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chromatography is being used for these analyses.

It appears that bacteria are involved in hydrogen embrittlement of metals in marine environments. We are studying this process in detail. We are constructing devices to measure absorption of bacterial hydrogen to metals and diffusion of the hydrogen into metals. Anaerobic bacteria are being tested in laboratory studies for their ability to produce hydrogen embrittlement. We are also studying the role of anaerobic bacteria other than *Desulfovibrio* in hydrogen sulfide associated corrosion. Bacteria capable of mediating transformations involving fumarate or carbon dioxide are being tested for their ability to cause corrosion.

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The Role of Microorganisms in Marine Corrosion Processes

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## I. OBJECTIVES

The intent of the proposed 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. Factors affecting the formation of microbial biofilms on corroding metallic surfaces; 2. The production of organic acids by bacteria and other microorganisms attached to metallic surfaces; 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 (Fontana and Greene, 1978). Corrosion in the marine environment represents a substantial portion of the total annual cost of corrosion and protection against corrosion.

The corrosion of metals in contact with seawater, and efforts to prevent corrosion, 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.

There is ample evidence of the enormous economic cost of biologically induced corrosion. Destruction of buried pipelines in the United States has been estimated to cost between 500 and 2000 million dollars a year (Greathouse and Wessel, 1954). Bacteria are thought to be responsible for more than 77% of the corrosion occurring in producing oil wells in the United States (Allred et al., 1969). Microorganisms have been estimated to be responsible for more than 50% of the failure of buried metal in cables and in pipelines (Booth, 1964).

Bacteria and other microorganisms play an integral role in marine corrosion, although the economic costs are not known (LaQue, 1975). A wide range of microbiological reactions causing corrosion occur 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 include

bacterial biofilm formation on corroding metals; anaerobic corrosion; hydrogen embrittlement; and, acid production. 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. BACKGROUND

#### A. Relationship to Fouling

The attachment and growth of marine organisms influence the rate of corrosion of metals in seawater (Mitchell and Benson, 1981). Deficiencies in oxygen supply within crevices and under discontinuous deposits are responsible for initiation of accelerated corrosion. Where the metals involved depend on oxygen induced passivity for their corrosion resistance, localized attack occurs, caused by differential aereation. The corrosion is propagated by powerful galvanic cells involving large differences in potential between the active and passive surfaces. Such corrosion cells can also be set up by invertebrates or by microorganisms. Microorganisms living beneath macrofouling layers also appear to stimulate general corrosion of the metal. Often, hydrogen sulfide produced within the biofilms stimulates the corrosion process.

Accelerated corrosion has been observed on copper nickel alloy surfaces in polluted waters. The regions attacked generally displayed distinct biofilms. It appears that the accelerated corrosion of these alloys is also the result of anaerobic processes occurring beneath the microbial films. Pitting of the copper nickel alloy was correlated with the production of hydrogen sulfide (LaQue, 1975).

Conversely, growth of marine microorganisms on surfaces sometimes acts to reduce corrosion by blocking the diffusion of oxygen to the metal surface. The controlling effect of oxygen availability through layers of microbial biofilms has been observed on stainless steel in seawater.

The close relationship between microfouling and corrosion processes has largely been ignored by corrosion engineers. Most marine corrosion studies either use artificial seawater or aged sterilized seawater. It is probable that the marine microflora plays an important role in the corrosion of metals

and that the biological nature of these processes is not understood in conventional corrosion studies. A surface film of microorganisms forms as soon as a metal is placed in seawater. This sequence of events has been described in detail (Mitchell, 1981). Primary microbial films consisting of growing microorganisms, their exo-polymers, trapped detritus and other organic matter, totally change the nature of metal surfaces. Metabolic processes occurring on the surface have a significant effect on a variety of different corrosion reactions.

Kalinenko (1959) found bacterial colonies on aluminum, brass and bronze plates immersed in natural seawater and suggested that the bacterial colonies accelerate the electrochemical process of metal corrosion. It has been suggested that bacteria enhance corrosion of stainless steel in seawater, by decreasing the protective effects of cathodic polarization. Conversely, bacteria may retard corrosion by sedimentation of calcium carbonate and magnesium hydroxide on the steel surface (Rozenberg and Ulanovskii, 1960). It has been reported that chrome manganese steel corrodes more rapidly in the presence of marine bacteria than in their absence. (Tawadse and Kemchadse, 1972).

