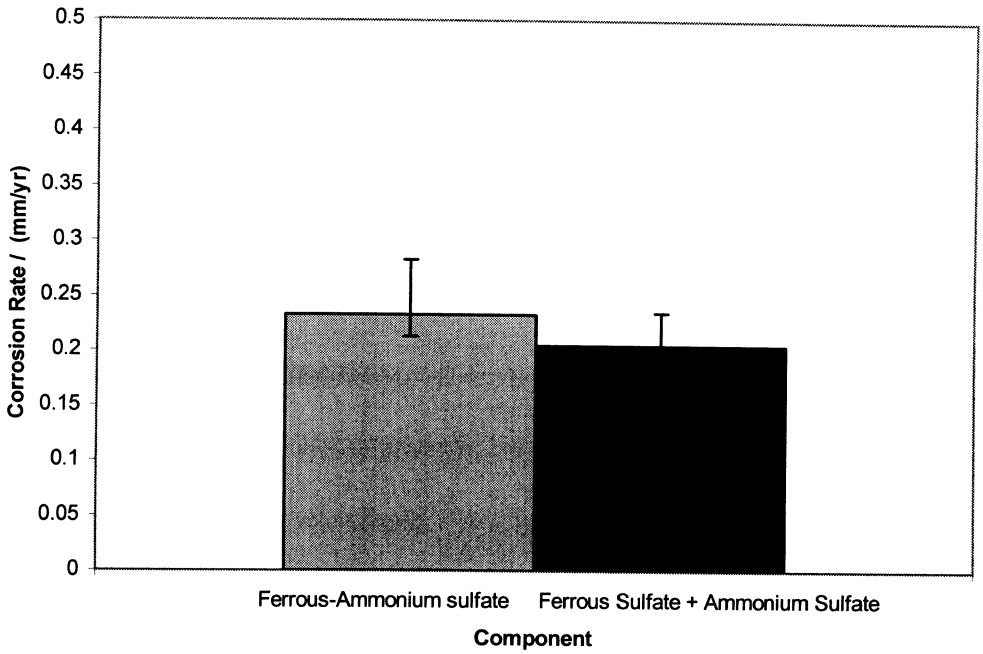


Figure 8. Effect of using ferrous ammonium sulfate versus (ferrous sulfate + ammonium sulfate) on corrosion rate monitored using weight loss for 7 days, T = 37 ^{o}C and pH = 7

5.2 Effect of temperature variation on MIC

Most species of SRB are mesophiles (Javaherdashti, 1999). They grow well in a temperature range from 25 °C to 40 °C. *Desulfovibrio desulfuricans* have been reported to grow most optimally at 37 °C. Most other SRB's grow in a temperature range of 5 °C to 50 °C (Javaherdashti, 1999).

Experiments were carried out at 5 °C, 25 °C and 37 °C to study the effect of temperature. As expected there was no cell growth at 5 °C. The growth was also very slow at 25 °C as compared to 37 °C. The slower growth resulted in lower corrosion rates (Figure 9). For most part of testing 37 °C was used henceforth. Inoculation was done only after the media cooled down to 40 °C or lower. The anaerobic bottles were incubated at 37 °C. For glass cell experiments the temperature was maintained using a hot plate and a thermocouple.

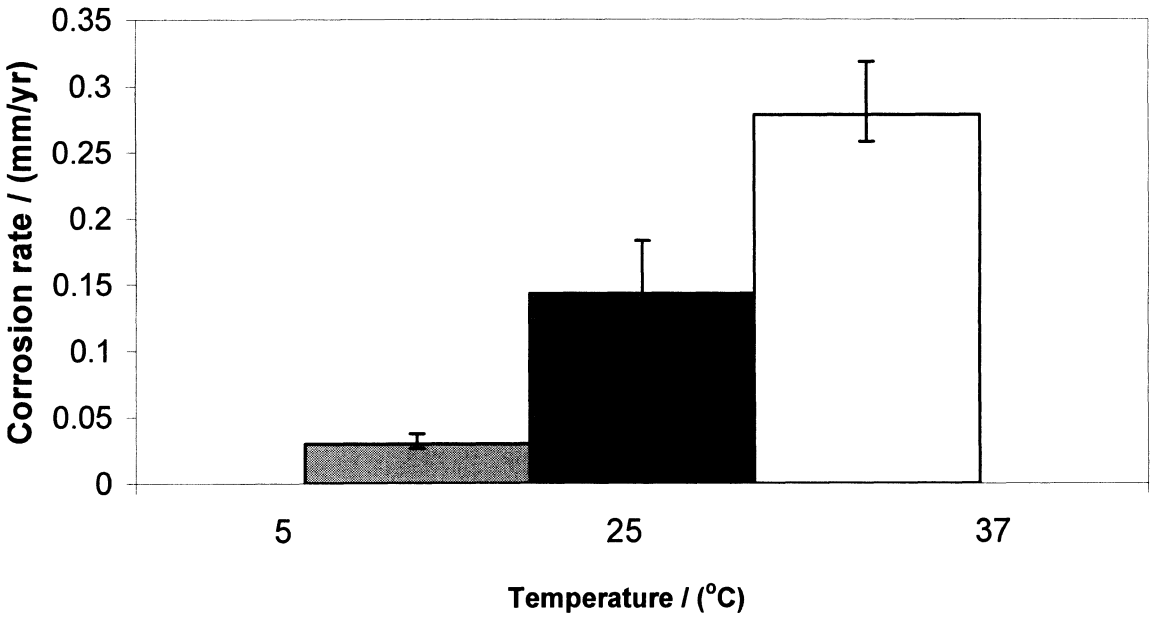


Figure 9. Effect of temperature on corrosion due to sulfate SRB, pH = 7

5.3 Study of effect of dissolved iron concentration variation

The influence of dissolved iron concentration on MIC of steel was investigated. Microbiologically influenced corrosion due to sulfate reducing bacteria could cause a localized attack on metal, but this has been in much debate in recent years. Some authors even claimed the fact that "MIC causes pitting corrosion" is a myth (Little et al., 1997).

