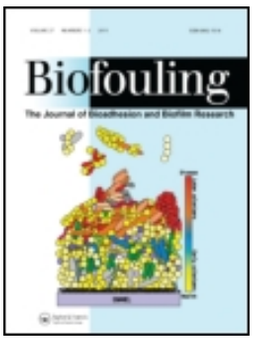

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CORROSION OF MILD STEEL IN AN ALTERNATING OXIC AND ANOXIC BIOFILM SYSTEM

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The effect of alternating oxic and anoxic conditions (12 h oxic–12 h anoxic) on sulfate reducing activity, iron-sulfur chemistry and the corrosion of mild steel, has been studied in biofilm reactors.

During the experiment (35 d) an increasing activity of sulfate reducing bacteria was observed. A part of the produced sulfide and iron sulfide (FeS) was oxidized during oxic periods and resulted in a mixture of acid volatile sulfides (mainly mackinawite, FeS), chromium reducible sulfur (mainly pyrite, FeS_2) and elemental sulfur (S^0). At the end of the experiment an amount of total S corresponding to 157 $\mu mol \cdot cm^{-3}$ was found within the deposit. Corrosion rates were measured electrochemically during the experiment and were found in the range of 3–5 mpy after 7 d to 120–160 mpy after 34 d.

An extended aeration of the biofilm system for 1 month without addition of any organics showed that the pools of Fe-S compounds in the deposit and the corrosion rate remained high. Microsensor studies of dissolved oxygen penetration through the biofilm and the deposit showed that even after 1 month of aeration oxygen did not penetrate to the metal surface. The limited oxygen penetration was caused by a very high oxygen consumption rate due to oxygenation of reduced chemical species originating from the dissolution of metal by the corrosion process (approximately 66 mmol $Fe \cdot m^{-2} \cdot h^{-1}$). Measurements of *in situ* sulfate reducing activity revealed high sulfate reduction rates within the anoxic part of the deposit and suggested that SRB activity was important as electron carrier from the metal surface to the oxic interface.

KEYWORDS: mild steel, corrosion, oxic-anoxic, sulfate-reducing bacteria, pyrite, biofilm, elemental sulfur

INTRODUCTION

Microbially influenced corrosion of steel in anaerobic environments is mainly considered to be due to sulfate reducing bacteria (SRB). They have been demonstrated to accelerate the anaerobic corrosion process significantly either directly by consuming hydrogen produced by the cathodic process (Table 1, Eqn 1) or indirectly by producing corrosive products. The products of the sulfate reducing activity is hydrogen sulfide (Eqn 2) and precipitates such as mackinawite, FeS (Eqn 4), which is corrosive due to its cathodic nature toward steel. This has been reviewed by several authors, *e.g.* Hamilton (1985) and Pankhania (1988).

In natural and industrial systems the sulfate reducers are almost always found in biofilms and SRB and their activity is often located close to the oxic-anoxic interface (Jørgensen, 1989). Thus, a complex mixture of partly reduced sulfur and iron-sulfur compounds can be found due to biological and chemical reactions and in such systems very high corrosion rates have been observed (Hardy & Bown, 1984; Hamilton *et al.*, 1991a, b; Moosavi *et al.*, 1991).

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Table 1 Equations for cathodic reactions (1–3), precipitation of FeS(4) and various important chemical reactions between oxygen, sulfides or iron compounds (5–8).

Equation Number		Reference
1.	$H^+ + e^- \rightarrow 0.5H_2$	King *et al.*, 1973
2.	$2H_2S + 2e^- \rightarrow 2HS^- + H_2$	Costello, 1974
3.	$S^0 + H_2O + 2e^- \rightarrow HS^- + OH^-$	Schaschl, 1980
4.	$Fe^{2+} + HS^- \rightarrow FeS + H^+$	Schmitt, 1991
5.	$2H_2S + O_2 \rightarrow 2S^0 + 2H_2O$	Schmitt, 1991
6.	$4FeS + 3O_2 + 2H_2O \rightarrow 4S^0 + 4FeO\,(OH)$	Pankov and Morgan, 1980
7.	$3H_2S + 2FeO(OH) \rightarrow 2FeS + S^0 + 4H_2O$	Berner, 1967
8.	$Fe^{2+} + HS_5S^- + HS^- \rightarrow FeS_2 + S_4S^{2-} + 2H^+$	Richard, 1975

From studies in abiotic systems it is known that oxygen will react with sulfide and produce elemental sulfur (Eqn 5) or other oxidized sulfur compounds such as thiosulfate, sulfite and sulfate (Chen & Morris, 1972; reviewed by Morse *et al.*, 1987). The reaction between FeS and oxygen produces mainly elemental sulfur at neutral pH (Eqn 6). Out-diffusing sulfide can also react with oxidized iron and form FeS and elemental sulfur (Eqn 7). Sulfide, elemental sulfur and other reduced sulfur compounds are also very efficiently oxidized by microbial activity. In biofilm systems the oxidation is often performed by very specialized microbial communities and the rates observed are usually much higher than the chemical oxidation rates (Jørgensen, 1989). Among these products elemental sulfur is very corrosive (Eqn.4, Schaschl, 1980; Schmitt, 1991) but also iron sulfide such as mackinawite (FeS), greigite (Fe_3S_4) and pyrite (FeS_2) are cathodic to mild steel (reviewed by Smith & Miller, 1975). Mackinawite is considered to be the major corrosion product found in SRB related anaerobic corrosion (McNeil & Little, 1990) but in the presence of oxidized compounds, mainly oxygen or FeO(OH), pyrite may be formed (Richard, 1975). The time scale in which pyrite can be formed at low temperature, ranging from hours or days to years, has been discussed by several authors, *e.g.* Luther (1991). Recently Luther (1991) also confirmed the findings of Richard (1975) that pyrite can be formed very rapidly if polysulfide is present (Eqn 8).

Hamilton *et al.* (1991a) has found that environmental factors and most notably oxygenation, are central for determining the rate and extent of SRB-related corrosion. High corrosion rates associated with pitting was found in aerobic environments close to the oxic/anoxic interface with a high proportion of non-acid volatile sulfides (NAVS), *e.g.* pyrite or elemental sulfur, (McKenzie & Hamilton, 1992). The corrosion rates were not found to correlate directly with SRB activity, but seemed to be more closely correlated to the amount of NAVS. Also Lee *et al.* (1993a,b) have shown that SRB activity in biofilms grown in the presence of oxygen in the bulk water is more corrosive than in total anaerobic systems. At present however, there are no investigations of how these Fe-S pools develop in biofilms systems, how dynamic the pools are or exactly how the pools are related to the corrosion. Furthermore, it is not known if it is possible to decrease the corrosion rate by suppressing SRB activity and oxidize the reduced sulfur compounds by an extended aeration of the biofilm system.

