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MICROBIAL CORROSION

Warren P. Iverson, et al

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For the Period
May 1, 1970, to April 30, 1971
and

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For the Period
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MICROBIAL CORROSION

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<p>In a continuing study of the anaerobic corrosion of mild steel by a marine strain of <i>Desulfovibrio</i> it was found that stalactite growths were formed from a mild steel electrode in a Trypticase Phytone seawater (TPSW) culture without added Fe^{++} ions. Analysis of this material indicated that it was composed of a compound of Fe and S with Fe in the Fe^{+++} state as well as free S. Heating the material in a vacuum at $1200^{\circ}C$ for 15 minutes revealed an iron-carbon alloy composed of 77% ferrite, 13% austenite, and 10% cementite. Attempts to reproduce this type of corrosion in the TPSW culture medium without added Fe^{++} as well as in the bacteria-free culture filtrate were unsuccessful. In the TPSW medium containing added Fe^{++} ions, corrosion of mild steel was stimulated, but the maximum corrosion rate was only about 1/10 that of the maximum corrosion rate of 255 mdd reported earlier in the same medium. In sterile TPSW medium, FeS (1.34 mM) chemically formed had little if any effect on the corrosion rate or potential of mild steel when the steel surface was facing the bottom of the vessel. In a TPSW culture filtrate from which the excess S^{--} ions were removed by precipitation with Fe^{++} ions, the corrosion rate of mild steel was extremely high. A corrosion rate of over 1000 mdd was recorded for an interval of a day. The evidence indicates that a depolarizing agent was produced by the bacterial cells which removed electrons from the metal surface. Trapping studies of the volatile products from a non-marine strain of <i>Desulfovibrio</i> has shown a compound to be trapped at $80^{\circ}C$ and one at $-196^{\circ}C$. IR absorption peaks of the compound trapped at $80^{\circ}C$ are at 695, 775, 910, and 990 cm^{-1}. The compound trapped at $-196^{\circ}C$ has peaks at 775 and 1740 cm^{-1}.</p>			

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Warren P. Iverson and Joan Churney
Corrosion Section
National Bureau of Standards
Washington, D. C. 20234



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IV

INTRODUCTION

The studies on microbial corrosion described in this report are a continuation of those initiated and reported in the last Technical Summary Report.⁽¹⁾ Emphasis continued to be placed on the mechanisms of anaerobic corrosion produced by sulfate-reducing bacteria.

Investigations of the corrosion rate of mild steel in Trypticase Phytone seawater (TPSW) medium inoculated with the marine strain of Desulfovibrio, both in the presence and absence of Fe^{++} ions, by polarization techniques, were continued and are described in this report.

Further investigations on the isolation and identification of the volatile product from a non-marine strain of Desulfovibrio are reported.

EXPERIMENTAL WORK

1. Physiological Studies

In the previous reports studies on a volatile phosphorous compound produced by Desulfovibrio^(1,2) and an unusual attack on Pyrex glass were discussed.⁽¹⁾

(a) Glass Degradation

Qualitative spectrochemical examination of one of the black deposits on the surface of a Pyrex glass joint showed faint traces of iron in addition to the constituents of the glass. Other metallic elements were not found. Further analysis using the electron probe will be done.

(b) Identification of Volatile Phosphorous Compound

After a delay, studies on the identification of the volatile phos-

phorous compound were again initiated. A new trap line was build facilitating trapped compounds to be moved to a high vacuum system where they could be fractionated and removed to IR and other cells for analysis.

Plates of the agar medium (2% yeast extract, 0.5% dipotassium phosphate + 2% agar) were inoculated with the API strain of Desulfovibrio desulfuricans and a stream of hydrogen carrier gas passed over them through a series of cold traps (ice + sodium chloride, CO₂ ice + ethanol, and liquid N₂). Growth of the organisms was generally poor on this medium and contamination was usually a problem. CO₂, ethanol, and acetone were the detectable products usually produced by these contaminants.

Two compounds, however, were produced by the strain of Desulfovibrio. One compound, immiscible in water, was found to be trapped at -80°C. The IR absorption peaks were at 695, 775, 910, and 990 cm⁻¹. The second compound, trapped at -196°C had absorption peaks at 775 and 1740 cm⁻¹. A distinct garlic-like odor was present from IR cells which contained the second compound. The first compound also had a distinct but dissimilar odor. It resembled that of a thio compound similar to the odor from vulcanized tires.

Further investigation of these compounds is in progress.

2. Corrosion Studies of Mild Steel by the Marine Isolate of Desulfovibrio

Simultaneous studies of the corrosion rate of mild steel in TSPW medium with and without added ferrous ions described in the last report⁽¹⁾ were completed.

Two further studies of the corrosion of mild steel in the absence of added ferrous ions and 5 studies in the presence of added ferrous ions

are described.

A study of the effect of chemically produced iron sulfide in sterile TPSW medium on the corrosion of mild steel is also reported.

(a) Experimental procedure

The procedures used in setting up the corrosion cell were in general described in the second report,⁽²⁾ with the following modifications.

Initially the test mild steel electrode (cylinder) and supporting rod were covered with heat shrinkable tubing. As it was found difficult to insure a tight fit around the steel test cylinder, the cylinder was embedded in epoxy resin. The excess epoxy was removed with a saw and a hole bored through the epoxy and the epoxy-filled threaded hole in the top of the cylinder. The hole was rethreaded using 5-40 #2 and 5-40 #3 taps. The supporting rod (5/32" diameter) was screwed into the cylinder top. A glass tube (9/32" O.D.) was inserted over the supporting rod and separated from the epoxy coated cylinder by a teflon washer and the nut at the end of the rod by a second teflon washer (cut from 1/4" teflon rod).

The nut was tightened with sufficient pressure to prevent leakage to the supporting rod. The epoxy coated cylinder was turned on a lathe so that the thickness of the epoxy layer around the cylinder was about 1/8" thick. The bottom or face of the cylinder was polished using progressively fine grades of emory paper (220, 320, 400, and 600), degreased in acetone, and the electrode including the glass rod immersed in 95% ethanol until used. A modification of the sterilization procedure consisted in immersing the electrode in a 1:1 mixture of conc. HCl and water, washing in alcohol, and removing the alcohol by flaming. The surface was repolished on sterile 600 emory paper.

The two polarization techniques employed (the "polarization break" method and the linear polarization method) were also described in the report.⁽²⁾ Although both techniques were employed, the results reported are those obtained with the "polarization break" method except when specified.

In addition to the basic circuit used for polarization described in Report No. 1⁽³⁾ a high impedance voltmeter (chopper amplifier), a 1-1/2 volt battery, and a variable resistance (1,000 ohms) were connected in series between the specimen electrode and the reference electrode. This was in addition to the pH meter which was used to measure the potential of the specimen electrode. By nulling the potential between the reference electrode and specimen electrode by means of the battery and variable resistance, the voltmeter reading could be adjusted to read 0 volts for the open circuit potential of the specimen electrode. When current was applied during polarization measurements, the readings on the voltmeter measured the overpotential or the potential difference before and after polarization or the addition of current. An indication of the sulfide ion activity was indicated (not standardized) by measuring the potential between a sulfide ion electrode and a saturated calomel electrode.

The same batch of aged seawater was employed for the medium (appendix) and the same marine strain of Desulfovibrio was used. It was maintained on petri plates of Trypticase soy agar plus seawater under a hydrogen atmosphere. Transfers were made at monthly intervals. Initially ferrous ions (0.05% ferrous ammonium sulfate) were incorporated in the agar but this was later omitted.