Experiments were planned out to cover a wide range of dissolved iron concentrations. The concentrations tested included 0, 5, 10, 25 and 50 ppm. Some experiments with higher iron concentrations including 140 ppm were done as per the modified Baar's media composition mentioned in the ATCC media composition. Iron was added to the system in the form of iron sulfate.

5.3.1 Determination of time duration required for the experiment

The experimental time duration was increased from seven days to fourteen days to study the effect of change in experiment duration. The results are show in Figure 10.

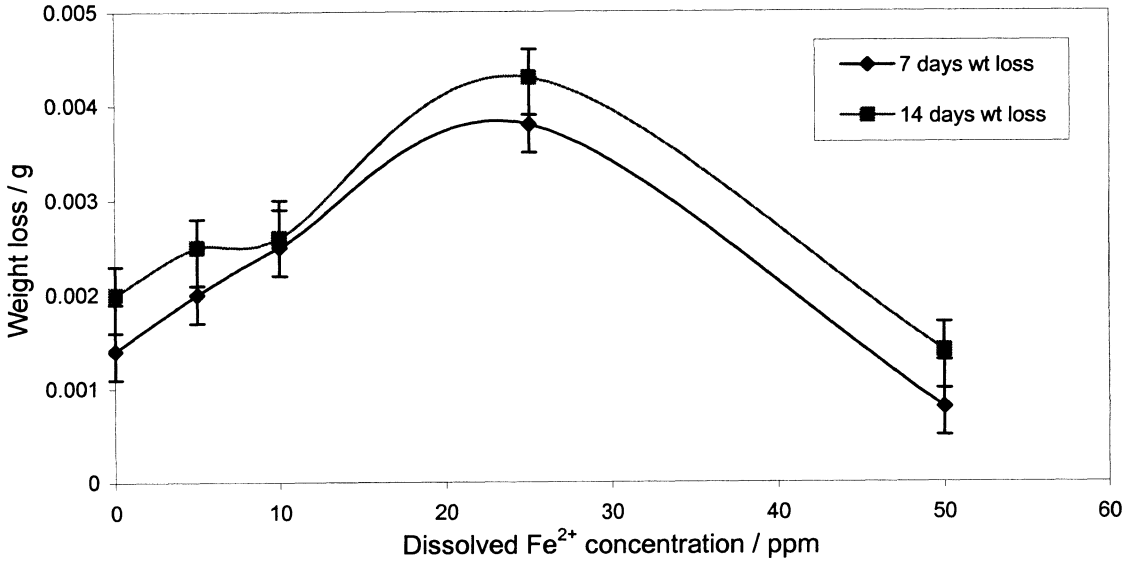


Figure 10. Weight loss results for 7 and 14 day experiments; T = 37 °C, pH = 7

The weight loss data indicated that the weight loss due to corrosion doesn't change much for fourteen days as compared to seven days (Figure 10). The limiting factor could either be lack of one of the media components or the toxicity of sulfides in the media. Hilton (1988) has discussed the sulfide-induced inhibition of anaerobic bacteria. The chances of the former being true are very weak. The sulfide in this case acts as an inhibitor by regulating the cell growth. The experimental duration was kept constant at seven days to study the dissolved iron effect.

5.3.2 Effect of dissolved iron concentration study

The experimental procedure was strictly followed as mentioned earlier for each set of experiments. The same amount of inoculum was used for all experiments. The stock culture was taken from the same bottle for each set of experiments to inoculate each bottle with equally viable culture. The coupons were weighed before and after the experiments. The coupons were weighed with and without the film to find the film weight. The coupons were cleaned with a series of distilled water and alcohol washes to remove all organic matter from the metal surface. The coupons were dried overnight and tested for elements present on the surface using an Energy dispersive spectroscopy (EDS). EDS scan in Figure 11 shows that iron and sulfur were amongst the most dominant elements on metal surface.

