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Anaerobic Corrosion: Metals and Microbes in Two Worlds*

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President Leathen, members of the Society for Industrial Microbiology, ladies, and gentlemen: It is indeed a great privilege for me to be a recipient of the Charles Thom Award for 1974; especially this year, the Silver Anniversary year of the Society for Industrial Microbiology. This award was most unexpected and I am most humbled and moved by this esteemed honor.

In Dr. Arthur Kaplan's Charles Thom Award address of 4 yr ago, he spoke briefly about two worlds, the real scientific world and the laboratory world. In the past and presently, my time and consideration are spent between these two worlds. I am sure this is also true for many of you, being in applied fields.

I have just come from this real world of sea, surf, and sand and must return to it later this afternoon (Fig. 1). For 6 yr now, I have been going to this spot on the Atlantic Coast at Dam Neck, Virginia, at a time which, unfortunately for me, coincides with this SIM meeting, for this is the time of year most favorable for these observations. Together with other members of the National Bureau of Standards staff, we have been measuring, by electrical techniques and visual observations, the effect of air and sea and surf and sand on these pilings, and their various protective systems. A practical approach, yes, but probably the only way of finding a reasonable answer to the main question we are asking, namely, which systems or system are the most effective?

I should like to tell you about another real world. This is the world of soil and silt which was encountered by Dutch investigators some 51 yr ago that was destroying their pipelines under conditions thought then to be unusual, namely, the almost total lack of oxygen. At this time, corrosion was inevitably associated with the presence of oxygen. These investigators in asking the question: "Why?" went to the laboratory world. In the area of corrosion, it often is quite difficult to do this and one may end up studying a corrosion process completely unrelated to the one which is taking place in the real world. This is especially true if one is asking the question: "Are biological agents involved?"

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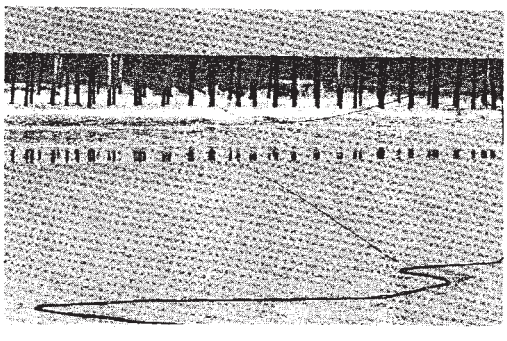

FIG. 1. National Bureau of Standards piling test site at Dam Neck, Va.

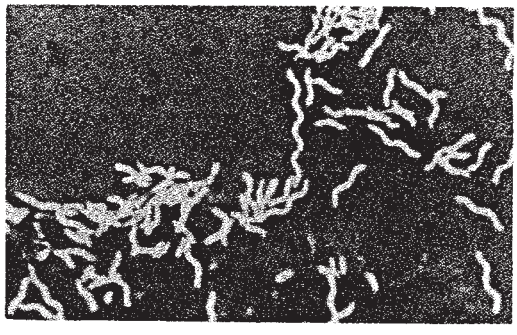

FIG. 2. Long spiral-shaped cells of *Desulfovibrio desulfuricans*, mag. 1800x (from Starkey and Wight 1945).

CATHODIC DEPOLARIZATION THEORY
of Von Wolzogen Kühr and Van Der Vlugt

I $8H_2O \longrightarrow 8OH^- + 8H^+$

II $4Fe \longrightarrow 4Fe^{++} + 8e$ (anode)

III $8H^+ + 8e \longrightarrow 8H$ (cathode)

IV $SO_4^{--} + 8H \xrightarrow{\text{(bacteria)}} S^{--} + 4H_2O$ (cathodic depolarization)

V $Fe^{++} + S^{--} \longrightarrow FeS$ (anode)

VI $3Fe^{++} + 6(OH)^- \longrightarrow 3Fe(OH)_2$ (anode)

$4Fe + SO_4^{--} + 4H_2O \longrightarrow FeS + 3Fe(OH)_2 + 2(OH)^-$

FIG. 3. Equation I is the ionization of water. Equation II represents the ionization or corrosion of iron through the removal of electrons. Electrons may be removed by the following mechanisms: (1) $2H^+ + 2e \rightarrow 2H \rightarrow H_2$, as in Equation III, (b) $H_2O + \frac{1}{2}O_2 + 2e \rightarrow 2(OH)^-$, (c) connecting the metal to the positive side of a battery. Equation III, the formation of hydrogen. Equation IV, the removal of hydrogen in an electrochemical cell by bacterial hydrogenase activity. Equation V and Equation VI, secondary reaction involving the formations of FeS or $Fe(OH)_2$.