The inoculum for the corrosion cells was prepared by scraping the growth from the surface of an agar plate and suspending it in sterile

The corrosion cell was inoculated by boring a hole through the petroleum paraffin mixture (vaspar) with a heated rod and the inoculum was introduced via a hypodermic needle. The hole in the vaspar was then sealed with melted vaspar. The corrosion cells were maintained at $28 \pm 1^\circ\text{C}$. All potential measurements of Fe electrodes reported were vs. S.C.E.

The divided cell employed in one experiment consisted of 2 large O-ring connectors (4 cm O.D.) bent at right angles and held together with a large clamp which also served as a base for support. A membrane filter with 0.2 μm pore size was held in place between the two connectors with an O-ring. After autoclaving at 15 psi for 15 minutes, 260 ml of medium was placed in each half, 2 rubber stoppers each holding the two electrodes (steel and platinum) and a luggin capillary were inserted in the ends of the connectors and melted vaspar poured through holes in the stoppers to form a layer about one inch thick on the surface of the medium.

(b) Results

(1) Corrosion of mild steel in the absence of Fe^{++} ions

The final observations of the experiment initially reported in Report No. 3 on the corrosion rate of mild steel in the seawater medium to which no Fe^{++} were added are shown in Table I. The potential of the steel electrode initially about -0.738 volts decreased (became more positive or more noble) to -0.401 after 263 days and then increased to -0.648 volts after 459 days. The corrosion rate was found to increase considerably with this change in potential. The redox potential became slightly lower over the entire interval of time from inoculation until 491 days, near the time that the cell was disassembled.

Polarization studies made during most of the interval indicated that the corrosion reaction was under mixed control. The breaks in the cathodic curve occurred at about the same current levels as the anodic curves.

As was indicated in Report No. 3, the fairly high rate of corrosion was associated with the formation of corrosion products in the form of black "stalactite" growths. Periodically they fell to the bottom of the cell (beaker) when it was disturbed and new ones formed. When the cell was disassembled, these formations produced a metallic sound when dropped to the bottom of the beaker. After drying in vacuo without heating, they had a tendency to crumble (Figure 1).

About 32 mg of material was obtained. Analysis of less than 1/3 of this material by x-ray diffraction studies indicated that it was amorphous.

Mössbauer studies of this material indicated that iron was present in a Fe^{+++} state. The compound was not identified but was a compound of iron and sulfur. Examination of the particles by the scanning electron microscope technique indicated a somewhat unusual topography (Figure 2).

Studies of the material with the scanning electron microscope coupled with an energy dispersive x-ray spectrometer indicated a compound of iron and sulfur with particles of free sulfur to be present.

Previously black products formed from ferrous ions in bacterial cultures which proved to be amorphous by x-ray studies were heated in vacuo to about 1,200°C to allow for possible atomic rearrangement to form a crystal structure which could then yield an x-ray diffraction pattern. The remaining (25.4 mg) material was heated in vacuo to about 1200°C for 15 minutes and allowed to cool to room temperature for a day. About 8.5 mg, or 34% was recovered. X-ray analysis of this material indicated a mixture of alpha

iron, gamma iron, and cementite. Mossbauer analysis indicated a percentage composition of 77% alpha iron (ferrite), 13% gamma iron (austenite), and 10% cementite.

When the corrosion cell was disassembled, several white particles were also noted in the bottom of the beaker as well as on the black "stalactite" material. Microscopic examination of this material indicated that the major part of it was probably composed of bacterial cells. Upon Gram straining this material, large numbers of Gram⁺ rods without spores were observed. Inoculation of a drop of the culture fluid on each of 8 agar plates of the Trypticase Phytone seawater agar medium and inoculation under a hydrogen atmosphere for about one week revealed numerous white colonies of Gram⁺, non-sporulating rods as well as colonies of Desulfovibrio to be present.

A similar experiment was repeated in an attempt to duplicate these results. The mild steel electrode, encased in epoxy, after polishing, was sterilized in dilute HCl according to the modification described under procedures. The results are presented in Table II. In contrast to the rapid decrease and increase in corrosion rate of the previous experiment, the corrosion rate fell only slightly and remained at this level (0.22-0.27 mdd) for the duration of the experiment. The potential fell very slowly to more positive values during this time. Although no further measurements were made, no stalactite formations were noted after a period of 10 months. The polarization curves indicated that the corrosion reaction was under mixed or slight cathodic control.

A third corrosion cell was prepared in which the microbial cell-free culture filtrate was employed instead of the bacterial culture. Four-

hundred ml of a 6-day-old culture of Desulfovibrio in the same medium incubated under H_2 was filtered through a Seitz asbestos filter to remove the bacterial cells. The pH of the filtrate was 7.0 and the potential of the sulfide ion electrode -0.620 volts. The filtrate was added to a sterile beaker and the sterilized electrodes, mounted in a rubber stopper, inserted into the filtrate. The results are indicated in Table III. The corrosion rate was found to increase to a little more than 7 times the initial rate (0.25 mdd) on the 8th day, then decreased to about the level of the initial rate (0.35 mdd) on the 39th day. The rate again increased to about 16 times the original corrosion rate (4.00 mdd) and then fell to 1 to 2 mdd at 128 days. The potential increased to more negative (active) values during the periods when the corrosion rate increased and then remained fairly constant at -0.572 volts for the remaining 30 days. The breaks in the polarization curves occurred most of the time at approximately the same current value, indicating that the corrosion was under mixed control. During the slight initial increase in corrosion rate, the breaks in the cathodic curves occurred before the breaks in the anodic curves, indicating cathodic control of the corrosion process. Contamination of the culture filtrate was noted after the 10th day.

(2) Corrosion of mild steel in the presence of added Fe^{++} ions

The final observations of the experiment initially described in Report No. 3 on the corrosion of mild steel in the presence of added ferrous ions are shown in Table I. The potential of the steel dropped slightly from -0.457 V on the 278th day to -0.438 V on the 495th day while the potential of the platinum electrode remained about the same during this period (-0.440 V, redox potential -0.190 V). Breaks in the polarization

curves indicated that the corrosion reaction was under mixed control during this period. Until the 39th day after inoculation the reaction was under cathodic control with the slope in the anodic curve being less after the break than before. From this time until the 495th day, the slope in the anodic curve after the break was greater than before the break. The corrosion reaction was at various times under mixed cathodic and anodic control until the 495th day.

A second corrosion cell was prepared with the same concentration of Fe^{++} (0.25% ferrous ammonium sulfate) as the previous cell. The results of the electrochemical measurements are presented in Table IV. The potential of the steel electrode dropped about 10 mV in a more active direction immediately after inoculation and remained fairly constant until 58 days after the cell was inoculated. The corrosion rate increased soon after inoculation and reached a maximum of 31 mdd, 28 days after inoculation. A definite decrease occurred after 74 days. The polarization curves indicated that the corrosion reaction was under cathodic control through the 58th day, with the slopes in the anodic curves after the breaks being less than before the breaks. On the 76th and remaining days the reaction was under mixed control, the anodic curves being similar in shape to the cathodic curves.

In a third experiment a divided cell was employed with a membrane placed between the two cell halves (A and B). The pore size of the membrane filter was small enough (0.2 μm) so that it would be expected to retain the bacterial cells in one cell (Cell A) and maintain the other cell (Cell B) sterile.

The object of the experiment was to determine whether the excess metabolic products of the organisms after reacting with the ferrous ions in Cell A and passing through the membrane would have any effect on the corrosion of steel in Cell B in the presence of ferrous ions.

