



Corrosion of mild steel under an anaerobic biofilm  
by Whonchee Lee

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in  
Chemistry

Montana State University

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Abstract:

Corrosion of mild steel under completely anaerobic conditions in the presence of a mixed population biofilm, including sulfate-reducing bacteria (SRB), has been studied in a continuous flow system. The open channel flow reactor was continuously fed with low concentration substrate at different dilution rates which influenced biofilm accumulation. No direct correlation was observed between corrosion and SRB activity in the absence of ferrous ion. Furthermore, corrosion of mild steel in the SRB environment was mainly determined by the nature of the metal and environmental conditions such as dissolved iron. When formation of iron sulfide film on mild steel was prevented before the biofilm accumulated, the metal surface retained its scratch lines after a 21-day experiment (SRB at  $2.6 \times 10^9/\text{cm}^2$ ). However, when iron sulfide film was formed before the accumulation of biofilm, visible localized corrosion appeared after 14 days and increased up to 21 days. Intergranular and pitting attack were found in the localized corrosion area. Inclusions (Al, Mn, and Fe) were usually found in the localized corrosion area. At high iron concentration (about 60 mg/L in the bulk water), all biogenic sulfide was precipitated and corrosion rate significantly increased. Intergranular attack was found over the entire metal surface.

The anaerobic corrosion processes of mild steel in the presence and absence of anaerobic biofilm, including sulfate-reducing bacteria, was compared. In the biotic experiment, biofilm accumulated on a chemically-formed iron sulfide film for 21 days. The abiotic experiment was designed to simulate this hydrogen sulfide environment which had similar hydrogen sulfide concentration, redox potential, and pH. There was no difference in the metal matrix at three days in the two conditions. However, corrosion propagation was different for the two cases after 3 days. Localized corrosion was found in the presence of biofilm while uniform intergranular attack was observed in the absence of biofilm.

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in

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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## ABSTRACT

Corrosion of mild steel under completely anaerobic conditions in the presence of a mixed population biofilm, including sulfate-reducing bacteria (SRB), has been studied in a continuous flow system. The open channel flow reactor was continuously fed with low concentration substrate at different dilution rates which influenced biofilm accumulation. No direct correlation was observed between corrosion and SRB activity in the absence of ferrous ion. Furthermore, corrosion of mild steel in the SRB environment was mainly determined by the nature of the metal and environmental conditions such as dissolved iron. When formation of iron sulfide film on mild steel was prevented before the biofilm accumulated, the metal surface retained its scratch lines after a 21-day experiment (SRB at  $2.6 \times 10^9/\text{cm}^2$ ). However, when iron sulfide film was formed before the accumulation of biofilm, visible localized corrosion appeared after 14 days and increased up to 21 days. Intergranular and pitting attack were found in the localized corrosion area. Inclusions (Al, Mn, and Fe) were usually found in the localized corrosion area. At high iron concentration (about 60 mg/L in the bulk water), all biogenic sulfide was precipitated and corrosion rate significantly increased. Intergranular attack was found over the entire metal surface.

The anaerobic corrosion processes of mild steel in the presence and absence of anaerobic biofilm, including sulfate-reducing bacteria, was compared. In the biotic experiment, biofilm accumulated on a chemically-formed iron sulfide film for 21 days. The abiotic experiment was designed to simulate this hydrogen sulfide environment which had similar hydrogen sulfide concentration, redox potential, and pH. There was no difference in the metal matrix at three days in the two conditions. However, corrosion propagation was different for the two cases after 3 days. Localized corrosion was found in the presence of biofilm while uniform intergranular attack was observed in the absence of biofilm.

## INTRODUCTION

Economic Impact of Sulfate-Reducing  
Bacteria (SRB).

The corrosion of iron and steel in the form of storage tanks, pipelines, pumps, and other systems by SRB is a serious problem faced by many industries including the petroleum industry. These bacteria also cause a decrease in the permeability of the fine pores of underground reservoirs, impeding the secondary recovery of petroleum by water injection. Recently, SRB have been suspected of contributing to failure of certain enhanced (tertiary) oil-recovery operations. Thus, almost every aspect of oil recovery can be hindered by SRB. SRB also are suspected to cause problems in pulp and paper industry, nuclear power plants, and other chemical industries. The cost related to SRB-mediated corrosion is estimated to be 1 to 2 billion dollars per year.

