



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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APPROVAL

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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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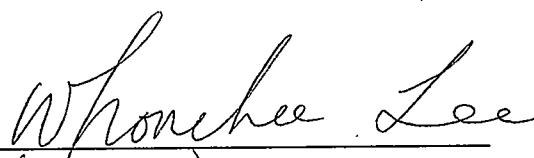
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ABSTRACT

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 μm . 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 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 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,	Growth HR. 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 ₁₂
<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

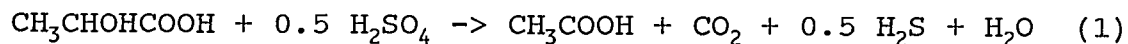
Stoichiometry and Kinetics of SRB

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.

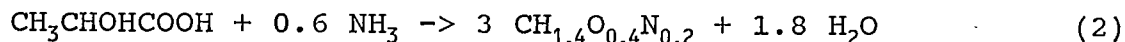
Stoichiometry of SRB

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:

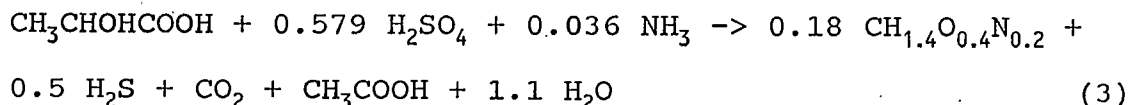
Energy with lactate



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

- μ = cellular specific growth rate (t^{-1})
- μ_{\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 $\mu = 1/2 \mu_{\max}$ ($M_s L^{-3}$)

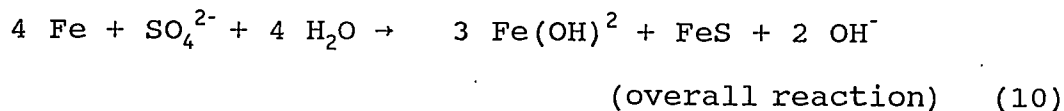
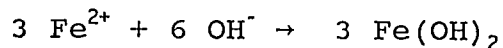
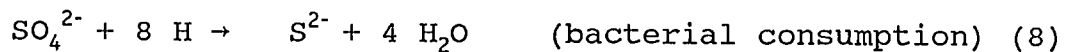
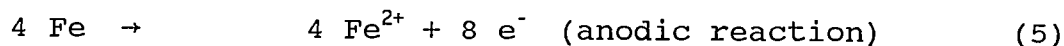
A number of information in terms of μ_{\max} and k_s for SRB are given in Table 2.

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

Bacterial species	μ_{\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, FeS_{1-x} to greigite Fe_3S_4 , resulting in an increase in product volume.

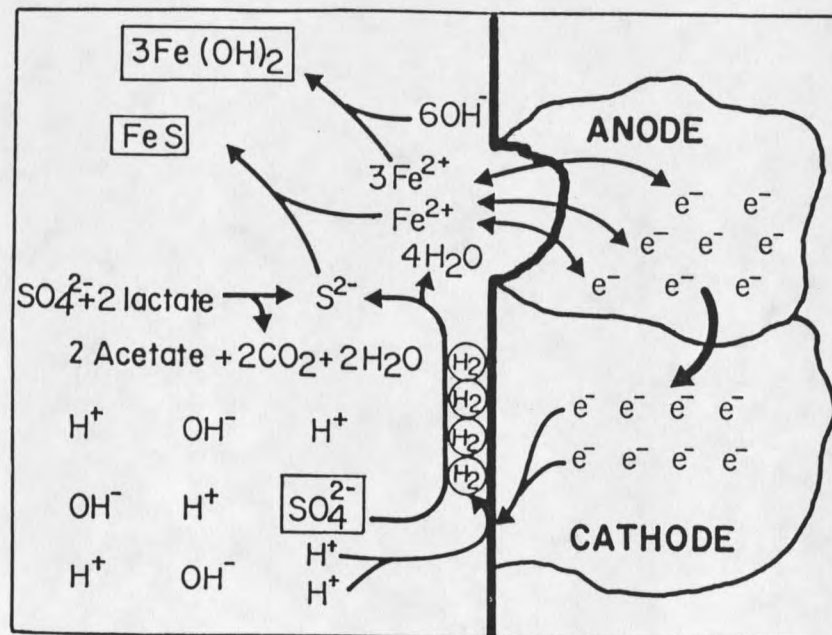


Figure 1. Proposed reaction of anaerobic corrosion in the presence of SRB on an iron surface.

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.

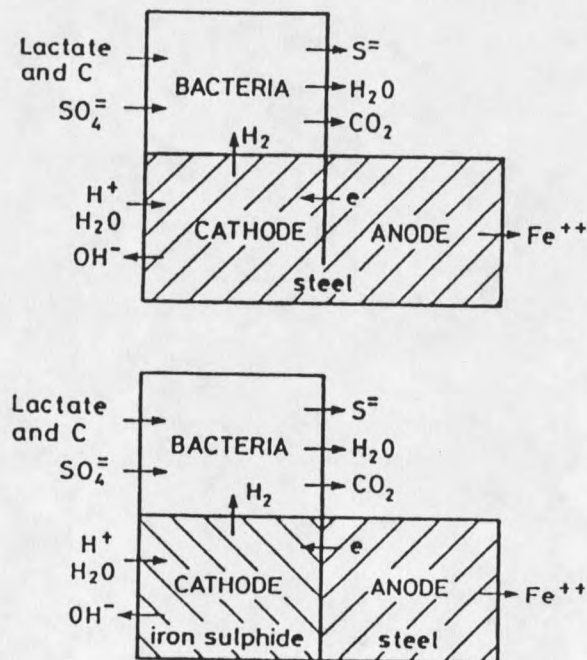


Figure 2. Schematic representation of the classical mechanism (top) and alternative mechanism (bottom) of microbial corrosion under anaerobic conditions (after Miller & King).

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

medium and a Desulfovibrio strain. Corrosion rates were determined by weight-loss measurements and by electrical resistance. Corrosion rates were low under totally anaerobic conditions, but increased with the addition of oxygen. Successive aeration-deaeration caused shifts in the corrosion rate: high rates were observed during periods of aeration. The attack was confined to areas beneath tubercles that consisted of loosely adherent material as opposed to the hard, tightly adherent films on uncorroded metal. The authors concluded that the presence of tubercles fixed the anode and forced the cathodic reaction to occur on the adherent sulfide film.

The situation in which aerobic and anaerobic conditions co-exist on metal surfaces can be achieved through the colonization of metal by aerobic micro- or macro- organisms, where due to their metabolic activities a rapid depletion of oxygen occurs. 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 (Figure 3). This anaerobic area then becomes the anode where the corrosion will take place, while the aerated region will provide the balancing cathodic reaction. Corrosion due to such oxygen concentration cells is quite common in industrial cooling water systems and heat exchangers.

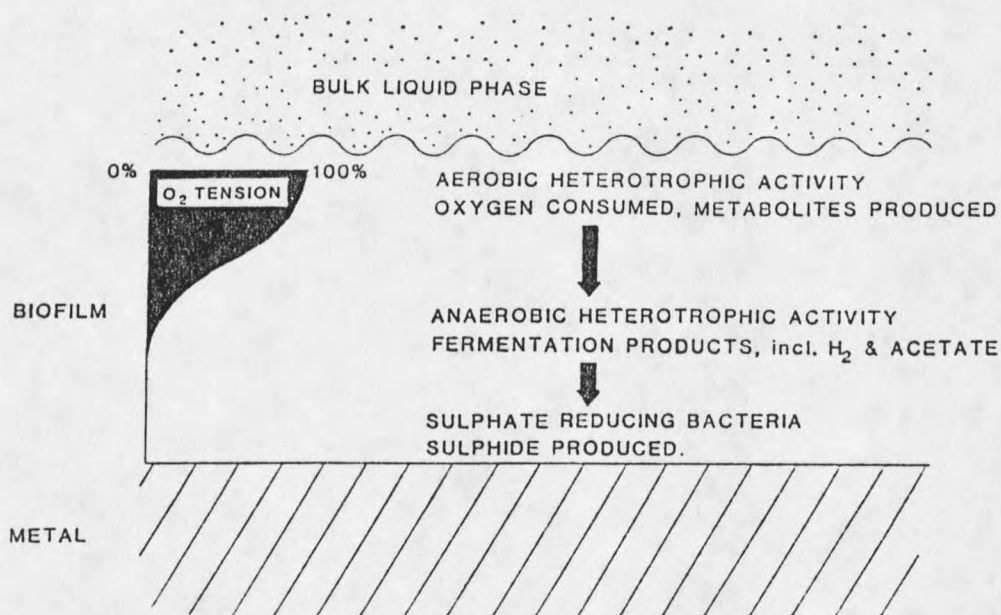


Figure 3. Model for microbial biofilm in oxygenic environment.

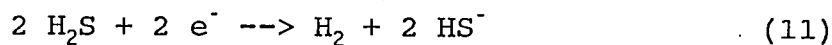
Mechanisms of H₂S Corrosion in the Absence of Bacteria

An iron sulfide film forms on the metal surface during corrosion of iron by hydrogen sulfide (Meyer et al, 1958). Surface analysis demonstrates that the type of film is dependent on pH, temperature, and hydrogen sulfide concentrations. The combined presence of CO₂ and H₂S often alters the type of film produced. Sardisco et al (1963) and Sardisco and Pitts (1965) found that iron corroding in a wet H₂S-CO₂ atmosphere at low H₂S partial pressure follows a seemingly parabolic rate expression, while at high H₂S partial pressure, distinction between linear and parabolic rate laws become impossible. Authors suggest that hydrogen sulfide pressure must affect the structure or permeability of the iron

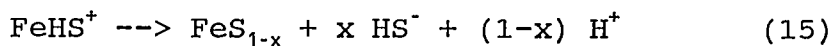
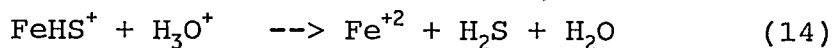
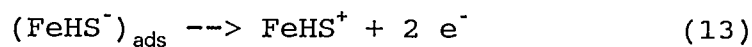
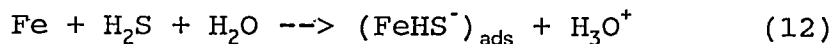
sulfide film. A number of authors have investigated the corrosion of iron by hydrogen sulfide as a function of pH (Sardisco et al, 1965; Gutzeit, 1968; Hutchison et al, 1961). All results indicate that the corrosion rate decreases with increasing pH between 4 and 7.

Mechanisms

In acid H₂S environment, the overall cathodic reaction has been suggested to be the following (Bolmer, 1965):



This reaction was found to be limited by H₂S diffusion and H₂ overpotential. It was suggested by the author that at regions beyond the diffusion current, the reduction of HS⁻ or H₂O was responsible for increased cathodic current. Iofa, Batrakov, and Ba (1965) proposed the following anodic dissolution mechanism in H₂SO₄ containing Na₂S at 25 °C:



In this mechanism, Fe is first converted to a catalyst

(FeHS^-) adsorbed on the iron surface, which then transforms to the hydrosulfide ion (FeHS^+). Subsequent to reaction (13), the species FeSH^+ may be hydrolyzed to yield a dissolved species (eg.14), or it may be incorporated directly into a growing layer of mackinawite (eg.15).