The medium containing Fe^{++} ions was the same as that employed in the previous experiments except that the additional NaCl (0.5%) was omitted. After an initial period of 21 days to allow the systems to equilibrate, Cell A was inoculated. The results of the electrochemical measurements are presented in Table V. As indicated in the table, the corrosion rate of the steel in Cell B was slightly greater than the corrosion rate of the steel in Cell A before it was inoculated. A few days after inoculation of Cell A, the corrosion rate started to increase and reached a maximum rate of 29 mdd on the 16th day and then decreased to 10-15 mdd for the remaining time the measurements were made. Up to and until the time the corrosion rate was maximum and started to decrease (17 days) the breaks in the polarization curves indicated that the corrosion reaction was under cathodic control. The slope in the anodic curve after the break was less than before the break. After the 17th day, the slope of the anodic curve after the break was greater than before the break and the corrosion reaction varied from mixed to slight anodic and cathodic control. The potential of the steel electrode decreased slightly in a more positive direction until the time when the corrosion rate was maximum (16 days) and then decreased much more rapidly from that time until the 27th day when it reached the lowest values (-0.552V). From that time it increased slightly and remained fairly stable with slight fluctuations throughout most of the time the measurements were made.

The corrosion rate of the steel in Cell B remained at its initial relatively low value until the 36th day when the rate was maximum (23 mdd). The corrosion rate dropped to about half that value by 42 days and stayed at that level or a little less for the remainder of the time. Again the potential of the steel electrode dropped to more positive values after the maximum corrosion rate. The polarization curves indicated that the corrosion process was under cathodic control up to and including the time when the maximum corrosion rate was observed. Again the slope in the anodic curves after the break was less than before the break. After the time of the maximum corrosion rate, the corrosion process, similar to the corrosion process in Cell A, was under mixed, slightly cathodic or anodic control.

It was suspected during the course of the experiment that the organisms moved through the membrane or a break in the membrane into Cell B, the uninoculated cell. These suspicions were confirmed when the organisms were cultured from Cell B on the 65th day after Cell A was inoculated. On disassembly of the cell the membrane was found to be extremely brittle and broke into several fragments.

(3) Effect of chemically prepared iron sulfide on the corrosion of Mild Steel

It appeared from the previous results that stimulation of corrosion by Desulfovibrio consistently occurred in the seawater medium when added ferrous ions were present. In view of the results by Booth et al.⁽⁴⁾ that FeS brought about cathodic stimulation of mild steel the possibility that this compound might be responsible for the stimulation in corrosion seemed apparent.

After electrochemical measurements were made on mild steel specimens in sterile medium with added ferrous ions (same concentration as used previously) sodium sulfide was added to form FeS and the measurements were continued for 14 days.

From the results presented in Table VI it appears that the chemically produced FeS had little if any effect on the corrosion rate or the potential of the steel.

(4) Effect of Fe⁺⁺ ions, added to a seawater culture filtrate, on the corrosion of mild steel

From the previous results, it appeared that an increase in the corrosion rate occurred when Fe⁺⁺ ions were present in the medium and a black precipitate was formed. The chemically produced FeS did not appear to affect the corrosion rate. To establish whether another black product (possibly Fe₃P) formed by reaction with the Fe⁺⁺ ions might be the corrosive agent, Fe⁺⁺ ions (ferrous ammonium sulfate) were added to a Seitz-filtered (to remove bacteria) culture. The electrochemical measurements of the electrodes in the filtrate are indicated in Table VIII. The amount of Fe⁺⁺ ions added to the filtrate was determined by titrating a small portion of the filtrate with a solution of ferrous ammonium sulfate using a sulfide ion electrode.⁽⁵⁾ The corrosion rate, initially about 4 mdd, dropped to 2.8 mdd on the 7th day, increased to 10.7 mdd on the 10th day, and then fell to about 2-3 mdd and remained at this level for the duration of the measurements. The corrosion process was under cathodic control until the 7th day with the characteristic slopes of the anodic curve before and after the breaks. After the 7th day the

corrosion process was under anodic control with the slopes after the break in the anodic curve being greater than before the breaks. The potential of the steel also fell fairly rapidly to more noble values after the changes in the anodic polarization curves. This appeared to indicate that a film was being formed on the steel surface due to the fresh formation of metabolic products including H_2S by the organisms. This was found to be the case upon disassembly of the cell. A strong odor of H_2S was apparent and measurement of a portion of the culture fluid by the sulfide ion electrode indicated that the sulfide ion activity in the filtrate was as great as that found in the filtrate immediately after Seitz-filtration. Culturing of the filtrate revealed the presence of Desulfovibrio.

The experiment was repeated adding $FeCl_2$ to the filtrate instead of ferrous ammonium sulfate. The results are indicated in Table VIII. After an induction period of 3 days during which the potential of the steel electrode decreased to more noble (positive) values, the corrosion rate increased greatly, reaching a maximum rate of over 1,000 mdd on the 10th day after the start of the corrosion process. This increase in corrosion rate was accompanied by a change in the redox potential to lower (more negative) values. A similar effect was noted previously⁽¹⁾ in the seawater culture containing Fe^{++} ions where a peak corrosion rate of 256 mdd was reported. The polarization curves indicated that the corrosion process was under mixed or anodic control after the 3rd day and that a film was produced on the surface of the steel. A black film which covered about 1/4 of the total surface area of the steel surface was observed when the electrode was removed (Figure 3).

To determine whether the corrosion of the steel was due to the black precipitate or a soluble component in the culture filtrate, Fe^{++} ions were added to a culture of the marine strain and the resulting organisms and black precipitate were removed by Seitz filtration. Five small mild steel nails were placed in the filtrate which was then sealed with vaspar. Five nails were also placed in sterile medium and sealed with vaspar to serve as a control. After an induction period of 3 days, the solution became completely black. Twenty days after the solution had turned black, the nails were removed, the black corrosion products removed by wiping with tissue paper, and the nails were weighed. The results are presented in Table IX. The total weight loss of the nails was about 75 mg. Approximately 180 mg of black dried product was recovered for analysis. The average corrosion rate of the nails was about 30 times that of the control nails.

(c) Discussion

It is evident from the results obtained that the marine strain of Desulfovibrio produces a strong depolarizing agent in the seawater medium and that the bacterial cells per se are not essential as originally proposed in the cathodic depolarization theory of von Wolzogen Kühr and van der Vlugt.

From the high corrosion rates obtained, it appears as if the depolarizing agent removes electrons directly from the metal itself rather than through direct utilization of hydrogen. The corrosion rate of iron based on the rate of hydrogen formation by the direct reduction of water as a function of the electrode potential has been calculated by Nelson to be from 0.05 to 0.4 $\mu A/cm^2$ (0.125 to 1.0 mdd), using potentials of

steel in bacterial cultures within the range of -0.45 to -0.6 V (NHE). Such values would be too low to explain the high corrosion rates obtained by direct utilization of hydrogen. A highly reduced compound appears to be formed in the medium as indicated by the lowering of the redox potential. A black compound was also formed which is presently being identified. The possibility is suggested that the depolarizing agent after removal of the electrons from the iron is in a highly reduced condition and transfers the electrons to an acceptor, in a manner similar to the electron transferring enzymes in biological systems. The black material may be the reduced acceptor.

Previously blackening of yeast extract broth, containing a steel coupon and inoculated with a non-marine strain of Desulfovibrio was reported.⁽³²⁾ The black corrosion product was initially amorphous but after heating in vacuo to 1230°C , Fe_2P was identified by x-ray diffraction studies.

