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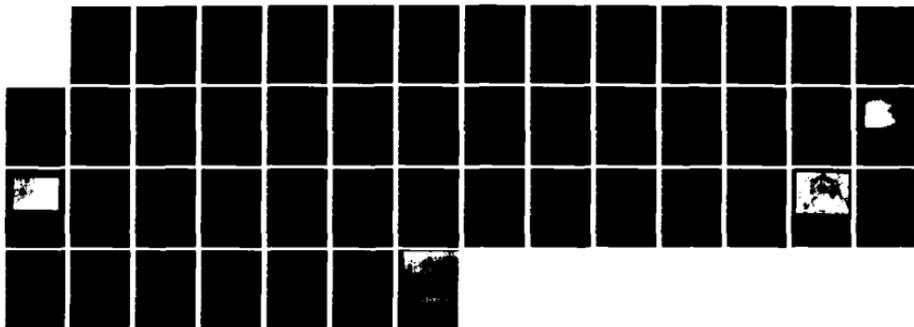
THE ROLE OF MICROORGANISMS IN MARINE CORROSION
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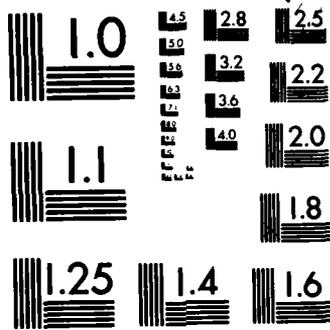
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The Role of Microorganisms in Marine Corrosion Processes

by

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I. OBJECTIVES

The intent of our research is to investigate the role of microorganisms in marine corrosion processes. The purpose of these studies is to develop a clear understanding of specific corrosion reactions that are mediated biochemically by microorganisms. The ultimate goal of the research is to gain the ability to control biologically induced corrosion.

Our research focuses on four areas of biologically induced marine corrosion. These are: 1. The attachment of bacteria to metals involved in corrosion; 2. Corrosion by extremely thermophilic bacteria; 3. The role of bacteria and other microorganisms in hydrogen embrittlement processes; and 4. Specific biochemically induced corrosion reactions mediated by strictly anaerobic bacteria, and the involvement of hydrogenase enzyme systems in marine corrosion processes.

II. PROJECT RELEVANCE

The annual cost of corrosion in the United States amounts to over 8 billion dollars. Corrosion in the marine environment represents a substantial portion of the total annual cost of this loss.

The corrosion of metals in contact with seawater, and efforts at prevention, are major sources of operational problems and economic loss for the Navy. Marine corrosion is a potential hazard to all alloys, and to virtually every structure in contact with the sea. Attack is frequently localized and failure may be the result of pitting, crevice corrosion, galvanic action or stress. The microbial contribution to marine corrosion remains largely unknown, despite the fact that biological corrosion processes were recognized almost 80 years ago.

Bacteria and other microorganisms play an integral role in marine

corrosion, although the economic costs are not known. A wide range of microbiological reactions causing corrosion occurs in marine habitats. Microorganisms also destroy protective coatings, causing accelerated corrosion. In addition, the production of organic acids by metabolic reactions of bacteria is a serious cause of microbial corrosion.

Prevention of microbially induced corrosion will require a detailed knowledge of the processes involved. Choice of protective methods, including protective coatings, appropriate alloys, and use of corrosion inhibitors will be dependent on the specific microbial process that is involved. For example, the use of alloys susceptible to sulfide induced corrosion should be discouraged in waters where organic pollution is an important factor. Chemical control of iron and manganese oxidizing bacteria needs to take into account the oxidative processes in which iron and manganese oxides are deposited. Those methods which fail to remove the oxide will not provide adequate protection against corrosion.

Our research program is aimed at studying microbiological processes involved in marine corrosion. We are studying the microorganisms and the chemical processes controlling microbial corrosion. Our research includes bacterial biofilm formation on corroding metals; corrosion by thermophilic bacteria; anaerobic corrosion; and hydrogen embrittlement. These studies will provide a more precise understanding of corrosion induced by microorganisms in marine habitats. The research will also allow the description of specific roles that bacteria play in biochemical corrosion reactions. Ultimately, this research may lead to the development of methods to inhibit or prevent the corrosion of metals by microorganisms in the sea.

III. INTRODUCTION

Marine corrosion is a potential hazard to all metals and alloys and to virtually every structure in contact with the sea. Corrosion of offshore structures is a major source of operational problems and economic loss. Frequently engineers fail to realize that a large proportion of marine corrosion may be initiated or assisted by the attachment and growth of bacteria and other marine organisms. Since the first recognition of biological corrosion processes nearly 80 years ago, corrosion of metals by microbial activity has emerged as an important economic problem. A wide range of corrosion phenomena in which microorganisms are involved has been recognized. For example, bacteria are thought to be responsible for more than 75% of the corrosion in productive oil wells in the United States and for over half of the failures of buried pipelines and cables. The economic costs of biologically-induced marine corrosion are not known, but fouling by marine micro- and macroorganisms can greatly alter the corrosion behavior of metals, as well as impair the efficient operation of marine engineering systems.

Recognition of the close relationship between biofouling and corrosion is important, since prevention of biological corrosion may require a unique set of tools; knowledge of the mechanisms involved will determine the choice of alloys, coatings, etc., to be used in a particular marine environment. In this report we will discuss the way in which films of microorganisms form on metals placed in the ocean and some of the specific corrosion reactions which may occur either directly or indirectly as a result of the activity of these organisms.

Biofilms on Corroding Metals

All solid surfaces, when exposed to seawater, are immediately colonized by microorganisms. Metal interfaces in aquatic systems represent sites of intense microbial activity. After immersion of only a week or two in the ocean, metal surfaces can harbor complex communities of growing bacteria, protozoa, and algae. All metals are susceptible to microfouling, even the "antifouling" copper-nickel alloys.

Our understanding of the mechanisms and significance of microbial attachment to surfaces has progressed rapidly in recent years, and the basic sequence of events in marine biofilm formation has been clarified by microbiologists. Immediately upon immersion of any solid in seawater, before any colonization by bacteria, a layer of organic macromolecules -- called a "conditioning film" -- is spontaneously adsorbed to its surface. This conditioning layer is important in establishing a base for subsequent buildup of the microfouling layer, for only after adsorption of this organic film can microorganisms attach effectively to the surface. The spontaneous adsorption of dissolved glycoproteins and other organic compounds from the aqueous phase alters the surface free energy of the solid, and these compounds may then serve as nutrients for the microorganisms that later colonize the surface.

Microorganisms arriving near the solid/liquid interface are subjected to short-range attractive forces (i.e., electrostatic, hydrophobic, and van der Waals forces) which balance the double-layer repulsive force and hold the bacteria weakly near the surface. Once short-range attractive forces have brought the microorganisms very close to a suitable surface, many of the cells secrete extracellular organic polymers which serve to anchor them to the substratum.

The development of methods for quantifying bacterial attachment and growth

on metals has allowed researchers in our laboratory and others to investigate the attachment of specific marine bacteria to alloys of known composition. We have found that both the number and kind of bacteria that attach is a function of the type of metal and the way in which its surface was prepared.

Copper-based alloys, for example, which have become attractive for seawater service because of their antifouling properties, are less rapidly fouled than other metals. However, in terms of microbial attachment, they have no advantage over other alloys once the base metal surface becomes obscured by microbial polymers or corrosion product films. This implies not only that biofilm growth is sensitive to continuing changes in the nature of the metal surface, but also that few, if any, metals are immune to the influence of microorganisms upon their corrosion behavior.

Metabolic processes mediated by biofilm at metal interfaces may have a significant effect on a variety of different corrosion reactions. Some of the basic mechanisms by which microorganisms initiate or accelerate corrosion include:

- 1) production of corrosive metabolic products, such as acids or hydrogen sulfide;
- 2) formation of discontinuous deposits on the surface, resulting in differential aeration and concentration cells;
- 3) disruption of natural and other protective films;
- 4) breakdown of corrosion inhibitors and coatings;
- 5) depolarization of cathodic or anodic reactions.

Our research is focused on development of a better understanding of these surface interactions.

Corrosion by Thermophilic Bacteria

Bacteria capable of living at extremely high temperatures are common in natural waters. Many of these bacteria grow well on surfaces. Typical extreme thermophiles thrive at temperatures in excess of 70°C. They are found in both acid and alkaline habitats and can utilize a very wide range of substrates.

The genus Thermus is common in hot springs, and survives at 90°C. It is filamentous, and attaches well to surfaces. It is an obligate thermophile, and fails to grow below 45°C. Isolates similar to Thermus are commonly found in water heater systems.