Film Diffusion Control in Corrosion Mechanism

Hausler et al (1972) demonstrated that the behavior of the iron sulfide film and its growth kinetics are principally controlled by environmental parameters such as pH and H_2S concentrations. At pH 4, they suggested that the rate-controlling film can reach a critical thickness. If the iron sulfide film grows beyond that thickness, the film breaks up and any additional thickness no longer affects corrosion rate. At pH 7, low rate of corrosion occurs due to formation of a coherent iron sulfide film. Shoesmith et al (1980) also showed the importance of pH in controlling the film properties under saturated hydrogen sulfide solutions. At pH 4, initially a solid-state reaction produces a layer of mackinawite, which readily cracks and spalls from the metal surface. This layer is replaced by a second solid-state growth mackinawite layer, which cracks again. Significant passivation was observed only at pH 7, when the initial mackinawite base layer remained virtually intact. Tewari et al (1979) concluded that corrosion of iron in saturated H_2S solution is governed by the dissolution rate of mackinawite. This dissolution is controlled by the chemical reaction between mackinawite and

H^+ and by the transport of the complexed $FeSH^+$ from the metal surface to the bulk. The developed noncoherent iron sulfide may accelerate corrosion by decreasing hydrogen overpotential but is limited by concentration-diffusion polarization (Booth et al, 1968 and Martin et al, 1981).

Localized Corrosion of Steel in the H_2S Environment

In addition to general corrosion, depending on sulfide level and pH, steel may undergo pitting corrosion. Gupta (1981) demonstrated that pitting corrosion of carbon steel was predominant at a medium sulfide level of 150 to 300 mg/L at pH 6 through 14. Brown (1970) proposed that localized corrosion is initiated and propagated due to the local acidity. The acidity is caused by hydrolysis of one or more corrosion products of the metal or alloy, and the acidity persists because of the restricted mass transfer between the corrosion cell and bulk environment caused by the porous corrosion products. Sulfide inclusions such as $(Mn,Fe)S$ in the steel can also act as sources of pit nucleation (Smialowska, 1972):

Use of Electrochemical Techniques for Studying Microbial Corrosion

Microorganisms influence corrosion by changing the electrochemical conditions at the metal surface. Theoretically, these changes may have many effects ranging from the induction of localized corrosion to a change in the

rate of corrosion, to corrosion inhibition. In every case, however, the process of corrosion is electrochemical and electrochemical techniques can generally be applied in the investigation of corrosion under both aerobic and anaerobic conditions.

Recently, a greater recognition of the complexity of the microbial corrosion process has developed. Microbial corrosion is rarely linked to a unique mechanism or to a single species of microorganisms. In the present state of knowledge, it is widely accepted that the growth of different microbial species within adherent biofilms facilitate the development of structured consortia that enhance the microbial effects on corrosion. Therefore, interpretation of electrochemical data from systems in which complex biofilm, consisting of microorganisms and their extracellular polymeric substances (EPS), must also consider the water chemistry in the biofilm.

Electrochemical methods have been used in complex electrolytes, such as microbiological culture media, where the type and properties of passive films are not fully known. The presence of high concentrations of aggressive anions and pitting inhibitors in these solutions make interpretation of the experimental results difficult. Microbial colonization of passive metals can drastically change the metal resistance to breakdown by locally changing the type and concentration of anions, pH, oxygen, paints and inhibitors. These changes may

result in significant modifications in the electrochemical behavior of the metal and in the electrochemical parameters used in laboratory experiments.

In many cases, laboratory studies have been made without considering the presence of different passive films on the metal before microbial colonization. The presence of a complex layer of corrosion products, cells and EPS may drastically modify the results of conventional electrochemical techniques. Some techniques must be used with special care at a biologically conditioned metal/solution interface, where classical electrochemical parameters are not always valid. However, the use of appropriate electrochemical methods coupled with a careful characterization of the microbiological components can lead to a better understanding of the corrosion mechanisms.

Open Circuit Corrosion Potential

The potential of a corroding metal can be measured by determining the voltage difference between that metal immersed in a corrosive medium and an appropriate reference electrode such as the saturated calomel electrode (SCE). The magnitude and sign of the corrosion potential are functions of the metal composition, as well as the composition, temperature and hydrodynamics of the electrolyte. The open circuit potential is measured using a potentiometer, or a high impedance digital voltmeter or electrometer. These instruments are capable of accurately measuring small voltages without drawing enough

current to polarize the reference electrode.

Because of its simplicity, measurement of the corrosion potential has been used in microbial studies for many years. The progress of open circuit potential has been used to assess the electrochemical behavior of steel specimens immersed in batch cultures of SRB (Hadley, 1943; Wanklyn and Spruit, 1952; Horvath, 1960). The initial potential decay observed in these media was attributed to cathodic depolarization of the steel electrode (Hadley) or to enhancement of the anodic process (Wanklyn and Spruit). Some years later, in similar experiments, Horvath attributed the steady-state corrosion potential after the initial drop to the formation of a semiprotective film of iron sulfide. Early use of open-circuit potential for studying in SRB corrosion were limited by the lack of complementary measurements. However, a plot of potential vs. time was useful for detecting the initiation of accelerated attack due to bacteria.

Redox Potential

The oxidation-reduction (or redox) potential is useful for predicting whether or not a metal will corrode in a given environment. Redox potential is the potential associated with the oxidation and reduction of hydrogen or oxygen in a corrosive environment and is a function of the oxygen concentration, temperature, and pH. At a given temperature and pH, the redox potential of any natural aqueous environment becomes more negative (or active) with decreasing dissolved

oxygen concentration. This type of redox potential measurement has been used frequently in microbiology to assess the suitability of a given environment for growth of SRB, SRB require an environment with a very active (negative) redox potential.

When measuring the redox potential, the concurrent measurement of pH is necessary because the experimental values have to be referred to a standard pH. Strictly speaking, the redox potential is only defined for an electrochemical reaction under equilibrium (zero current flow) conditions, and it must be measured by special electrochemical techniques under carefully controlled equilibrium conditions. Such equilibrium conditions are not usually encountered in living systems, such as within the microbiological films of interest here. Nevertheless, steady-state conditions do occur in microbial cultures and films, and the redox potential measured under these conditions can provide useful information on the redox characteristics of that environment and any environmental changes resulting from bacterial metabolism. Generally, a noble (positive) redox potential value for a given environment indicates conditions favorable for corrosion. If a noble redox potential occurs together with a noble corrosion potential and an active (negative) pitting potential for a particular alloy, it does indicate that the environment is very aggressive for that alloy.

Potentiostatic Polarization

The current-potential (Evans) diagram is a graphical method for demonstrating the relationship between corrosion rate and the polarization of anodic and cathodic reactions in which both reactions are presented as linear E-i curves that converge and intersect. This intersection point corresponds to the corrosion potential and the corrosion current density. The corrosion current density cannot be measured directly since the current involved is one that flows between numerous microscopic anodic and cathodic sites on the surface of the corroding metal. A convenient practice is to employ a potentiostat in conjunction with a reference electrode to polarize the specimen first in the active (or cathodic) direction and then in the noble (or anodic) direction. In this procedure, the specimen is initially made to act as a cathode in the electrochemical cell and the potential-current density curve is measured over several hundred millivolts active to the open circuit corrosion potential, varying the applied potential in discrete steps (25 mV every 5 minutes is common). At the corrosion potential, the applied potential is zero, and the measured cathodic current density approaches zero.

Pitting Potentials

Microorganisms often stimulate localized forms of corrosive attack, such as pitting, depending on the passive film formed on the metal or alloy. Thus, different methods for determining characteristic pitting potential values can be

useful in defining the tendency of microorganisms to accelerate pitting in a given metal/solution system. "Pitting potential", as used in the literature, is a non-specific term related to the onset of pitting corrosion. The numerical value obtained for the "pitting potential" depends strongly on the experimental technique used to measure it. Strictly speaking, the value measured by each of these techniques should be called by a different name. As an example, the "critical pitting potential," E_{cpp} , is usually defined as the most noble potential at which the passive current density remains stable and pits do not nucleate (on a crevice-free surface). E_{cpp} is measured potentiostatically in the laboratory under carefully controlled conditions. If measured carefully, E_{cpp} is independent of the geometry of the specimen and the test apparatus. The "breakdown potential," E_p , is defined as the potential at which the anodic polarization curve shows a marked increase in current, leading to breakdown of the passive film and pit initiation. E_p is usually measured by potentiodynamic polarization sweep techniques and depends on both the sweep rate and experimental geometry.

Several laboratory studies (de Mele et al., 1979; Salvarezza et al., 1979; Salvarezza and Videla, 1980; Videla, 1986a; Gaylarde and Videla, 1987) indicate that the presence of certain microorganisms decreases (makes less noble) the E_p values of several metals and alloys. In one study (Salvarezza and Videla, 1980), SRB and sulfide in artificial sea water

resulted in equivalent E_p values of 100-200 mV more active than in sea water alone. These experimental results suggest that, since planktonic SRB behave in a similar manner to chemically added sulfide, the action of anaerobic bacteria on the corrosion of mild steel is a consequence of metabolic sulfide production. In addition, the breakdown of passivity was accomplished more easily in deaerated solutions where lower levels of sulfides were needed.

Alternating Current Methods

In recent years, electrochemical impedance techniques have been used for measuring the polarization resistance and other characteristics of a corroding metal surface. Problems of accuracy and reproducibility frequently encountered in the application of direct current methods have led to an increasing use of electrochemical impedance spectroscopy (ECIS). The rapid development of commercially available instrumentation allows one to take measurements over a wide range of frequencies from 10^{-3} to above 10^3 Hz.

Direct current techniques assess the overall corrosion process occurring at a metal surface but treat the metal/solution interface as if it were a pure resistor. The ECIS technique measures not only the resistive, but also the capacitive and inductive components of the overall interfacial impedance. Thus, ECIS is particularly useful in the presence of non-conducting and semi-conducting surface films such as organic paints and many metal oxide films. The organic and

microbiological films that adsorb to all surfaces immersed in natural aqueous environments are also non-conducting, so ECIS techniques are potentially useful in their presence. Some caution is required in data interpretation, however, because the adsorbed organic and biological films tend to provide patchy, rather than continuous surface coverage.

ECIS techniques are also able to distinguish between electrochemical reactions based on their relaxation times. A given electrochemical reaction will only respond to an alternating current signal whose period is longer than the characteristic time for the rate limiting step for that reaction to occur across the interface. The evaluation of the impedance spectrum of an electrode over a wide range of frequencies can then be used to distinguish among the various processes taking place at the interface between the metal and the electrolyte or between the metal and a film or coating.

ECIS techniques in microbial corrosion research should be used with caution: a) The interpretation of ECIS data depends largely on the development of equivalent electrical circuits for modeling of the solid/electrolyte interface. These equivalent circuits are difficult to formulate for complex interfaces involving partial coverage by microbial films. b) The combination of microbial film and corrosion products often encountered in microbial corrosion causes the impedance to become very high at low frequencies, thus requiring extrapolation. c) Microbial films are not stable

like paint films or even metal oxide films. Rather, microbial films are dynamic entities, within which changes are continually occurring as a result of metabolism. Such changes can cause short-term fluctuations in the electrochemistry at the metal/film interface, causing localized attack under the film that is not easily detected. d) The period of these fluctuations can be shorter than the time necessary to gather ECIS data when using a full frequency sweep, meaning that not all frequencies of the applied signal are responding to the same electrochemical conditions (Lee, 1986). e) In addition, it must be emphasized that the continuous changes occurring at the metal solution interface due to biofilm formation and detachment, and microbial activity within the biofilm, cannot be adequately interpreted through equivalent electrical circuits.