As H_2S is also produced by Desulfovibrio the results presented in this paper as well as by others^(8,9,10,11) appear to indicate that a film of iron sulfide is formed on the surface of the steel if there is no material (Fe^{++} ions) present to remove it. This film appears to act as a protective film thereby preventing corrosion. When this film becomes detached, the ensuing corrosion is probably a result of the action of the depolarizing agent also produced by Desulfovibrio. If this protective sulfide film is prevented from forming by previous removal of the sulfide, the depolarizing agent has access to the metal surface and can initiate the corrosion reaction. This appears to happen when Fe^{++} ions are present in the medium. The corrosion rate at first increases and then decreases (Figure 7⁽¹⁾ and Table IV). The decrease in corrosion rate may be due

to additional H_2S which was produced from the ammonium sulfate after the sulfide ions initially produced were removed by the Fe^{++} ions. Although Booth et al⁽⁴⁾ demonstrated cathodic depolarization of mild steel by chemically produced FeS (0.6 - 5.0 mM FeS) little if any increase in the corrosion rate of steel was noted by 1.34mM FeS. The steel surfaces used by Booth were in such a position (facing the top of the vessel and vertically) that FeS could accumulate on their surfaces. The surface of the electrode used in these studies was facing the bottom of the cell, having less possibility for FeS accumulation on the surface and less possibility for the formation of FeS-Fe couples.

SUMMARY

1. Analysis of the black corrosion products, which formed from a steel surface as stalactite growths in Trypticase Phytone seawater medium by Mossbauer analysis and the scanning electron microscope indicated it to be an amorphous compound of Fe and S which has not yet been identified. The Fe was present in the Fe^{+++} state. Some free S was also found.
2. Heating the corrosion product in vacuo to 1200°C yielded an iron-carbon alloy composed of 77% alpha iron, 13% gamma iron, and 10% cementite.
3. Two further attempts to reproduce a similar type of corrosion, one in a culture and the other in a culture filtrate of the seawater medium, were unsuccessful. The corrosion process appeared to be inhibited by a film and was under a combination of mixed, slightly anodic, or cathodic control.
4. In the presence of added Fe^{++} ions, the corrosion of mild steel appeared to be fairly reproduceable. Maximum corrosion rates obtained were 25-30 mdd. The corrosion process was under cathodic control.

5. In a divided cell, separated by a membrane, and containing the seawater medium plus added Fe^{++} ions, the corrosion of steel initially developed in the inoculated half and later in the cell which was not inoculated. The "leak" of organisms into the uninoculated side was probably due to the deterioration of the membrane.

6. The presence of FeS in the sterile seawater medium had little if any effect on the potential of the steel or its corrosion rate.

7. The addition of Fe^{++} ions to a Seitz-filtered seawater culture produced a maximum corrosion rate of about 1000 mdd. The potential of the steel fell to more noble values and the redox potential to negative values at the time when the corrosion rate accelerated. Mild steel nails lost about 2% of their original weight during 20 days in a Seitz-filtrate of a culture to which Fe^{++} ions were added.

8. Further studies of the volatile products from the marine strain of Desulfovibrio have shown the presence of two compounds. One is trapped at -80°C and the other at -196°C . The first compound has IR absorption peaks at 695, 725, 910, and 990 cm^{-1} . The second compound trapped at 196°C has absorption peaks at 775 and 1740 cm^{-1} .

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Appendix

Trypticase Seawater Medium

Trypticase	15.0 gm
Phytone	5.0 gm
Sodium Chloride	5.0 gm
Aged Seawater	1000.0 ml

pH adjusted to 7.0 ± 0.1 with NaOH. When the medium contained ferrous ions, 2.5 gm of ferrous ammonium sulfate in aged seawater solution were Seitz filtered and added immediately after sterilization of the above medium. For cultivation of the Desulfovibrio strain on agar plates, 20 gm agar was added. Ferrous ammonium sulfate (0.05%) initially was added to the plating medium. This was later omitted.

Table 1

The Effect of Added Ferrous Ions on the Instantaneous Corrosion Rates of Mild Steel in a Trypticase-Seawater Culture (cont'd).

Cumulative Time (days)	No Ferrous Ions						Ferrous Ions					
	Potential ⁺ (volts)		Corrosion Rate (mdd) ^{**}		Potential ⁺ (volts)		Corrosion Rate (mdd) ^{**}		Potential ⁺ (volts)		Corrosion Rate (mdd) ^{**}	
	Steel	Platinum ⁺⁺	PB [°]	PA [°]	Steel	Platinum ⁺⁺	PB [°]	PA [°]	Steel	Platinum ⁺⁺	PB [°]	PA [°]
278	-.574	-.425	13.35	21.60	-.457	-.438			-.457	-.438	8.32	4.32
308	-.609	-.441	-	41.60	-.452	-.452			-.452	-.452	-	2.07
318	-.611	-.451	-	288.25	-.448	-.447			-.448	-.447	-	4.32
320	-.610	-.448	-	271.20	-.450	-.451			-.450	-.451	-	4.32
321	-.612	-.447	-	241.12	-.458	-.452			-.458	-.452	-	5.60
327	-.617	-.458	-	187.55	-.450	-.448			-.450	-.448	-	4.56
334	-.620	-.458	-	-	-.450	-.450			-.450	-.450	-	-
335	-.620	-.453	-	-	-.461	-.456			-.461	-.456	-	-
350	-.622	-.465	-	277.76	-.458	-.461			-.458	-.461	-	3.15
351	-.622	-.461	-	197.76	-.454	-.452			-.454	-.452	-	1.77
356	-.622	-.467	-	185.12	-.448	-.452			-.448	-.452	-	1.44
361	-.623	-.420	-	158.64	-.455	-.457			-.455	-.457	-	0.88
362	-.622	-.469	-	180.03	-.459	-.459			-.459	-.459	-	2.40
377	-.630	-.463	-	-	-.457	-.466			-.457	-.466	-	-
459	-.648	-.571	-	434.00	-.441	-.441			-.441	-.441	14.55	2.87
460	-.638	-.521	151.20	434.00	-.440	-.449			-.440	-.449	-	-
462	-.557	-.398	-	120.75	-.439	-.441			-.439	-.441	-	1.44
465	-.581	-.425	-	66.00	-.439	-.441			-.439	-.441	-	3.18
466	-.587	-.430	-	13.04	-.440	-.441			-.440	-.441	-	1.68
467	-.588	-.430	-	56.60	-.438	-.440			-.438	-.440	-	2.08
469	-.590	-.436	-	46.24	-.439	-.441			-.439	-.441	-	1.44
488	-.618	-.461	312.40	-	-	-			-	-	-	-
489	-.613	-.461	-	52.00	-	-			-	-	-	-
490	-.610	-	-	43.30	-	-			-	-	-	-
491	-.609	-.457	39.68	43.60	-	-			-	-	-	-
495	-	-	-	-	-.438	-.441			-.438	-.441	1.70	1.18

Table 1 (cont'd)

" mdd - "milligrams/square decimeter/day
 " Pp - "polarization break" method
 " FA - linear polarization method
 " potential vs sat calomel half cell
 " Redox-potential may be calculated by adding +0.250 volts to the values. As the final pH was near 7.0, no
 " correction for pH is necessary.