The relationship between these extreme thermophiles and corrosion of metals in contact with hot water is not known. There is anecdotal evidence that bacteria are involved in corrosion of hot water systems. Recent work at NORDA has provided evidence that bacteria may be involved in failure of cooling systems. We have isolated a large number of extreme thermophiles and have begun a study of their implication in corrosion. Emphasis is being placed on brazed surfaces, which appear to be especially sensitive to thermophilic bacteria.

Anaerobic corrosion

The presence of extensive fouling communities on structures submerged in seawater can easily limit diffusion of oxygen to the surface of metals used in their construction. Although growth of microorganisms on surfaces sometimes acts to reduce corrosion by providing a continuous barrier to the diffusion of oxygen to the surface, this deficiency in oxygen supply may be responsible for the initiation of localized corrosion caused by differential aeration, particularly if the fouling has resulted in discontinuous deposits on the surface. Such corrosion cells can be set up by macrofoulers (such as barnacles and mussels), as well as by microorganisms.

Anaerobic conditions existing beneath microbial biofilms, on metal surfaces in contact with anaerobic sediments at the bottom of the ocean, in stagnant, nutrient-rich water in pipes, or in heavily polluted seawater, may also stimulate the growth and activity of bacteria which live only in anoxic environments. Some of these anaerobic bacteria, such as those of the genus Desulfovibrio which reduce sulfate to form hydrogen sulfide, have been associated with extensive corrosion of a wide variety of metals in the sea. Usually the corroding metals tend to pit, resulting in local perforations. Accelerated corrosion of copper-nickel alloys, for example, has been observed in polluted, oxygen-deficient waters, and the regions of greatest attack generally showed distinct biofilms. It appears that accelerated corrosion of these alloys resulted from anaerobic microbial processes within the biofilms, and pitting of the copper-nickel alloys could be correlated with bacterial production of hydrogen sulfide. However, anaerobic corrosion appears to involve other anaerobic microbial processes unrelated to the activities of Desulfovibrio. We are studying these processes in detail.

Hydrogen embrittlement

Hydrogen embrittlement is weakening of a metal resulting from the absorption of atomic hydrogen. In susceptible metals this results in a loss of ductility and tensile strength and often in premature failure of the material. This phenomenon is especially important in high-strength steels, although other alloys also are susceptible. It constitutes a serious hazard, since catastrophic failures can occur with no prior warning. Microorganisms may play a significant role in hydrogen damage processes, and several mechanisms may be involved.

It is well established that hydrogen gas can cause embrittlement in a

variety of materials, including steels, titanium alloys, and nickel alloys. A wide range of bacteria produce molecular hydrogen as an end product from the fermentation of carbohydrates. When this hydrogen is produced by organisms within the biofilm on a metal surface, it may, under certain conditions, be absorbed into the metal if dissociation into atomic hydrogen occurs.

Furthermore, acid production by fouling microorganisms may influence hydrogen embrittlement. Not only can the pH change associated with acid production affect surface conditions relevant to hydrogen entry into the metal, but also reduction of microbially derived protons (H^+) may be coupled to anodic dissolution of the metal, resulting in the formation of hydrogen atoms. If recombination of this atomic hydrogen into molecular hydrogen is prevented (by sulfides or other hydrogen evolution poisons), then the uncombined hydrogen atoms accumulate at the metal surface, and the probability of their absorption into the metal lattice increases. Alternatively, bacteria which consume hydrogen (e.g. Desulfovibrio) may actually suppress the absorption of hydrogen by removing it from the metal surface before it diffuses into the metal itself.

Certain substances such as sulfide ions, phosphorous-containing ions, arsenic compounds, and cyanides are known to be effective poisons of the conversion of atomic to molecular hydrogen. In the presence of such compounds uncombined hydrogen atoms increase in number on metal surfaces, and hence the probability that they will be absorbed into the metal also increases. Many of these poisons are found in petroleum process streams, which is the reason that hydrogen damage of metals is a major problem in the petroleum industry.

Hydrogen sulfide (H_2S) production by sulfate-reducing bacteria in anaerobic marine environments thus may stimulate the absorption of atomic hydrogen into metals by preventing its recombination into hydrogen gas. As described before, these nearly ubiquitous microorganisms are active in the

anoxic conditions existing beneath microbial biofilms on metal surfaces, inside tubercles or under deposits, in crevices, on metals surfaces in contact with anaerobic muds or sediments, and in stagnant, polluted waters. Whenever atomic hydrogen is produced by proton reduction in the presence of H_2S , embrittlement of sensitive materials must be expected. H_2S produced in aqueous environments does, in fact, limit the use of hardened, high-strength steels in oil and gas equipment and in sour gas wells. Embrittlement and failure of offshore structures might be expected to occur at the sediment-water interface, where H_2S production by sulfate-reducing bacteria is greatest. Use of materials which are sensitive to hydrogen should also be discouraged in waters heavily polluted by organic matter.

We are studying the microbial processes involved in hydrogen embrittlement. The activities of hydrogen producing bacteria in *biofilms on metals* is being investigated. Biochemical analysis is being used to determine the activity of these bacteria on surfaces. Methods are being developed to determine the significance of bacterial hydrogen production in embrittlement of metals.

IV. WORK COMPLETED IN OUR LABORATORY 1982-83

A. MARINE BACTERIAL BIOFILM FORMATION ON CORRODING METALS

Background

Metal surfaces in aquatic systems are sites of intense microbial activity, which can result in the enhancement of corrosion processes. After immersion of only a week or two in the ocean, these surfaces can harbor complex communities of growing bacteria, protozoa, algae, and their extracellular products. This biofilm changes the physical/chemical nature of the metal surface and can have a significant effect both on the kinetics of corrosion and the type of corrosion occurring on the metal. However, the relationship between biofouling and corrosion is poorly understood.

Research during the past decade on marine biofilm formation has dealt mainly with the qualitative aspects of bacterial attachment to surfaces. Many studies have provided visual records of the attachment of various microorganisms to metallic and non-metallic surfaces in the sea (Gerchakov et al., 1976; Zachary et al., 1980; and others). Most of these reports have concentrated on the temporal spacing of events in the development of marine microbial biofilms more specifically (Corpe, 1972), and have depended on old techniques that allow only a portion of the microbial population to be analyzed.

Data are particularly scarce concerning the quantitative behavior of specific film-forming bacteria in responses to metals of known composition. Studies in this area often have used artificial seawater as the test medium. Evidence strongly suggests that results of experiments on marine biological fouling and corrosion differ when the analyses are conducted in natural

seawater.

We have been using recently developed methods for quantifying bacterial attachment and growth on metals to investigate the attachment of specific marine bacteria to alloys of known composition. We have also begun to study in more detail some of the factors responsible for observed differences in the attachment behavior of bacteria on different types of metal.

Work Completed and Continuing Research

All metals are susceptible to fouling by microorganisms, even the "antifouling" copper-nickel alloys. Different types of metals, however, appear to be susceptible to microfouling in different degrees, at least upon initial immersion in the water. Furthermore, different metals may harbor quite different types of bacterial flora.

In the research initiated in 1981-82, we began to quantify the attachment of specific marine bacteria to metals and alloys of known composition. The purpose of these experiments was to develop a clear, quantitative understanding of: 1) the specific rates at which known marine bacteria attach to various commonly used metals; 2) the rates at which bacteria that have attached to metals grow under specified, marine and estuarine environmental conditions; 3) the ultimate bacterial population densities that develop on specific metals in seawater; and 4) the interactions that occur within multispecies microbial films on metal surfaces in the sea. These experiments have been continued during 1982-83 with the emphasis placed on the relationship between biofilms and corrosion.

In our initial experiments we compared the attachment of pure cultures of marine bacteria to metals that either have, or do not have, surface-associated organic matter. In those experiments sanded, degreased, sterile metal samples

were immersed for 5 minutes in an organic rich broth containing peptone and yeast extract and then were transferred to a dish containing approximately 10^6 cells/ml of a specific bacterial suspension in seawater. Control samples were placed directly into an identical dish of the same bacterial suspension without prior exposure to organic matter. Metals were removed from the bacterial suspensions after 5 minutes, rinsed in sterile seawater to remove unattached bacteria, and incubated for 24 hours in filter-sterilized seawater to follow increases in cell number. Specimens were removed at various times during the incubation, preserved in formalin, and stained with acridine orange dye. The bacteria attached to the metal surface were then counted by epifluorescent microscopy. Four different alloys were used in these experiments -- 316 stainless steel, titanium, 90-10 copper-nickel (CA 706), and aluminum bronze D (CA 614).