EXPERIMENTAL

Experimental Reactor

All experiments were conducted in a continuous open channel flow reactor with dimensions 1.00 m x 0.15 m x 0.30 m and containing a working volume of 4.5 liters and a surface area of 3.5 m² (Figure 4).

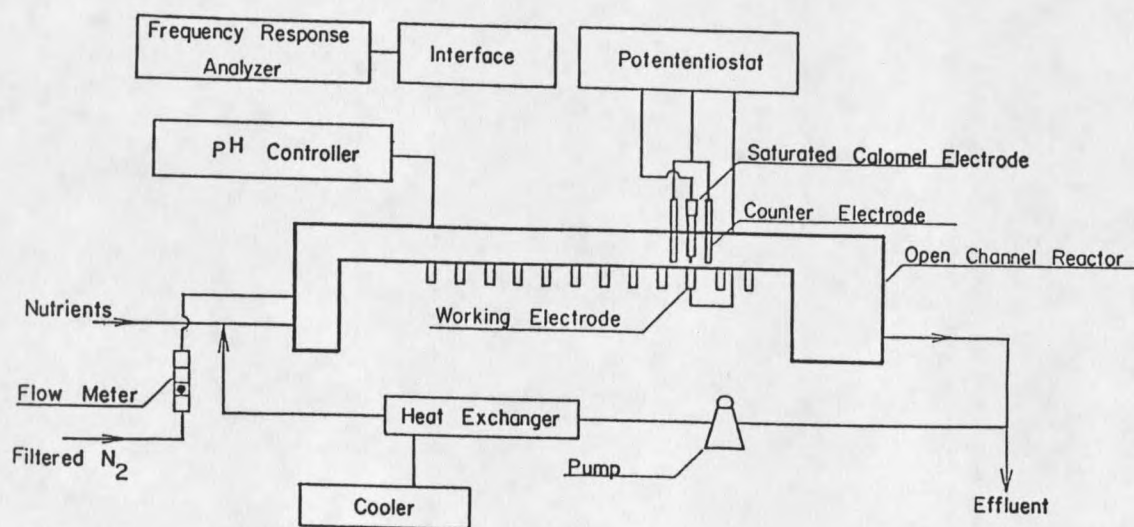


Figure 4. Continuous open channel flow reactor with dimensions 1.00 m x 0.15 m x 0.30 m and with a working volume of 4.5 liters and a surface area of 0.35 m².

The reactor has a recirculation loop to provide sufficient mixing and a flow velocity of approximately 0.3 m/s. The whole experimental system is sparged with nitrogen to minimize air permeation. The purge gas was first passed through hot copper filings to remove traces of oxygen. The reactor contains twenty-four (24) corrosion coupons which are connected to a potentiostat.

Media and Biofilm Growth Conditions

Bacteria were grown in 1/10 strength artificial seawater, 200 mg/L lactate acid, 50 mg/L yeast extract, 50 mg/L NH_4Cl , and 5 mg/L Na_2HPO_4 . The ionic composition of 1/10 strength artificial seawater is listed in Table 4. Ferrous iron was absent in the media except for selected experiments. The pH was controlled at 8 with 0.1 N NaOH and 0.1 N HCl. The stock solution of both NaOH and HCl were flushed with nitrogen and reduced by adding 0.013% Na_2SO_3 . Temperature was controlled at 30° C by a heat exchanger and cooler. Desulfovibrio desulfuricans (ATCC 5575) was inoculated into the open channel flow reactor which, for the first week, was operated as a batch reactor until surface colonization occurred. Thereafter, substrate was continuously fed to the reactor. The experiment was not conducted aseptically in order to simulate natural marine environments in which the SRB grow.

Table 4. The ionic compositions of 1/10 artificial seawater.

ION		PPM @ 3.4%
Chloride	[Cl ⁻]	1,878.8
Sodium	[Na ⁺]	1,042.4
Sulfate	[SO ₄ ⁼]	257.7
Magnesium	[Mg ⁺⁺]	126.5
Calcium	[Ca ⁺⁺]	39.8
Potassium	[K ⁺]	37.1
Bicarbonate	[HCO ₃ ⁻]	14.5
Borate	[BO ₃ ⁼]	2.8
Phosphate	[PO ₄ ⁼]	0.14
TOTAL		3,399.74ppm

Coupons

Disks of AISI 1018 mild steel (Metal Samples, Inc., Mumford, AL.) 15.9 mm in diameter were cast in acrylic resin to minimize crevice corrosion. The coupons were polished with grit paper (120, 200, 320, 400, 600) followed by ultrasonic cleaning in 100% alcohol for 1 minute and dried in air at room temperature. The elemental composition of AISI 1018 mild steel are presented in Table 5.

Table 5. Elemental composition of AISI 1018 mild steel

Element	C	P	S	Mn	Fe
%	0.20	0.01	0.014	0.76	Balance

Corrosion Measurements

Corrosion potential, polarization, AC impedance, and weight loss were measured in the open channel flow reactor periodically for up to 21 days. DC measurements were made using a Princeton Applied Research Corporation (PARC) 273 Potentiostat/Galvanostat and AC measurements using a Solartron 1250 Frequency Response Analyzer through a Solartron 1186 Electrical Interface.

The potential of the working electrode was measured against a saturated calomel electrode (SCE). Two graphite rods were used as counter electrodes. Cathodic polarization measurements were performed from E_{corr} to 400 mV in the cathodic direction at a scan rate 0.2 mV/s. Pitting potential measurements were performed beginning at -800 mV in the anodic direction with the same scan rate as cathodic polarization. AC impedance measurements curve made using a sine wave voltage with an amplitude of 7.5 mV at frequencies between 2 mHz and 10 KHz. Ten frequencies were examined per decade. At the end of each experiment, coupons were removed from the reactor and cleaned in an ultrasonic bath with an inhibited acid solution for 30 seconds prior to the weight loss measurement.

Water Chemistry

Desulfovibrio desulfuricans uses lactate as a carbon and energy source and oxidizes it to acetate coupled with the reduction of sulphate to sulfide. As a consequence, the

chemical analysis of the reactor fluid included lactate, acetate, sulfate, total dissolved sulfide, and redox potential. Samples were taken from both influent and effluent to complete material balances.

Lactate was determined by an enzymatic method (Boehringer Mannheim GmbH). Acetate was measured with a Varian 3700 gas chromatograph. Sulfate concentration was determined using the barium chloride turbidimetric method (Standard Method, 1971). Total dissolved sulfide was determined by adding zinc acetate to precipitate sulfide followed by use of methylene blue method (Cline, 1969). The redox potential was determined by measuring the potential of a platinum electrode using the PARC 273 Potentiostat/Galvanostat against a saturated calomel electrode.

Biological Analysis

SRB numbers in the biofilm were determined by the five (5) tube multiple dilution MPN method using Postgate B broth. A metal coupon containing a biofilm was removed from the reactor and transferred to an anaerobic tent in which the biofilm was removed by brushing into 30 ml of sterile 1/10 artificial seawater and homogenized to disperse the biofilm. A dilution series to 10^{-10} is prepared from 1 ml suspended biofilm using the normal procedure. SRB number in the bulk water phase were determined by the same MPN method for a 1 ml sample.

Numbers of GAB both in the biofilm and bulk water were determined by the same procedure as that for SRB but used fluid thioglycollate medium.

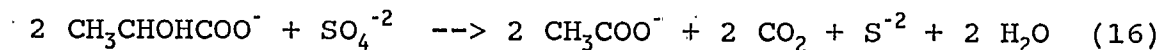
Surface Analysis of Biofilm and
Corrosion Products

A biofilm sample was transferred anaerobically to the anaerobic tent and fixed for 8 hours in 2% glutaraldehyde diluted with filter-sterilized artificial seawater. Then, it was dehydrated in a graded series of alcohol (15 minutes in alcohol 30% - 95%, and 30 mins. in 100%) washes. The sample was then dried using a Balzers CPD-020 critical point dryer for scanning electron microscopy (SEM). Finally, the sample was coated with Au-Pd alloy and examined with a ISI-40 scanning electron microscope. Composition of the corrosion products was analyzed with the SEM and a Princeton Gamma-Tech System 4 Energy-dispersive X-ray Spectrometer (EDS).

RESULTS

Stoichiometry of a Mixed Population SRB
Biofilm with Corrosion Coupon

Stoichiometric determinations were conducted in batch conditions after biofilm had accumulated in the open channel flow reactor. The exhaustion of lactate corresponded to plateau values for sulfate reduction and sulfide production (Figure 5). The decrease in sulfide concentration was due to the continuous purge of nitrogen in the reactor. Both sulfate reduction and sulfide production agreed with the theoretical calculations based on the stoichiometry of lactate oxidation:



The stoichiometric ratios provide strong evidence that SRB dominated the biofilm under the experimental conditions.

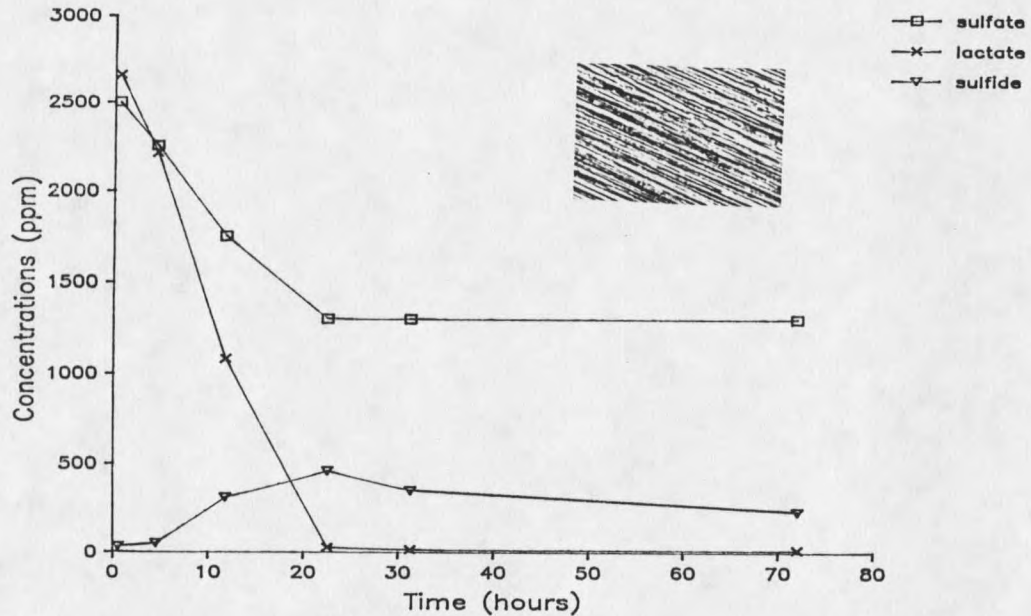


Figure 5. Sulfide production, lactate consumption, and sulfate degradation in mixed culture containing SRB. Attached picture (x50) indicates no iron sulfide corrosion products at the end of experiment.