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Table II

The Effect of a Seawater Culture of *Desulfovibrio*, without Added Fe^{++} ,
on the Corrosion of Mild Steel

Time (days)	Potential (volts)		Corrosion	
	Fe	Redox	Current Density ($\mu A/cm^2$)	Rate (mdd)
0	-0.732	+0.055	-	-
1	-0.715	+0.160	0.32	0.80
2	-0.722	+0.175	0.15	0.37
6	-0.728	+0.182	0.11	0.27
<u>Inoculated⁺ on 6th Day</u>				
0	-0.721	+0.130	-	-
2/3	-0.750	-0.100	0.12	0.30
2	-0.740	-0.137	0.13	0.32
3	-0.721	-0.142	0.06	0.15
4	-0.710	-0.143	0.18	0.45
8	-0.684	-0.210	0.16	0.40
9	-0.682	-0.216	0.10	0.25
14	-0.671	-0.213	0.10	0.24
15	-0.671	-0.232	0.11	0.27
17	-0.650	-0.219	0.12	0.30
18	-0.148	-0.216	0.09	0.22
22	-0.631	-0.215	0.03	0.07
23	-0.629	-0.218	0.09	0.22
25	-0.622	-0.215	0.06	0.15
28	-0.613	-0.238	0.08	0.20
30	-0.605	-0.218	0.09	0.22
32	-0.600	-0.238	0.09	0.22
35	-0.598	-0.220	0.09	0.22
36	-0.599	-0.220	0.09	0.22
38	-0.591	-0.221	0.05	0.12
42	-0.580	-0.188	0.08	0.20
43	-0.572	-0.221	0.09	0.22
45	-0.575	-0.221	0.09	0.22
46	-0.572	-0.222	0.11	0.27
49	-0.570	-0.220	0.11	0.27
50	-0.569	-0.231	0.11	0.27
53	-0.566	-0.222	0.10	0.25
60	-0.561	-0.222	0.09	0.22
64	-0.561	-0.222	0.10	0.25

⁺ Inoculum consisted of the growth from an 11-day-old culture on a Trypticase-Phytone seawater agar plate without added ferrous ions.

Table III

The Effect of a Seawater Culture Filtrate⁺ of Desulfovibrio
on the Corrosion of Mild Steel

Time (days)	Potential (volts)		Corrosion	
	Fe	Redox	Current Density (uA/cm ²)	Rate (mdd)
0	-0.670	-0.110	-	-
1	-0.642	-0.192	0.10	0.25
2	-0.650	-0.200	0.11	0.27
3	-0.683	-0.201	0.19	0.47
7	-0.742	-0.207	0.64	1.60
8	-0.746	-0.210	0.73	1.82
9	-0.753	-0.229	0.49	1.62
10	-0.735	-0.215	-	-
29	-0.582	-0.220	0.42	1.05
30	-0.572	-0.220	0.48	1.54
36	-0.565	-0.221	0.42	1.05
37	-0.565	-0.228	0.20	0.50
38	-0.568	-0.228	0.22	0.55
39	-0.569	-0.221	0.14	0.35
42	-0.560	-0.221	0.22	0.55
45	-0.614	-0.269	0.96	2.40
49	-0.618	-0.225	1.36	3.40
50	-0.619	-0.215	1.60	4.00
53	-0.621	-0.225	0.96	2.40
56	-0.629	-0.223	0.54	1.35
58	-0.631	-0.216	0.72	1.80
59	-0.632	-0.228	1.02	2.55
63	-0.639	-0.288	0.83	2.07
65	-0.635	-0.228	0.96	2.40
66	-0.634	-0.228	0.88	2.20
71	-0.633	-0.221	0.85	2.12
73	-0.629	-0.221	0.56	1.40
78	-0.597	-0.230	0.99	2.47
81	-0.589	-0.229	0.71	1.77
87	-0.581	-0.231	0.54	1.35
91	-0.579	-0.230	0.46	1.15
93	-0.579	-0.231	0.41	1.02
98	-0.577	-0.228	0.74	1.85
100	-0.571	-0.241	0.69	1.72
101	-0.573	-0.228	0.82	2.05
105	-0.570	-0.28	0.83	2.20
108	-0.572	-0.231	0.56	1.40
112	-0.580	-0.281	0.3	1.82
119	-0.572	-0.289	0.74	1.85
128	-0.571	-0.231	0.61	1.52

⁺ Seitz filtrate of 6-day-old culture.

Table IV

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The Effect of a Seawater Culture of *Desulfovibrio*, with Added Fe^{++} ,^o
on the Corrosion of Mild Steel

Time (days)	Potential (volts)		Corrosion	
	Fe	Redox	Current Density ($\mu\text{A}/\text{cm}^2$)	Rate (mdd)
0	-0.699	-0.099	-	-
1	-0.699	-0.108	-	-
2	-0.699	-0.101	0.92*	2.30
5	-0.697	-0.091	1.34	3.35
6	-0.699	-0.189	1.06	2.65
7	-0.699	-0.220	0.93	2.36
9	-0.696	-0.181	1.12	2.80
12	-0.692	-0.082	1.08	2.70
Inoculated ^o on 12th Day				
0	-0.685	-0.139	-	-
1	-0.698	-0.155	2.20	5.50
2	-0.681	-0.201	1.48	3.70
3	-0.678	-0.187	2.78	6.95
4	-0.680	-0.180	3.62	9.05
7	-0.683	-0.191	3.52	8.81
8	-0.675	-0.205	4.90	12.25
9	-0.676	-0.179	6.20	15.50
11	-0.677	-0.211	6.40	16.00
14	-0.676	-	7.75	19.37
16	-0.680	-0.251	7.45	18.62
17	-0.679	-0.251	5.25	16.10
18	-0.672	-0.249	6.52	16.30
22	-0.674	-0.229	6.56	16.32
23	-0.677	-0.238	7.75	19.35
24	-0.675	-0.240	9.89	24.72
25	-0.675	-0.214	9.20	23.00
28	-0.678	-0.238	12.42	31.06
29	-0.677	-0.223	7.70	19.15
30	-0.681	-0.221	6.85	17.12
36	-0.681	-0.221	7.20	18.00
38	-0.683	-0.245	9.47	23.67
39	-0.685	-0.223	9.50	23.75
42	-0.682	-0.223	9.86	24.65
43	-0.683	-0.231	9.15	22.87
44	-0.686	-0.249	9.26	23.15
46	-0.687	-0.226	8.95	22.32
49	-0.688	-0.227	10.30	26.15
56	-0.691	-0.227	10.21	25.50
58	-0.668	-0.232	10.53	26.32
74	-0.617	-0.190	5.86**	14.65
92	-0.620	-0.194	4.80	12.00
101	-0.610	-0.201	4.16	10.40

* Cathodic Control

** Mixed Control

^o 0.25% ferrous ammonium sulfate^o Inoculum consisted of the growth from a 7-day-old culture on a Trypticase Phytone seawater agar plate without added ferrous ions.

Table V

Corrosion of Mild Steel⁺⁺ in Two Corrosion Cells, of Seawater Medium
Plus Fe⁺⁺, Separated by a Membrane

Time (days)	Cell A				Cell B			
	Potential		Corrosion		Potential		Corrosion	
	Fe	Redox	Current Density (uA/cm ²)	Rate (mdd)	Fe	Redox	Current Density (uA/cm ²)	Rate (mdd)
0	-0.738	-0.151	-	-	-0.716	-0.037	-	-
1	-0.749	-0.154	-	-	-0.716	-0.105	-	-
2	-0.718	-0.127	-	-	-0.700	-0.181	-	-
3	-0.717	-0.177	0.70	1.75	-0.703	-0.089	1.70	4.25
4	-0.717	-0.140	0.78	1.95	-0.708	-0.088	1.46	3.65
7	-0.709	-0.111	0.89	2.22	-0.711	-0.101	1.22	2.95
9	-0.714	-0.121	0.72	1.80	-0.711	-0.111	1.29	3.22
14	-0.712	-0.111	0.74	1.85	-0.711	-0.102	1.28	3.20
15	-0.709	-0.119	0.82	2.05	-0.709	-0.111	1.47	3.67
21	-0.702	-0.130	0.86	2.15	-0.711	-0.122	1.25	3.12
<u>Inoculated* on 22nd Day</u>								
1	-0.712	-0.190	0.96	2.40	-0.721	-0.110	1.40	3.50
2	-0.698	-0.360	3.62	9.05	-0.722	-0.102	1.41	3.52
3	-0.700	-0.330	5.34	13.35	-0.723	-0.093	0.98	2.45
6	-0.703	-0.288	4.70	11.75	-0.726	-0.078	1.59	3.97
7	-0.719	-0.300	4.86	12.15	-0.741	-0.108	1.38	3.45
8	-0.695	-0.279	6.24	15.60	-0.721	-0.067	1.16	2.90
9	-0.692	-0.279	6.94	17.35	-0.720	-0.075	1.98	4.97
10	-0.689	-0.289	8.77	21.92	-0.720	-0.119	1.43	3.57
13	-0.687	-0.299	10.66	26.65	-0.722	-0.061	1.31	3.27
14	-0.687	-0.300	10.66	26.65	-0.724	-0.070	1.41	3.52
15	-0.687	-0.301	-	-	-0.721	-0.068	-	-
16	-0.643	-0.284	11.62	29.05	-0.721	-0.068	1.34	3.35
17	-0.637	-0.280	9.60	24.00	-0.722	-0.068	1.22	3.05
20	-0.619	-0.278	4.45	11.12	-0.722	-0.061	1.54	3.85