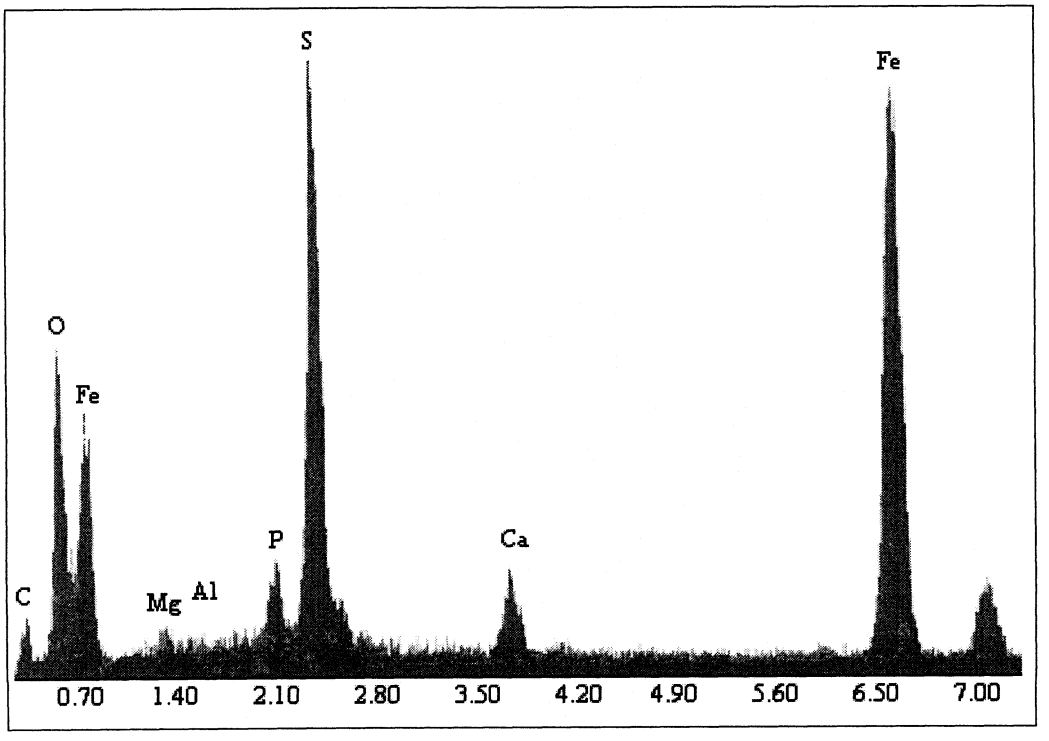


Figure 11. EDS scan of metal surface showing the presence of iron and sulfur

As can be seen from Figure 12 high corrosion rates were observed for intermediate iron concentrations. The corrosion rate was highest at 25 ppm, 37 °C and a pH of 7. Also the nature of corrosion was more severe at 25 ppm. The metal coupon exposed to 25 ppm iron concentration had undergone severe localized attack as can be seen from Figure 14. High iron concentrations of 50 ppm and very low iron concentrations of 5 ppm and less did not show localized attack.

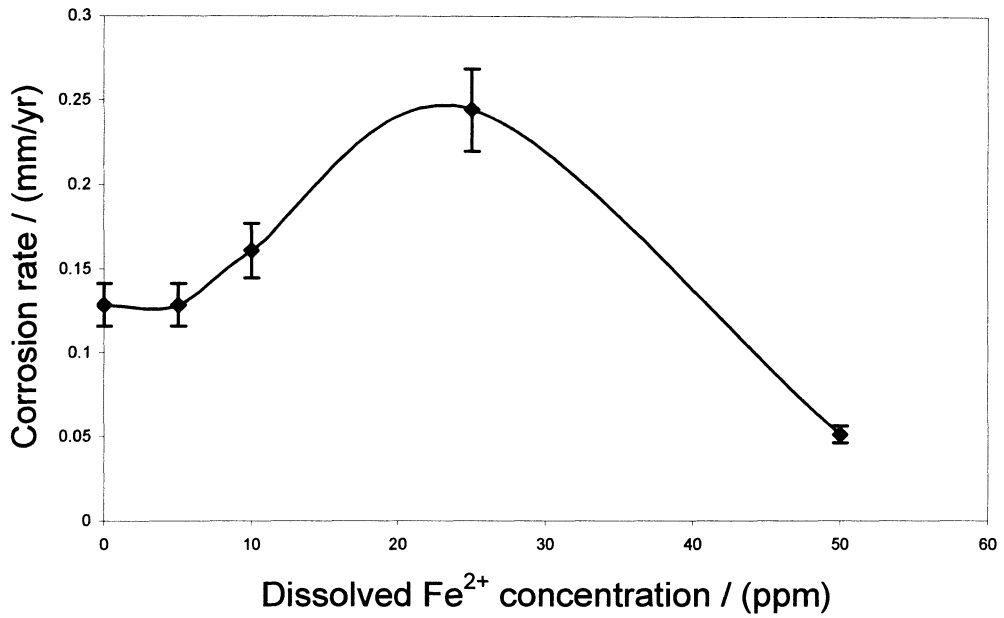


Figure 12. Graph showing the corrosion rate variation with the change in dissolved iron concentration at T = 37 °C, pH = 7

At different dissolved iron concentrations the metal is covered by different forms of films. At very low dissolved iron concentrations the solution reaches super-saturation rather late. The experiment with 25 ppm iron had undergone complete super-saturation while 0 ppm experiment is cloudy but not super-saturated (Figure 13).

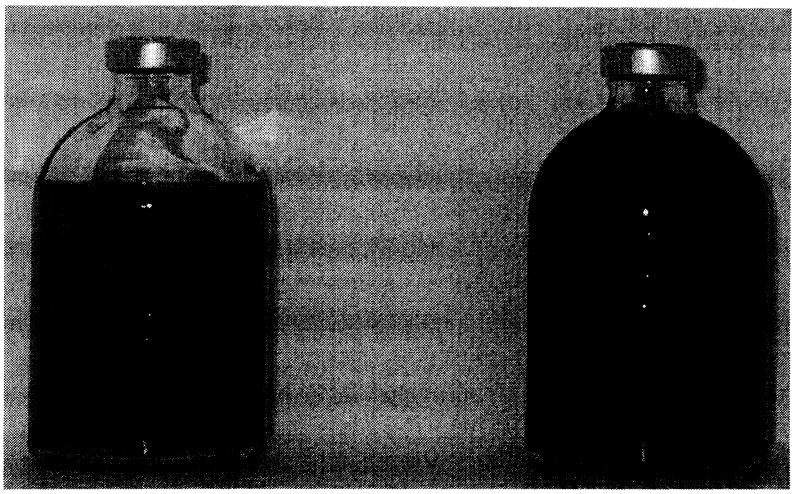


Figure 13. Difference in super-saturation levels at 0 ppm and 25 ppm

The metal underwent severe localized corrosion at intermediate concentrations of dissolved iron as can be seen from Figure 14.