This paper describes how microbial activity in a biofilm creates highly corrosive conditions for mild steel in an alternating oxic-anoxic environment. The aim was to follow biofilm accumulation, measure microbial activity, analyze and quantify the different iron-sulfur pools, describe their dynamics and finally to observe the related corrosion rate. Whether an extended aeration of the system had any effect on Fe-S pools, SRB activity and corrosion rate was also investigated.

MATERIALS AND METHODS

Reactor System

The experiments were conducted in two parallel continuous channel reactors slightly modified from the description given by Lee *et al.* (1993a). The reactors were made of transparent PVC and each had a total volume of 1.95 l and a biofilm covered area of 0.068 m^2. Biofilm growth on all other surfaces (cover, walls, tubings) was periodically removed. A recirculation loop provided mixing and a turbulent flow across the biofilm with a flow velocity of approximately 0.3 $m \cdot s^{-1}$. The dissolved oxygen (DO) concentration in the biofilm reactor was controlled by the recirculation flow in a separate recirculation loop, which was connected to an aeration tower with vigorous air bubbling. Each reactor contained 48 corrosion metal coupons each with a diameter of 1.0 cm. At the beginning of the experiments the mild steel coupons accounted for 5% of the total biofilm covered area in the reactor.

Media and Biofilm Growth Conditions

The medium consisted of 0.35% (w/w) artificial seawater, 20 $mg \cdot l^{-1}$ yeast extract and 21 $mg \cdot l^{-1}$ sodium lactate, 10 $mg \cdot l^{-1}$ NH_4Cl and 2 $mg \cdot l^{-1}$ Na_2HPO_4 in anoxic periods and only seawater in oxic periods. The medium was flushed with nitrogen to prevent any oxygen supply to the reactors during the anoxic periods. The pH in the reactor was 6.5 ± 0.3. Total organic carbon (TOC) was 14.6 $mg \cdot l^{-1}$ and the organic loading in the anoxic period was 477 mg $TOC \cdot m^{-2} \cdot h^{-1}$. *Pseudomonas aeruginosa*, *Kleibsiella pneumonia* and *Desulfovibrio desulfuricans* were used as inocula but the reactors were not conducted aseptically. The reactors were run in the dark to prevent any growth of phototrophic organisms. The temperature was controlled to 30°C by a heat exchanger. The reactors were run as batch for 1 d, the corrosion coupons were inserted and the operation was switched to continuous flow with a dilution rate of $1 \cdot h^{-1}$.

The experimental conditions were divided into: 1) a 35 d period with alternating oxic/anoxic conditions in a cycle of 24 h and subsequently 2) a 36 d period with only oxic conditions, here referred to as the extended aeration period. On a daily basis part 1 with alternating oxic/anoxic conditions included an anoxic period of 12 h (no oxygen present) but with organic substrate for the first 11 h. This was followed by an oxic period of 12 h with a bulk DO around 125–190 μM but without any supply of organics. In part 2 with extended aeration the reactors were switched to continuous aeration with a bulk water DO of 230 μM (saturation). No organics, N or P were supplied to the reactors during this period.

One reactor was used for measurements of corrosion products, while the other was used for electrochemical measurements. Removal of organics, oxygen removal rates and sulfide production rates were measured in both reactors and only minor differences were observed. The data presented on biological activity are from the reactor for electrochemical measurements.

Biofilm Activity

Removal of dissolved organic carbon (DOC) and sulfide production rate in the reactors were determined from mass balances on the reactors (Lee *et al.*, 1993a). Total organic carbon (TOC) was determined using a DC-80 TOC Analyzer and dissolved organic carbon (DOC) after a filtration through a 0.22 μM filter. Lactate was analyzed enzymatically (L-lactic measurement, Boehringer, Mannheim, Germany). Dissolved

sulfide in bulk water was determined colorimetrically in accordance with Cline (1969). The oxygen uptake rate in the reactors was determined from initial oxygen removal rates when the reactor was totally closed, *i.e.* no substrate addition or recirculation from the oxygen supply recirculation loop. Microelectrode measurements of oxygen profiles within the deposit were performed in accordance with Lewandowski *et al.* (1989).

Measurement of *in situ* sulfate reduction rate during the extended aeration period with oxygen saturated bulk water was performed using (^{35}S) sulfate radiotracer assay. While the recirculation and reaeration loop remained on, the flow of medium to the reactor was stopped, 0.1 $\mu Ci \cdot ml^{-1}$ radiotracer was added and the reactor was incubated for 10–12 h. The reactor was flushed several times with non-radioactive medium, and coupons were taken out and immediately fixed in 10% ZnAc and frozen until analysis. ^{35}S-H_2S produced was trapped on filters after a distillation of H_2S, FeS and FeS_2 according to methods described later and measured with a liquid scintillation counter. The sulfate reduction rate was calculated in accordance with Fossing and Jørgensen (1989, 1990). Sulfate was measured by ion chromatography (Nielsen, 1991).

Corrosion Coupons

Disks of AISI 1018 mild steel from Metal Samples, Incorporated (Mumford, AL USA) 10.0 mm in diameter were cast in acrylic resin to avoid crevice corrosion. The coupons were polished with a series of grit papers (120, 200, 320, 400, 600), followed by ultrasonic cleaning in 100% ethanol for 1 min, then dried in air at room temperature.

Corrosion Measurements

In situ corrosion measurements were conducted by positioning a graphite counter electrode above the metal coupon and a saturated calomel electrode (SCE) next to the metal coupon. Open circuit corrosion potential, cathodic and anodic polarization and AC (alternating current) impedance were measured on different metal coupons every week during biofilm accumulation. DC (direct current) measurements were made using a computer controlled Princeton Applied Research Corporation (PARC) 273 Potentiostat/Galvanostat. Instantaneous corrosion rate was calculated using PARC Model 352C SoftCorr software. AC measurements were performed using a Solartron 1250 Frequency Response Analyser in combination with a computer controlled PARC 273 Potentiostat with a 92 electrochemical interface. Measurements were carried out at the corrosion potential over a frequency range of 1 mHz to 10 KHz (10 points per decade). The applied voltage was 10 mV (rms). Polarization resistance (Rp) from AC measurements was determined using the CIRFIT software (Kending *et al.*, 1983).

Measurements of Fe-S Compounds in Biofilm

Biofilm samples were sequentially analysed for elemental sulfur (S^O) by extraction in ethanol, distillation of H_2S and FeS as acid volatile sulfide (AVS) and distillation of FeS_2 as chromium reducible sulfur (CRS). The methods described by Rosser and Hamilton (1983) and Fossing and Jørgensen were modified (1989) so that small biofilm samples could be analysed with a high sensitivity.