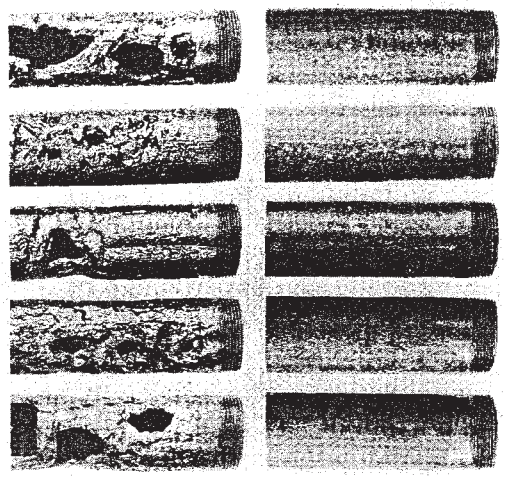

FIG. 4. Cast iron pipes, exposed for approx. 11 yr to highly corrosive soils, showing graphitization before (right) and after removal of corrosion products (left) (from Romanoff 1957).

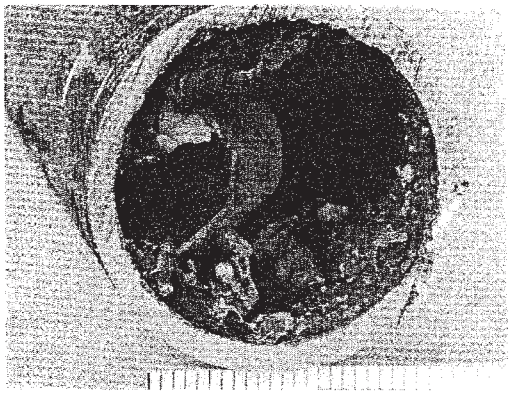

FIG. 5. Interior of steel water pipe showing extensive tuberculation (from Iverson 1972).

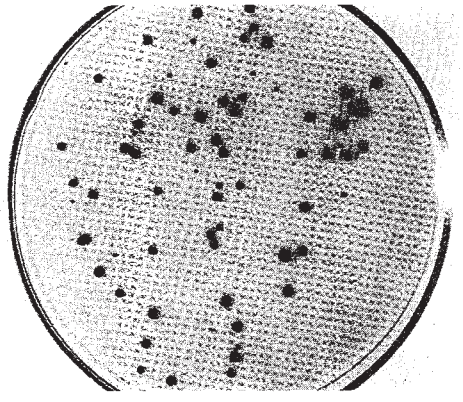

FIG. 6. Colonies of *Desulfovibrio desulfuricans* (Mid-Continent, A strain) on Trypticase Soy Agar plus Salts. Plates incubated 10 days at room temperature in a hydrogen atmosphere and exposed to air for 2 h (from Iverson 1966).

Generally, the evidence in the form of dessicated, corroded specimens arrives at the laboratory long after corrosion has been discovered and stopped.

Nevertheless, after some excellent scientific investigation, two Dutch scientists, von Wolzogen Kühr and van der Vlugt (1934) presented some pretty good evidence that (anaerobic) sulfate-reducing bacteria were implicated in the corrosion of these pipelines. Sulfate-reducing bacteria (Fig. 2), discovered by Winogradsky in 1895, can obtain their energy by the oxidation of hydrogen and subsequent reduction of sulfate to sulfide. von Wolzogen Kühr and van der Vlugt (1934) even proposed a theory to account for this anaerobic or oxygen-free type of corrosion. This theory, in brief, proposed that these sulfate-reducing bacteria removed hydrogen and/or electrons from the surface of iron, thereby causing iron to corrode or dissolve in the form of ferrous ions.