Table V (cont'd)

Time (days)	Cell A					Cell B				
	Potential		Corrosion		Rate (mdd)	Potential		Corrosion		Rate (mdd)
	Fe	redox	Current Density ($\mu\text{A}/\text{cm}^2$)	Rate (mdd)		Fe	Redox	Current Density ($\mu\text{A}/\text{cm}^2$)	Rate (mdd)	
21	-0.611	-0.274	3.87	9.67	-0.722	-0.069	1.22	3.05		
22	-0.604	-0.270	4.32	10.80	-0.722	-0.069	1.64	4.10		
23	-0.595	-0.250	4.32	10.80	-0.722	-0.063	1.01	2.52		
24	-0.587	-0.254	0.67	1.67	-0.722	-0.068	0.96	2.30		
27	-0.552	-0.244	2.08	5.20	-0.725	-0.066	0.96	2.30		
30	-0.579	-0.242	-	-	-0.732	-0.065	-	-		
31	-0.560	-0.235	-	-	-0.732	-0.081	-	-		
36	-0.591	-0.230	1.60	4.0	-0.682	-0.267	9.47	23.67		
37	-0.595	-0.222	-	-	-0.611	-0.288	-	-		
38	-0.601	-0.242	-	-	-0.599	-0.280	-	-		
39	-0.592	-0.237	-	-	-0.592	-0.237	-	-		
42	-0.600	-0.237	5.50	13.75	-0.553	-0.240	5.40	13.00		
43	-0.590	-0.239	4.80	12.00	-0.561	-0.229	3.30	8.25		
44	-0.576	-0.240	-	-	-0.562	-0.209	-	-		
45	-0.588	-0.238	3.98	9.95	-0.581	-0.201	4.00	10.00		
46	-0.578	-0.238	4.32	10.80	-0.598	-0.197	4.38	10.95		
49	-0.582	-0.241	-	-	-0.590	-0.190	-	-		
50	-0.592	-0.239	3.42	8.55	-0.579	-0.189	3.52	8.80		
51	-0.581	-0.244	4.25	10.60	-0.551	-0.191	4.40	11.00		
52	-0.606	-0.246	4.90	12.25	-0.540	-0.190	-	-		
53	-0.582	-0.241	4.75	11.87	-0.542	-0.178	3.29	8.22		
57	-0.589	-0.241	4.60	11.50	-0.494	-0.188	2.80	7.00		
58	-0.587	-0.242	-	-	-0.569	-0.191	-	-		
59	-0.579	-0.239	4.85	10.52	-0.535	-0.189	2.94	7.25		
64	-0.570	-0.244	5.12	12.80	-0.535	-0.191	2.60	6.50		
65	-0.601	0.249	-	-	-0.535	-0.193	-	-		
66	-0.589	-0.238	-	-	-0.530	-0.147	-	-		
67	-0.573	-0.235	4.70	11.75	-0.568	-0.169	2.08	5.20		
70	-0.559	-0.190	5.35	12.37	-0.567	-0.180	2.68	6.70		
71	-0.586	-0.240	4.85	12.12	-0.557	-0.192	3.58	8.95		

Table V (cont'd)

Time (days)	Cell A				Cell B			
	Potential		Corrosion		Potential		Corrosion	
	Fe	Redox	Current Density ($\mu\text{A}/\text{cm}^2$)	Rate (mdd)	Fe	Redox	Current Density ($\mu\text{A}/\text{cm}^2$)	Rate (mdd)
74	-0.561	-0.240	-	-	-0.500	-0.190	-	-
79	-0.582	-0.238	-	-	-0.551	-0.190	-	-
80	-0.563	-0.234	-	-	-0.528	-0.191	-	-
81	-0.557	-0.237	-	-	-0.533	-0.190	-	-
85	-0.568	-0.242	-	-	-0.554	-0.209	-	-
86	-0.561	-0.239	-	-	-0.517	-0.201	-	-
91	-0.573	-0.257	-	-	-0.591	-0.200	-	-
92	-0.568	-0.232	-	-	-0.538	-0.199	-	-
93	-0.570	-0.231	-	-	-0.580	-0.198	-	-
101	-0.587	-0.231	-	-	-0.569	-0.211	-	-
102	-0.581	-0.239	-	-	-0.567	-0.201	-	-
105	-0.551	-0.248	-	-	-0.549	-0.201	-	-
106	-0.564	-0.242	-	-	-0.551	-0.199	-	-
107	-0.571	-0.239	-	-	-0.540	-0.200	-	-
112	-0.568	-0.241	-	-	-0.561	-0.200	-	-
113	-0.572	-0.233	-	-	-0.561	-0.200	-	-
114	-0.564	-0.236	-	-	-0.556	-0.199	-	-
115	-0.563	-0.237	-	-	-0.554	-0.205	-	-
116	-0.551	-0.241	-	-	-0.551	-0.201	-	-
119	-0.561	-0.240	-	-	-0.515	-0.218	-	-
120	-0.576	-0.238	-	-	-0.518	-0.211	-	-
122	-0.575	-0.240	-	-	-0.519	-0.202	-	-
127	-0.573	-0.241	-	-	-0.499	-0.201	-	-
133	-0.584	-0.240	-	-	-0.538	-0.206	-	-
135	-0.554	-0.239	-	-	-0.528	-0.204	-	-
137	-0.566	-0.238	6.08	15.20	-0.521	-0.203	-	-
141	-0.562	-0.251	5.76	14.40	-0.528	-0.209	5.48	14.98
143	-0.560	-0.248	-	-	-0.511	-0.210	-	-
144	-0.570	-0.239	-	-	-0.511	-0.205	-	-

Table V (cont'd)

Time (days)	Cell A				Cell B			
	Potential		Corrosion		Potential		Corrosion	
	Fe	Redox	Current Density (uA/cm ²)	Rate (mdd)	Fe	Redox	Current Density (uA/cm ²)	Rate (mdd)
147	-0.591	-0.237	-	-	-0.532	-0.211	-	-
156	-0.532	-0.238	-	-	-0.489	-0.211	-	-
162	-0.550	-0.237	-	-	-0.557	-0.210	-	-

* Inoculum consisted of the growth from a 25-day-old culture on a Trypticase-phytone seawater agar plate without added ferrous ions.