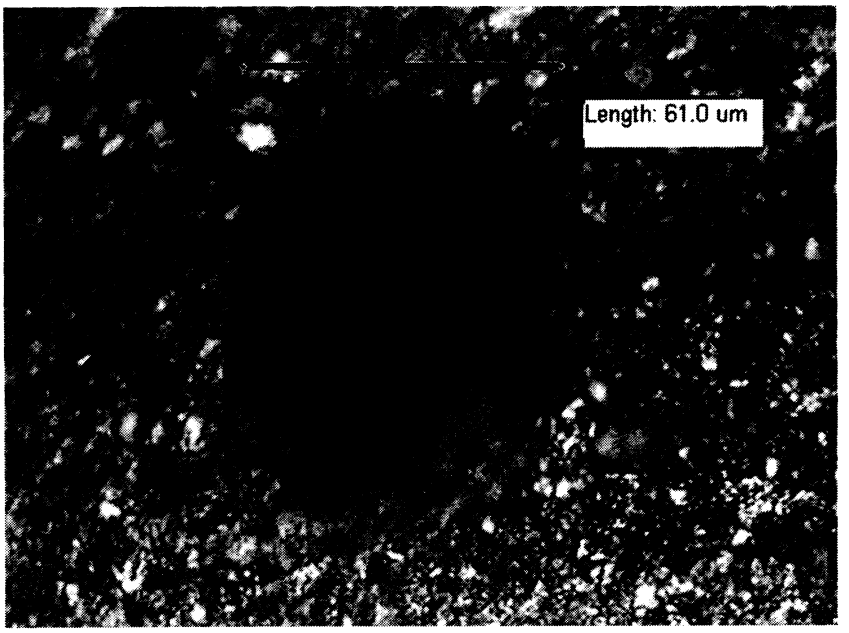


Figure 14. Microscopy image showing a pit on the coupon surface at 600X magnification, Fe^{2+} = 25 ppm (initial)

Figure 15 shows a polished metal surface. Figure 16 shows the metal surface after corrosion. Some areas in Figure 16 show clear signs of corrosion while other areas still show the polishing marks similar to the ones in Figure 15.

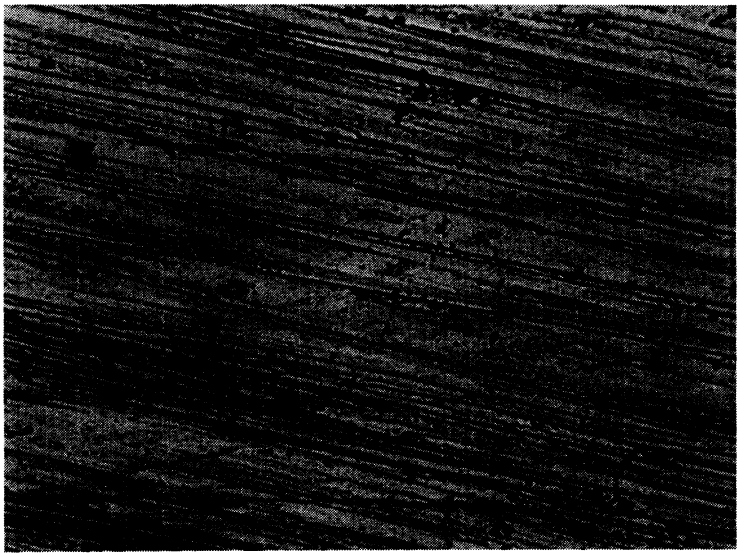


Figure 15. Polished C1018 steel surface at 400X magnification

The film formation process influences the corrosion process. The type of film and the nature of film will predict whether localized corrosion will occur or not. For the case of a bacterial biofilm combined with iron sulfide film the system is more complex. The interaction between the two types of film influences the corrosion process.

For systems with low dissolved iron concentrations the bacterial biofilm may cover up most of the metal surface before the solution reaches super-saturation. Iron sulfide will then deposit on top of the bacterial biofilm. The metal surface will remain covered with bacterial biofilm. The metal corrodes uniformly underneath the biofilm. There is no permanent separation of anode and cathode on metal surface.

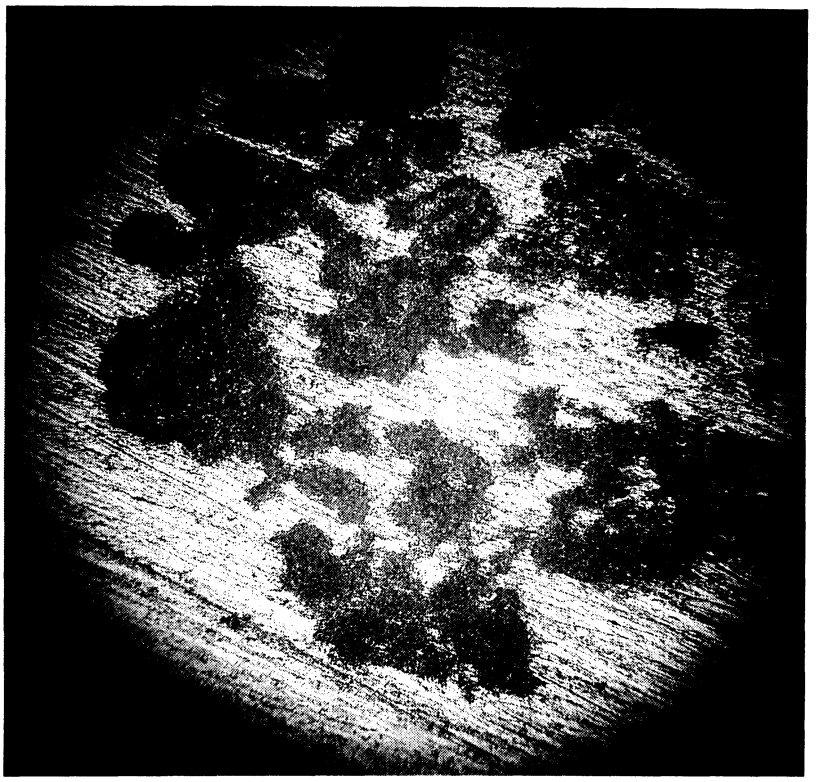


Figure 16. Coupon surface after cleaning at 400X showing localized attack in an experiment with 25 ppm dissolved iron concentration

The reverse is true for very high concentrations of dissolved iron. At concentrations greater then 50 ppm the solution reaches super-saturation almost immediately. All iron sulfide formed begins to precipitate on the metal surface. Most of the metal is covered with an iron sulfide film before the bacterial biofilm can develop. Hence, the metal is protected against localized corrosion.