This theory was based primarily on the findings of Stephenson and Stickland (1931) that these bacteria utilize hydrogen for the reduction of sulfate to sulfide. They named their theory the cathodic depolarization theory since the removal of hydrogen was thought to take place at the cathodic portion of an electrochemical cell (Fig. 3). Since then, several investigators began reporting on their evidence supporting or disproving this theory.

However, the problem of bacterial corrosion is still with us today. For example, Dr. Guy Booth (1964) stated that 50% or more of all pipeline corrosion he examined in England was due to bacterial action. Although it is now possible to protect the outside of underground steel pipe through the use of protective coatings and cathodic protection, it is still very costly. Fig. 4 shows an example of bacterial action on cast iron pipe called graphitization. The iron is leached out leaving a residue of carbon which still gives the pipe an intact appearance.

Corrosion, however, may also take place from the inside of a pipe. Witness this "arteriosclerotic" water pipe (Fig. 5), which is plugged with tubercles, masses of corrosion products. There are indications that this type of corrosion may also involve the action of sulfate-reducing bacteria.

My first exposure to this area of corrosion occurred while I was a graduate student of Dr. Waksman at Rutgers University. Dr. Starkey (1957) and his students in the same department were working with these sulfate-reducing organisms, the same ones involved in the cathodic depolarization theory. The world of antibiotics was expanding, and the black, foul-smelling bottle cultures of *Desulfovibrio desulfuricans* seemed to have little appeal at the time.

After a brief interval of work in antibiotics at Parke, Davis & Co., I was involved for some 15 yr in teaching a variety of courses in microbiology at the U.S. Army Biological Laboratories. While there, contamination of jet fuel became a serious problem in the Air Force and I became involved in a taxonomic study of bacteria, mostly *Pseudomonas* strains, isolated from this jet fuel. Associated with this contamination was an even more serious problem, namely, the corrosion, often catastrophic, of the aluminum tanks which held the contaminated fuel.

While examining a corrosion pit in a small aluminum tank which had contained contaminated fuel, some spiral forms, not unlike those of *Desulfovibrio,* were observed in the debris. Unfortunately, this dried material was unsuitable for cultivation. At about this same time, it was found that a commercially available medium would permit excellent agar surface growth of these organisms, thus eliminating the use of liquid and semi-solid medium which made contamination difficult to detect (Fig. 6). Using this medium, it was possible to isolate and obtain a pure culture from pits in the next available tank.

Although these organisms were associated with corrosion pits in aluminum tanks, it was most difficult to induce them to initiate corrosion. The corrosion of iron and study of its mechanism therefore appeared more attractive. It seemed essential again to test the validity of the cathodic depolarization theory. A little experiment was devised to simulate as closely as possible the conditions proposed in the theory.

Two iron electrodes, electrically connected, were placed on the surface of an agar plate containing the redox-dye benzyl viologen (BV) and an organic buffer to maintain a pH of near neutrality (Fig. 7). The BV was used as an electron acceptor in place of sulfate, as proposed in the theory, to avoid the evolution of hydrogen sulfide which would complicate the corrosion process. Underneath one electrode was placed a heavy paste of bacterial cells (*Desulfovibrio desulfuricans,* mid-Continent, A strain). This was to be the cathode and the other electrode, with no bacteria in contact with it, the anode. After placing the plate with the electrodes in an inert atmosphere for about 17-24 h, the electrodes were removed and a reduced area of violet benzyl viologen was found under the electrode in contact with the cells (similar to Fig. 8a). Soluble iron appeared at the anode (similar to Fig. 8b) after developing the plate with ferricyanide, as indicated by the theory. These observations indicated that an electrochemical cell was formed and it actually was possible to measure the corrosion current (Fig. 9). Extrapolated to a pipeline, this corrosion current would not be large enough to account for the perforation of a quarter-inch thick pipe as reported by Dr. Guy Booth in England. Furthermore, if sulfate, the electron acceptor proposed in the theory, was substituted for the BV, no reaction occurred.