Apparatus

FeS and FeS_2. A modification of the method described by Rosser and Hamilton (1983) for determination of FeS using passive distillation in which the H_2S gas diffuses into a

trap in a closed reaction flask was used. All surfaces in the reaction chamber were of Teflon as some H_2S gas was found to adsorb to glass surfaces. This caused significant errors at very low sulfide concentrations. A 50 ml transparent Teflon Erlenmeyer flask (Nalgene, FRP) equipped with a Teflon lid containing 3 pieces of Teflon tubing was used for FeS and/or FeS_2 distillation. One piece of tubing was used to add acid to the flask, the second held the H_2S trap within the flask and a filter was placed at the tip of this piece of tubing. The filter was wetted in a 10% zinc acetate solution before distillation. The third piece of tubing was used as an outlet for nitrogen gas under flushing when the gas was added through the second piece of tubing (which also held the filter). For FeS determination the presence of oxygen in the flask did not influence the recovery of FeS, so the distillation was performed without an initial nitrogen flushing. Determination of FeS_2 requires anoxic, reduced conditions and the sample was flushed with nitrogen of high purity for 15 min before FeS_2 distillation was initiated. During the distillation of FeS and FeS_2 the flask were gently shaken for 15 and 20 min respectively.

Elemental sulfur. Samples extracted in ethanol were analysed on HPLC using a UV detector at 254 nm. A reversed phase column, Partisil 5, ODS-3 (Whatman), was used with 100% HPLC grade methanol as eluent and a flow rate of 1 $ml \cdot min^{-1}$. The sample injection volume was 25 µl. Various extraction solvents were tested (ethanol, acetone, carbon disulfide and carbon tetracloride) and all showed the same extraction efficiency.

Extraction Procedures for S-compounds in Biofilms

Elemental sulfur. 0.1–1.0 ml homogenized biofilm suspensions fixed in 10% ZnAc were centrifuged in polypropylene microcentrifuge tubes, a known amount of the supernatant was removed and 0.10–1.00 ml 96% ethanol was added. The samples were extracted with gentle shaking for 24 h at room temperature. One extraction was found to be sufficient to extract all elemental sulfur. If maximum sensitivity was desired, the extract was concentrated by evaporation before analysis.

H_2S and FeS. The extracted biofilm suspension was washed twice in ethanol to remove all elemental sulfur, resuspended in 10% ZnAc and a subsample added to the distillation flask. A filter wetted in 10% ZnAc was placed in the flask which was closed to keep the gas inside, 1–2 ml of 6 N HCl were added and the flask was shaken for 15 min. The filter was removed and placed in 1–10 ml 1% ZnAc depending on the amount of sulfide to be analysed. Sulfide was measured colorimetrically by the methylene blue method according to Cline (1969).

FeS_2. Pyrite was determined by reduction of Cr^{2+} in cold acid (Fossing & Jørgensen, 1989) on the suspension remaining from the ethanol extraction and the FeS distillation. A new wetted filter was inserted and the flask was flushed with nitrogen for 15 min. It was then sealed and 2.5 ml of 1 M Cr^{2+} in 0.5N HCl were added. The flask was gently shaken for 20 min at room temperature and the trapped hydrogen sulfide was measured as previously described.

The detection limit for the method was 1 nmol for FeS and FeS_2-S and 6 nmol for S^0 without preconcentration. It corresponded to a concentration of 2 µM FeS and FeS_2-S and 12 µM S^0 in a 0.5 ml wet biofilm suspension. The recovery of known amounts of S^0, FeS and FeS_2 added to biofilm suspensions was above 90 %. The presence of Fe^{3+} did not affect the distillation of FeS. All chemicals were prepared in accordance with Fossing and Jørgensen (1989).

RESULTS

1. Alternating Oxic and Anoxic Conditions

Biofilm Activity

During the 35 d run with alternating oxic-anoxic conditions the deposit (biofilm and corrosion products) increased to a thickness of approximately 2 mm. As the biofilm received organic substrate only during anoxic periods, the biofilm was relatively thin and difficult to distinguish from the corrosion products. In order to maximize the oxygen penetration of the deposit during the oxic period, organic substrate was added to the reactor only during the anoxic period. The purpose was to create as much partly oxidized Fe-S compound as possible. At the end of the experiment the deposit on the metal coupon protruded into the bulk water beyond the surrounding biofilm.

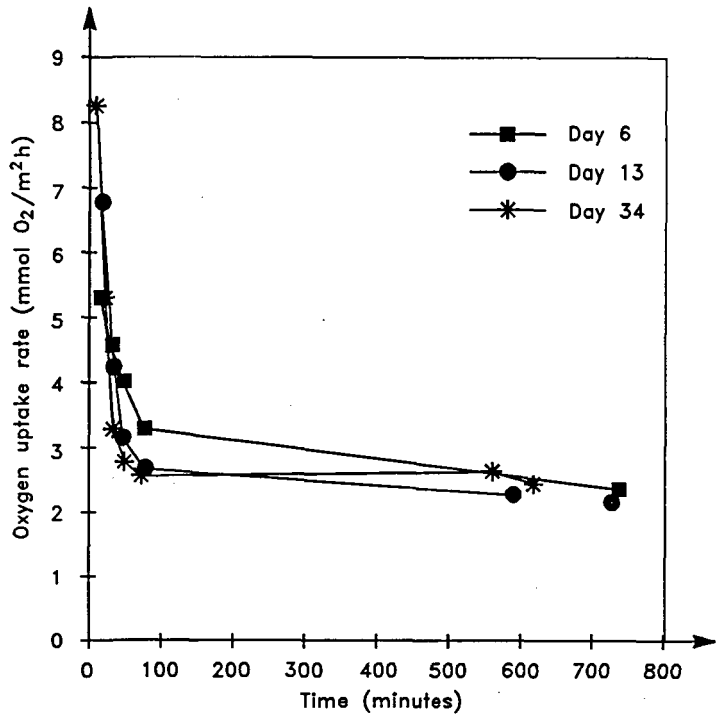
The development in microbial activity for the entire biofilm in the reactors reached steady state for organic carbon removal and oxygen removal after a few days in the reactors. The concentrations of dissolved organic carbon (DOC) in the effluent remained between 0.9–2.0 during the oxic period and 9.1–11.0 $mg \cdot l^{-1}$ during the anoxic period. Figure 1a shows that the oxygen consumption rate was high for the first 1–2 h after changing from anoxic to oxic conditions due to the presence of some organic substrate from the previous period and also, later in the experiment, due to oxidation of sulfide. The oxygen consumption rate in the following 10 h remained constant. Sulfide was not observed in the bulk water until day 26–27 because of precipitation or oxidation in the iron rich deposit (see later). In the following days the sulfate reducing activity increased significantly, and after day 32 a rate of approximately 3.2 mmol $TS \cdot m^{-2} \cdot h^{-1}$ was observed in most of the anoxic period, where TS expresses the total dissolved sulfide concentration ($H_2S + HS^- + S^{2-}$) (Fig. 1b). This production rate produced a sulfide concentration of 0.10 mM in the reactor (and in the effluent). The sulfate reducing activity was most likely limited by the availability of organic matter as no lactate was detectable in the effluent in the anoxic period. DOC, however, remained between 9.1–11.0 $mg \cdot l^{-1}$. The observed molar ratio between consumed lactate and produced sulfide was 1:1.9. In the last hour of the anoxic period the supply of organics was shut off and a decrease in sulfide level was observed (Fig. 1b, days 27 and 34).