To validate the above argument the biofilm was washed away and the metal was observed under a scanning electron microscope (SEM) (Figure 17) to see the nature of residual iron sulfide film on the metal surface.

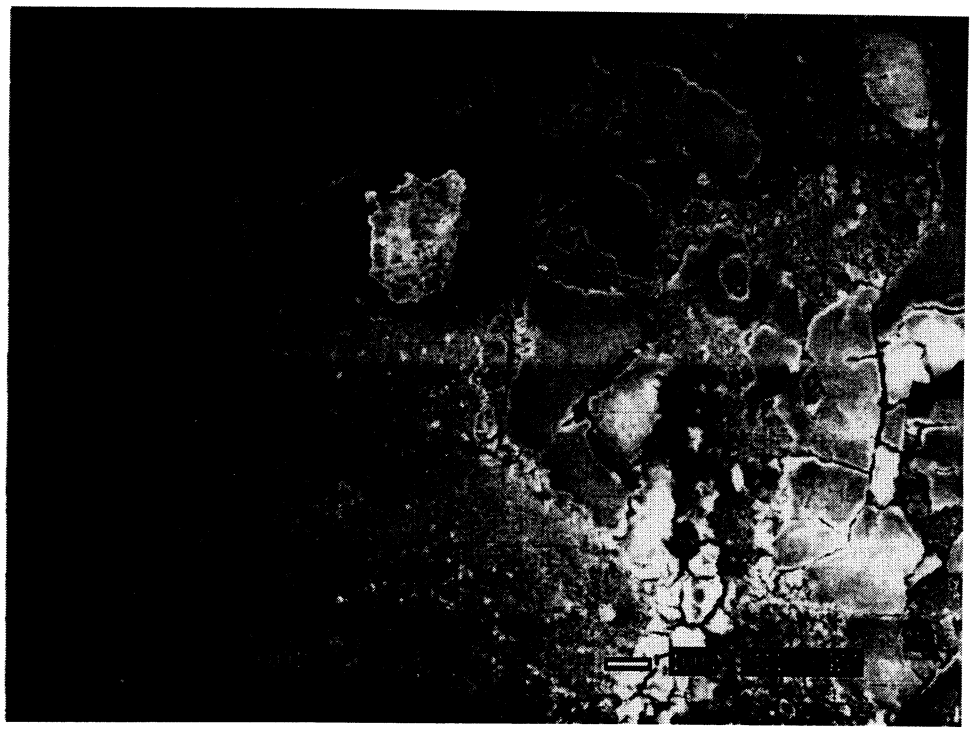
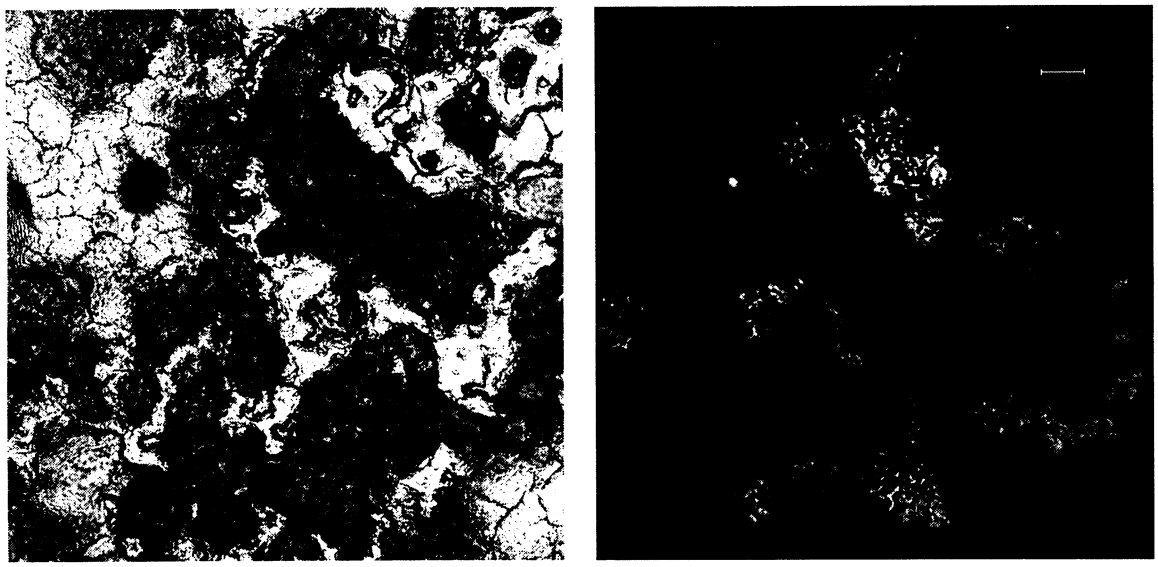


Figure 17. SEM image of metal surface after cleaning with water and alcohol; Fe^{2+} = 25 ppm, T = 37 °C, pH = 7

The localized corrosion at intermediate iron concentrations can be attributed to simultaneous biofilm growth along with iron sulfide precipitation and film formation. The biofilm starts to develop around the single cell attached to metal surface. The metal could be covered partly with iron sulfide and partly with the biofilm. The image from confocal microscope in Figure 18 shows the different types of film on metal surface.

The area underneath the biofilm becomes the anode. The iron sulfide film being conductive helps in the loss of electrons. Hence, areas covered by the iron sulfide film act as the cathode. The anodes and cathodes are now permanently separated on the metal surface. Metal is lost continuously at anodes whereas film formation progresses effectively at cathodes.