Table VI
Effect of FeS^+ on Corrosion of Mild Steel
in Sterile Seawater Medium

Time (days)	Potential (volts)		Corrosion	
	Fe°	Redox	Current Density ($\mu\text{A}/\text{cm}^2$)	Rate (mdd)
0	-0.705	-0.000	-	-
1	-0.698	-0.012	0.33*	0.82
2	-0.695	-0.003	0.38	0.95
6	-0.690	-0.001	0.28	0.70
Addition of Na_2S^+				
0	-0.702	-0.020	0.38	0.95
1	-0.705	-0.040	0.46	1.15
2	-0.705	-0.080	0.48	1.07
3	-0.705	-0.090	0.32	0.80
6	-0.703	-	0.51	1.25
14	-0.705	-0.100	0.38	0.95

$^\circ$ Potential of iron vs. S.C.E.

$^+$ 0.3 gms $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ added to 200 ml Trypticase Phytonc seawater medium containing 0.5 gm ferrous ammonium sulfate (23.6 mg FeS or 1.34 mM FeS recovered). Ferrous ions were found to be in excess.

* Corrosion under cathodic control.

Table VII

Effect of Addition⁺ of Fe⁺⁺ to a Seawater Culture Filtrate
of Desulfovibrio on the Corrosion of Mild Steel

Time (days)	Potential (volts)		Corrosion	
	Fe	Redox	Current Density ($\mu\text{A}/\text{cm}^2$)	Rate (mdd)
0	-0.689	-0.348 ⁺	2.02 [*]	5.05
1	-0.725	-0.150	1.50	3.75
2	-0.720	-0.175	1.44	3.60
3	-0.713	-0.177	1.64	4.10
6	-0.698	-0.332	1.66	4.15
7	-0.678	-0.319	1.12 ^{**}	2.80
8	-0.664	-0.282	1.18	2.95
9	-0.630	-0.272	3.52	8.80
10	-0.629	-0.250	4.62	10.75
13	-0.568	-0.220	2.88	7.20
14	-0.554	-0.217	2.18	5.45
15	-0.545	-0.207	2.02	5.05
16	-0.540	-0.217	1.63	4.07
17	-0.533	-0.236	1.18	2.95
20	-0.530	-0.237	0.62	1.55
22	-0.525	-0.241	0.77	1.91
28	-0.515	-0.215	1.34	3.35
30	-0.510	-0.230	1.41	3.52
36	-0.508	-0.225	1.12	2.80
43	-0.507	-0.221	0.99	2.47
53	-0.507	-0.220	1.55	2.87
64	-0.512	-0.240	1.25	3.12
99	-0.501	-0.225	1.12	2.80

^o Potential of iron vs S.C.E.

^{oo} Corrosion current measured using "polarization break" method.

⁺ Based on potentiometric titration of culture filtrate with 0.01 M ferrous ammonium sulfate using silver sulfide ion electrode (0.55 gm ferrous ammonium sulfate added to 200 ml culture filtrate). Ferrous ions were in excess.

^{*} Corrosion reaction under cathodic control.

^{**} Corrosion reaction under anodic control.

Table VIII

Effect of Addition⁺ of Fe⁺⁺ to a Seawater Culture Filtrate^{*}
of Desulfovibrio on the Corrosion of Mild Steel

Time (days)	Potential		Corrosion			
	Fe	Redox	Current Density [°] ($\mu\Delta/\text{cm}^2$)	Rate (mdd)	Current Density [°] ($\mu\Delta/\text{cm}^2$)	Rate (mdd)
0	-0.695	-0.240	-	-	-	-
1	-0.796	-0.062	1.1	2.7	2.3	5.7
2	-0.784	-0.097	1.0	2.5	2.3	5.7
3	-0.762	-0.295	1.0	2.5	2.0	5.0
3-1/3	-0.662	-0.335	-	-	20.8	52.0
4	-0.595	-0.318	-	-	115.5	288.7
5	-0.589	-0.320	-	-	172.8	432.0
6	-0.578	-0.319	134.4	336.0	172.8	432.0
7	-0.574	-0.315	114.2	285.5	154.2	385.5
8	-0.570	-0.310	192.0	480.0	172.8	432.0
9	-0.565	-0.303	307.0	767.5	198.4	496.0
10	-0.562	-0.300	255.0	637.5	153.6	384.0
13	-0.548	-0.278	452.5	1131.2	460.8	1152.0
14	-0.550	-0.192	102.1	255.2	106.6	226.5
15	-0.542	-0.223	71.8	192.0	69.4	173.5

⁺ 0.383 gm FeCl₂·4H₂O in 4 ml sterile seawater added to 200 ml filtrate.

^{*} 8-day-old culture of Desulfovibrio. Potential of sulfide ion electrode -0.620 V. pH of 6.7 after addition of FeCl₂·4H₂O.

[°] Polarization "break" method.

• Linear polarization (polarization admittance) method.

Table IX

Effect of Filtrate⁺ of *Desulfovibrio* Culture plus Fe⁺⁺
on the Corrosion of Mild Steel Nails

Nail (#)	Before (gm)	After (gm)	Filtrate		Corrosion Rate [*] (mdd)
			Loss (gm)	Loss (%)	
1	0.8159	0.8023	0.0136	1.67	30.5
2	0.7627	0.7457	0.0170	2.23	39.7
3	0.7835	0.7639	0.0196	2.50	34.0
4	0.8050	0.7953	0.0097	1.20	22.2
5	0.7731	0.7573	0.0158	2.04	35.2
Average			0.0151	1.92	33.3
			Control ^c		
1	0.7704	0.7698	0.0006	0.08	1.40
2	0.7750	0.7749	0.0007	0.09	1.62
3	0.7645	0.7640	0.0005	0.06	1.15
4	0.7761	0.7755	0.0006	0.08	1.40
5	0.7523	0.7519	0.0004	0.05	0.92
Average			0.0006	0.07	1.29

⁺ 0.3803 gm FeCl₂·4H₂O added to 200 ml of a 7-day-old seawater culture of *Desulfovibrio*.

^{*} Based on a 20-day period

^o 1.82 mm av. dia.; 39 mm av. length.

^o Nails in sterile uninoculated seawater medium sealed with vaspar.

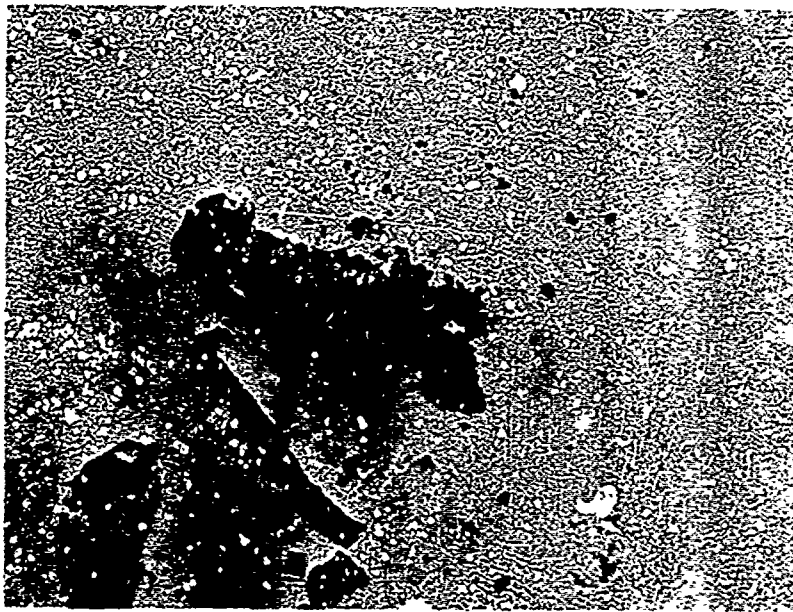
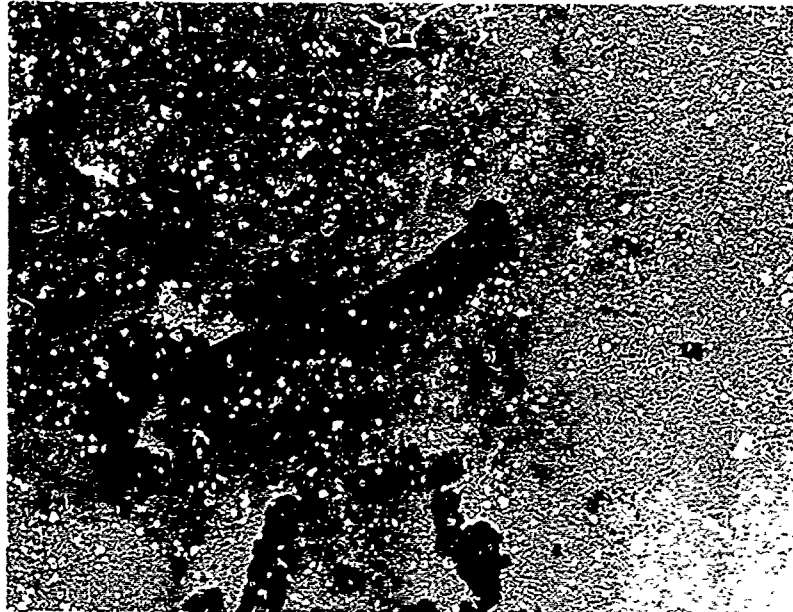