Corrosion Rate and Electrochemical Measurements

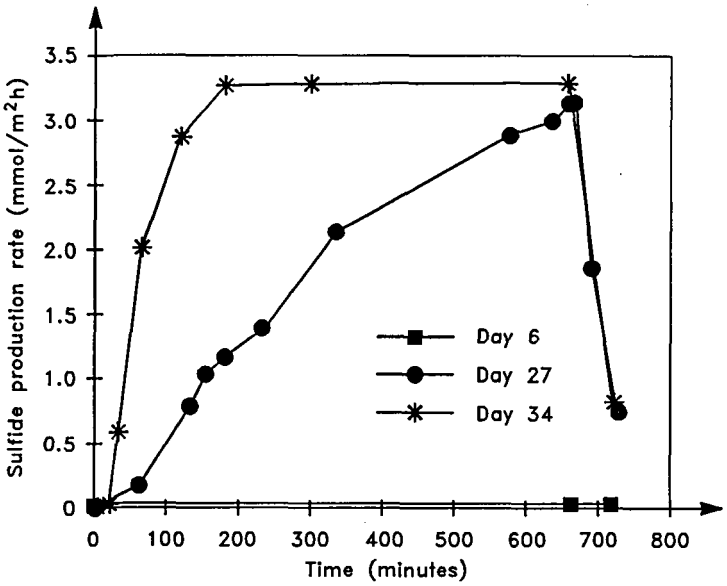
From DC polarization measurements the cathodic current both in the anoxic (Fig. 2) and oxic (Fig. 3) periods increased rapidly from the end of the first week to day 35, while the anodic currents remained nearly unchanged. The corrosion rate calculated from DC polarization curves in the same period showed an increase from 6 to 121 mpy in anoxic periods and from 27 to 162 mpy during the oxic periods (Fig. 4). The polarization resistance (R_p) obtained from the AC impedance measurements decreased rapidly in the same period from 2000 to 350 $ohms \cdot cm^{-2}$ for the anoxic and from 1850 to 200 $ohms \cdot cm^{-2}$ for the oxic period (Fig. 5). Since polarization resistance is inversely proportional to the corrosion rate, these results confirmed an increase of corrosion rate with time.

Corrosion Products

The pools of elemental sulfur, FeS and FeS_2 showed a general increase in the deposit with time (Fig. 6). Following detection of sulfide in the bulk water after day 26, the pools



a)



b)

Fig. 1 Oxygen uptake and sulfide production rates over 35 d with alternating oxic and anoxic periods. (a) oxygen uptake rates in oxic periods; (b) sulfide production rates in anoxic periods based on measurements of sulfide production into the bulk water.

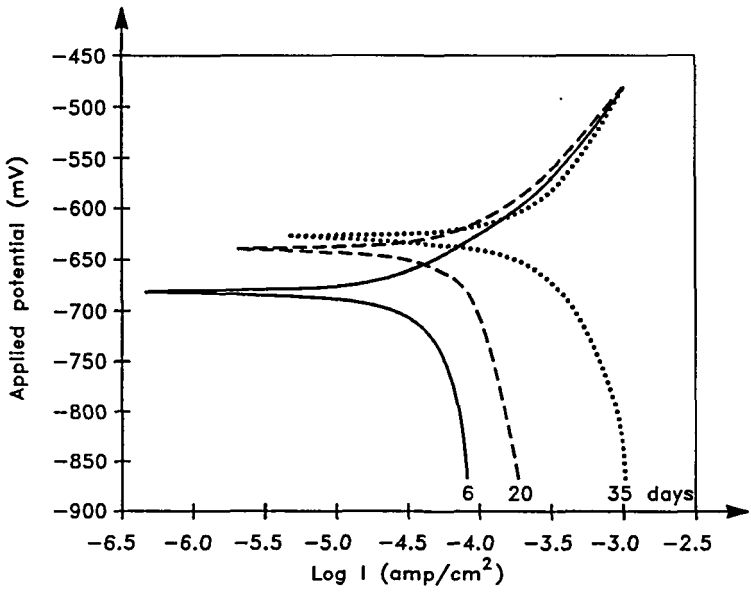


Fig. 2 Anodic and cathodic polarization for the oxic period over 35 d with alternating oxic and anoxic periods.

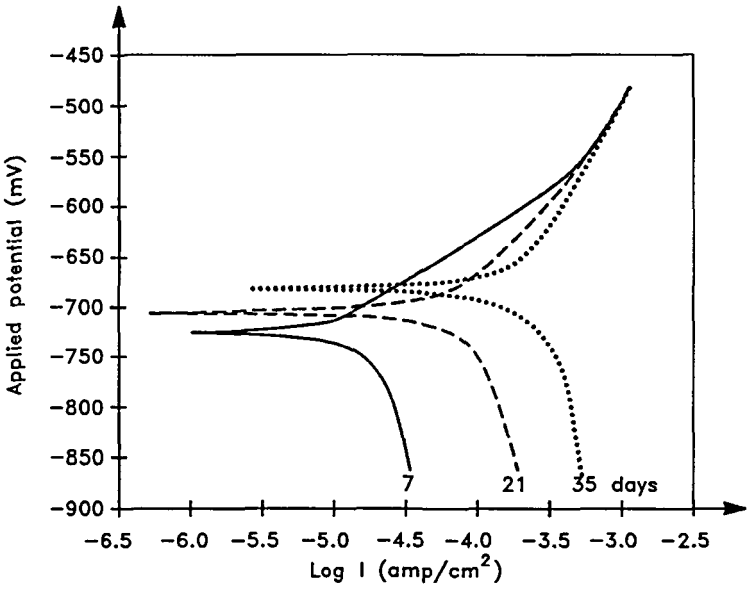


Fig. 3 Anodic and cathodic polarization for the anoxic period over 35 d with alternating oxic and anoxic periods.