(A) (B)

Figure 18. Untreated film under a confocal microscope at 400 X magnification showing the different layers on metal surface with average film thickness of 15 microns

5.4 Study of flow effect on corrosion due to SRB in glass cell

The study of flow effect was done in a glass cell as shown in Figure 4. Procedure was as described previously. The coupons were mounted on a shaft and rotated using a speed control unit. Experiments were done at two different rotational speeds 1000 and 2000 rpm. The base line experiments were done at 0 rpm. The results were compared to see the shift in the limiting current with the increase in rotational speed. The experiments were conducted in batches. The experiments being similar to anaerobic fermentation experiments relied to some extent on the viability of the stock culture.

Rotating cylinder flow geometry has been studied by Yang (2002) and some other authors (Nesic et al., 1995; Silverman, 1984). The rotational speed was used to calculate the velocity at the metal surface as per the correlation proposed by Silverman (1984) (Figure 19).

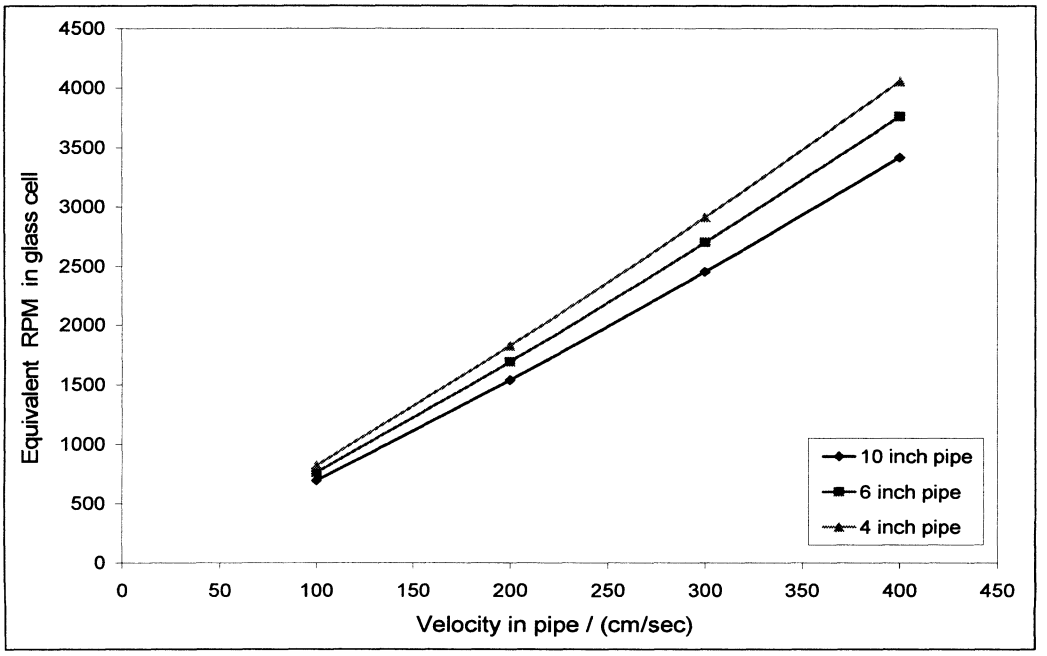


Figure 19. Relation between rotational speed in glass cells and surface velocity in a straight pipe flow

Lee and Characklis studied the anaerobic corrosion of iron at 0.35m/s. The current work extended the flow regime to higher velocities. The increase in velocity restricted the cell growth process. The cells did not have a stationary surface to adhere and propagate. At 3000 rpm the growth was much slower as compared to other velocities. The solution did not reach super-saturation until six days even with the same iron concentration in the solution. Most growth was found at the bottom of the cell where the disturbance due to flow was minimal. A sudden drop in corrosion rate was observed when the solution reached super-saturation. After reaching super-saturation the corrosion rate remained almost stable. Experiments were done for longer time periods initially to check the time required by the system to reach a stable corrosion rate. Figures 20 and 21 below show the results of electrochemical measurements.

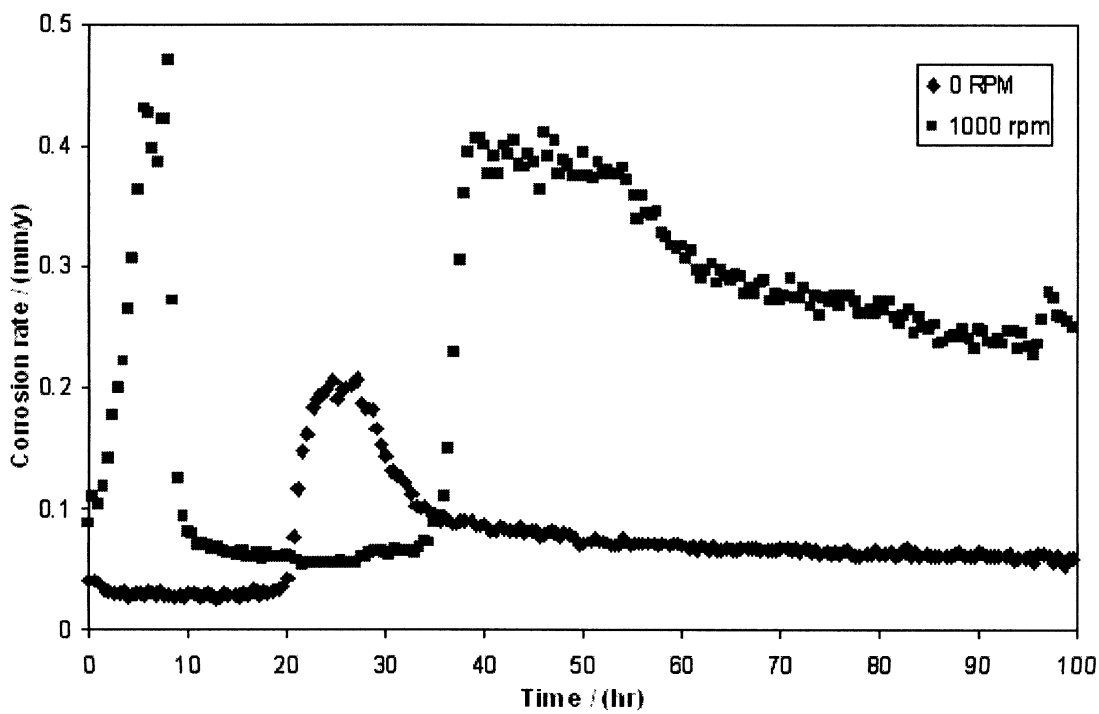


Figure 20. Effect of velocity on corrosion at 0 and 1000 rpm; T = 37 °C and pH = 7