Results of experiments using 316 stainless steel and titanium indicated that fewer bacteria attach initially to metals which have organic material associated with the surface than to identical organic-free metal samples. However, the bacteria that do attach to filmed metals seem to multiply more rapidly and reach higher population densities than those on the organic-free metals. It appears that bacteria attached to organic-filmed surfaces quickly shift their metabolism to utilize the organic nutrients on the surface and increase in number rapidly, with growth rates similar to those seen in high nutrient culture broth. Conversely, bacteria attached to metals having a very low level of surface-associated organic matter may run out of reserve nutrients, resulting in a corresponding dramatic decrease in growth.

When the same experiments were carried out using 90-10 copper-nickel and aluminum bronze, we generally observed a decrease in cell number with time. Figure 1 shows data for aluminum bronze. The data suggest either a toxic

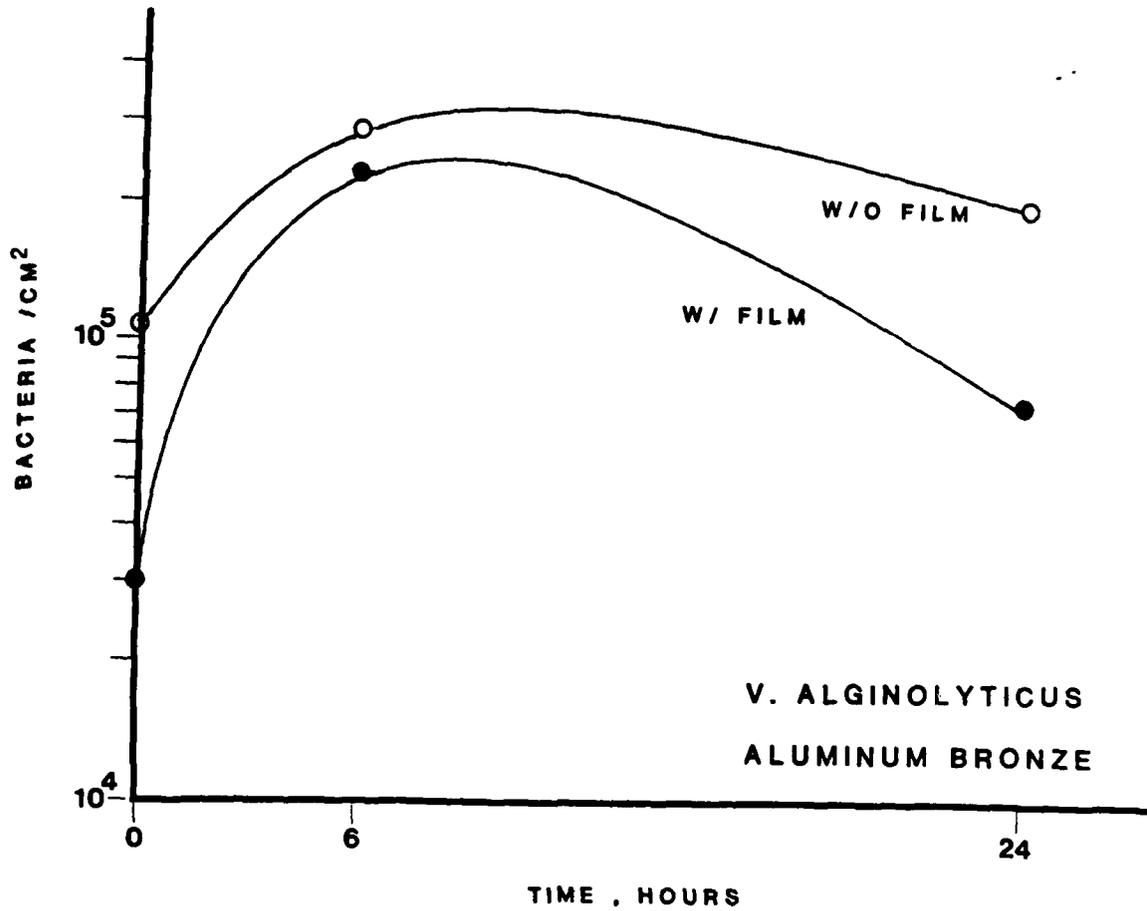


FIGURE 1: Growth of the common marine bacterium, *Vibrio alginolyticus* on aluminum-bronze surfaces with and without an organic film on the metal surface. Note that the decline in the bacterial population is stimulated by the organic film on the metal surface.

effect from copper ions leaching from these alloys or perhaps a loss of cells due to sloughing-off of loosely bound corrosion products. The organic film accentuates this effect. Bacteria do attach to these copper alloys in significant numbers, however, despite the reputation that these metals have as "antifouling" materials.

Our pure culture experiments, such as those just described, are designed to monitor bacterial growth on metal surfaces, after the bacteria have attached. Growth is monitored initially by following increases in cell numbers on the metal surfaces during incubation in filter-sterilized seawater; increases in numbers of bacteria result from cell multiplication and not from new recruitment in this system. Cell numbers are monitored using epifluorescent microscopy and scanning electron microscopy. The experiments are carried out in an apparatus which we designed in 1981-82 to allow multiple replicate metal specimens to be moved aseptically from one test fluid to another, thus allowing direct comparison between samples. The apparatus has been used successfully in our laboratory for over a year now.

In the summer of 1982 we conducted field experiments at the Marine Biological Laboratory in Woods Hole, Massachusetts. Samples of the four metals listed above were cleaned, degreased, and sterilized, and hung from styrofoam floats in Eel Pond. Since previous experiments had indicated that removal of the oxide film from metal surfaces can significantly alter bacterial attachment and growth, both sanded and unsanded specimens of each type of metal were prepared. After exposure to the seawater for varying time periods ranging from 30 minutes to 14 days, the metals were removed and examined using acridine orange direct counts (AODC) and scanning electron microscopy (SEM).

Data from those experiments indicated that both qualitative and quantitative differences in the attached microbial communities occur in

response to different types of metal surfaces. In general, the two copper alloys supported smaller and apparently less active populations of bacteria than did similarly treated stainless steel and titanium. The differences in populations among the four metals, however, appeared to diminish with time, as attached bacteria became increasingly insulated from the base metal by corrosion product films and the accumulating biofouling layer. Thus, in terms of protection from bacterial fouling, any advantage that copper alloys might hold over stainless steel or titanium would seem to be short-term.

After exposure to seawater for one day, unsanded metal samples were found to have up to one or two orders of magnitude more attached bacteria than sanded samples. This difference was most pronounced for the copper alloys, although both stainless steel and titanium also showed this behavior to a lesser degree. The difference also seemed to be more pronounced when the metals were exposed to flowing seawater in the laboratory than when they were incubated in situ. With time, numbers of bacteria on metals treated in the two different ways became more similar. Apparently the metal oxide films, which are removed when the metals are sanded, are in some way more suitable surfaces for bacterial attachment and growth than the bare, unprotected metal. They may, for example, provide protection from toxic effects of the sanded metal surface. The observation of a greater protective effect for the copper alloys would seem to support this idea.

We have begun to study in more detail some of the factors responsible for the differences in bacterial attachment and growth behavior that we observed on the different types of metal. We wish to know what properties of metal surfaces and/or their adsorbed organic films have the greatest influence on attachment and how these properties differ among the various alloys that we have studied. The influence of substratum characteristics on the attachment of

marine bacteria has been extensively studied (Dexter et al., 1975; Fletcher and Loeb, 1979; Loeb, 1977). Many of these experiments have shown that hydrophobicity, both of the substratum and of the cell surface, can significantly influence the attachment of bacteria at interfaces (Dexter et al., 1975; Pringle and Fletcher, 1983; and others). Weiss et al. (1982) demonstrated that bacteria which adhere to metal prostheses placed in the human mouth tend to be very hydrophobic.

We are conducting experiments to determine whether substratum and/or cell surface hydrophobicity are important in the attachment of marine bacteria to metal surfaces. If differences can be found in the overall hydrophobicity of those bacteria which attach to different metals, then this would provide evidence that the surface properties of the various metals and their adsorbed organic conditioning films are quite different. During the past several months we have isolated between 150 and 200 strains of aerobic, heterotrophic bacteria from the surfaces of metals exposed to various marine environments. We have used 316 stainless steel, titanium, 90-10 copper-nickel, aluminum bronze, and pure copper -- both sanded and unsanded for these studies. The bacteria were removed from the metals by mild sonication and then grown on 2216 marine agar (Difco). Colonies were picked randomly from these plates and purified by restrycking 2 to 3 times. Environments sampled included Boston Harbor and at the New England Aquarium in Boston. Bacteria were isolated from these metals after 24 hours and 7 days of exposure to seawater.