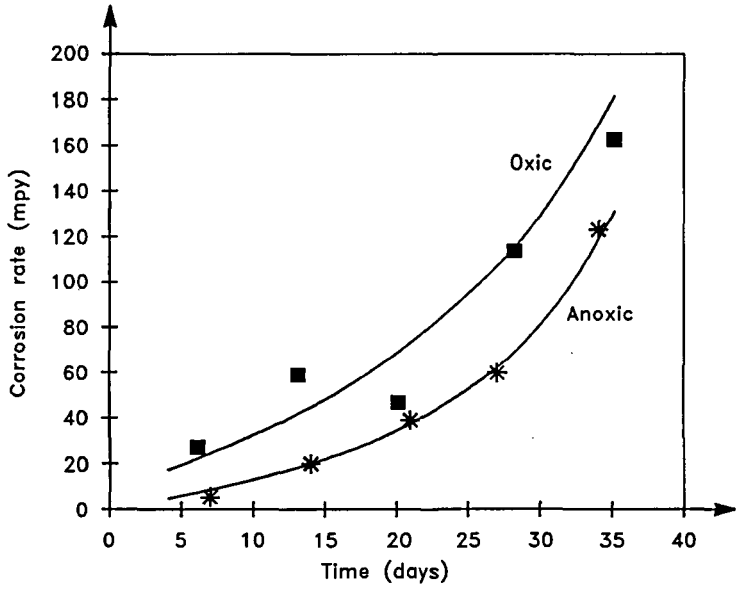


Fig. 4 Corrosion rate over 35 days with alternating oxic and anoxic periods (average for cathodic polarization (CP) and anodic polarization (AP).

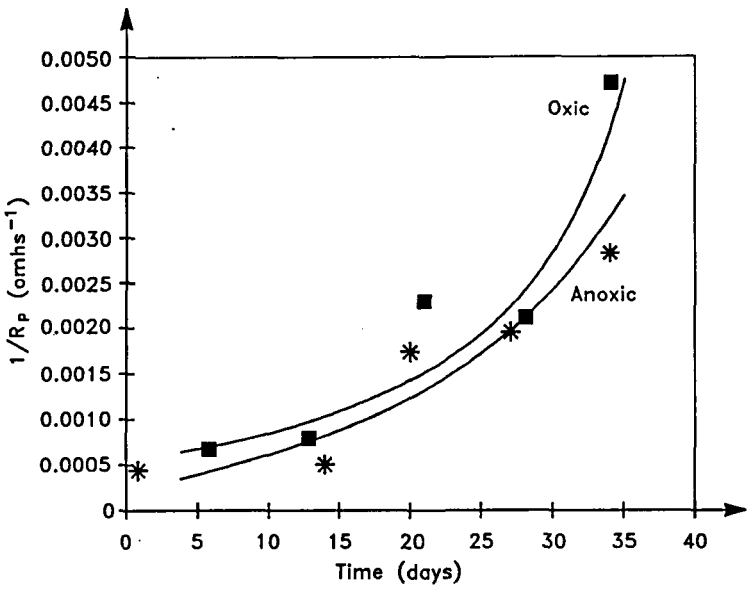


Fig. 5 Change in polarization resistance with time over 35 d with alternating oxic and anoxic periods.

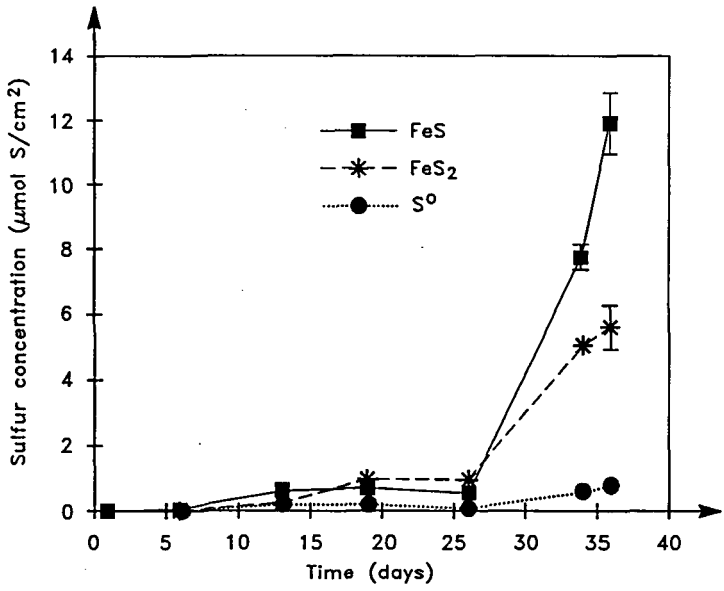


Fig. 6 Development in the pools of FeS, FeS_2 and S^0 with time. The results are averages of 2–3 metal coupons from the oxic and 2–3 metal coupons from the anoxic period within one cycle (1 d). Bars = error of the mean.

increased significantly due to a sulfide saturation of all reactive iron in the deposit which turned black. In order to follow the dynamics of possible FeS oxidation and S^0 production within one oxic period, the pools of S^0, FeS and FeS_2 were measured at the end of both the oxic and the anoxic period throughout the experiment. However, no differences in the pools were found within one cycle except for the last week. In this week the concentration of FeS was 20–30% higher at the end of the anoxic period relative to the end of the oxic period. This was probably due to oxidation of some FeS in the uppermost layer of the deposit during the oxic period.

The concentration of the various sulfur compounds within the deposit reached high values. The average concentration for the pools and a total S concentration as high as 157 $\mu mol \cdot cm^{-3}$ was found. AVS is usually assumed to include amorphous FeS, crystalline FeS and greigite while CRS includes pyrite and possibly marchasite.

2. *Extended Aeration*

After 35 d operation the reactors were switched to continuous aeration with a bulk water DO of 230 µM (saturation). No organics, N or P were supplied. The oxygen consumption rate in the reactors decreased from approximately 2.5 to 1.2–1.6 mmol $O_2 \cdot m^{-2} \cdot h^{-1}$ during the first few days and thereafter it remained constant at this level for 1 month. No large effect on the FeS pools was observed (Fig. 7). After an initial decrease in the FeS pool the amount of FeS increased towards the end of the experiment. The pools of FeS_2 remained relatively constant and S^0 increased slightly during the aeration period. Some variability occurred in the pools which was caused by detachment of exposed parts of the deposits. During the aeration the deposit continued to increase in size due to a very high corrosion rate.

The corrosion rate remained around 160 mpy. Both anodic and cathodic current remained constant from day 34 throughout the extended aeration period. R_p decreased from 200 to 100 ohms·cm^{-2}.

The penetration of oxygen into the deposit after 1 month of aeration is shown in Figure 8. The upper 1 mm of brown oxidized deposit showed oxygen saturation and approximately 1 mm above the metal surface, the oxygen concentration dropped to zero over a distance of less than 100 μM. Assuming an effective diffusion coefficient of 2×10^{-6} cm^2·s^{-1} for oxygen (Lewandowski *et al.*, 1989) and a linear decrease in concentration over 100 μM, the oxygen flux into the deposit was calculated to be approximately 15.6 mmol O_2·m^{-2}·h^{-1}. This value is approximately 10 times higher than the overall uptake rate from the entire biofilm in the reactor, which shows that the oxygen uptake rate from the metal coupons was much higher than from the biofilm elsewhere in the reactor. As the metal coupons only covered a minor part of the reactor (5.5% at the beginning and less than 1% at the end of the experiment) their contribution to the overall rate was negligible.