Corrosion of Mild Steel at Different Substrate Loading Rates

Biofilm accumulation was strongly dependent on substrate loading rate. At constant substrate concentration, there was enough residence time (25 hours) for the organisms to grow in the bulk liquid at low dilution rate (0.04 h^{-1}) and resulted in a thin biofilm. It appears that bulk liquid cells compete effectively with biofilm for substrate. However, there was not enough time for organisms to grow in the bulk liquid at high dilution rate (0.75 h^{-1}) which resulted in a thick biofilm. The formation of iron sulfide precipitates on the mild steel surface was deliberately prevented before the

biofilm accumulation to reduce the interference of iron sulfide with the cathodic depolarization reaction. Dissolved sulfide in the bulk liquid and hydrogen sulfide in the gas phase were flushed away after some initial biofilm had accumulated in the open channel flow reactor. Then, corrosion experiments were initiated by insertion of clean metal coupons into the reactor followed by normal substrate input. The continuous flow experiments were conducted for 21 days.

Biofilm accumulation was a function of substrate loading rate (Figures 6 to 11). Biofilm thickness reached about 1000 μm at the high substrate loading rate while accumulating to only about 5 μm at the low substrate loading rate after 21 days. At the high loading rate, the biofilm was mainly composed of cells with a small amount of extracellular polymeric substances (EPS) (Figure 12). Much higher SRB populations were found both in the bulk liquid (Figures 13 to 14) and biofilm (Figures 15 to 16) at the high substrate loading rate. The high numbers in the bulk liquid were a result of detachment from the biofilm. The GAB numbers in the bulk water decrease as the substrate loading rate increases. Chemical analysis of the bulk water showed similar concentration of sulfide and acetate and redox potential for the two loading rates. However, there was residual lactate in the high substrate loading rate experiment (Tables 6 and 7).

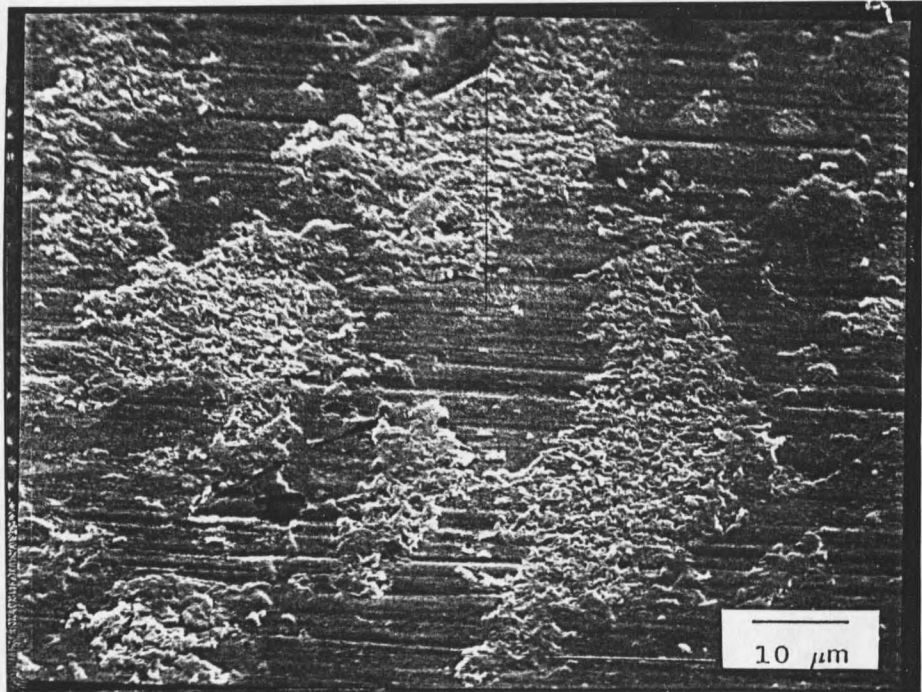


Figure 6. Scanning electron micrographs of biofilm grown on a mild steel surface for 3 days, x1300, at low substrate loading rate.

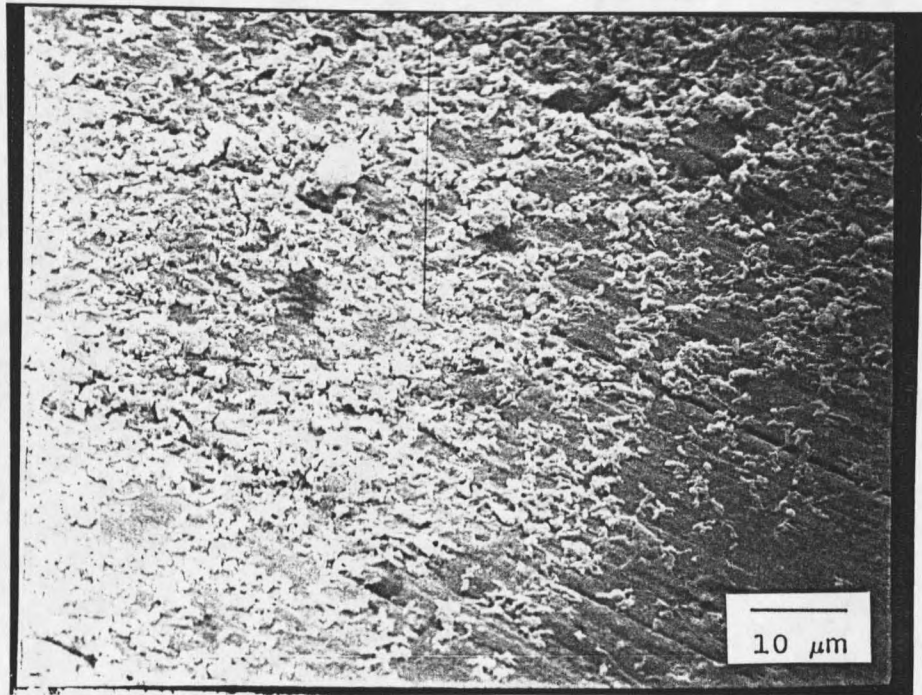


Figure 7. Scanning electron micrographs of biofilm grown on a mild steel surface for 7 days, x1300, at low substrate loading rate.

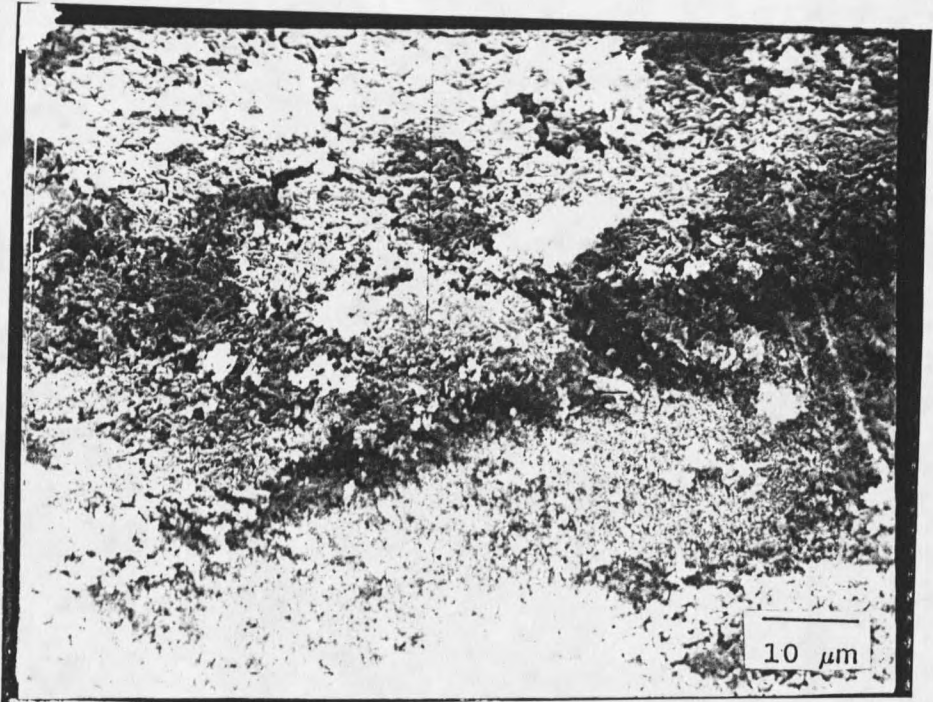


Figure 8. Scanning electron micrographs of biofilm grown on a mild steel surface for 21 days, x1300 at low substrate loading rate.

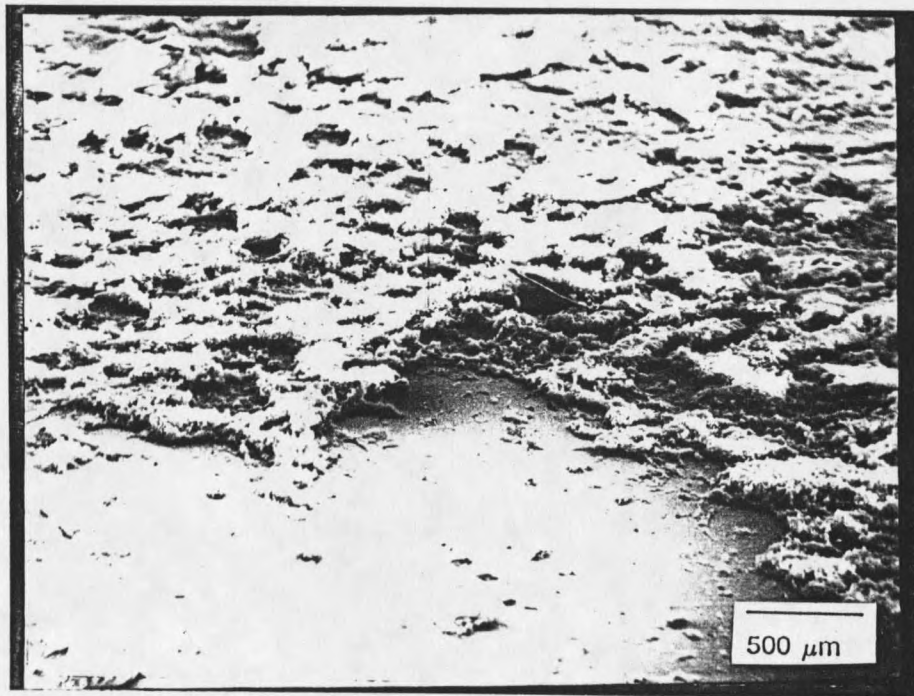


Figure 9. Scanning electron micrographs of biofilm accumulated on a mild steel surface for 3 days, x30, at high substrate loading rate.