We have tested the cell surface hydrophobicity of a number of these isolates using a hexadecane separation technique developed by Rosenberg et al., (1980). In this method cell suspensions are mixed with varying volumes of hexadecane; the degree to which the cells disappear from the aqueous phase to the hydrocarbon phase is a measure of the hydrophobicity of the cells. Using

this technique, nearly all of the strains of bacteria isolated from our metal samples have proved to be very hydrophilic. The method of Rosenberg et al. (1980), though, is known to be relatively insensitive to small differences in cell-surface hydrophobicity (S. Kjelleberg, pers. comm.). We are repeating the experiment using the method of hydrophobic interaction chromatography (HIC) (Dahlback et al., 1981), a much more sensitive technique. HIC uses [³H]-labeled cells run through a column packed with Octyl-Sepharose CL-4B gel, and the relative hydrophobicity of the cells is expressed as the ratio, g/e, between the radioactivity of the gel and the eluate. The higher the value of g/e, the higher is the tendency to hydrophobic interaction. These experiments should be completed early this summer.

B. CORROSION BY THERMOPHILIC BACTERIA

Background

Studies of the microbial flora of natural geothermal habitats, such as hot springs, have shown that a wide variety of bacteria thrive at temperatures from 60 to over 90°C (Brock, 1978). Some types apparently can grow even in boiling water (Brock, 1978). Bacteria also are commonly found in man-made thermal habitats such as hot-water heaters (Brock and Boylen, 1973; Ramaley and Hixson, 1970). The organisms isolated from such high-temperature environments generally are obligately thermophilic; that is, they not only tolerate high temperatures but, in fact, require them. For example, the bacterium Thermus aquaticus, commonly isolated from both natural and man-made thermal sources, has an optimal temperature for growth of from 70 to 75°C and will not grow at all below 45°C (Brock and Freeze, 1969). These bacteria probably grow on the walls and surfaces of hot water systems, rather than in the free water. Large

populations may build up on such surfaces, even in the presence of extremely low concentrations of organic matter (1 to 2 mg.L⁻¹) (Brock, 1967).

The practical significance of bacteria growing in man-made hot-water systems has not been adequately explored. In particular, metal surfaces which become covered with fouling films of thermophilic microorganisms may become sites of biologically-induced corrosion, even at near boiling temperatures. The demonstration of a connection between bacterial activity and corrosion of high-temperature aqueous systems such as heat-exchangers would have enormous practical implications.

Recently Brenda Little (Oceanography Division, NORDA, Bay St. Louis, MS) observed abundant growth of filamentous bacteria in corroding heat exchanger systems through which distilled water flowed, in a closed loop, at 60°C. Failures in the nickel tubing occurred at brazing points, and bacteria often were observed to be growing preferentially near the brazes. Furthermore, time to failure of the brazes appeared to be correlated with growth of the bacteria. We have begun a cooperative effort with Little to examine in more detail the role of thermophilic bacteria in the corrosion of high-temperature systems.

Work Completed and Continuing Research:

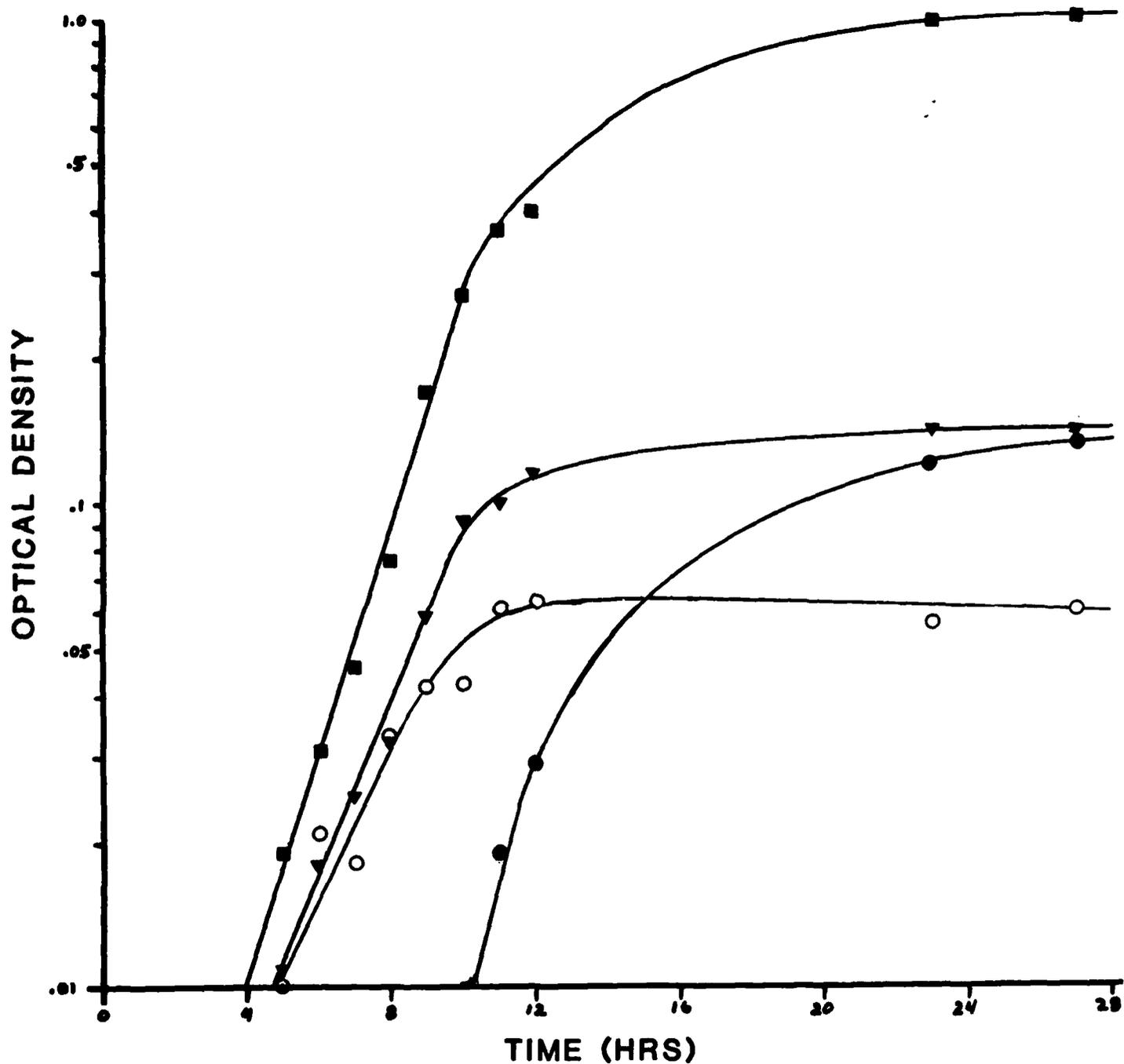
We are currently studying the impact of thermophilic microorganisms on crevice corrosion and brazing failure in heat exchanger systems operating in the temperature range of 60 to 80°C in cooperation with NORDA. Construction of model heat exchanger and electrochemical corrosion measurements are being carried out at NORDA, while microbiological analyses are being done in our laboratory at Harvard.

During the past several months, we have succeeded in isolating strains of bacteria capable of growing at 70°C from a number of hot-water sources, mostly

domestic hot water heaters. Nearly all of the strains isolated so far are pleomorphic, filamentous, nonsporulating rods, similar in morphology and cultural characteristics to bacteria of the genus Thermus, which apparently are common inhabitants of hot-water heaters (Brock and Boylen, 1973). One strain is a short, spore-forming rod, probably a Bacillus species. All grow well in very low concentrations of organic nutrients and appear to be obligately thermophilic. Figure 2 shows typical data. The Thermus-like strains characteristically grow in flocs of long, thin filaments which appear to adhere readily to surfaces (Figure 3). Experiments are in progress to characterize these bacteria more completely and to determine their growth characteristics under different environmental conditions.

We are growing several of the strains of thermophilic bacteria that we have isolated in 70°C flowing water systems. These systems, constructed of plastic and glass tubing and driven by peristaltic pumps, are designed to simulate conditions which might be encountered in real heat exchanger systems. Glass and metal baffles placed in the water flow allow us to investigate the growth behavior of these microorganisms on surfaces. At low nutrient concentrations, populations of bacteria living free in the water remained extremely low. But when the glass and metal baffles were examined microscopically, large numbers of bacteria were observed to be attached to these surfaces. Cultures of these bacteria have been sent to NORDA.

A model heat exchanger system that contains sections of brazed nickel 201 tubing and is connected to a potentiostat for doing cyclic potentiodynamic anodic polarization measurements to determine the presence of crevice corrosion has been designed by Little. Comparison of corrosion rates in sterile systems and those inoculated with pure cultures of our isolates of thermophilic bacteria will allow us to determine the impact of these organisms on crevice



NUTRIENT CONC.

- | | |
|--------|----------|
| 1.0% ● | 0.01% ▼ |
| 0.1% ■ | 0.001% ○ |

FIGURE 2: Growth curves of an extremely thermophilic bacterium (T01-SWM-B) isolated from a hot water heater. The organism was grown at 70°C. The curves show that it grows better at 0.1% of tryptone and yeast extract than at 1.0% of the added nutrients.