Corrosion of Mild Steel Under Anaerobic  
Conditions in the Absence of Bacteria

Thermodynamically and abiotically, there is very little, if any, corrosion of steel in the absence of oxygen under neutral or alkaline conditions. However, anaerobic corrosion may occur in the presence of sulfide due to cathodic depolarization ( $2 \text{H}_2\text{S} + 2 \text{e}^- \rightarrow 2 \text{HS}^- + \text{H}_2$ ). The attack

occurs by dissolved sulfide as well as iron sulfide (Smith et al, 1975). Iron sulfide causes cathodic depolarization by decreasing the hydrogen overpotential.

The anaerobic corrosion process of mild steel has been studied in a saturated hydrogen sulfide solution (Tewari et al, 1979; Shoesmith et al, 1980). Initially, a solid-state reaction produces a layer of mackinawite, which readily cracks and spalls from the metal surface and results in a loose iron sulfide precipitate. The corrosion rate is mainly controlled by the stability of the iron sulfide film in the initial stage. The saturated hydrogen sulfide (approximately 3150 mg/L hydrogen sulfide) represents an extreme condition unlikely to exist in a natural environment containing SRB. The levels of hydrogen sulfide released by the anaerobic decay of a marine fouling deposit (Edyvean et al, 1985) is about 5 to 150 mg/L. Therefore a more realistic assessment of the corrosion effects of hydrogen sulfide induced by fouling deposits on the corrosion behavior can be obtained by testing at low levels of hydrogen sulfide.

#### Corrosion of Mild Steel Under Anaerobic Conditions in the Presence of Bacteria

Biotic production of sulfide may lead to corrosion of mild steel in anaerobic environments. For example, chemostat (planktonic cells) investigations indicate that microbial sulfide production leads to cathodic depolarization primarily due to the iron sulfide reaction. Booth et al. (1966, 1967)

reported that no correlation exists between hydrogenase activity and corrosion rate when experiments were undertaken in semi-continuous and continuous culture in low or high concentration ferrous medium. Low rates of corrosion were observed in low level iron medium due to the formation of a protective iron sulfide film. However when these films ruptured, very high rates of corrosion occurred. In field investigations, Starkey (1985) also reported that only slight corrosion of a completely buried steel pipe was detected in anaerobic mud supporting an active community of SRB.

Von Wolzogen Kühr and van der Vlugt (1934) proposed the cathodic depolarization theory for microbial (SRB) corrosion based on batch (closed) reactor investigations. They suggested that hydrogenase catalyzed the hydrogen reduction reaction on the iron surface. King and Miller (1973) supported the hydrogenase theory but proposed that the cathodic depolarization occurred on the iron sulfide instead of the iron surface. Costello (1974) reports that Desulfovibrio desulfuricans, in a batch reactor, produces  $H_2S$  which directly depolarizes the cathode. In summary,  $H_2S$  corrosion is under kinetic control and the iron sulfide film seems to play an important role in the anaerobic corrosion process both in biotic and abiotic systems. There is evidence for cathodic depolarization as an important mechanism in the anaerobic corrosion of iron and mild steel. However, the exact mechanism, including the role of hydrogenase and reaction site

of hydrogen reduction, is not definitively understood.

Most microbial corrosion in nature and in technological systems occurs in association with microbial aggregates or biofilms. Hamilton (1985) has developed a qualitative biofilm model of anaerobic microbial corrosion where the growth of biofilm SRB requires that the appropriate physicochemical conditions be supplied by other facultative and anaerobic organisms within the biofilm. To address the anaerobic microbial corrosion problems in natural environments, the activities and behavior of biofilm or sessile SRB, not planktonic SRB, must be considered.