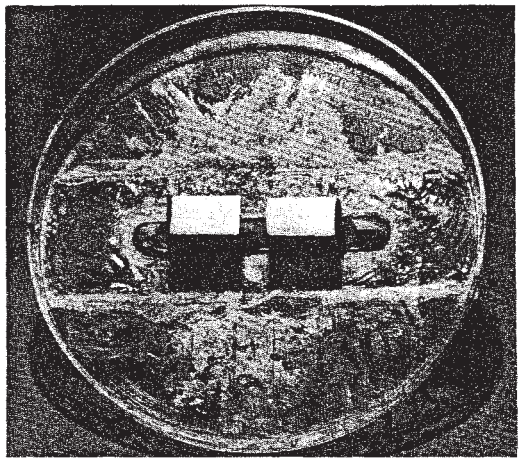

FIG. 7. Mild steel electrodes mounted in plastic petri dish cover (from Iverson 1972).

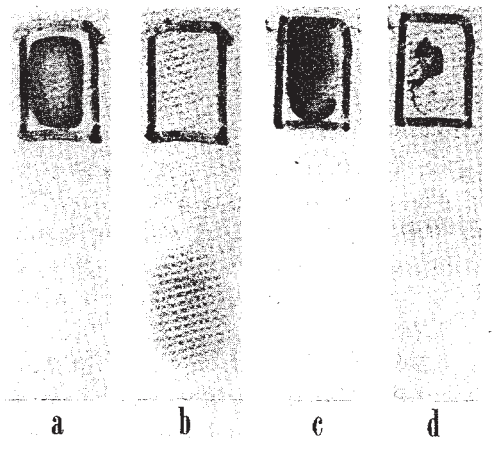

FIG. 8. Areas in agar under coupon indicating location of iron and reduced benzyl viologen (BV). (a) Yeast extract (YE) agar plus BV surface immediately after removal of steel coupon. Dark area (cathode) caused by reduction of BV by *Desulfovibrio* cells. (b) Same plate, 15 min after addition of aqueous potassium ferricyanide (10% w/v) indicating a concn of Fe^{++} ions at the anode (no cells). (c) YE agar minus-BV immediately after removal of steel coupon showing dark area under cells. (d) same plate 15 min after addition of aqueous potassium ferricyanide showing concentration of Fe^{++} ions (from Iverson 1968).

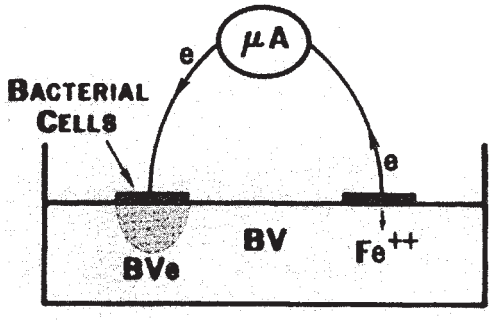

FIG. 9. Measurement of "Cathodic Depolarization Current."

In some previous studies, it was found that yeast extract with hydrogen as the electron donor and sulfate as the electron acceptor provided excellent growth of this organism. If yeast extract at pH 7.0 was substituted for the buffer, BV was again reduced at the cathode and ferrous ions were found at the anode (Figs. 8a and 8b). If sulfate was substituted for BV, another reaction occurred. Blackening in the agar was found to occur underneath the cathode (Fig. 8c), and ferrous ions were found only at this cathode upon development with ferricyanide (Fig. 8d). This same reaction also occurred if the electron acceptor, sulfate, was omitted.

To carry this a little further, if one placed a strip of sterile mild steel in yeast extract broth inoculated with the bacteria and maintained anaerobic conditions, blackening of the broth took place within a matter of a few hours. This appeared to be a most unusual reaction. One's first impression was that this black material was iron sulfide, but hydrogen sulfide does not normally react with metallic iron in this fashion at neutral or near neutral pH values. In the presence of hydrogen sulfide, a film of iron sulfide usually forms on the surface of the iron leaving the solution clear. If the bacterial cells and the black material were removed from this solution by filtration and a fresh piece if iron was placed in the clear filtrate under anaerobic conditions, darkening again occurred. I was able to collect a small speck of this material before the project terminated. The corrosion rate of the iron was so low that it could not be detected by the instrumentation used at that time. Most fortunately, through the efforts of Dr. Jerome Kruger in the Section at the National Bureau of Standards (NBS) and some additional funding from ONR through the efforts of Dr. Robert Acker, I was able to resume this work at NBS within a year's time. NBS had been involved in underground corrosion since 1910 when it had been commissioned to study stray-current corrosion by an Act of Congress.