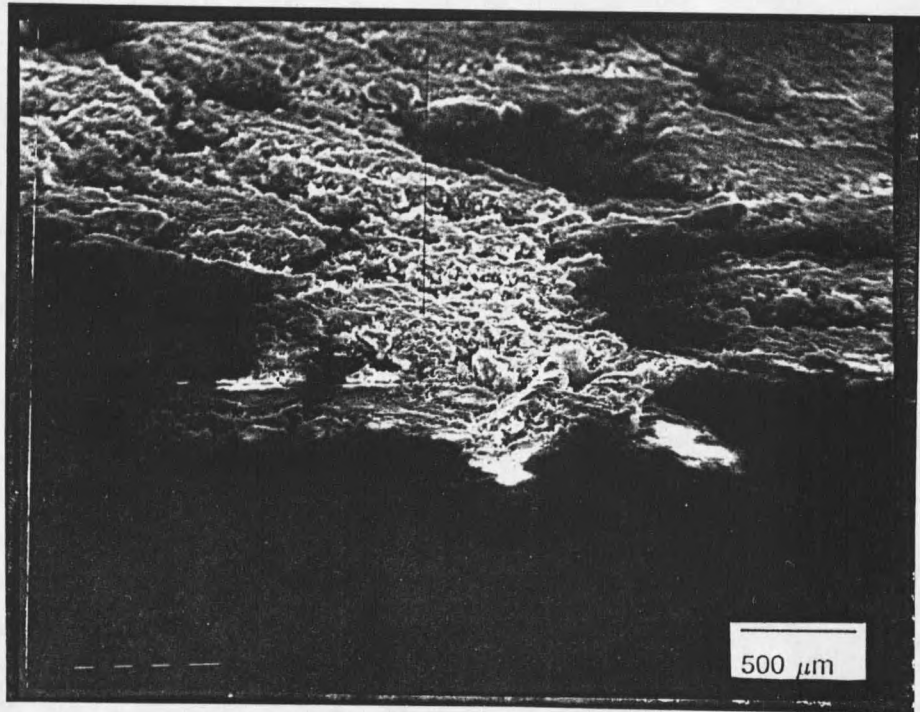


Figure 10. Scanning electron micrographs of biofilm accumulated on a mild steel surface for 7 days, x30, at high substrate loading rate.

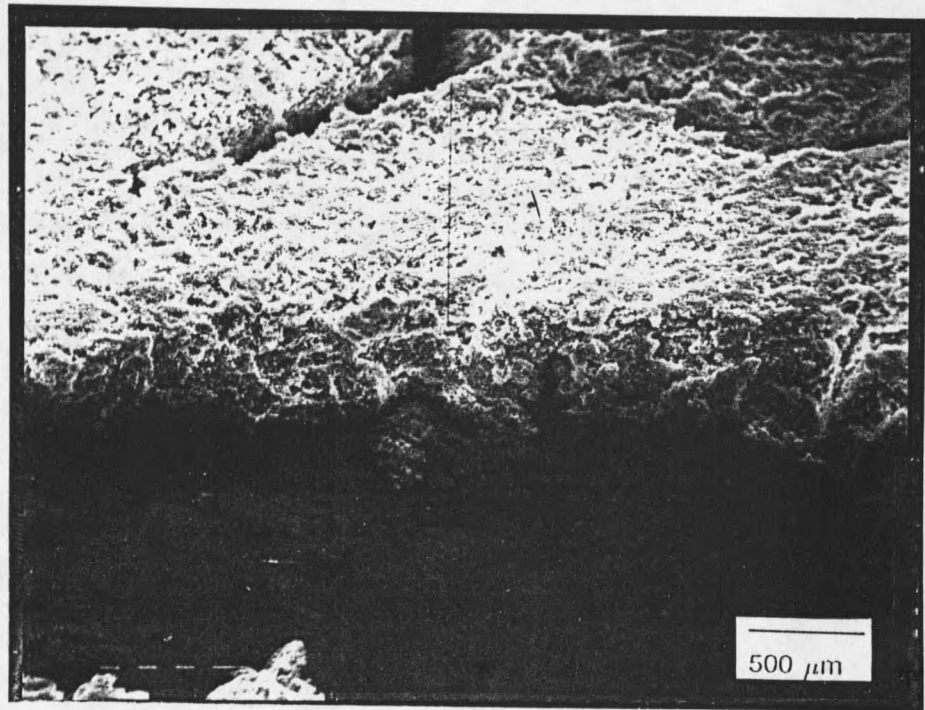


Figure 11. Scanning electron micrographs of biofilm accumulated on a mild steel surface for 21 days, x30, at high substrate loading rate.

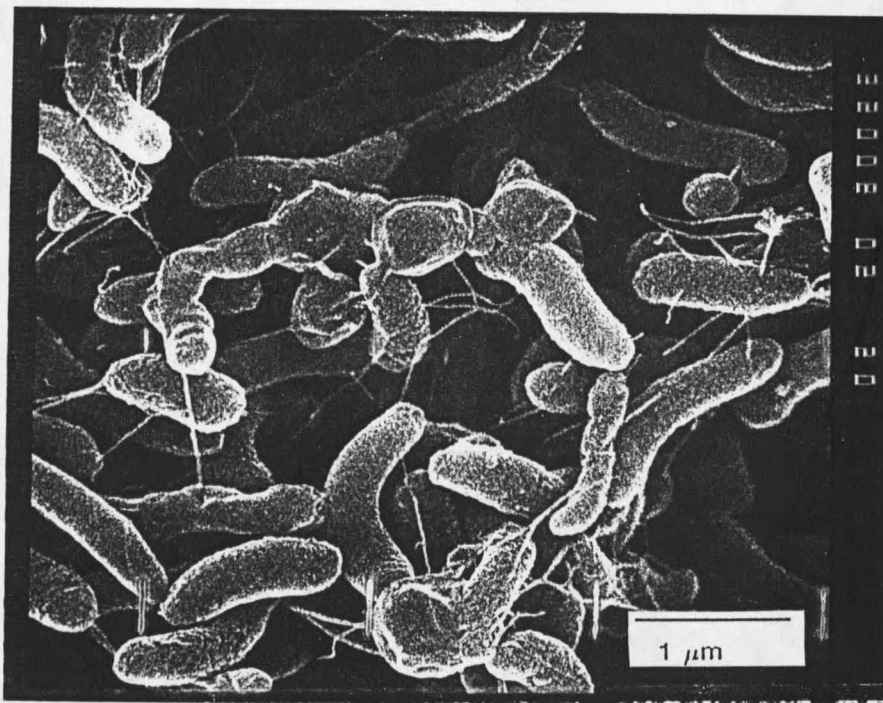


Figure 12. Scanning electron micrograph of a biofilm accumulated on a mild steel surface for 21 days at high substrate loading rate. x20,000

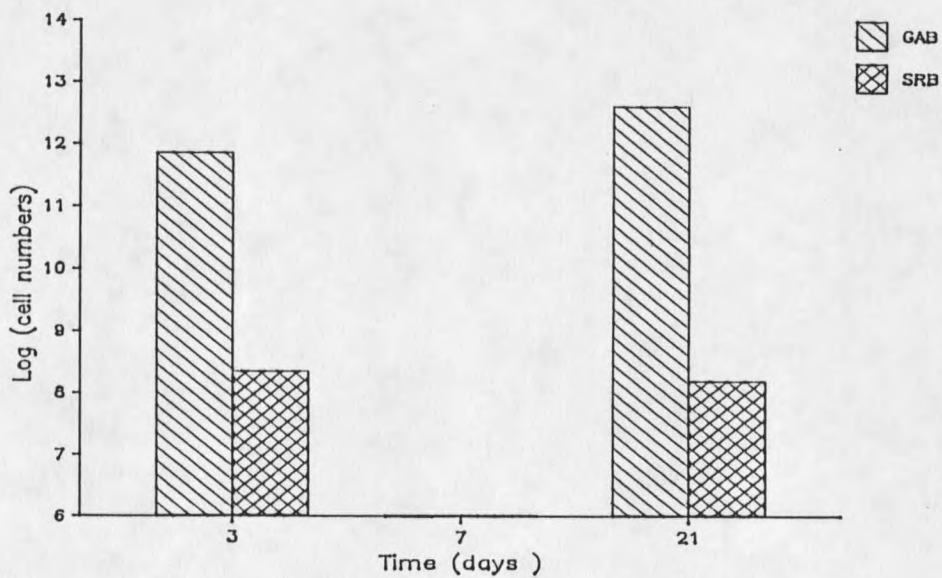


Figure 13. MPN for GAB and SRB at low substrate loading rate in the bulk water at different exposure times.

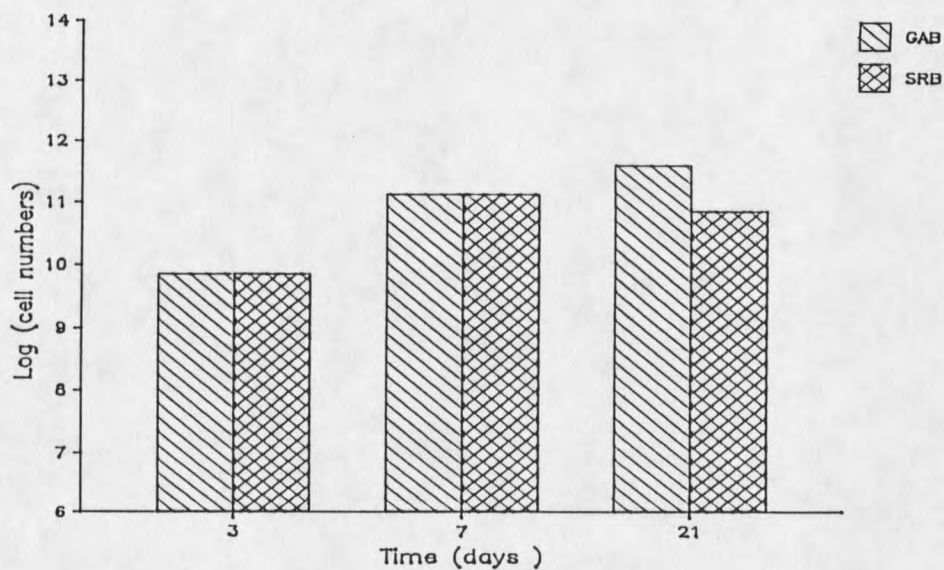


Figure 14. MPN for GAB and SRB at high substrate loading rate in the bulk water at different exposure times.

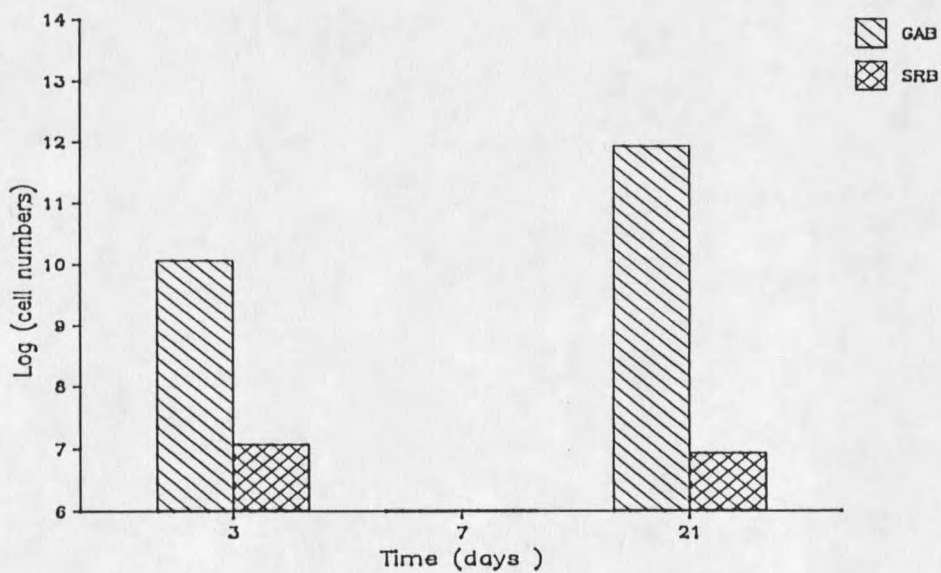


Figure 15. MPN for GAB and SRB at low substrate loading rate in the biofilm at different exposure times.