There is very little quantitative information available related to the rate and extent of microbial sulfide production in biofilms. Nielsen (1987) reports from a continuous flow SRB biofilm reactor that sulfate diffusion in the biofilm is rate-limiting for SRB biofilm activity.

#### The Problem

The relation between SRB activity and corrosion rate of mild steel is still not well established despite decades of research. Progress in microbial corrosion research has been limited by at least two factors: 1) avoidance of experimental methods which simulate biofilm processes in continuous systems and 2) analytical techniques which can be used in a biofilm systems and while providing adequate characterization of the corroding metal surface.

### Research Goal

The goal of this research was to obtain a fundamental understanding of corrosion of mild steel under an anaerobic biofilm. To accomplish this goal, the following objectives and tasks were established.

#### Objectives

1. Determine the effects of anaerobic biofilms on the corrosion behavior of mild steel.
2. Determine the impacts of environmental parameters on the corrosion behavior of mild steel in the presence of an anaerobic biofilm.

#### Tasks

1. Determine the effect of substrate loading rate on the corrosion of mild steel in the absence of ferrous iron and relate SRB biofilm activity with the cathodic depolarization reaction.
2. Assess the influence of suspended iron sulfide on the corrosion of mild steel in the presence of a mixed SRB biofilm.
3. Evaluate the effect of the iron sulfide film on the corrosion of mild steel in the presence of a mixed SRB biofilm.
4. Assess the influence of low concentrations of hydrogen sulfide on the corrosion behavior of mild steel in the absence of bacteria.

## LITERATURE REVIEW

Types of SRB and Their Substrates

The most common cell morphologies of SRB are curved and oval to rod-shaped; their diameters usually range from 0.5 to 2  $\mu\text{m}$ . Many SRB are actively motile by flagella. Other forms are spheres and long multicellular filaments. Several types of SRB tend to grow in clumps or cell aggregates and stick to surfaces.

Nutritionally, SRB may be divided into two major groups. Species of the first group carry out an incomplete oxidation of organic substrates with acetate as an end product. Species of the second group oxidize organic substrate, including acetate, completely to  $\text{CO}_2$ .

Most incompletely oxidizing SRB may grow rather fast under optimum conditions and reach doubling times of about 10 hours. The best-studied representatives are Desulfovibrio species that can be easily isolated from nearly every aquatic sediment (Postgate, 1984). For most, lactate is an excellent substrate that is oxidized to acetate and  $\text{CO}_2$ .

The completely oxidizing SRB grow relatively slowly, with optimum doubling times seldom shorter than 15 hours. The nutritionally specialized Desulfobacter species prefer acetate as substrate, the quantitatively most important organic

fermentation product; higher fatty acids are not used. Other completely oxidizing SRB are nutritionally more versatile; they may oxidize propionate, higher fatty acids, lactate, alcohol; and even aromatic organic acids. Some of the completely oxidizing SRB can use  $H_2$  as electron donor and synthesize cell material from  $CO_2$  as the sole carbon source. The growth requirements and growth rates of selected SRB are given in Table 1.

Table 1. Growth requirements of selected sulfate-reducing bacteria

Species	Electron Donor			Doubling Time, HR.	Growth Range	Temp. °C Optimum	Growth pH		Other Requirements
	Acetate	Propionate	Butyrate				Range	Optimum	
Incomplete Oxidation									
<i>Desulfobulbus propionicus</i>	No	Yes	No	appx. 10	10-43	39 (28-39)	6.0-8.6	7.2	p-aminobenzoic acid
<i>Desulfovibrio sapovorans</i>	No	No	Yes	NR	15-38	34 (30-36)	6.5-9.3	7.7	NR
Complete Oxidation									
<i>Desulfobacter postgatei</i>	Yes	No	No	15-20	10-37	32	6.2-8.5	7.3	salt-0.7%, p-aminobenzoic acid and biotin
<i>Desulfotomaculum acetoxidans</i>	Yes	No	Yes	12-24 (15-30)	20-40	35	6.6-7.6	7.1	vitamins
<i>Desulfococcus multivorans</i>	Yes	Yes	Yes	appx. 24	NR	35	NR	NR	NR
<i>Desulfosarcina variabilis</i>	Yes	Yes	Yes	NR	15-38	33 (38-33)	6.7-9.0	7.4	salt-1.5%, p-aminobenzoic acid and biotin
<i>Desulfonema limicola</i>	Yes	Yes	Yes	30-100	15-36	29 (30)	6.5-8.8	7.6	salt-1.5%, surface, biotin
<i>Desulfonema magnum</i>	Yes	Yes	Yes	30-100	15-37	32	6.6-7.5	7.0	salt-2%, surface, biotin, aminobenzoic acid, vitamin B <sub>12</sub>
<i>Desulfovibrio baarsii</i>	Yes	Yes	Yes	NR	20-43	35-39	6.5-8.2	7.3	NR
Mixed culture	Yes	NR	NR	32	NR	NR	NR	NR	NR