Figure 1. "Stalactite" growths after drying in vacuo.

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Figure 2. Scanning electron
micrograph of corrosion products
(mag ca 2100X).

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Figure 3. Surface of mild steel electrode exposed to seawater culture filtrate plus F_{ett} ions.

Review Summary

Physiological Studies

1. The inability to grow non-marine sulfate reducers (Desulfovibrio) on the agar surface of media (Baar's and yeast extract) used for their cultivation has been found to be due to the inhibitory effect of the indicator salt (ferrous ammonium sulfate) for hydrogen sulfide production in the media.
2. An agar plating medium on which good surface growth of marine sulfate reducers (Desulfovibrio) occurs has been devised. It consists of Trypticase (1.5%), Phytone (0.5%), agar (2%), NaCl (0.5%), and aged seawater. Ferrous ammonium sulfate (0.05%) may be added as an indicator salt for selective isolation.
3. A marine isolate of Desulfovibrio used in the corrosion studies was isolated from detritus on a steel piling off-shore near Dam Neck, Virginia, and obtained in pure culture through use of this medium.
4. Growth of four non-marine strains of Desulfovibrio on the surface of Trypticase-soy broth medium to which is added 2% agar was found to be greatly stimulated by the substitution of aged seawater for distilled water.
5. An agar (2%) medium consisting of yeast extract (2%) in distilled water was found to be useful as a screening agent for hydrogen (electron donors and acceptors) for the growth of non-marine strains of Desulfovibrio.
6. Growth of the marine Desulfovibrio strain in the Trypticase, Phytone seawater medium containing 0.25% ferrous ammonium sulfate resulted in the formation of a black precipitate which was heated in vacuo at 120°C. The resulting material was identified by x-ray diffraction studies to be a mixture of iron sulfide (troilite) and iron phosphide (Fe_3P schreiberite) in a weight ratio of 1.7:1. Spectrographic analysis of the schreiberite revealed the major elements to be Fe and P (>10%) and possibly potassium. Elements in the 0.1-1.0% range were Ni, Cu, and Mg. Schreiberite and troilite are commonly found associated with each other in iron-nickel meteorites.
7. Crystals of vivianite [$\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$] were found to develop on mild steel specimens in yeast extract broth inoculated with a non-marine strain of Desulfovibrio after 1 month incubation in a hydrogen atmosphere. In this medium a black product which normally forms has been identified as Fe_2P .
8. A volatile phosphorous compound was detected during the growth of a non-marine strain of Desulfovibrio on yeast extract agar containing K_2HPO_4 . The presence of the compound was confirmed by the formation of ammonium phosphomolybdate (APM) in a solution of ammonium molybdate exposed in the hydrogen atmosphere of growing yeast extract,

phosphate, and Trypticase Phytone seawater cultures of Desulfovibrio strains (marine and non-marine). Reduction of the APM, probably by H_2S , occurred. Exposure of $HgCl_2$ to the atmosphere of growing cultures of a non-marine strain of Desulfovibrio on yeast extract, phosphate agar resulted in a tan colored product, identified by x-ray diffraction patterns as a compound quite similar to H_3PO_4 . In attempts to isolate the volatile phosphorous material by passing a stream of hydrogen over yeast extract phosphate cultures of a non-marine strain of Desulfovibrio through a series of cold traps, two compounds were obtained. One compound, trapped at $80^\circ C$ showed IR absorption peaks at 695, 775, 910, and 990 cm^{-1} . The second compound, trapped at $-196^\circ C$ showed IR peaks at 775 and 1740 cm^{-1} . The two compounds had distinctive odors. The mass spectrum of the first compound indicated a series of fragments up to a mass of about 158.

9. An unusual attack upon pyrex glass surfaces exposed to room air was observed during isolation of the phosphorous compound. It was characterized by discoloration and pitting of the glass.

Corrosion Studies

1. In preliminary studies to determine the effect of microorganisms on cathodically protected steel, it was found that light has an effect upon the current received for protection. On the steel specimen employed, it was found that about 1.5 times the amount of current, required in the dark, was needed to maintain the same potential (0.8 V) in the light. The corrosion rate in indirect sunlight was found to be about 1.3 times the corrosion rate in the dark.
2. Electrochemical studies on the corrosion of mild steel in Trypticase-Phytone aged seawater medium without added Fe^{++} ions indicated that either severe corrosion may occur or that very limited corrosion will take place. When extensive corrosion occurs, the corrosion at first decreases to a rather low rate and then increases to a very high rate for a prolonged period of time. During the increase in corrosion rate, marked fluctuations in the open circuit potential occur. The increase in corrosion rate also appears to be associated with the breakdown and detachment of an FeS film as evidenced by direct observation and by changes in potential and polarization curves. During the prolonged period when the corrosion rate was high, the formation of corrosion product or products occurred in the form of visible "stalactites" or "whiskers" from the steel surface. X-ray diffraction studies of this material indicated a non-crystalline structure. Analysis by Mössbauer technique and the scanning electron microscope indicated a compound of iron and sulfur with iron in the Fe^{++} state as well as free sulfur. Heating the compound to $1200^\circ C$ in vacuo produced an alloy of 77% alpha iron, 13% gamma iron, and 10% cementite. An attempt to produce corrosion in the bacteria-free culture filtrate was unsuccessful.

3. In the same seawater medium containing added Fe^{++} ions, the corrosion rate in one instance increased to a maximum of 255 mdd and then fell to much lower rates. A marked fall in the redox potential (to more reducing conditions) occurred during the rapid increase in corrosion rate. In subsequent experiments the corrosion rate rose, but only to about 1/10 of this extremely high rate
4. The formation of chemically produced FeS to the sterile culture medium had little if any effect on the corrosion rate.
5. When Fe^{++} ions were added to a Seitz-filtered culture, the corrosion rate, after a 3-day induction period, reached a peak of 1100 mdd on the 10th day after the end of the induction period. Small mild steel nails when placed in a Seitz-filtrate of a culture to which Fe^{++} ions were added lost about 2% of their original weight during a corrosion period of 20 days.
6. These results suggest that the corrosion by the marine strain of Desulfovibrio is due to a soluble depolarizing agent produced as a result of the metabolism of the organism.

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