Gerchakov and his co-workers have discussed the importance of periphytic microorganisms on the corrosion of metal surfaces in some detail (Gerchakov and Sallman, 1977). They concluded that the corrosion rate of metals in natural waters is a function of the metallurgical characteristics and of environmental factors such as temperature, velocity and chemical make-up of the water. They observed that metal surfaces in natural waters became populated with periphytic bacteria in as short a time as four hours. Subsequent colonization by a variety of microorganisms produced a complex microfouling layer. This biofilm has 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.

#### B. Anaerobic Corrosion

The anaerobic conditions existing beneath microbial biofilms on metal surfaces, on metal surfaces in contact with the anaerobic marine sediment layers, or in heavily polluted seawater, stimulate the activities of sulfate reducing bacteria. The consequent production of hydrogen sulfide is associated with extensive corrosion of a wide variety of metals in the sea. The corroded metal tends to be pitted rather than evenly corroded, usually leading to localized perforations. Popplewell has described localized corrosion of copper alloys in anaerobic seawater. He noted that the mechanism of attack appeared to be by the formation of cuprous sulfide regions either during initial sulfide exposure, or at defects in the cuprous oxide film. These regions act as local anodes and promote rapid corrosion during periods of oxygenation (Popplewell, 1980) Metals in contact with marine sediments are particularly susceptible to anaerobic corrosion (King, 1979). The activity of the sulfate reducing bacteria under these conditions is controlled by the organic matter content, the nitrogen and phosphorus concentrations and the temperature of the water.

Sulfate reducing bacteria are a particular hazard on oil tankers. The presence of sulfur dissolved in crude oil provides an excellent substrate for these bacteria, resulting in development of corrosion pits in the metal. Examination of the pits and analysis of corrosion products on adjacent surfaces indicates that the accelerated attack is a result of microbial activity (Munger, 1975). Apparently the oxidation/reduction cycle between air/seawater, and the production of hydrogen sulfide provides a mechanism for continuous corrosion, which causes the rapid pitting corrosion experienced on these

surfaces. The biological processes involved have not been studied.

The sulfate reducing bacteria are equally effective as a source of pitting of aluminum in seawater (Tiller and Booth, 1968). Cathodic behavior of aluminum in the presence of sulfate reducing bacteria is identical to that for steel. The corrosion rates are similar to steel and appear to be the result of cathodic depolarization.

Steels and copper alloys are particularly sensitive to corrosion in the presence of anaerobic polluted seawater. Stagnant nutrient rich water in pipes, or metals in contact with polluted waters present a significant hazard (Efird and Lee, 1979). Corrosion under these conditions has been associated with anaerobic microorganisms and with the evolution of hydrogen sulfide. Exposure to fresh seawater under turbulent conditions stimulates corrosion initiated under anaerobic conditions. The degree of corrosion acceleration is dependent on the length of exposure to anaerobic condition prior to re-exposure to aerobic conditions. This observation, which is common in studies of anaerobic corrosion, remains unexplained. It appears that the anaerobic bacteria initiate a corrosion process which continues at a more rapid rate when the metal is re-exposed to aerobic conditions.

The mechanism of anaerobic corrosion has been studied for many years and will not be described in detail in this report. Postgate (1980) has described this process in detail in his recent book. The distinctive feature of corrosion by these bacteria is the cathodic depolarization by Desulfovibrio or other related bacteria. These bacteria apparently utilize the hydrogen produced at the cathode for sulfate reduction. The enzyme hydrogenase may be involved, although some workers argue that non-hydrogenase producing Desulfovibrio strains stimulate corrosion. It has been suggested that ferrous sulfides are responsible for anaerobic corrosion.

We intend to compare the corrosive effects of known Desulfovibrio strains which are positive or negative for hydrogenase production. In addition, studies are required in which the direct effect of ferrous sulfide is investigated. The common observation that corrosion is accelerated following re-aeration of metals also needs investigation. No hypothesis has been presented in the literature. The theories suggested to explain anaerobic corrosion i.e., cathodic depolarization and the production of ferrous sulfide have been summarized in a recent paper (Crombie et al., 1980).