The *in situ* sulfate reduction rate was found to be high within the deposit at saturated bulk oxygen concentration after 1 month of aeration. Using radiotracer assay (^{35}S-sulfate) the rate was found to 2.0 μmol SO_4^{2-}·h^{-1} per metal coupon with a relative standard deviation of 26% for two coupons. This rate corresponded to approximately 25.7 mmol SO_4^{2-}.m^{-2}·h^{-1} or to a biofilm volume activity of 25.5 μmol SO_4^{2-}·cm^{-3}·h^{-1}

DISCUSSION

The alternating operation of the experimental system created the expected very corrosive environments for mild steel with corrosion rates up to 160 mpy. Other studies of similar

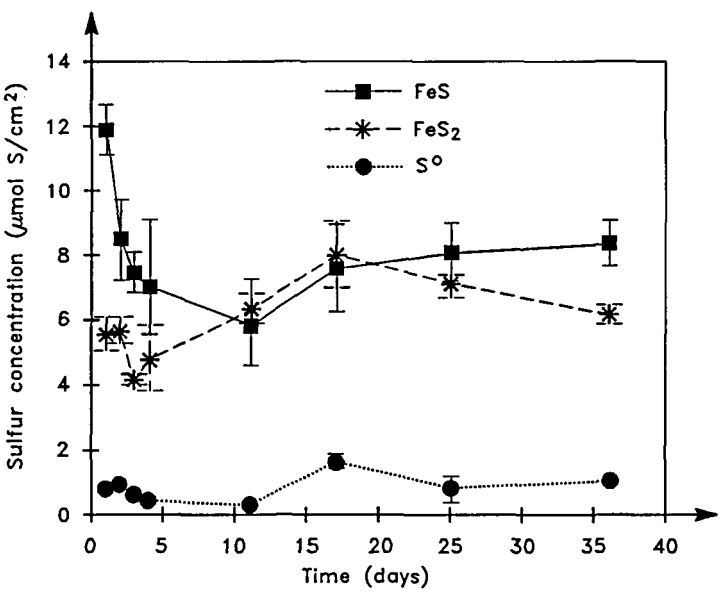


Fig. 7 Development in the pools of FeS, FeS_2 and S^0 with extended aeration time. The results are averages of 2–3 metal coupons. Bars = error of the mean.

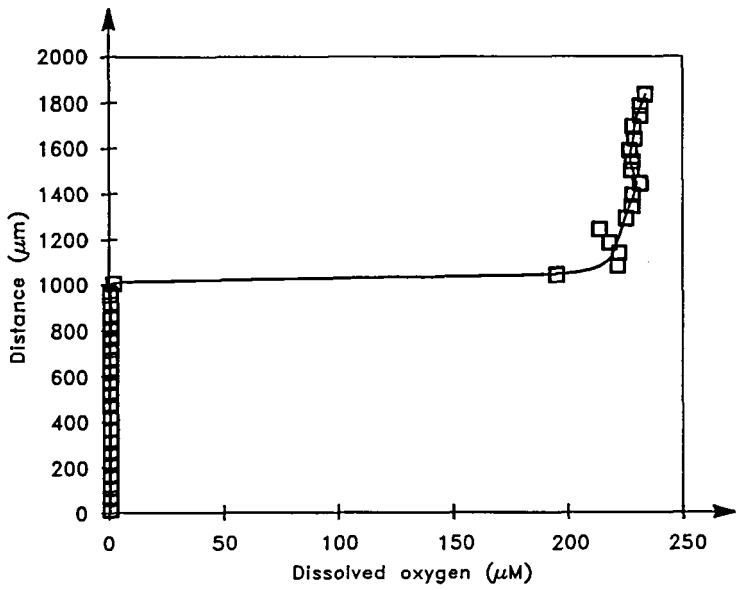


Fig. 8 Oxygen penetration through deposit/biofilm after 1 month of extended aeration (microsensor measurements). The y-ordinate shows distance from metal surface.

biofilm and reactor systems with continuously anoxic or oxic bulk conditions showed considerably lower corrosion rates. Lee and Characklis (1993) and Lee *et al.* (1993a, b) observed corrosion rates around 10 mpy after 2 months in systems with constant bulk oxygen and even lower corrosion rates in totally anaerobic systems with high SRB activity. Only a few other aerobic/anaerobic biotic systems have been studied and relatively high corrosion rates have been observed with maximum rates in the range 13–53 mpy (Moosavi *et al.*, 1991; McKenzie & Hamilton, 1992). The observation of the insignificant effect of extended aeration on the Fe-S pools and the corrosion rate seems to be important. It indicates that once the high corrosion rate in the deposit is established, it is difficult to slow down the corrosion process by oxygenation of the system.

Biofilm Activity

Overall activities in the entire reactors such as oxygen uptake rate and substrate removal reached a steady state after a few days. Sulfide in bulk water was not observed until just before the extended aeration was started. The SRB activity in the reactor was most likely limited by the lactate concentration as the calculated ratio between consumed lactate and produced sulfide of 1:1.9 was close to that found for a pure culture of *D. desulfuricans* of 1:2.3 (Okabe *et al.*, 1993). Also hydrogen, acetate or yeast extract could have been potential electron donors for SRB activity.

It is important to note that the overall activity measured in the reactors was not representative of the microbial activity on the metal coupons, where much higher microbial activity was found. It resulted in a 5 to 10 fold difference between the oxygen uptake rate and the sulfate reduction rate for these two types of substratum. It implies that if the ratio between metal area and other areas in the bioreactor is very small, the mass balance on the entire reactor is not an adequate measurement for the activities in the biofilm on the metal coupons.

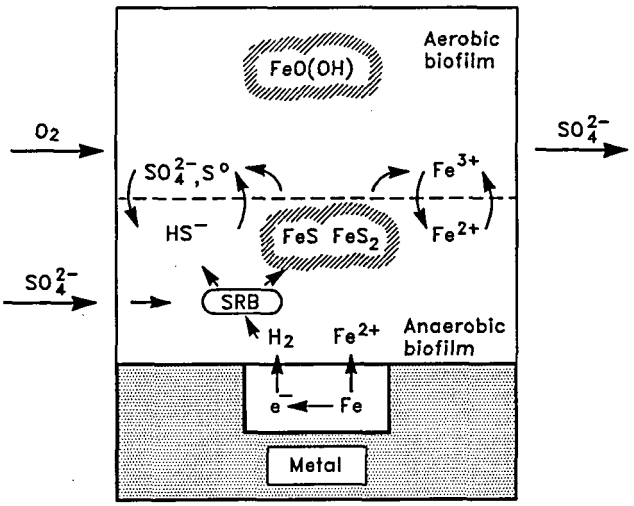


Fig. 9 Hypothetical model of microbial activity and corrosion within deposit.