Getting back to the black material again, X-ray analysis at first indicated it to be amorphous. It was thought that by heating it to a high temp (ca. 1200 C) in a vacuum oven, an arrangement of the atoms to a crystalline structure might occur. This did happen, and the X-ray diffraction pattern fitted that of iron phosphide (Fe_2P). The black material also had become magnetic. Another form if iron phosphide, namely, schreibersite (Fe_3P), and troilite (iron sulfide) also were formed from a similar heat treatment of a black precipitate formed in a seawater culture of a marine strain of *Desulfovibrio* containing ferrous ions. This strain was isolated from one of the pilings at Dam Neck, although this has no special significance since these organisms abound in the seawater all along the coast.

Interestingly, these two materials, troilite and schreibersite, are found closely associated together in meteorites, particularly iron-nickel meteorites (Fig. 10) and tektites (Fig. 11), glass bodies of rounded but indefinite shapes containing metal spherules of iron

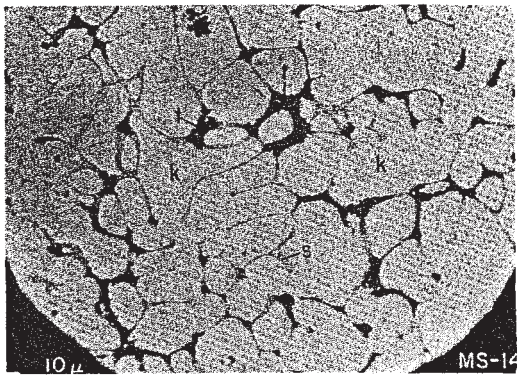

FIG. 10. Enlarged view of polished section of nickel-iron spheroid showing both the interstitial schreibersite (S) and troilite (t). The matrix is kamacite. Meteor Crater, Arizona. Reflected light (from Chao et al. 1964).

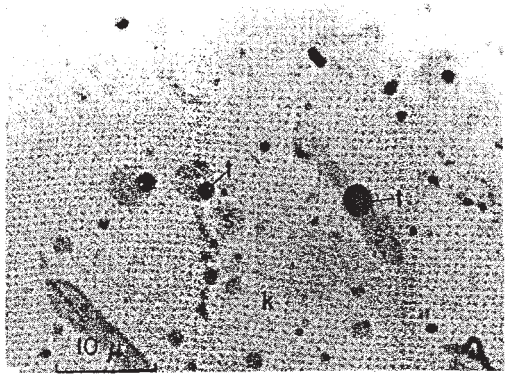

FIG. 11. Enlarged view of a polished section of a nickel-iron spherule (Phillipine tektite) showing blebs of trolite (t) in schreibersite (S). Reflected light (from Chao et al. 1964).

and nickel and of unknown origin found in the Netherlands, Indies, Australia, and elsewhere. It is not uncommon, however, to find an association of α-iron with schreibersite (Fe_3P) and troilite (FeS) in iron and steel manufacture. Phosphorus also appeared in another form. If strips of mild steel were kept in a 2% yeast extract culture as before for a month or more, crystals were found growing on them (Figs. 12 and 13). No corrosion was observed under the crystal growths. These crystals turned out to be vivianite or ferrous phosphate ($Fe_3(PO_4)_2 \cdot 8H_2O$).

Going back to the real world, Booth et al. (1962) at the National Chemical Laboratory at Teddington, England, examined some nails of an early 16th-century origin found on an archeological site of high corrosivity in St. Neots, Huntingdonshire, which were remarkably free of corrosion. These nails were found to carry a highly adherent and compact coating of this vivianite. After studying these nails electrochemically, Dr. Booth concluded that their relative freedom from corrosion was due primarily to the vivianite coating which was severely restrictive of the passage of ferrous ions from the metal into solution.

Thus, these are two examples of what one sometimes finds in the laboratory world and then finds in the real world or "outer world."

Since ONR was partially funding the work at NBS, I turned to the real world, the sea, for a sulfate-reducing strain which I isolated in pure culture. This strain of *Desulfovibrio*, which I used for the remaining corrosion studies to be described, seemed to grow best in seawater which was fortified with enzymatic digests of soybeans and casein at a pH of 7.0 or near 7.0.