These biochemical processes are controlled by the environment to which the microorganisms are exposed. Conditions at the metal/water interface are critical. The presence of water in oil or in atmospheric condensates is of enormous importance. The chemical structure and concentrations of sulfur compounds and organic materials necessary for the growth of bacteria almost certainly control the form of corrosion developing on the metal surface. All of these factors need more extensive research.

Iverson (1974) reported that mercaptans exert an important corrosive action on iron. These reduced sulfur compounds are common on metal surfaces beneath biofilms, in marine sediments and in polluted seawater. It is probable that the mechanism of attack is similar to hydrogen sulfide. Sulfur-containing amino acid can be expected to behave in a similar manner to mercaptans. The importance of these compounds in the anaerobic corrosion process requires investigation.

### C. Hydrogen Embrittlement

Stress corrosion cracking is a result of the combined effects of corrosion and tensile stress (LaQue, 1975). This form of cracking frequently involves hydrogen embrittlement as part of the cracking mechanism. It is particularly

important in stainless steels. The role of microorganisms in this corrosion process is not understood. However, since the production of atomic hydrogen is identified as being a major factor in hydrogen embrittlement it can be assumed that microbial transformations involving the production of atomic hydrogen would be resevant.

Stainless steels seem to be particularly susceptible to hydrogen embrittlement in seawater flowing at low velocities (Werchniak and Gudas, 1981). The same alloys were immune to stress corrosion at high velocities. The mechanism is not understood. However, at low velocities microbial activity would be expected to be much greater, and biologically produced hydrogen embrittlement should occur.

It has been suggested that hydrogen embrittlement results from the formation of atomic hydrogen from molecular hydrogen at defect points in the metal. The hydrogen stimulates the formation of metal plasticity resulting in turn in the entry of additional hydrogen into the critical cracking regions. Experiments using hydrogen gas have shown that both low grade and high grade alloy steels are susceptible to this form of corrosion cracking (Chu et al., 1980).

We have begun to study the role of microorganisms in hydrogen embrittlement. A wide range of bacteria produce hydrogen gas as a normal metabolic product (Stanier, 1974). Under anaerobic conditions organic acids typically yield formic acid as a metabolic product. Many bacteria possess the enzyme formic hydrogenlyase which splits formic acid to carbon dioxide and hydrogen. In the obligately anaerobic group of bacteria, the clostridia, hydrogen is formed as a direct product of pyruvic acid cleavage. The environmental conditions necessary for the production of hydrogen at a seawater/metal interface needs to be investigated.

The importance of sulfate reducing bacteria in the generation of hydrogen also needs investigation. Chu and his co-workers showed that hydrogen sulfide is an important factor in stress corrosion cracking. They postulate that the hydrogen sulfide reacts with ferrous iron to yield ferrous sulfide and molecular hydrogen. The absorbed molecular hydrogen dissociates into atomic hydrogen, causing embrittlement. Presumably under natural conditions in anaerobic seawater the sulfate reducing bacteria would produce hydrogen sulfide and the reducing conditions would yield ferrous iron. One would expect that the resulting conditions would yield serious problems of embrittlement of stainless steels. Stress corrosion cracking occurs actively in the presence of anaerobic conditions (F. LaQue, personal communication). The literature on this subject is contradictory. A controlled series of experiments using known sulfate reducing bacteria combined with the measurement of molecular and atomic hydrogen would help to clarify the many questions that remain unanswered. It is our intention during the next year to initiate these experiments.

#### D. Iron and Manganese Bacteria

In well aerated seawater the corrosion of alloys containing iron and manganese is common. Ferric and manganic chlorides are an important source of corrosion. The presence of microbial films on surfaces provides the potential for oxidation of iron and manganese by iron and manganese bacteria. A number of iron bacteria have been associated with corrosion processes (Lutey, 1980). The stalked bacteria, Gallionella, Siderocapsa and Sphaerotilus have been associated with oxidative corrosion. These bacteria are responsible for a tubercles seen on steel surfaces. Frequently sulfate reducing bacteria predominate inside the tubercles, where oxygen transfer is inhibited. Other organisms which may be involved in the corrosion of iron include Thiobacillus

ferrooxidans, which obtains its energy from the oxidation of ferrous irons. The role of these bacteria in corrosion is unknown (Iverson, 1974). No information is available about the microorganisms involved in manganese oxidation.