Figure 9 shows the hypothetical corrosion mechanism taking place within the deposit after one month of aeration. Since the Fe-S pools remained constant the last month (no net production of Fe-S) and no organics were added, an overall mass balance on the production of ferrous ions and the oxygen uptake can be made. If all released iron is oxidized to ferrihydroxide the overall mass balance would be:

$$4Fe + 3O_2 + 2H_2O \rightarrow 4FeO(OH) \qquad \text{(Eqn 9)}$$

Within the deposit the SRB mediated corrosion reactions will be (without taking the production of pyrite and elemental sulfur into account):

$$4Fe + SO_4^{2-} + 8H^+ \rightarrow FeS + 3Fe^{2+} + 4H_2O \qquad \text{(Eqn 10)}$$

These reduced compounds (mainly Fe^{2+}, which can diffuse, but also FeS, FeS_2 and S^O) will be transported to the oxic/anoxic interface and create an equivalent oxygen uptake rate over a certain area and thus act as an electron carrier from the metal to the surface. If no loss of reduced substances (such as Fe^{2+} and FeS) into bulk water takes place, the oxygen consumption rate should be proportional to the corrosion rate (Eqn 9).

In this study a lot of dissolved Fe(II) was generated as a result of a corrosion rate of 160 mpy. It is equivalent to a metal dissolution rate of 66 mmol $Fe \cdot m^{-2} \cdot h^{-1}$. This is equivalent to 132 mmol electrons $(e^-) \cdot m^{-2} \cdot h^{-1}$ for oxidation to Fe(II) (Eqn 10), or 198 mmol $e^- \cdot m^{-2} \cdot h^{-1}$ for oxidation to Fe(III) (Eqn 9). The oxygen uptake rate, based on microsensor measurements, was approximately 15.6 mmol $O_2 \cdot m^{-2} \cdot h^{-1}$ or 62.4 mmol $e^- \cdot m^{-2} \cdot h^{-1}$. This rate was considerably lower than expected from the corrosion rate if all iron was oxidized to Fe(III) (Eqn 9). Several reasons are possible. Reduced substances may have been released into bulk water without causing any oxygen consumption and since the microelectrode measurement is site specific and the oxygen profile has a low spatial resolution, the oxygen uptake rate may have been underestimated. On the other hand, an oxygen uptake rate of 15–20 mmol $O_2 \cdot m^{-2} \cdot h^{-1}$ is very high and only found in systems with high flow velocity, high organic loading, and high oxygen concentration (Nielsen *et al.*, 1992). The very high oxygen uptake rate may be due to chemical or most

likely microbial oxidation (Jørgensen, 1989) of the reduced chemical species, mainly Fe^{2+} but also FeS, FeS_2, S^O and maybe H_2. To what extent sulfide diffuses to the interface and causes an oxygen uptake is not known, as Equations 4 and 10 indicate that most sulfide was precipitated due to the high ferro ion concentration.

The observed corrosion rate created a high oxygen demand which limited dissolved oxygen penetration into the deposit. It produced favourable conditions for sulfate reducing bacteria within the deposit. The function of the SRB can be regarded as electron carrier from the metal surface to the biofilm anoxic/oxic interface. The electrons are transferred from the corroding metal to form sulfide, which is transported toward the oxic interface. Due to the constant pool of Fe-S compounds, the sulfate mass balance remains unchanged.

In this study measurements of "*in situ*" sulfate reduction rates in a system with aerobic bulk water has been demonstrated without incubation of the coupons under anaerobic conditions, as is usually performed (McKenzie & Hamilton, 1992). The calculated rate may underestimate the real activity due to internal reoxidation of produced sulfide to sulfate or S^O (Fründ & Cohen, 1992). This is assumed to be of minor importance with the continuous dissolution of Fe^{2+} by the corrosion process and the resulting precipitation of FeS, the large FeS pool present in most samples and a fast isotopic exchange from ^{35}S-sulfide to the large FeS pool (Fossing & Jørgensen, 1990). The radioactive sulfide in the S^O pool was most likely not included in the measurement which was performed using a cold chromium distillation, and this could also cause an underestimation.

The sulfate reduction rate within the anaerobic part of the deposit was estimated to be 25.6 mmol $SO_4^{2-}.m^{-2}.h^{-1}$ or a volumetric rate of 25 μmol $SO_4^{2-}.cm^{-3}.h^{-1}$. Similar activities are measured in wastewater systems (Nielsen, 1987; Nielsen & Hvitved-Jacobsen, 1988; Kühl & Jørgensen, 1992), where organic substrate, but not Fe, was used as the electron donor. As a comparison, a pure culture of *D. desulfuricans* in biofilms with lactate as electron donor has volumetric sulfate reduction rates 10 times higher than reported here (Okabe *et al.*, 1993). The observed sulfate reduction rate was approximately 200 mmol $e^-.m^{-2}.h^{-1}$, which was higher than the calculated corrosion rate of 132 mmol $e^-.m^{-2}.h^{-1}$ for oxidation of iron to Fe(II).

The same range of sulfate reduction rates was found in other similar biofilm systems and varying incubation periods confirmed that it is possible to give a good estimate of "*in situ*" sulfate reduction rate. The measured SRB activity was near the detection limit due to the small sample volumes. Consequently, the calculated sulfate reduction rate has some uncertainty, but it is important to note that the SRB activity was relatively high. The activity was measured after 1 month of aeration without the addition of organics. Most likely the electrons (hydrogen) derived from the corrosion process contributed to this high activity. Such high activities may also have taken place earlier in the experiment, but due to the small ratio of metal coupon to the entire biofilm surface area, it was not significant in the monitored overall sulfide production rate for the entire reactor.

Corrosion Products

The Fe-S pools increased during the experiment. The maximum total sulfur concentration found in the deposit was 157 μmol $S.cm^{-3}$. High concentrations of Fe-S compounds alone can be corrosive, when an electrical contact with the metal surface is provided (Lee *et al.*, 1993a). In many ecosystems similar high Fe-S concentrations are encountered. Typical Fe-S concentrations in marine sediments are in the range of 100–200 μmol $S.cm^{-3}$. It is predominantly pyrite, whereas FeS and S^O in sediments typically were at a maximum of 10–20 μmol $S.cm^{-3}$ each (Troelsen & Jørgensen, 1982; Fossing & Jørgensen, 1990).

It is believed that the rapid formation of pyrite in the present biofilm system is an important observation as pyrite is one of the most corrosive Fe-S compounds (King *et al.*, 1973). The rapid pyrite formation correlates with results from abiotic experiments (Richard, 1975, Luther, 1991) from marine sediments (Howarth & Jørgensen, 1984; Oenema, 1990). Polysulfide is a key intermediate and can be produced by a reaction between S^O and sulfide, by oxidation of HS^- or FeS by oxygen, or by oxidation of HS^- by Fe(III) (Luther, 1990). The fluctuating redox conditions in this experiment created excellent conditions for pyrite formation in the deposit.