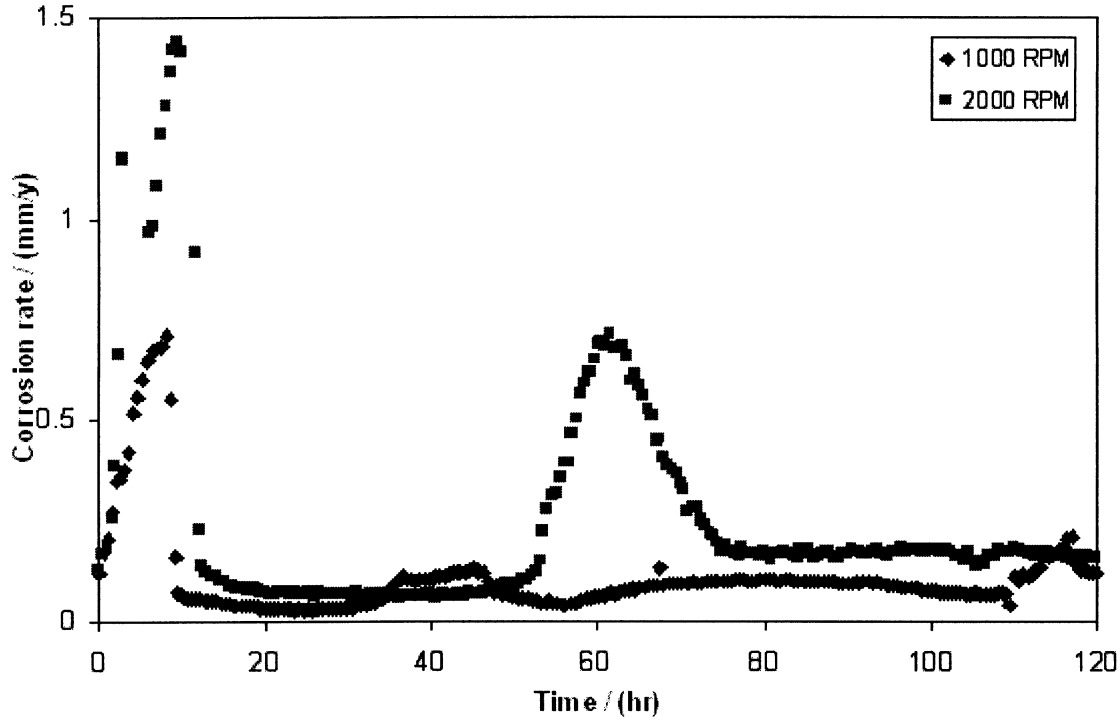


Figure 21. Effect on velocity on corrosion at 1000 and 2000 rpm; T = 37 °C, pH = 7

Figures 20 and 21 show the results of electrochemical measurements in glass cell. There was a significant increase in the corrosion rate at 1000 rpm as compared to stagnant condition (0 rpm). This could be attributed to an increase in the mass transfer rate of corroding species (HS^- and H_2S) from the bulk of the solution to the metal surface and the increased availability of nutrient species to the sessile SRB cells in the biofilm on the coupon surface. The corrosion rate did not increase significantly as the rotation speed increased from 1000 to 2000 rpm. It was also noted that when the rotational speed was sufficiently large, SRB growth and corrosion rate were inhibited. It indicates that fluid shear can be a potential MIC mitigation method. Further experiments are currently underway.

5.5 Determining minimum inhibitory concentration level of biocide

The best way of combating biocorrosion problems is the use of biocides. The use of simple organic compounds as biocides is becoming increasingly popular. The effectiveness of these biocides in the part per millions is quite appealing. Glutaraldehyde a common organic aldehyde was tested to determine its effectiveness. A wide series of concentration ranging from 0 to 250 ppm were tested to determine the most effective lethal dosage. The procedure for these experiments remained the same as described earlier. The minimum inhibitory concentration required to control cell growth completely was found to be 50 ppm (Figures 22 and 23). At concentration lower then 50 ppm viz. 30 ppm and 10 ppm the culture was found to grow effectively (Table 4). At 30 ppm the growth rate was slower then in the absence of biocide.