As a result of some meticulous studies by Schwerdtfeger (1957) at NBS, it was now possible to measure the instantaneous corrosion rate using the electrochemical technique which he had developed. This technique eliminated the necessity for removing metal specimens, thereby disturbing the system for weight loss measurements. This is the same technique which we are using to measure the corrosion of the steel pilings at Dam Neck (Fig. 1). A three-electrode system, comprising a cylindrical specimen of mild steel (the test specimen) with only the end exposed, an agar bridge (Luggin capillary), and a platinum gauze electrode or working electrode was employed. This electrode also served for redox-potential measurements. After placing the electrodes in freshly autoclaved

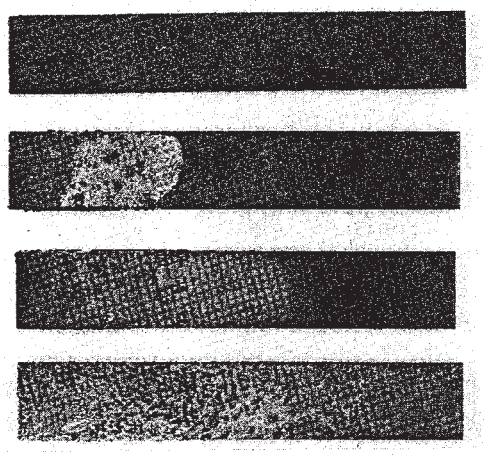

FIG. 12. Crystals of Vivianite on 1010 steel coupons x 1.25. Coupons were in a vertical position while in the yeast extract broth (right →left=top→bottom).

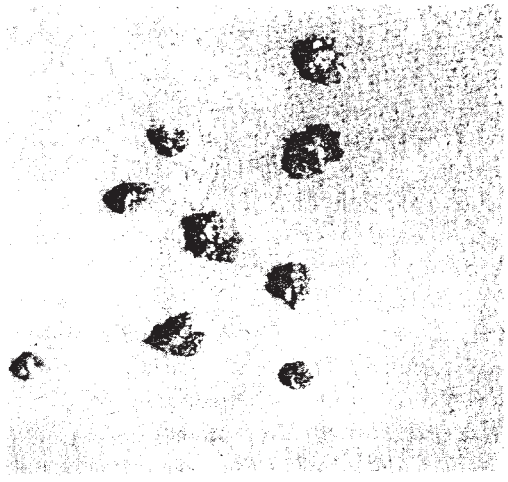

FIG. 13. Enlarged view of vivianite crystale x10.

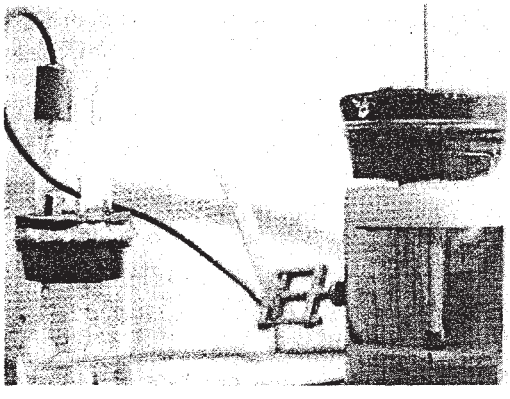

FIG. 14. Polarization cell and calomel half-cell (from Iverson 1973).

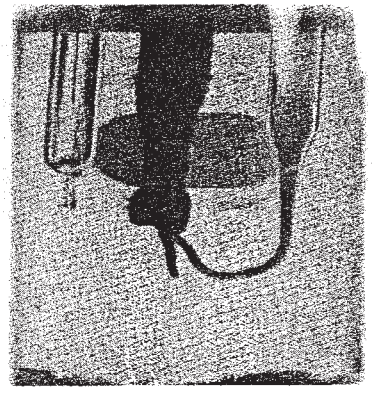

FIG. 15. "Stalactite" formation from mild steel electrode in sea water medium culture without added Fe^{++} ions.

FIG. 16. Electron scanning micrograph of "stalactite" fragment (magnification ca. 1050x) (from Iverson 1973).