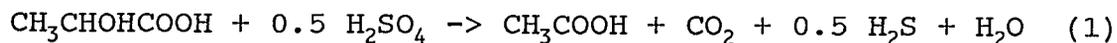
### Stoichiometry and Kinetics of SRB

There are two aspects of the microbial reaction of science and engineering interest; stoichiometry and kinetics. Stoichiometry provides what changes will occur and to what extent. Kinetics describes how fast the change will occur.

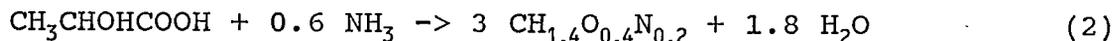
#### Stoichiometry of SRB

Dissimilating sulfate reduction can be represented by a pair of chemical equations that describe the oxidation of an organic carbon source and the synthesis of bacterial cells. For example, in the case that lactate is an electron donor and energy source, stoichiometric equations of sulfate reduction are described as following:

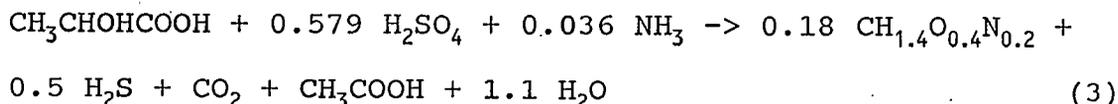
Energy with lactate



Synthesis with lactate



Overall stoichiometry



Overall stoichiometric equation was balanced using the experimental data concerning biomass yield and carbon dioxide production of D. desulfuricans culture determined by Traore et al (1982). D'Alessandro et al (1974) reported very similar stoichiometry of sulfate reduction by D. vulgaris. In both cases, lactate and sulfate were consumed in stoichiometric ratio of 2 : 1.

#### Kinetics of SRB

Bacterial cells reproduce by binary fission. For a given set of environmental conditions (e.g., temperature and pH) the rate of reproduction due to binary fission depends on the concentration of nutrient which are available for growth. If all required nutrients are supplied in excess except one, the growth limiting nutrients, the rate of cellular reproduction can be empirically related to the concentration of the limiting nutrient. The equation most widely used to describe this relation was originally proposed by Monod (1949) to describe the growth of Escherichia coli on glucose. The Monod equation is written as follows:

$$\mu = \frac{\mu_{\max} S}{k_s + S} \quad (4)$$

where

- $\mu$  = cellular specific growth rate ( $t^{-1}$ )
- $\mu_{max}$  = maximum cellular specific growth rate ( $t^{-1}$ )
- $s$  = concentration of the limiting nutrients, commonly referred to as the substrate ( $M_s L^{-3}$ )
- $k_s$  = saturation coefficient, numerically equal to the substrate concentration at  $\mu = 1/2 \mu_{max}$  ( $M_s L^{-3}$ )

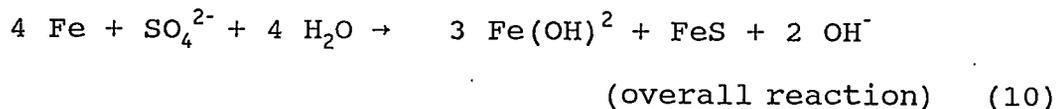
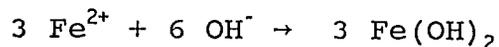
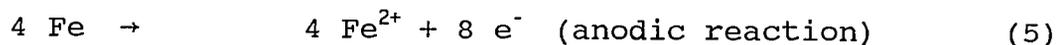
A number of information in terms of  $\mu_{max}$  and  $k_s$  for SRB are given in Table 2.