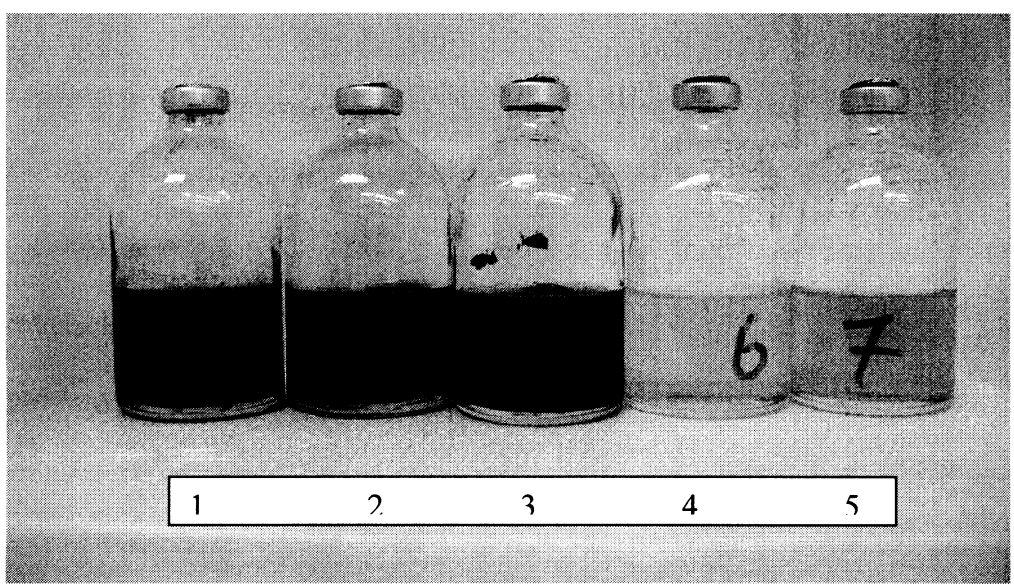


Figure 22. Effect of glutaraldehyde on the bacteria after 4 days 1.) 0 ppm 2.) 10 ppm 3.) 30 ppm 4.) 50 ppm 5.) 100ppm; T = 37 ^{o}C, pH = 7

Table 4. Results of glutaraldehyde tests for effective biocide concentrations

Experiment Number	Glutaraldehyde	Growth
1	10	Yes
2	10	Yes
3	30	Yes
4	30	Yes
5	50	No
6	50	No
7	100	No
8	100	No
9	250	No
10	250	No

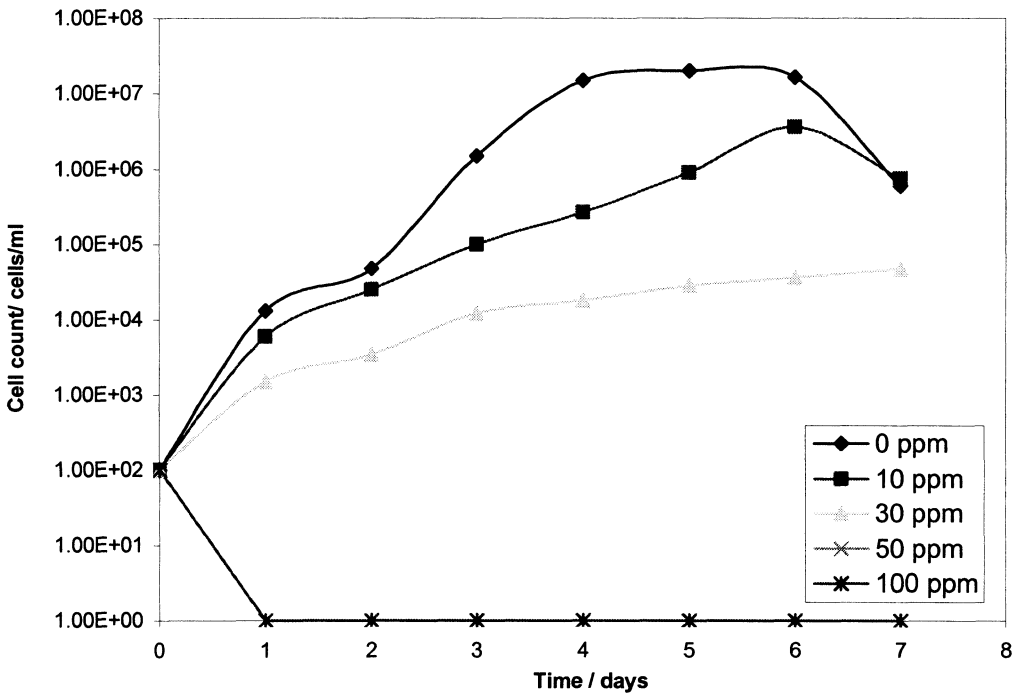


Figure 23. Planktonic cell count for media with different concentrations of glutaraldehyde, T = 37 °C, pH = 7

CHAPTER 6: CONCLUSIONS

The physical characteristics of a biofilm and its interaction with an iron sulfide film were studied in anaerobic bottles and glass using a culture of sulfate reducing bacteria species *Desulfovibrio desulfuricans*. The following conclusions were derived based on this study.

- *Desulfovibrio desulfuricans* needs sulfate for growth. Growth is restricted in the absence of sulfates.
- The corrosion rate is lowered with a decrease in temperature. At lower temperatures the cell growth rate is also much slower as compared to the growth rate at 37 ^{o}C.
- Iron sulfide films can affect the nature of corrosion due to SRB. At intermediate iron concentrations the corrosion becomes localized.
- Flow affects the corrosion process. The overall corrosion rate increases with increase in flow. The corrosion is more uniform under flowing conditions as compared to stagnant conditions.
- Glutaraldhyde is an effective biocide at low concentrations of parts per million.

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The role of a biofilm and its characteristics in microbiologically influenced corrosion of steel. (66pp.)

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A class of anaerobic bacteria, sulfate reducing bacteria (SRB), is able to survive by deriving energy from reducing sulfates to sulfides. Sulfides produced as a consequence are very harmful to iron and steel structures. Uncontrolled SRB growth in oilfield operations often leads to severe corrosion problems.

Current mitigation methods for Microbiologically Influenced Corrosion (MIC) mostly rely on toxic biocides. To develop new simpler and safer mitigation methods we need to develop an understanding of the SRB biofilms.

This work studied the physical characteristics of SRB biofilms to develop a clearer understanding of the role played by the SRB biofilms in the corrosion of iron and steel. The ATCC 7757 strain of *Desulfovibrio desulfuricans* was used in this work. It is a common SRB species.

Removal of a key nutrient from the system will restrict the cell growth and hence control corrosion. It was found that the absence of sulfates stops the growth of SRB. Removing the sulfates will thus help control bacterial growth in the system.

Effect of temperature on SRB growth was studied in the range of 5 ^{o}C to 37 ^{o}C. The growth rate and corrosion rate are much slower at 25 ^{o}C than at 37 ^{o}C.

The effect of dissolved iron on the nature and extent of corrosion is important. Experiments with different initial Fe^{2+} concentrations revealed that intermediate