The alternating oxic and anoxic conditions had very little effect on the composition of the various Fe-S pools within one cycle (24 h). Pyrite was not expected to show great variation in concentration, as it is very stable under totally reduced conditions and is oxidized slowly in oxic environments by dissolved oxygen or Fe(III) (Moses *et al.*, 1987; De Haan, 1991). The pools of FeS and S^O remained the same within one oxic-anoxic cycle until after 34 d, when all reactive iron was saturated with sulfide and the deposits turned black. In the following oxic period only the uppermost part of the black deposits was oxidized and even after the extended aeration, the pools remained constant. The oxygen profiles may explain the constant concentration of FeS. The depth of oxygen penetration was limited by the high oxygen uptake rate in the biofilm due to continuous production of reducing compounds within the deposit. Even though FeS can be oxidized chemically by oxygen (Eqn 6, Table 1), it did not occur as the oxygen did not penetrate into the deposit.

Corrosion Mechanism

The results indicated positive correlation between the amounts of corrosion products and the corrosion rate. It was not possible to establish a direct linear correlation between the amount of Fe-S compounds and the corrosion rate. The corrosion products and the corrosion rate were not measured on coupons in the same reactor, so possible differences among the coupons and the reactors could make it difficult to establish a correlation. The corrosion rate expressed per amount of Fe-S compounds was between 10–25 mpy·(μmol S)$^{-1}$·cm^{-2} (or 0.3–0.8 mpy·(μg S)$^{-1}$·cm^{-2}) after 10 d of operation and throughout the experiment, being highest at the beginning. Similar numbers were also found around 25 mpy·(μmol S)$^{-1}$·cm^{-2}, in a stable aerobic/anaerobic biofilm (Lee *et al.*, 1993b), indicating that a quantitative correlation exists. However, in a marine aerobic/anaerobic laboratory system over 100 times more Fe-S compounds per cm^2 were found on mild steel coupons with corrosion rates of only 10–17 mpy (McKenzie & Hamilton, 1992), so other factors must also be important.

Not only the presence but also the exact physical and chemical nature of the compounds are important factors in the corrosion of mild steel. For adherent surface sulfides, corrosivity seems to be minimal with pyrite (Tapping *et al.*, 1983). In contrast, work with suspended Fe-S compounds tends to show steadily increased corrosion rates with increased sulfur to iron ratio (King *et al.*, 1973). For suspensions of sulfides, cathodic polarization changes accompanied by minor changes in anodic behaviour are recorded (Martin & Annald, 1981). The present experimental results always showed shifts in cathodic and almost no change in anodic behaviour. The acceleration of the cathodic process only was accompanied by an increase of loosely accumulated iron sulfide and elemental sulfur within the fouling deposits. Therefore, corrosion acceleration by accumulated sulfides can be attributed to cathodic depolarization, apparently by stimulation of hydrogen ion reduction.

The sulfate reducing activity within the deposit in the absence of external organic substrate indicates that SRB consumed hydrogen from the corrosion process. However, it is not known if this activity enhanced the corrosion rate or if the bacteria utilized hydrogen as a substrate. Several authors have found that the oxidation of iron is not directly stimulated by hydrogen removal but by the sulfide produced by SRB, see Equation 2 (Costello, 1974; Cord-Ruwisch & Widdel, 1986; Deckena & Blotevogel, 1992). If the removal of hydrogen or the production of sulfide is essential for enhancing the corrosion process, the SRB are not only important for the initiation of the corrosion process, such as proposed by McKenzie and Hamilton (1992), but also for the continuation and increase in the process.

Oxygen seemed not to be directly involved in the corrosion process as a rate limiting step since it was not possible to measure a large difference in the corrosion rate during the oxic and anoxic period. The role of oxygen was most likely to create the partly oxidized Fe-S compounds and to oxidize the out-diffusing reduced compounds (*e.g.* Fe^{2+}) at the anoxic/oxic interface. A direct role of oxygen could be a reaction of oxygen with hydrogen atoms accumulated within the deposit, which has been suggested by Uhlig (1971), or oxygen could be consumed by aerobic hydrogen consuming microorganisms. Recently, it has been shown that SRB are able to consume oxygen (Dilling & Cypionka, 1990), and hydrogen can be consumed at very high rates by oxygen respiration (Fitz & Cypionka, 1991). This implicates two possible mechanisms of hydrogen removal by the SRB in alternating oxic/anoxic systems, *viz* sulfate reduction and aerobic respiration.

A relatively large pool of corrosive S^O was present in the deposit. Elemental sulfur and polysulfide are corrosive to mild steel and may contribute to the observed high corrosion rate. It may be reduced by the corrosion process (Eqn 3) and regenerated at the oxic interface, transporting electrons toward the oxic part of the deposit. Thus, an autocatalytic process is possible once the environmental conditions are established.

The characteristic type of pitting attack beneath the tubercle reported by Hardy and Bown (1984) was not observed in the present experiment. The steel surface was corroded uniformly and covered with a thick deposit (about 2 mm) at the end of the experiment. This can be attributed to differences in structure and composition of the corrosion product. Hardy and Bown reported that the corrosion rate was 7.2 mpy and that the metal surface was covered with a glutinous black precipitate before air was introduced into the system. After air exposure the corrosion rate increased to 25.6 mpy and the sulfide film was differentiated into loosely adherent material and a protective tarnish. In the present experiment no significant adherent layer was observed. This was probably due to the large amounts of iron hydroxide which covered the metal surface and which was produced by a predominantly aerobic corrosion process at the start of the experiment. It was later converted to iron sulfide when the SRB became active.

CONCLUSIONS

Alternating oxic and anoxic environments created highly corrosive conditions for mild steel after one month, where corrosion rates reached up to 120–160 mpy. Large pools of S^O, FeS and FeS_2 were detected within the deposit with a total S concentration of approximately 160 $\mu mol \cdot cm^{-3}$.

Extended aeration of the biofilm system for one month without addition of any organics had no effect on the system. The Fe-S pools and corrosion rate remained high and constant. The high corrosion rate created a high oxygen consumption rate which prevented penetration of dissolved oxygen to the metal surface. Measured "*in situ*"

sulfate reduction rates after extended aeration showed high activities within the anaerobic part of the deposit. The oxygen uptake rate showed a rate comparable to the corrosion rate. The activity of the sulfate reducers thus seemed to act as electron carrier from the metal surface to the oxic deposit surface.

It was concluded that the corrosion mechanism was related to the large Fe-S pool which provided surface area and hydrogen to SRB as well as potentially corrosive elemental sulfur. The rate limiting step for corrosion remains unclear.

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