In recent years the study of stalked and filamentous metal oxidizing bacteria has progressed significantly. New organisms have been isolated which are found in the sea and are capable of oxidizing either manganese or iron. These include the genera Caulobacter, Hyphomicrobium, and Metallogenium. Studies of manganese nodules on the ocean floor have yielded a complete family of manganese bacteria capable of oxidizing manganous to manganese dioxide (Ehrlich, 1981). Fungi may also be involved in the formation of organic iron and manganese complexes. No attempt has been made to determine the function of these unusual microorganisms in corrosion processes. We intend to isolate pure cultures of these microorganisms from iron and manganese containing alloys in seawater and to determine their significance. Kinetic studies will be undertaken with specific microorganisms, and an attempt will be made to determine the function of specific oxidative forms of iron and manganese in the corrosion process.

#### E. Acid Production

The production of organic and inorganic acids by microorganisms is a major source of corrosive. Sulfuric acid is produced by the thiobacilli in the oxidation of reduced sulfur compounds. Rapid corrosion in the presence of these bacteria has been reported (Starkey, 1966). In seawater the thiobacilli usually are found in association with sulfate reducing bacteria. When the reduced sulfides are exposed to oxygen they are re-oxidized to yield sulfuric acid.

Most heterotrophic bacteria yield organic acids during the fermentation of

organic substrates. The corrosion of steel piping has been associated with this process. Organic acids from a wide range of gram negative bacteria have been shown to cause extensive corrosion of steel (Ehlert, 1967).

There is little information available about the relationship between specific marine microorganisms or their acid production and characteristic forms of corrosion. No studies have been performed to determine the relationship between acid metabolism of microorganisms and corrosion processes. Nor is there any knowledge about the effect of environmental conditions on acid induced corrosion. We have begun a series of experiments to answer some of these questions, using both natural marine microbial populations and pure cultures of marine microorganisms, including fungi, on a variety of different metal alloys.

#### F. Methods

Standard electrochemical methods for the study of corrosion are available. It is not our intension in this study to attempt to modify these procedures. However, in recent years new methods have become available for the study of microbial processes on surfaces. These biochemical techniques would be well suited to our study of corrosion. Biochemical methods are now available to quantatively and qualitatively assess the kinetics of microbial growth on metal surfaces (Burke et al., 1981). Biomass can be estimated from extractable lipid phosphate, and total extractable palmitic acid. All microbial cells contain membranes in which phospholipids are essential components. Measurement of the total extractable lipid phosphate provides a quantitative assay to estimate the microfouling film biomass. Palmitic acid is found in all lipids, phospholipids in membranes, and fats; thus, it is a measure of all fats. The eucaryotes (i.e., protozoa, algae and fungi) contain fats while the bacteria do not.

Since all microbes contain phospholipids the ratio of lipid phosphate to palmitic acid can provide an estimate of the bacteria to eucaryotes. The eucaryotes but not the bacteria also contain polyenoic fatty acids, that is, fatty acids with three or more double bonds. Examination of the types and proportions of fatty acids allows comparison between components making up the microbial community that is independent of the total biomass.

It should be possible to make use of these techniques to analyze biochemically the development of specific microfloras on individual alloys under different environmental conditions. This would enable us to determine accurately the relationship between microbial processes and corrosion reactions. For example, it would be possible for us using these methods, with the aid of high pressure liquid chromatography, to differentiate between types of microfloras on submerged metallic surface. We would be capable of separating sulfate reducing microorganisms from hydrogen producing microorganisms. Specific products produced by iron and manganese bacteria would enable us to separate them from other microorganisms in the biofilms. We have begun to make use of these techniques and to determine their significance in the study of microbially induced corrosion.