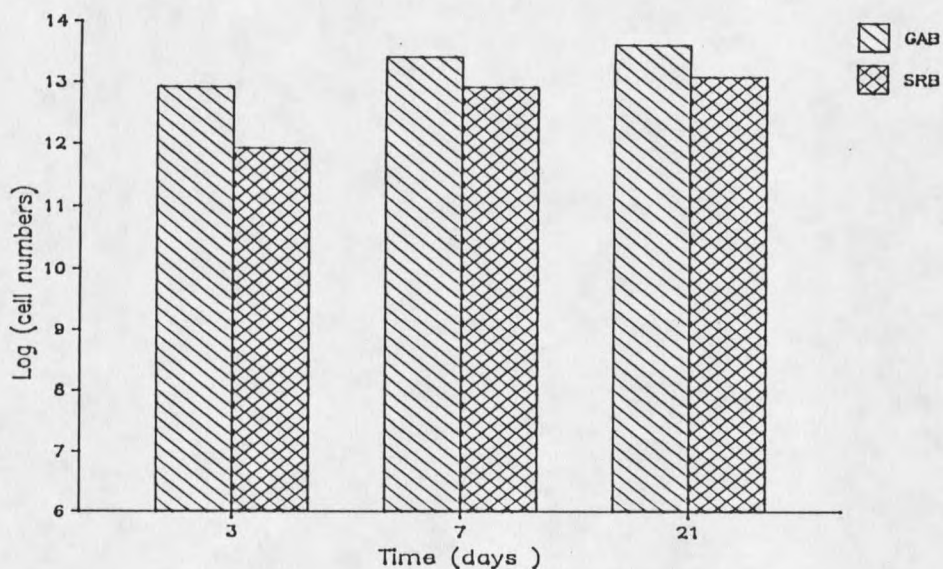


Figure 16. MPN for GAB and SRB at high substrate loading rate in the biofilm at different exposure times.

Table 6. Sulfate, lactate consumption and sulfide acetate production occurred at 25 hrs resident time with medium contained no ferrous ions.

chemicals time	sulfate consumed	lactate consumed	sulfide produced	acetate produced	redox potential
3 days	125 ppm	182 ppm	-	-	-.495V
7 days	115 ppm	202 ppm	-	-	-
21 days	120 ppm	230 ppm	58 ppm	50 ppm	-.465V

Table 7. Sulfate, lactate consumption and sulfide, acetate production occurred at 1.33 hr resident time with medium contained no ferrous ions.

chemicals time	sulfate consumed	lactate consumed	sulfide produced	acetate produced	redox potential
3 days	90 ppm	110 ppm	46 ppm	40 ppm	-.440V
7 days	120 ppm	120 ppm	46 ppm	43 ppm	-.420V
21 days	100 ppm	127 ppm	47 ppm	41 ppm	-.483V

There was no detectable difference in weight loss due to change in loading rate. The scratch lines on the coupon can be seen after 21 days under the biofilm and no iron sulfide film formed (Figure 17). An X-ray dot map (Figure 18) indicated no iron in the biofilm. No cathodic depolarization occurred either at low (Figure 19) or high (Figure 20) substrate loading rate in the absence of ferrous iron. The pitting potential becomes more noble as the biofilm become thicker both at low (40 mV, Figure 21) and high (150 mV, Figure 22) substrate loading rates. There was no pitting or localized corrosion observed on the metal surface under open circuit conditions after 21 days.

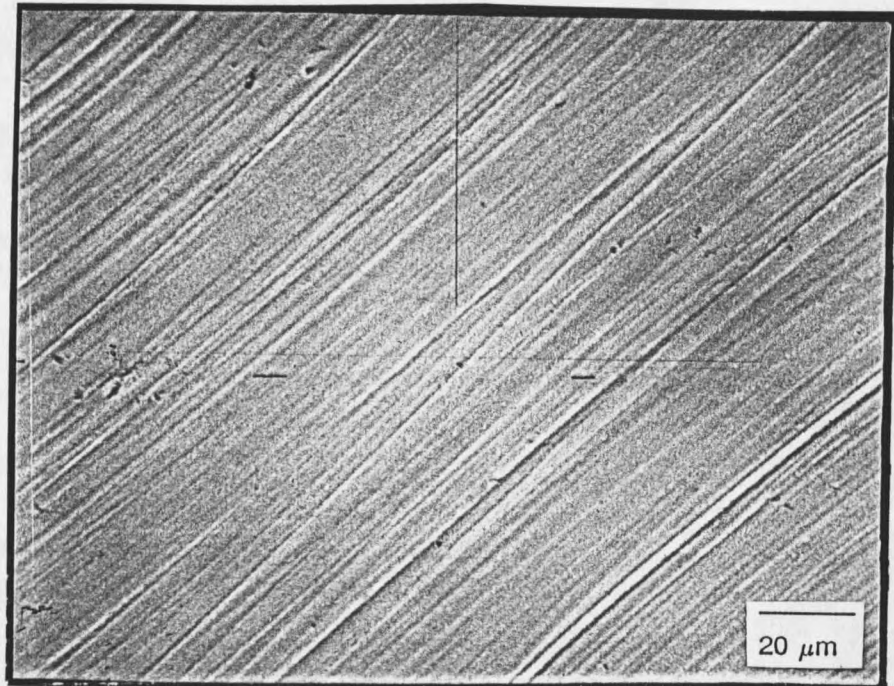
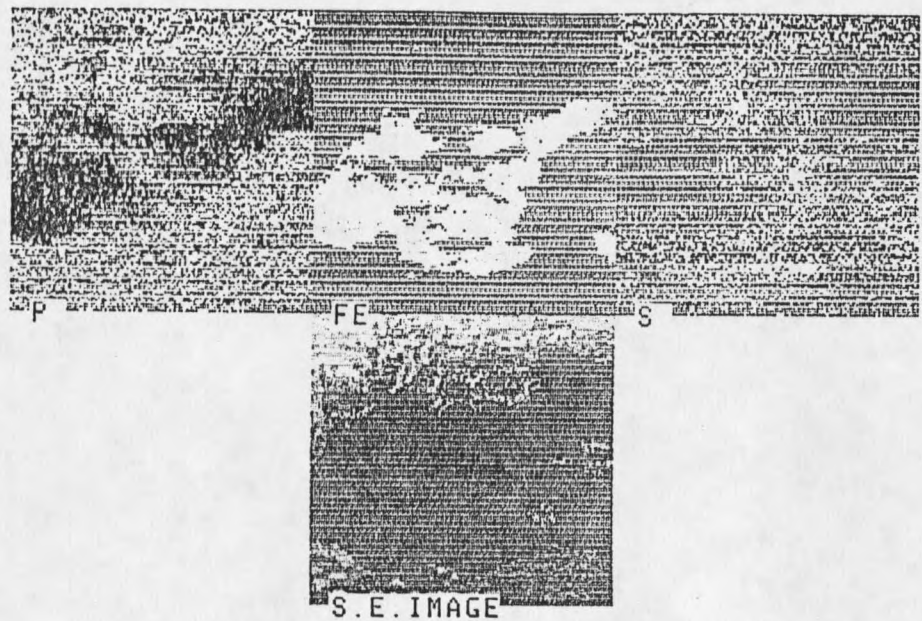


Figure 17. Scanning electron micrograph of a mild steel surface after biofilm has been removed at 21 days. x700



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Figure 18. X-ray dot map indicating the elemental distribution of iron, sulfur, and phosphorous on the top of biofilm and the cross section of biofilm and metal surface.

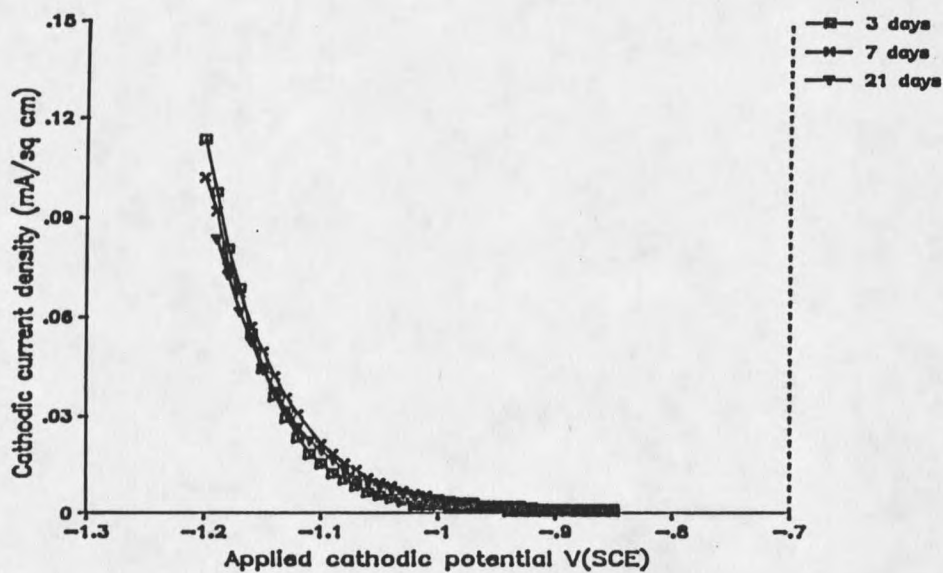


Figure 19. Cathodic depolarization curves of mild steel at low substrate loading rate at different exposure times.

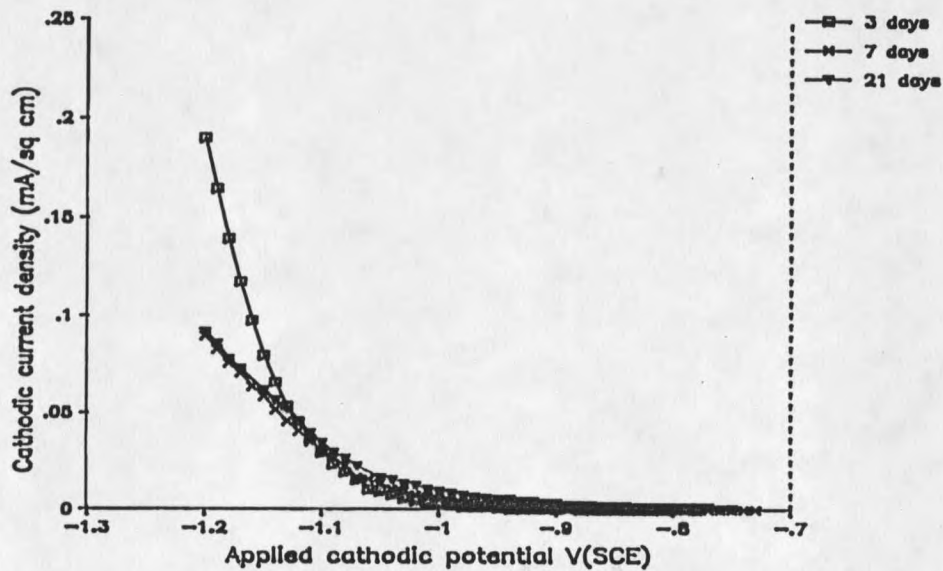


Figure 20. Cathodic polarization curves of mild steel at high substrate loading rate at different exposure times.