FIGURE 3: Photomicrographs of Thermus-like strains of obligately thermophilic bacteria isolated from hot water systems.

- a) Filamentous growth on a surface.
- b) Development of a flocculent microcolony.

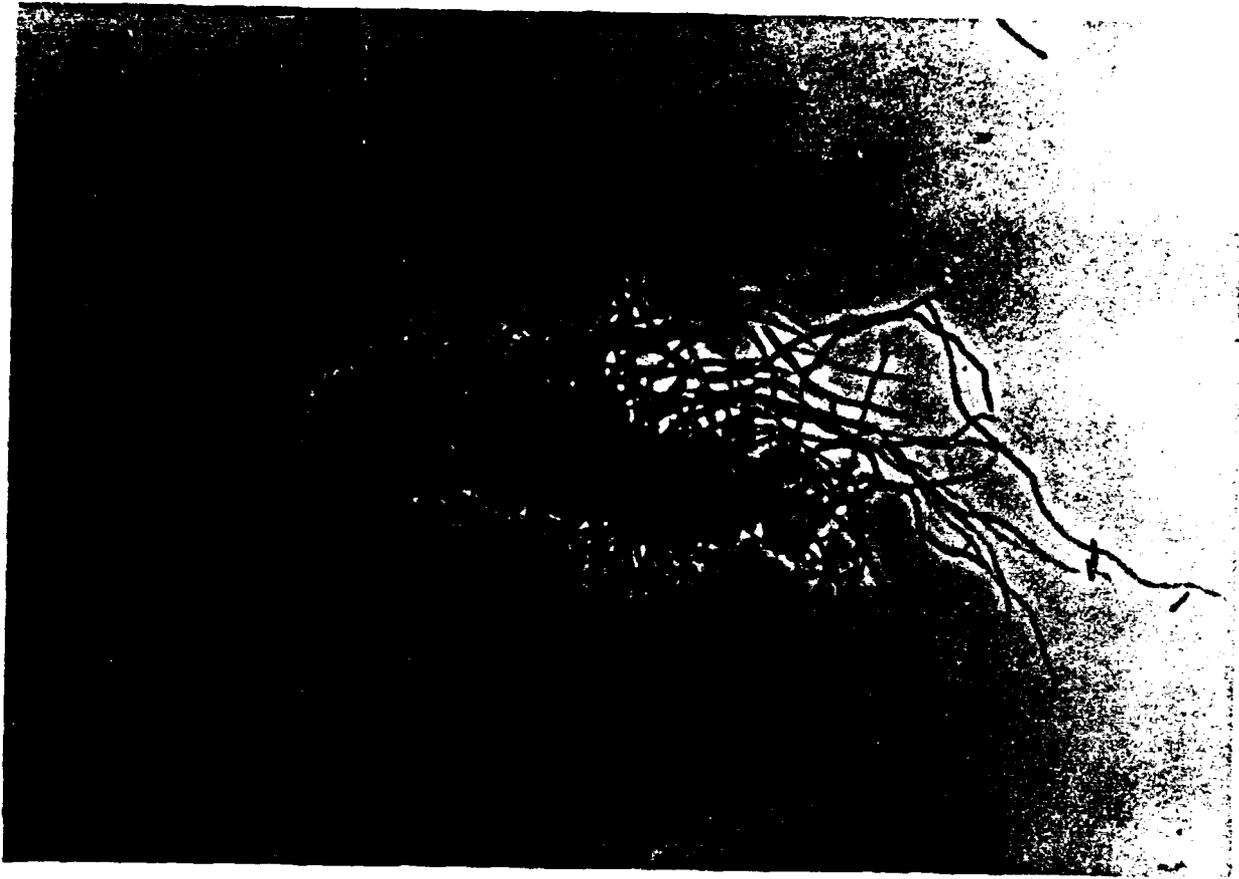


FIGURE 3a) Filamentous growth on a surface.



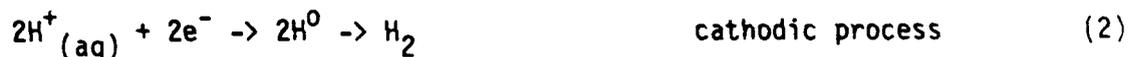
FIGURE 3b) Development of a flocculent microcolony.

corrosion and brazing failure. Further experiments will be conducted with strains found to enhance corrosion of the nickel tubing to try to determine the nature of the interaction between the bacteria and the metal surface and the reason for preferential settlement of the organisms in the brazed fillets.

C. THE ROLE OF HYDROGEN-CONSUMING BACTERIA IN ANAEROBIC CORROSION PROCESSES

Background

Current theory states that bacteria (mainly sulfate reducers) enhance corrosion by removing hydrogen from the metal surface:



This is known as the cathodic depolarization theory.

In nature several kinds of bacteria, in addition to sulfate reducers, could play an important role in cathodic depolarization. Examples of potential mechanisms are:



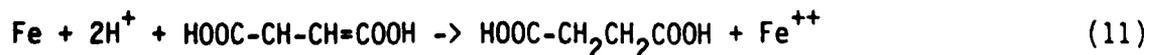
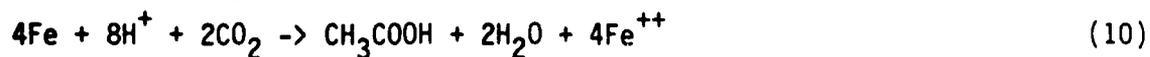
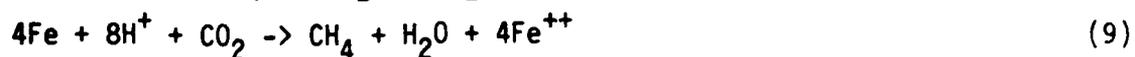
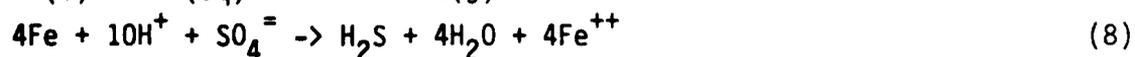
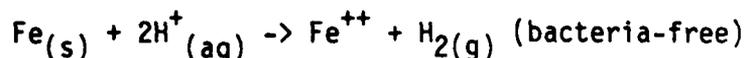
We are currently examining these mechanisms and developing methods to determine the relationships between these anaerobic processes and corrosion.

Work Completed and Continuing Research

One of the weakest points of the cathodic depolarization theory is that the anaerobic corrosion rates obtained in the laboratory are significantly lower than those observed in the field. We have been designing an experimental system to provide defined and optimum growth conditions for the bacteria, the main goal being to see whether we can obtain corrosion rates comparable to those observed in the field.

For this purpose we are testing a new approach for measuring anaerobic corrosion rates. Electrodes have traditionally been used to measure rates of corrosion. Their use has a serious disadvantage when they are used to measure anaerobic corrosion. They are poisoned by sulfide. Rather than using electrochemical methods, we are utilizing direct chemical analysis to measure corrosion rates.

The equations below show the chemical reactions in which the iron oxidation reaction has been added to the hydrogen consuming one mediated by particular bacteria. Since there is a stoichiometric coupling between the disappearance of elemental iron and the appearance in the medium of bacterial metabolites, the rates of corrosion can be determined by measuring the production of specific metabolites.



Corrosion rates can be measured several ways; by the appearance of iron,

methane, hydrogen, acetate, succinate or hydrogen sulfide in the medium, or by the disappearance of sulfate or fumarate. We have the means to measure these substances by using gas chromatography, atomic absorption spectrophotometry or colorimetry.

Of central importance in these studies is the assumption that the bacteria will be able to utilize the hydrogen coming off the metal as their sole energy source. Preliminary experiments suggest that this is indeed the case with Desulfovibrio vulgaris. These data are shown in Figure 4.

Stronger evidence for our proposed methods to measure corrosion can be seen in Figure 5. In these experiments we have used a strain of Desulfovibrio vulgaris capable of substituting sulfate for fumarate as the terminal electron acceptor, while growing on H_2 as the sole energy source (Eq. 7). Figure 5a shows a chromatogram of non-volatile fatty acids produced in uninoculated medium in the presence of mild steel. Figure 5b illustrates the same medium inoculated with D. vulgaris. The fumarate was reduced yielding succinic acid.