#### IV. WORK COMPLETED IN OUR LABORATORY 1981-82

##### A. Marine Bacterial Biofilm Formation on Corroding Metals

Research over the past decade concerning marine biofilm development has dealt generally 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 (e.g. 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. Yet, little information exists on the specific identity of microorganisms beyond the level of "bacteria", "yeasts", "diatoms", etc. A few efforts have been made to identify the microorganisms involved in the formation of a natural biofilm (Corpe, 1972), although most of these studies have depended on old techniques that allow only a portion of the microbial population to be analyzed.

Data are scarce concerning the quantitative behavior of specific film-forming bacteria in response to metals of known composition. Studies in this area have generally been qualitative, and, often, have utilized artificial seawater as the "defined" test medium. The assumption that marine bacteria require only sodium chloride and perhaps a few other salts is false. Recent evidence suggests strongly that results from experiments on marine biological corrosion differ when the analyses are conducted in natural seawater.

In the first phase of the research initiated during the past year, we have begun to quantify the attachment of specific marine bacteria to metals and alloys of known composition. The intent of these experiments is 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.

During the past year we have developed and modified techniques to observe and quantify the attachment of bacteria to metals. We have constructed a series of test chambers that allow multiple replicate metal samples to be submerged collectively in various solutions. The apparatus allows all specimens in a series to be moved in unison from one test fluid to another, thus allowing direct comparison between samples. This simple apparatus is made by fixing plastic coated wires to the inner surface of a standard glass petri dish top. The wires have hook-shaped bottoms, and each can hold an individual metal sample (1 x 2 cm, with 2 mm hole at one end). The replicate metal samples are submerged by placing the cover on a standard glass storage dish (Corning) which contains a particular test solution. The cover, to which all replicate samples are attached, can be moved easily and aseptically from one storage dish to another to alter test conditions and test solutions. The entire apparatus is autoclavable.

The final development of the test apparatus described above required a significant effort, in order to yield a simple device that would allow: 1. The handling of directly comparable, multiple replicates; 2. Easy removal of samples during time-series experiments; 3. Sterilization of the chamber and samples; and, 4. The easy movement of samples from one fluid to another in unison. The test chambers have been in use for several months now, and we have begun to collect baseline data on the rate of attachment of specific bacteria to known metals.

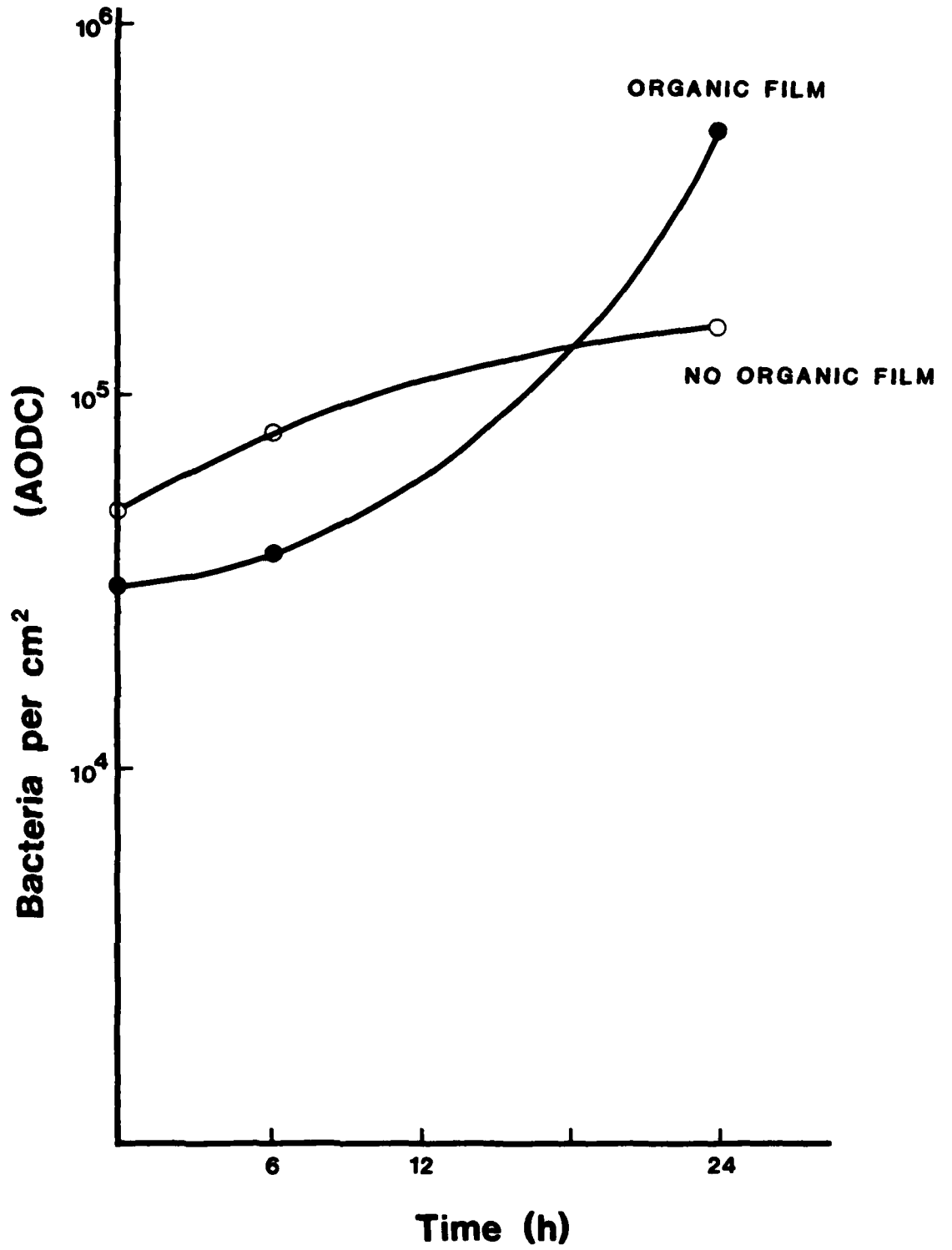
Our initial experiments have been directed at comparing the attachment of known marine bacteria to metals that either have, or do not have,