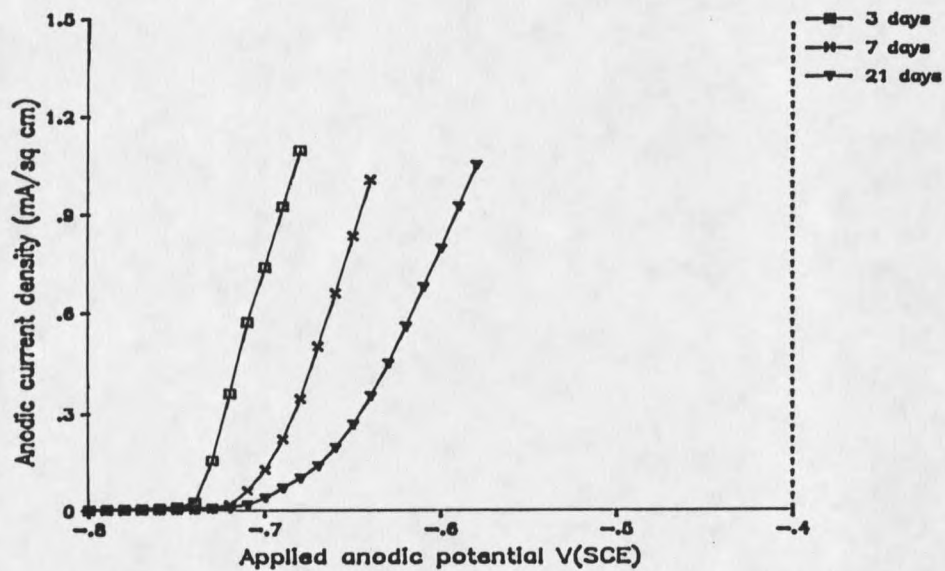


Figure 21. Anodic polarization curves of mild steel at low substrate loading rate at different exposure times. Pitting potential becomes more noble (40mv) during 21 days experiment.

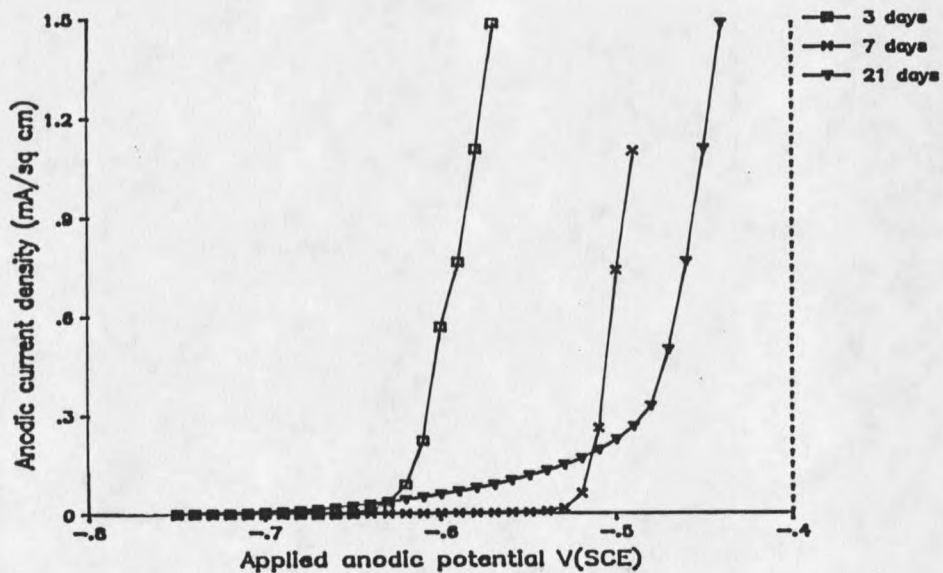


Figure 22. Anodic polarization curves of mild steel at high substrate loading rate at different exposure times. Pitting potential becomes more noble (150mv) during 21 days experiment.

Corrosion of Mild Steel with a Precoated Biofilm
at Different Ferrous Ions Concentration

The mild steel coupons were precoated with biofilm at a high substrate loading rate for one week in the absence of ferrous ions followed by weekly step increases in ferrous ion to 1, 10, and 60 mg/L.

There were higher populations of SRB and general anaerobic bacteria (GAB) in the biofilm than that in the bulk water during the experiment (Figures 23 to 24). The total dissolved sulfide concentration decreased in the bulk water as ferrous ion concentration increased in the influent and reached zero when ferrous ion concentration was 60 mg/L. There was residual lactate during the experiment (Table 8)

Table 8. Sulfate, lactate consumption and sulfide, acetate production occurred at 1.33 hr resident time with different ferrous ions concentrations.

chemicals time	sulfate consumed	lactate consumed	sulfide produced	acetate produced	redox potential
7 days	65 ppm	70 ppm	50 ppm	30 ppm	-.450V
14 days	90 ppm	98 ppm	50 ppm	35 ppm	-.450V
21 days	83 ppm	71 ppm	40 ppm*	25 ppm	-.440V
28 days	85 ppm	84 ppm	0 ppm*	-	-

* Sulfide ions in the bulk water were precipitated with ferrous ions and cannot be detected accurately.

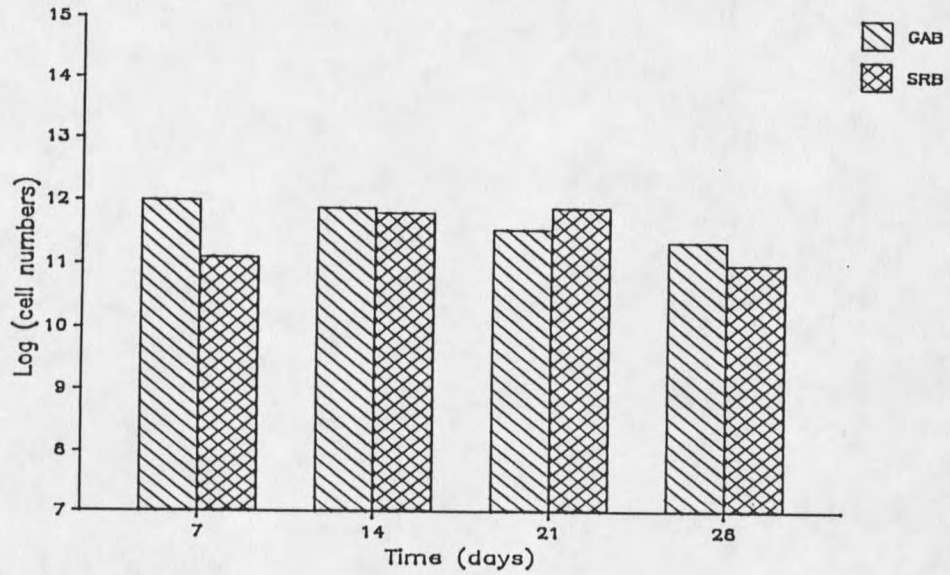


Figure 23. Most probable number (MPN) for GAB and SRB in the bulk water at different ferrous ion concentrations.

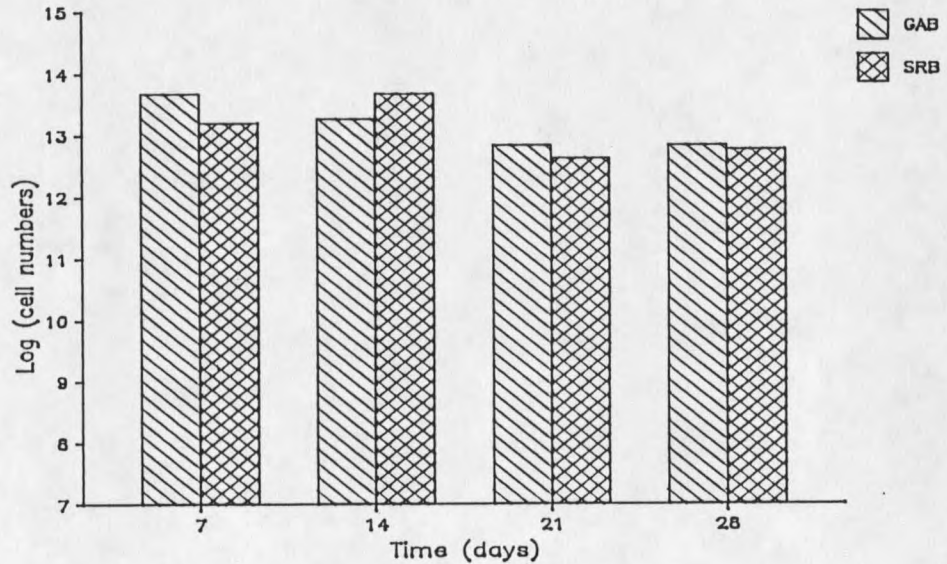


Figure 24. Most probable number (MPN) for GAB and SRB in the biofilm at different ferrous ion concentrations.

The accumulation of iron sulfide in the biofilm at different ferrous ion concentrations is illustrated in Figures 25 to 27. Very little corrosion occurred at zero or low ferrous ion concentration (0, 1 and 10 mg/L). Some iron sulfide particles accumulated in the biofilm but no direct contact occurred between iron sulfide particles and the mild steel surface. However, iron-rich media (60 mg/L which is enough to precipitate all the biogenic sulfide) caused large amounts of precipitation of iron sulfide (Figure 28). An X-ray dot map showed the iron sulfide particles penetrate through the biofilm and contact the metal surface (Figure 29). Cathodic (Figure 30) and anodic (Figure 31) currents increased dramatically as ferrous ions increased from 10 to 60 mg/L. Nyquist plots indicated that corrosion rate was much higher at 60 mg/L Fe^{+2} than at 10 Fe^{+2} . The charge transfer resistance (R_{ct} , the diameter of the semicircle, which is inversely proportional to the corrosion rate) decreased dramatically as ferrous iron concentration increased from 10 mg/L Fe^{+2} to 60 mg/L Fe^{+2} (Figure 32). There was no coherent sulfide film formed under the biofilm in the iron rich media. Uniformly intergranular attack was observed over the entire metal surface after precipitated iron sulfide and biofilm was removed (Figure 33).