Table 2. Typical values for the maximum specific growth rate,  $\mu_{max}$ , and the saturation constant,  $k_s$ , for lactate-utilizing SRB in lactate, sulfate medium at temperature = 30 ° C.

Bacterial species	$\mu_{max}$ ( $h^{-1}$ )	$k_s$ (mg/L)	pH	Reference
<u>D. desulfuricans</u>	0.041	-	6.5	Cork et al (1979)
<u>D. desulfurucans</u>	0.360	-	7.4	Cappenberg (1975)
<u>D. desulfuricans</u>	0.104	-	-	Traore (1982)
<u>D. vulgaris</u>	0.011	0.461	7.2	Ivgvorsen (1984)
<u>D. sapovorans</u>	0.007	0.700	7.2	
<u>D. salexigens</u>	0.021	7.363	7.2	

Mechanisms of Metal Corrosion by SRB

Anaerobic bacteria, particularly sulfate reducers, have been the most studied microorganisms in biological corrosion investigations. The early work of Von Wolzogen Kühr and van der Vlugt (1934) suggested the following electrochemical reactions:



The overall process was described as "depolarization" based on the theory that these bacteria remove hydrogen that accumulates on the iron surface. The electron removal as a result of hydrogen utilization results in cathodic depolarization forcing more iron to be dissolved at the anode. The direct removal of hydrogen from the surface is equivalent to lowering the activation energy for hydrogen removal by providing a "depolarization" reaction (Figure 1). The enzyme, hydrogenase, synthesized by many species of Desulfovibrio, may be involved in this specific depolarization process. Booth et al. (1962) using polarization techniques and weight loss

measurements versus hydrogenase activity have provided additional evidence to substantiate this theory based on batch culture experiments. However, Booth et al (1966, 1967) further proved that this relationship does not exist in low and high ferrous iron medium in semi-continuous or continuous culture experiments. Low rates of corrosion were observed in low iron concentration medium due to the formation of a protective iron sulfide film. However when these films ruptured, very high rates of corrosion occurred. Mara and Williams (1972) observed the same trend and concluded that film breakdown resulted from sulphidation of the primary corrosion product mackinawite,  $\text{FeS}_{1-x}$  to greigite  $\text{Fe}_3\text{S}_4$ , resulting in an increase in product volume.

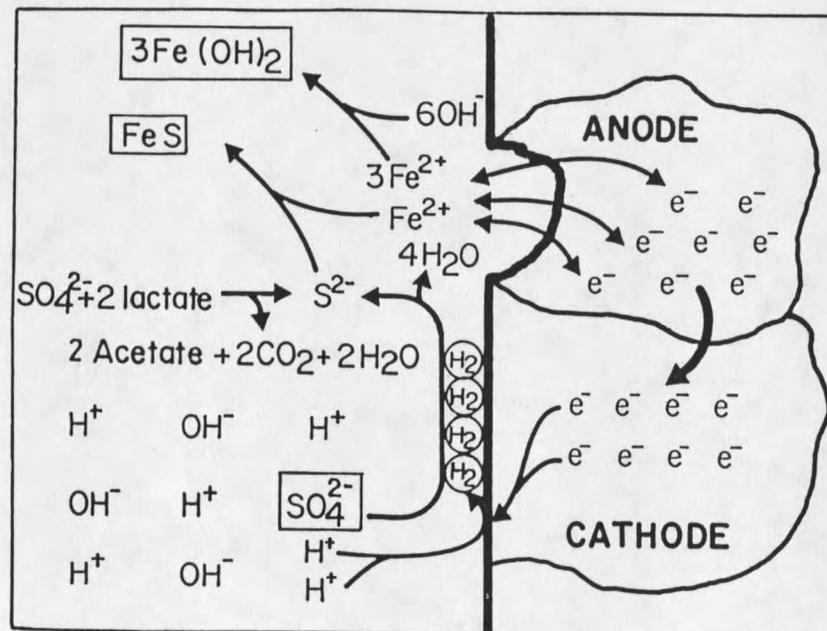


Figure 1. Proposed reaction of anaerobic corrosion in the presence of SRB on an iron surface.