surface-associated organic matter. The preliminary results of these experiments are very interesting. Metal samples are prepared for the experiments by cleaning with detergent, and rinsing with water and alcohol. They are then pickled, rinsed and sterilized. One half of the stainless steel (No. 316) samples are immersed for 5 minutes in an organic rich broth containing peptone and yeast extract. These organic-contaminated samples then are transferred to a dish containing a specific bacterial suspension in seawater. The bacterial suspensions approximate normal marine bacterial cell densities (ca.  $10^6$  cells/ml). A parallel set of metal samples are placed directly into an identical dish of the same bacterial suspension without prior exposure to organic matter. Metal specimens are removed from the bacterial suspension following an exposure of 5 minutes; they are dipped gently in a rinse dish containing sterile seawater to remove bacteria that are not attached, and are preserved in formalin. Subsequently, each specimen is stained with acridine orange, and the bacteria attached to the metal surface are counted by epifluorescence microscopy.

It appears that fewer bacteria attach initially to metals which have organic matter associated with the surface than to identical organic-free metal samples. However, the bacteria that do attach to organic-filmed metals appear to multiply more rapidly and reach higher population densities than their counterparts on organic-free metals. The data seem to indicate that bacteria attached to metals which possess a surface-associated organic film quickly shift their metabolism to utilize the organic matter present on the surface. They increase in number rapidly, with growth rates similar to those seen in high nutrient culture broth. Conversely, bacteria that attach to metals with a very low level of surface organic matter rapidly run out of reserve nutrients resulting in a correspondingly dramatic decrease in growth [See Figure 1].

Figure 1: Growth of marine bacteria (a pure culture of Vibrio alginolyticus) attached to the surface of 316 stainless steel. Bacterial enumeration by acridine orange direct count (AODC) using epifluorescence microscopy. Two sets of metal samples were exposed to bacteria at normal estuarine cell density (approx.  $10^6$  cells/ml) for 5 min; they were then rinsed gently to remove unattached bacteria, and were incubated for 24 hr. in filter-sterilized seawater to follow increases in cell number. The first set of samples consisted of cleaned, sterile metal (No Organic Film). The second set was identical, except that there were immersed for 5 min. in a protein-rich broth and then rinsed gently immediately prior to exposure to the bacteria (Organic Film). Bacteria on the organic-filmed metals increased in numbers at a logarithmically increasing rate, while those attached to metals not filmed with organic matter showed a clear decrease in doubling rate as they ran out of nutrients.