Our objective in this phase of the research is to determine if we can achieve anaerobic corrosion rates comparable to those observed in the field. For this purpose we must create conditions that favor the removal of H_2 from the metal surface. One of the main obstacles is the formation of iron precipitates on the surface of the metal being studied. We are approaching this problem in several ways:

- (1) using chelators to keep the Fe^{++} in solution;
- (2) passing all media stock solutions through an ion exchange column to remove the iron normally present as a contaminant;
- (3) controlling the pH in the growth vessels;
- (4) cleaning the metal to be used;

1 D. vulgaris growing in H₂ in the absence of mild steel.



2 D. vulgaris growing on the H₂ coming off mild steel as sole energy source.

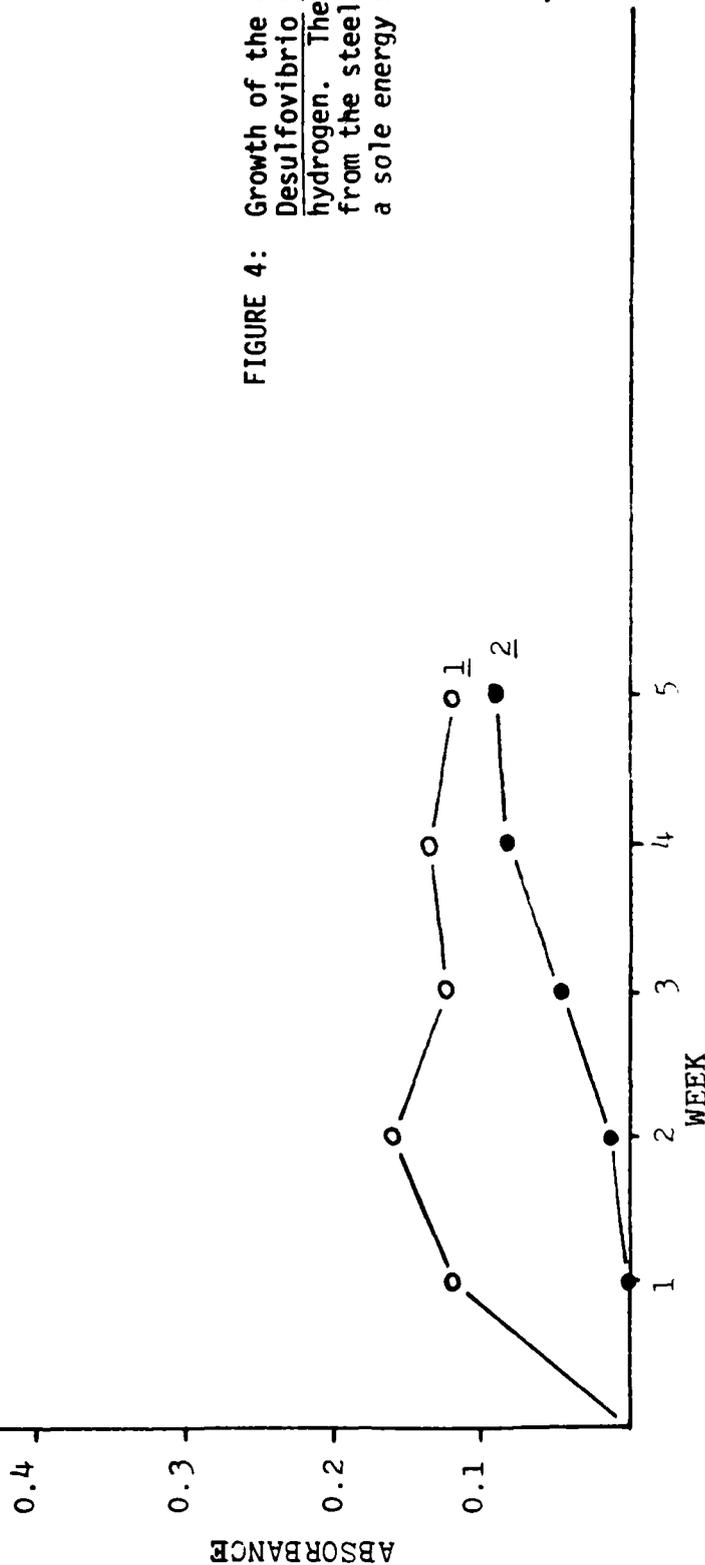
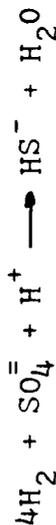


FIGURE 4: Growth of the anaerobic bacterium Desulfovibrio vulgaris on hydrogen. The hydrogen evolved from the steel is being used as a sole energy source.

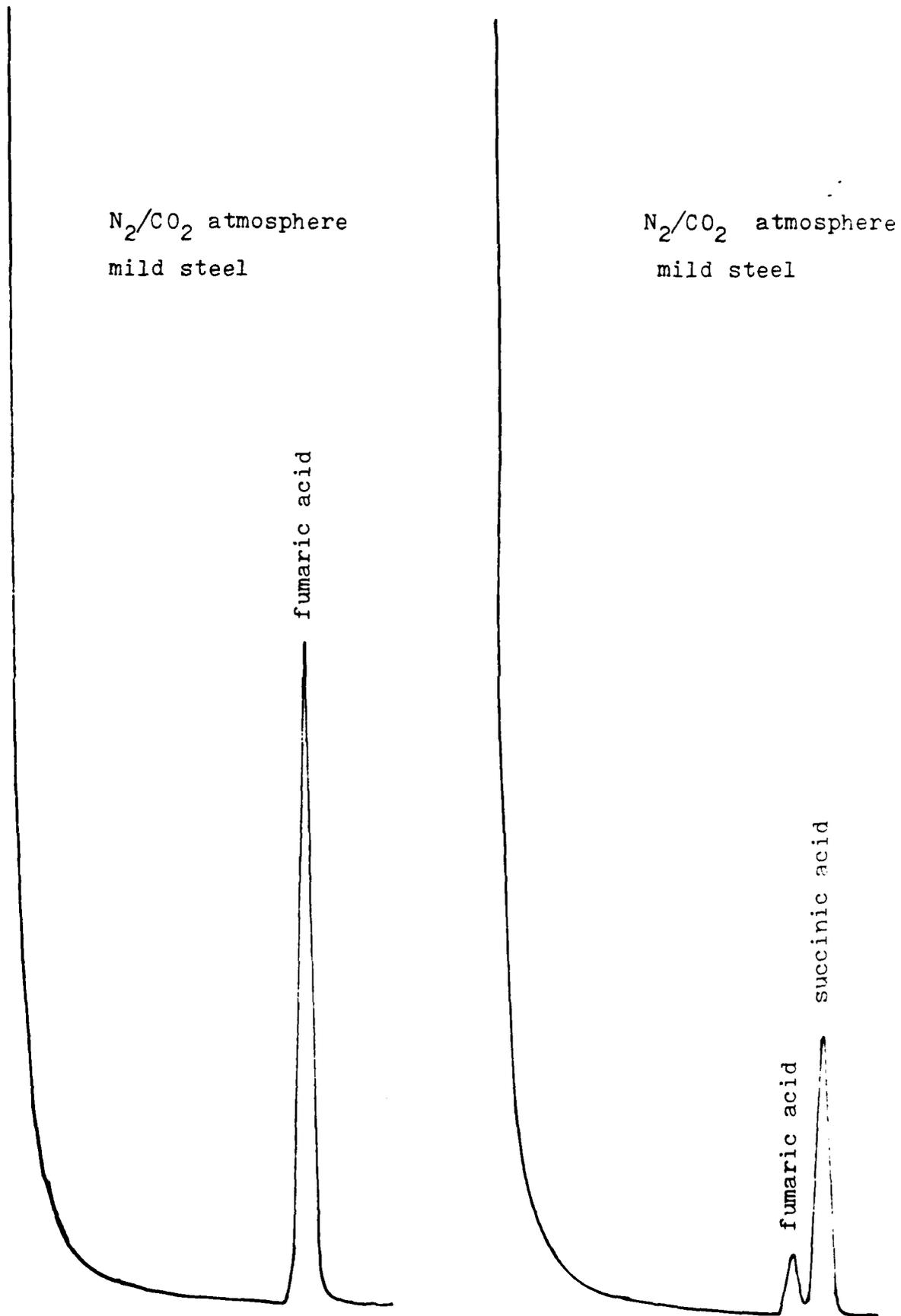


Figure 5. a. Un-inoculated

5 b. Inoculated with D. vulgaris

Figure 5: Chromatograms showing the utilization of fumarate as the terminal electron acceptor in the growth of Desulfovibrio vulgaris on hydrogen. The fumarate was reduced yielding succinate, while hydrogen coming off the metal was utilized as the sole energy source.

- (5) precipitating excess iron in the media by adding the metal and then dispensing it into the growth vessels by sterile filtration.