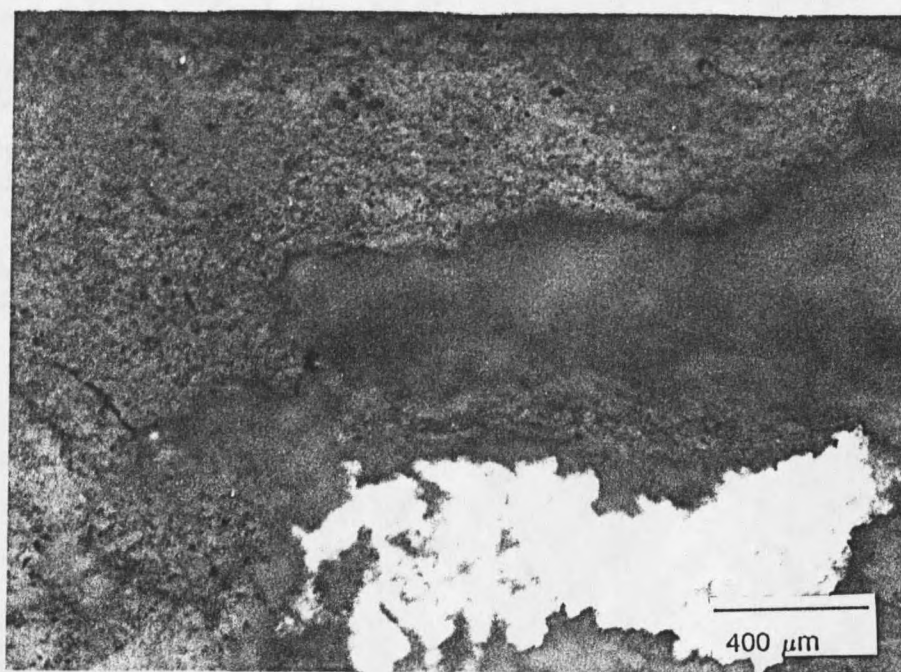


Figure 25. Accumulation of iron sulfide on a precoated biofilm at 1 mg/L ferrous ion concentration. x50

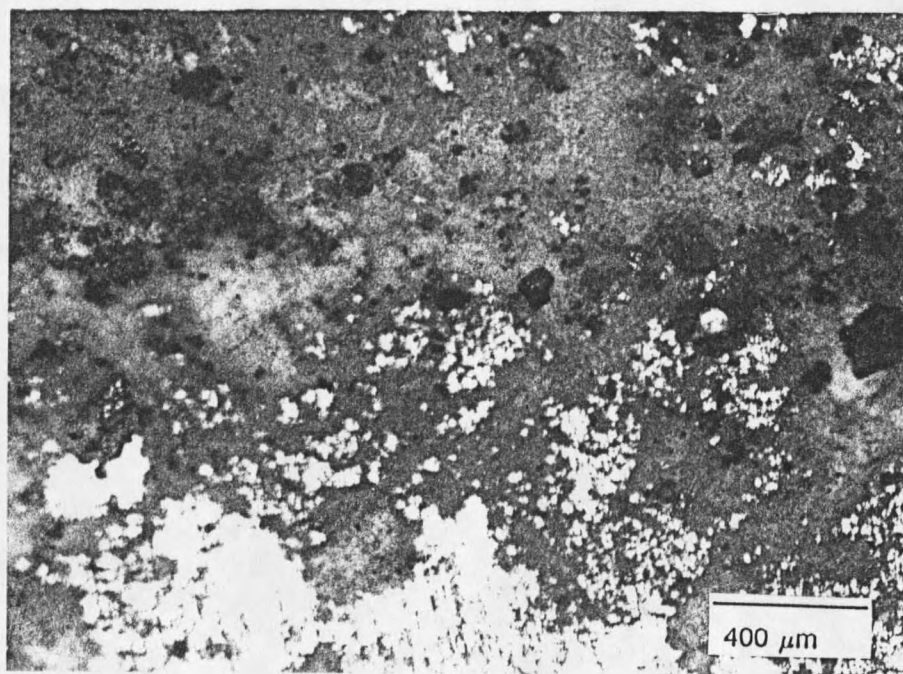


Figure 26. Accumulation of iron sulfide on a precoated biofilm at 10 mg/L ferrous ion concentrations. x50

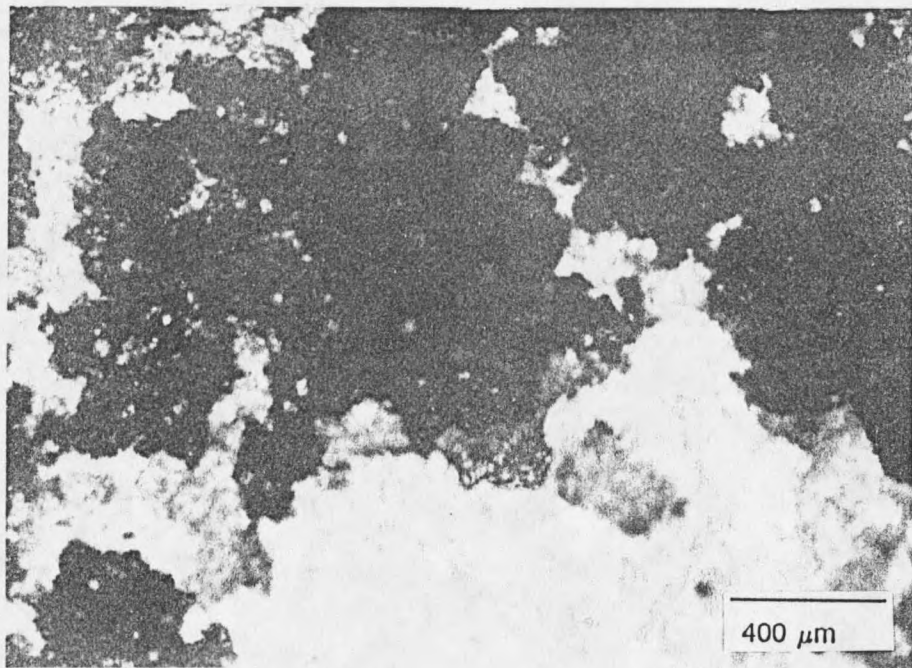


Figure 27. Accumulation of iron sulfide on a precoated biofilm at 60 mg/L ferrous ion concentrations. x50

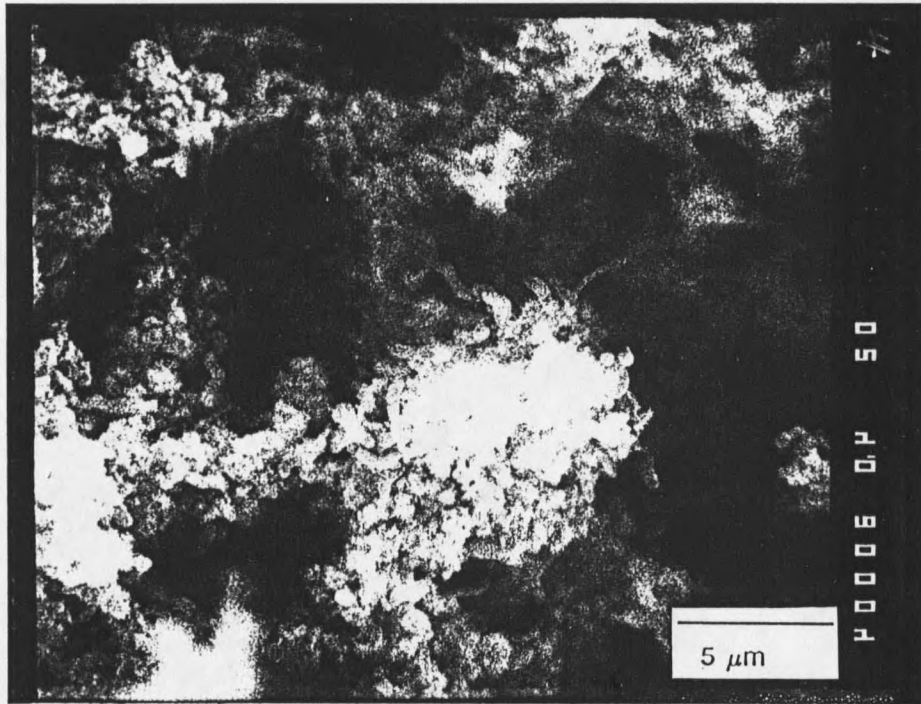
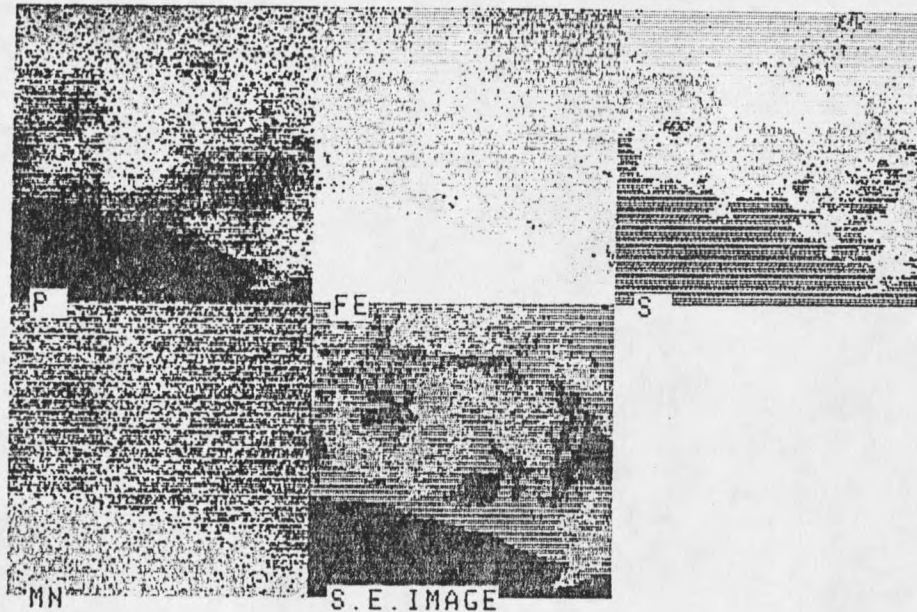


Figure 28. Scanning electron micrograph of biofilm when the media contain 60 mg/L ferrous ion concentration. x4,000



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Figure 29. X-ray dot map indicating the elemental distribution of iron, sulfur, and phosphorus on the top of biofilm and metal substratum.

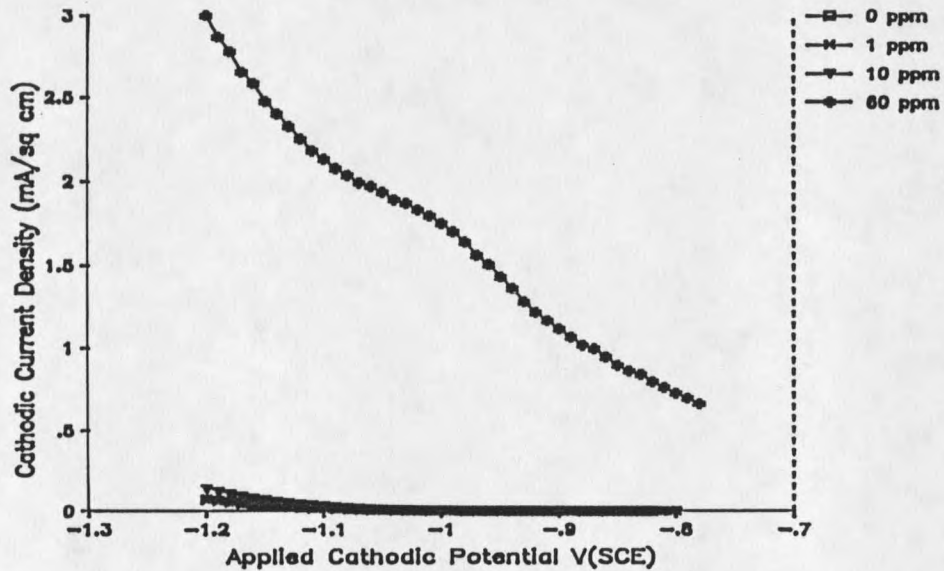


Figure 30. Cathodic polarization curves of mild steel on a pre-coated biofilm followed by step increase in ferrous ion concentrations.