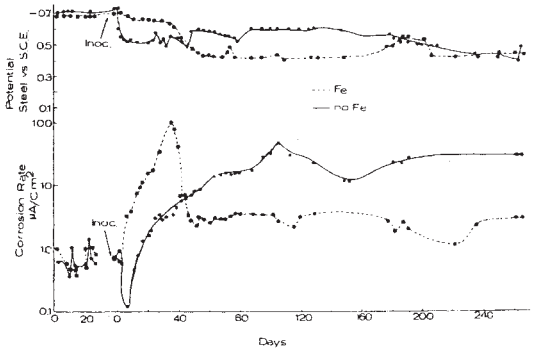

FIG. 17. Corrosion rates and open circuit potentials of mild steel vs. time in seawater medium culture with and without added Fe^{++} ions (2.5% ferrous ammonium sulfate).

seawater medium,[1] the surface was sealed with a mixture of paraffin and petroleum jelly, and the system was allowed to come to electrochemical equilibrium (Fig. 14). When equilibrium was reached and there was no sign of visible contamination, the bacterial cells were introduced through the seal.

It was found generally that the corrosion rate of the steel in the presence of the bacteria decreased to some low level and remained so for as long as a year, due to a protective film of some form of iron sulfide. On one occasion, however, the film was weakened or gave way so that the corrosion rate increased to a relatively high level and remained so for several months. The corrosion products appeared in the form of little stalactites which grew, dropped off when the cell was touched, and regrew again (Fig. 15). These growths were not totally unlike the tubercles formed on the inside of the steel water pipe which you saw earlier. A scanning electron micrograph of this growth presents a somewhat unusual appearance (Fig. 16). The instantaneous corrosion rates associated with formation of these stalactites are shown by the solid line in Fig. 17.

Some evidence from both worlds indicated that iron in the form of ferrous ions[2] stimulated corrosion and indeed it did, in an accelerated fashion as indicated by the broken line (Fig. 17). This was not usually the case although the corrosion rates were still high.

Was this increase in corrosion rate caused by some form of iron sulfide as thought by some investigators? After further studies, this hypothesis did not seem tenable. An alternative possibility which suggested itself was that the organisms were producing some corrosive metabolite.

A corrosion cell was prepared in which the electrolyte consisted of a bacteria-free filtrate of a culture from which sulfide ions were removed by the addition of ferrous ions. The resulting iron sulfide was again removed by filtration. Also it was possible to do the operation in one step, namely, add ferrous ions to the culture and filter both the bacteria and the iron sulfide.

The resulting clear bacteria and sulfide-free filtrate remained clear until the third day when it started to turn dark. Marked changes in the potential of the steel electrode and

[1]Trypticase-Phytone Seawater (TPSW) medium: Trypticase 15.0 g; Phytone 5.0 g; Sodium Chloride 5.0 g; seawater 1 liter.

[2]2.5 g ferrous ammonia sulfate per liter to (TPSW) medium.

TABLE 1. *Effect of TPSW culture filtrate $+Fe^{++}$ ions on the corrosion of mild steel*

Time (Days)	O.C. Potential Steel (V)	Redox Potential (V)	Corrosion	
			Current Density ($\mu A/cm^2$)	Rate (mdd)
1	−0.796	−0.162	2.3	5.7
3	−0.762	−0.295	2.0	5.0
$3\frac{1}{3}$	−0.662	−0.335	20.8	52.0
4	−0.595	−0.318	115.5	288.7
6	−0.578	−0.319	172.8	432.0
9	−0.565	−0.303	198.4	496.0
13	−0.548	−0.278	460.8	1152.0
15	−0.542	−0.223	69.4	173.5

8-day old (Trypticase-Phytone Seawater) culture seitz-filtered and 0.3835 g $FeCl_2 \cdot 4H_2O$ (dissolved in 4 ml sterile seawater) added. Open circuit potentials of mild steel vs. saturated calomel half-cell (volts). Redox potential (corrected to pH 7.0 and hydrogen electrode). Corrosion rate expressed in $mg/dm^2/day$ (mdd) assuming 1 $\mu A/cm^2$ = 2.5 mdd for $Fe \rightarrow Fe^{++}+2e$.

the corrosion rate started to occur. For the next 3 or 4 days, the corrosion rate reached a peak corresponding to about 40 times the rate of mild steel in aerated seawater (Table 1). The evidence indicates that the corrosion of mild steel by the marine strain of sulfate-reducer is due primarily to some oxidizing agent which is extracellularly liberated by the bacterial cells, and not by contact of the bacterial cell with the metal surface and removal of hydrogen as postulated in the cathodic depolarization theory.