Our results to date show that with the use of chelators and in the absence of the metal, we can obtain a high cell density of sulfate reducing bacteria ($> 10^7$ cell ml^{-1}) without any iron sulfide precipitation. In the presence of the metal, a combination of approaches (1) + (5) prevents the initial formation of the precipitate. We have found that although we can prevent the formation of precipitate, the growth of the bacteria is inhibited. This is probably due to the removal of other metals which are essential for their growth. We are now experimenting with other ways to prepare the medium to prevent inhibition of growth resulting from loss of essential substrates.

We are interested in carrying out anaerobic corrosion experiments under highly defined conditions. For this purpose we need to utilize bacteria that grow on a simple salts mineral medium with H_2 as the sole energy source. We are only aware of two strains of Desulfovibrio vulgaris capable of growing under these conditions. We have obtained both strains from culture collections. Experiments are in progress in which these strains are being utilized.

We also have enrichment cultures of H_2 -consuming sulfate reducers obtained from well water, an anaerobic lake, a tropical marine environment and a coal gasification project. We are currently in the process of isolating and purifying several H_2 -consuming methanogens and a bacterium that consumes H_2 but does not reduce SO_4^{2-} or produce CH_4 .

Previous studies indicate that corrosion enhancement by bacteria can be correlated with the presence of hydrogenases. These enzymes can catalyze both the formation and/or consumption of hydrogen. We know that there are hydrogenase-positive bacteria that can only consume hydrogen, others that can only

produce it and others that can carry out both processes. Thus, the presence of hydrogenase per se cannot be an indication of the corrosion enhancement potential of a bacterium if the removal of hydrogen from the surface of the metal is the main corrosion-causing mechanism.

An interesting aspect of the reactions that produce H_2 is that many of them are thermodynamically unfavorable or close to the point of chemical equilibrium. If the hydrogen is removed by another bacterium the reaction becomes more favorable and the H_2 -producing bacterium is allowed to grow. We have carried out experiments with Desulfovibrio vulgaris to illustrate this phenomenon (Figure 6).

This type of interaction has implications for the understanding of microbially-caused corrosion in nature. It illustrates how sulfate reducers can proliferate in sulfate depleted environments. It also shows that even though they can be isolated from corroding environments, other bacteria, e.g., methanogens, may actually be responsible for cathodic depolarization. We are presently carrying out experiments to test this hypothesis.

D. HYDROGEN EMBRITTLEMENT

Background

Hydrogen embrittlement is weakening of a metal due to the absorption of atomic hydrogen. In susceptible metals this results in a loss of ductility and tensile strength and often in premature failure of the material. This phenomenon is especially important in high-strength steels, although other alloys also are susceptible (Bernstein and Thompson, 1974). It constitutes a serious hazard, since catastrophic failures can occur with no prior warning.

Microorganisms can play a significant role in hydrogen damage processes, and several mechanisms may be involved. These include: 1) production of molecular

- 1 Growth of D. vulgaris on sulfate medium
pyruvate + sulfate → acetate + sulfide
- 2 Growth on sulfate-free medium
pyruvate → acetate + H₂
- 3 Growth in coculture with M. formicicum
pyruvate → acetate + H₂ D. vulgaris
H₂ + CO₂ → methane M. formicicum

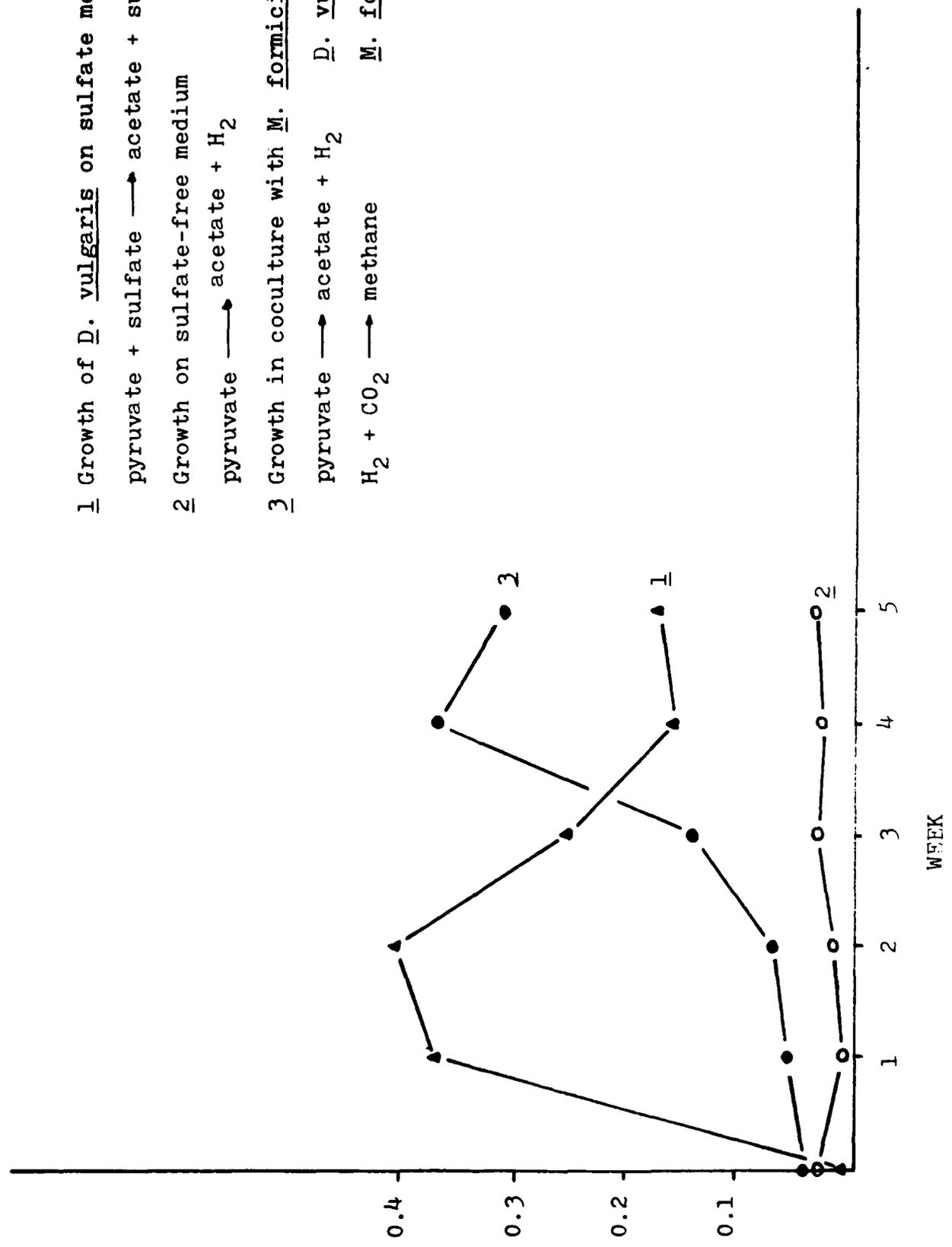


Figure 6: Production of hydrogen from pyruvate by Desulfovibrio. The reaction is driven by the removal of the hydrogen by a second bacterium, Methanobacterium formicum.

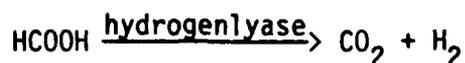
hydrogen, which may be dissociated into atomic hydrogen and absorbed into the metals; 2) production of hydrogen ions which may be reduced to form hydrogen atoms at cathodic areas of corroding metals; 3) formation of hydrogen sulfide; which may stimulate the absorption of atomic hydrogen into metals by preventing its recombination into hydrogen molecules; 4) destabilization of metal oxide films, which, depending upon conditions at the metal surface, may either stimulate or inhibit the embrittlement process. Bacteria may also suppress hydrogen absorption in some cases by removing it from the metal surface. We are using biochemical and electrochemical methods to investigate the microbial processes and the environmental conditions which control hydrogen production and consumption on metal surfaces.

Work Completed and Continuing Research

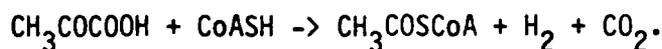
The importance of hydrogen-induced failure of metals has been widely recognized, but the role of bacteria in this corrosion process is not well understood. We are investigating several mechanisms by which microorganisms may be involved in hydrogen embrittlement of metals and are using biochemical and electrochemical methods to study the microbial processes and environmental conditions which control hydrogen production and consumption on metal surfaces.