Miller and Tiller (1970) have proposed cathodic "depolarization" induced by microbially produced FeS as a corrosion mechanism. King and Miller (1973) demonstrated that weight loss of steel as proportional to the concentration of ferrous sulfide and the stoichiometry of the particular ferrous sulfide minerals. They concluded that accelerated corrosion of mild steel in the presence of SRB was due principally to the formation of iron sulfide. Because iron sulfide is not a permanent cathodic depolarizer, sustained corrosion rates were found to be dependent on the removal of the bound hydrogen by the action of bacterial hydrogenase. The comparison of the two mechanisms is shown in Figure 2.

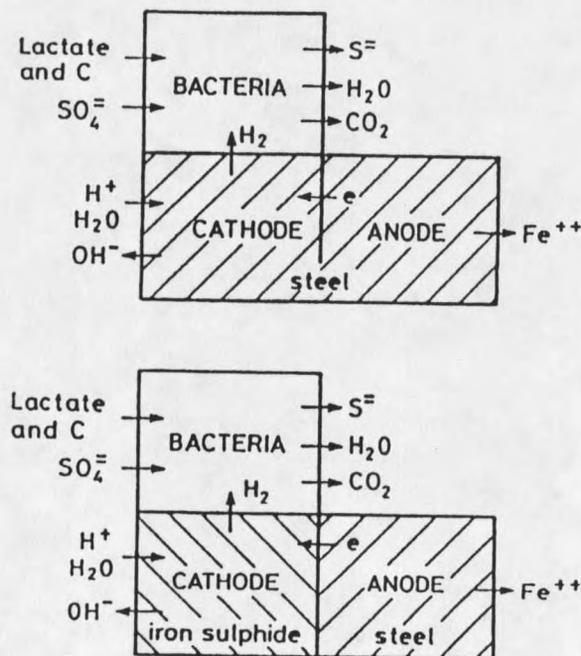


Figure 2. Schematic representation of the classical mechanism (top) and alternative mechanism (bottom) of microbial corrosion under anaerobic conditions (after Miller & King).

Costello (1974) proposed that dissolved  $H_2S$  produced by SRB is responsible for the cathodic depolarization. Further evidence was provided by Togano et al (1975) who demonstrated that the corrosion rate was proportional to the concentration of  $H_2S$  produced by SRB, and it was suggested that  $H_2S$  accelerated both anodic and cathodic reactions. The cathodic depolarization theories are summarized in Table 3.

Table 3. The history of cathodic depolarization theories.

Yr	Experimen- ter	Type of Bacteria	Type of Culture	Medium	Depol- arizer	Mater- ial	Experi- mental period
'34	Von Wolzo- gen Kuhr	D.desul- furicans	batch	wet soil	hydro- genase	iron	/
'62	Booth and Tiller	D.sale- xigens	batch	rich*	hydro- genase	mild steel	11 days
'68	Booth et al.	SRB	Semi & contin.	rich*	FeS	mild steel	60 weeks
'72	Mara and Milliams	D.vul- igaris	contin.	rich*	FeS	mild steel	10 weeks
'73	King and Miller	D.desul- furicans	batch	rich*	hydro- genase & FeS	/	/
'74	Costello	SRB	batch	rich*	$H_2S$	mild steel	8 days

\* rich means the media has carbon between 1000 to 2000 mg/L.

The impact of oxygen on obligate anaerobic SRB was examined by Hardy and Brown (1984) using a synthetic seawater

































































































































































