Thus this organism produces two agents, a corrosion inhibitor, hydrogen sulfide, and a corrosive agent. Whether or not corrosion occurs in the real world would seem to depend on the selective availability of these agents at the metal surface. A corrosive environment may be one in which only the hydrogen sulfide is prevented in reaching the metal surface, by selective adsorption or absorption in the soil, for example. If this is indeed a generalized phenomenon among the sulfate reducers and if the corrosive agent could be demonstrated in the real world, the question of "How?" may be partially answered.

Since this is a Charles Thom address and since Dr. Thom was an eminent mycologist, I cannot conclude without describing a phenomenon which has nothing to do with microbes. Yet that which occurs appears very similar to fungal growth. This all happened as a result of attempting to devise a microscopic test for metallic iron. If one places a small piece if iron or steel in a slightly acidified solution of ferricyanide or ferrocyanide, small hollow tubules or "whiskers" sprout from the metal surface at various places. The diameter and speed with which they form can be controlled by the concn of the ferrocyanide or ferricyanide.

Fig. 18 shows an enlarged view of these hollow whiskers which have sprouted from a piece of fatigued nichrome wire. Fig. 19 shows a more enlarged view of some of these whiskers. Morphological features of fungi are apparent such as branching and possibly separation. They also appear to have been stained. The bifurcated tips are pink and the remainder of the tubules are green or blue. The greenish coloration is due to the color of ferrous ferricyanide and the pink coloration is due to the ferricyanide complex of another metal, possibly cobalt. This reaction takes place with any metal the ions of which form a complex with ferrocyanide or ferricyanide. The reaction appears to be due to streams of

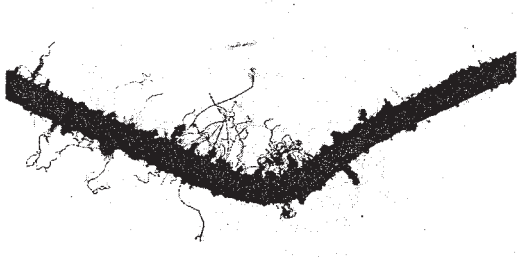

FIG. 18. Tubules formed from fatigued nichrome wire in acidified 10% potassium ferricyanide solution (x10) (from Iverson 1967).

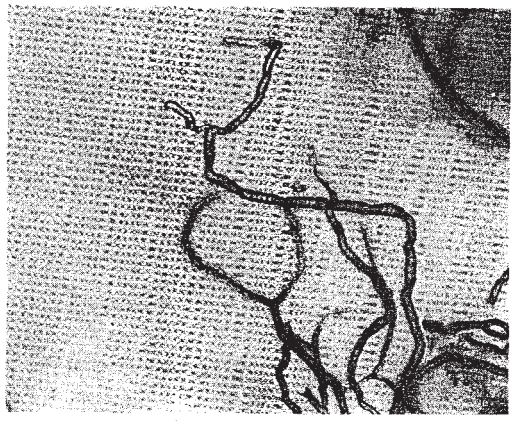

FIG. 19. End of tubules formed in acidified 10% potassium ferricyanide solution. Tips are pink, probably due to a cobalt ferricyanide complex while remainder of tubule is blue-green due to a ferrous ferricyanide complex (ca. x135) (from Iverson 1967).

ions which arise from the surface of the metal when electrons are removed from the metal. The metal ions react with the ferricyanide or ferrocyanide to form a "lake" which comprises the wall of the tubule.

In now taking leave from these two worlds for awhile, I should like to recognize all those persons in the field without whose contributions these results and observations which I described to you would hardly have been possible; particularly Dr. Robert Starkey and Dr. Guy Booth, my previous and present administrators, for providing the proper kind of "milieux" in which science can be carried out, my instructors and advisors who gave me an appreciation of the way science is done, my many colleagues, past and present, who gave me encouragement along the way, all of the technical personnel at NBS and the U.S. Army Biological Laboratories who performed the different types of analysis required in these investigations, and the Office of Naval Research for support of some of these studies.

"No man is an island, entire of itself, every man is a piece of the continent" (John Donne, *Devotions* XII).

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