It is well established that external hydrogen gas can cause embrittlement in a variety of materials, including steels, titanium alloys, and nickel alloys (Johnson, 1974). Both low- and high-pressure hydrogen can be effective embrittling agents, provided that dissociation of H_2 occurs, thereby allowing for the absorption of atomic hydrogen into the metal lattice. A wide range of bacteria produce molecular hydrogen as an endproduct from the fermentation of carbohydrates. Organic substrates are fermented under anaerobic conditions with subsequent release of a mixture of organic acids, typically consisting largely of formate, acetate,

propionate, lactate, and butyrate. Many of these bacteria possess the enzyme hydrogenlyase, which splits the formic acid into equimolar quantities of carbon dioxide and hydrogen:



Bacteria which carry out this type of fermentation include those of the genera Escherichia coli, Proteus, Salmonella, Enterobacter, and Aeromonas. Formation of molecular hydrogen as an endproduct of carbohydrate fermentation is also characteristic of the spore-forming bacteria of the genera Bacillus and Clostridium. In these organisms, however, hydrogen is formed directly from the cleavage of pyruvic acid



The fermentation balances for some species of hydrogen-producing bacteria are shown in Table 1. It is evident that significant amounts of hydrogen may be produced, particularly by Clostridium. One would expect to find large populations of such fermenting bacteria on the surface of any metal that has accumulated a layer of fouling microorganisms. Even a relatively thin biofilm can inhibit oxygen transfer to such an extent that conditions at the metal surface are virtually anaerobic. Anoxic conditions also exist beneath tubercles and deposits, in crevices and pits, and in wet soils and sediments to which metals may be exposed.

In our studies of biofilms on surfaces we frequently find microcolonies of bacteria. Totally unrelated bacterial populations appear to develop in the anaerobic regions beneath the surface film of bacteria. An example of these discontinuous populations is shown in Figure 7. The scanning electron micrograph shows a colony of bacteria growing beneath the surface biofilm on stainless steel in seawater. The molecular hydrogen that is produced by bacteria in these anaerobic regions of the metal surface may, under certain conditions, be absorbed

Table 1. Fermentation and products from some species of hydrogen-producing bacteria.

Product	mol formed / 100 mol glucose fermented				
	<u>Escherichia coli</u>	<u>Enterobacter aerogenes</u>	<u>Clostridium butyricum</u>	<u>Clostridium perfringens</u>	<u>Clostridium acetobutylicum</u>
formate	2.4	17.0	-	-	-
acetate	36.5	0.5	42	60	14
lactate	79.5	2.9	-	33	-
succinate	10.7	-	-	-	-
ethanol	49.8	69.5	-	26	7
2,3-butanediol	0.3	66.4	-	-	-
CO ₂	88.0	172.0	188	176	221
H ₂	75.0	35.4	235	214	135
butyrate	-	-	76	34	4
butanol	-	-	-	-	56
acetone	-	-	-	-	22

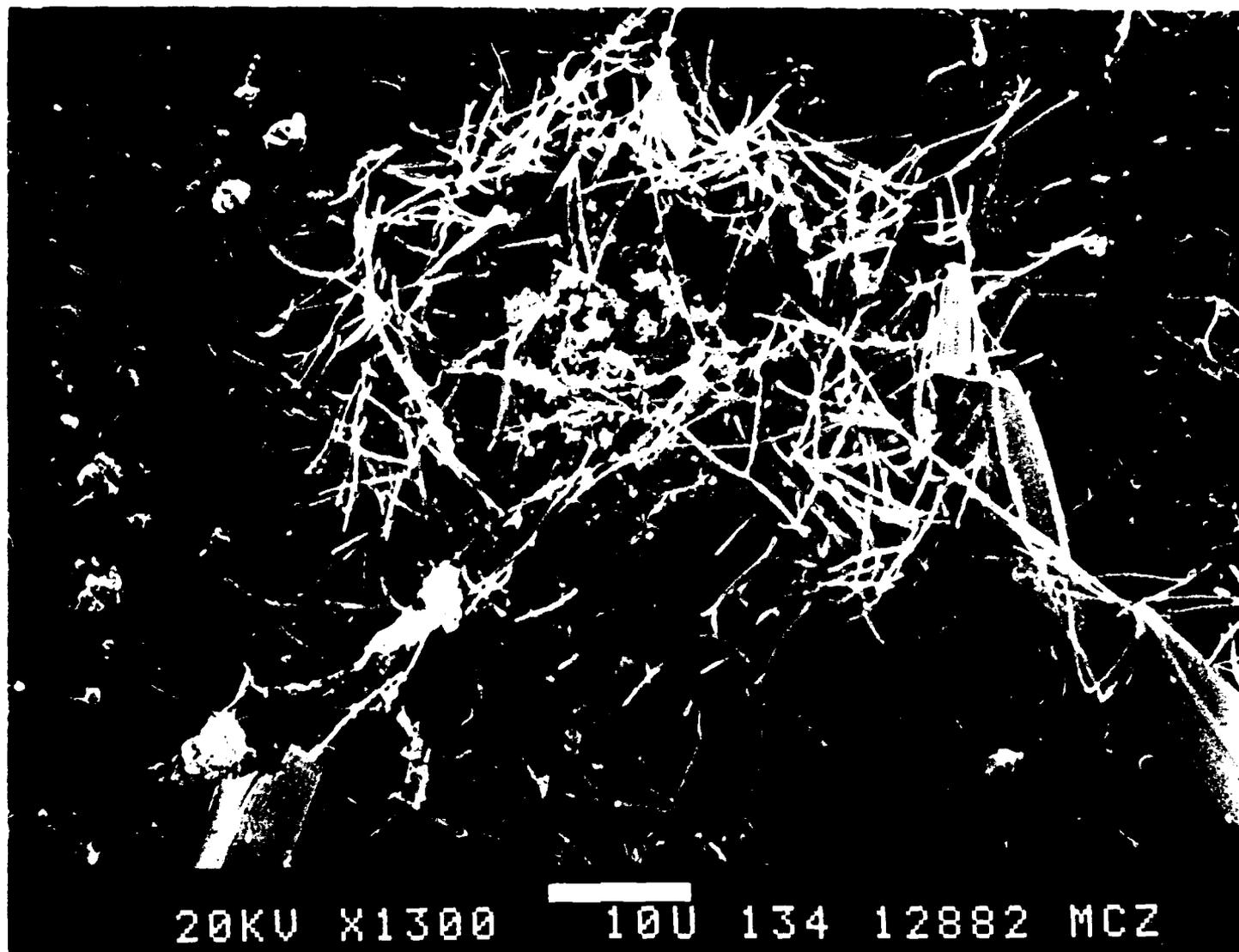


FIGURE 7: A scanning electron micrograph showing growth of a colony of bacteria in the anaerobic zone beneath the surface biofilm on stainless steel in seawater.

into the metal if dissociation into atomic hydrogen occurs.

We are currently using pure cultures of anaerobes to determine the appropriate bacteria to use in our embrittlement studies. In order to accurately assess the importance of hydrogen producing reactions in embrittlement we need to determine both growth rates and hydrogen production rates on metal surfaces. These tests are currently under way. The bacteria being tested are clostridia and strains of Selenomonas. In addition, we have isolated marine hydrogen producing bacteria capable of growing well on metal surfaces. These are being assessed for their activity in biofilms as candidates for our embrittlement studies.

We have been working for the past year on a method to measure the production of atomic and molecular hydrogen within microbial biofilms. In addition, it is our intention to measure microbially produced hydrogen, accumulating within metal lattices in contact with the biofilms.

Permeation of hydrogen into metals is normally determined electrochemically using palladium membranes. This method shows the amount of hydrogen accumulating at the metal surface, providing a measure of potential hydrogen embrittlement. We are modifying an electrochemical apparatus in cooperation with Prof. R. Latanision of MIT's Department of Material Science and Engineering. The modified apparatus will have palladium foil in strictly anoxic seawater with films of hydrogen-producing bacteria growing on the surface. It should be possible, using this technique, to assess the rate of bacterially produced hydrogen accumulation at the metal surface under a range of environmental conditions.

In order to determine the diffusion of bacterial hydrogen into metals we are also developing, together with Prof. Latanision, a method based on uptake of radiolabelled hydrogen by the metal. The technique is based on the use of tritium labelled organic substrates. Anaerobic bacteria will be grown on these media to yield tritiated hydrogen as a product. The bacteria used in these experiments

will be chosen from previous studies to maximize growth and hydrogen production on metal surfaces. These tests are currently being undertaken.

In an extension of these studies we will grow anaerobic bacteria on different metal surfaces in the presence of tritium labelled substrates. Following growth we will strip the biofilm from the surface and measure the concentration of tritium that has diffused into the metal lattice. The tritium concentration in the metal can be determined by driving off the gas at 300°C and trapping it on a palladium foil. The quantity of hydrogen absorbed by the metal can be calculated from measurements of the tritium released.

Our observations of hydrogen accumulation on metal surfaces and of hydrogen absorption into the metal lattices should permit us to assess the potential of bacterial hydrogen to produce embrittlement. During the next year we intend to extend our research to determine the relationship between accumulation of bacterial hydrogen and the embrittlement process, testing metals commonly in use in marine environments.

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