316 STAINLESS STEEL



We are currently conducting experiments to monitor bacterial growth on metal surfaces, once the bacteria have attached. Bacterial growth is being monitored initially by following increases in cell numbers on the metal surfaces during incubations in filter-sterilized seawater; increases in bacterial cell numbers result from cell multiplication and not from new recruitment in this system. In these studies we are monitoring increases in cell numbers using epifluorescence microscopy and scanning electron microscopy. Figure 2 shows the development of marine bacterial microcolonies of Vibrio alginolyticus on 316-stainless steel surfaces over a 24 hour incubation period. We are conducting similar experiments presently on 316 stainless steel, 90:10 copper nickel; aluminum bronze; titanium and aluminum using a range of bacteria that we have isolated from marine habitats and identified for use in these studies. Field experiments will be initiated during summer 1982.

#### B. Microbial Production of Organic Acids on Metal Surfaces in the Sea

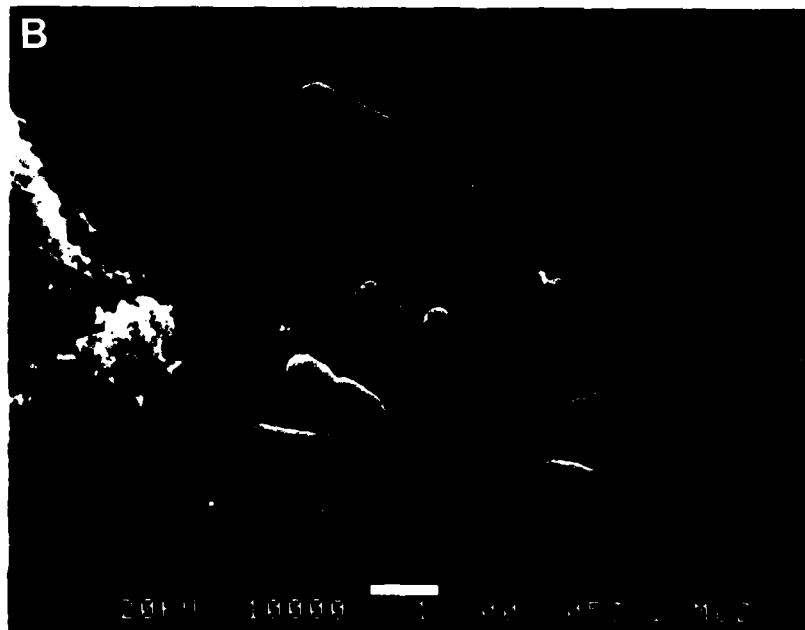
Many microorganisms ferment organic substrates under anaerobic conditions with the subsequent release of organic acids. In many of these fermentations the resulting metabolites are a mixture of organic acids, consisting most commonly of formic, acetic, propionic, lactic and butyric acids.

Organic acids are corrosive to iron-containing metals. They are produced by a variety of bacteria and fungi, and by algae as well. The types and amounts of organic acids produced during anaerobic microbial metabolism depends on the organisms that are present and on the substrate molecules that are available.

Methanogenic bacteria can utilize organic acids as an energy source for growth. At first consideration, then, methanogenic bacteria might appear to lower the concentration of acidic corrosives on the metal surface; however,

Figure 2: Growth of marine bacteria attached to 316 stainless steel (samples were fixed in 2.5% glutaraldehyde with 0.1 M sodium cacodylate in 0.22 micron filtered seawater; they were then dehydrated in acetone, critical point dried and coated with gold-palladium for viewing under the scanning electron microscope).

- A) Marine bacteria (Vibrio alginolyticus) attached to 316 stainless steel after 5 min. immersion of the metal in a bacterial suspension at normal estuarine cell density (approx.  $10^6$  bacteria/ml).
- B) Microcolony of marine bacteria on the surface of 316 stainless steel following a 24 hr. incubation (in bacteria-free seawater) of a replicate of the sample shown in A above.



methanogens reduce carbon dioxide and they may initiate cathodic depolarization by disrupting the film of hydrogen present at the metal surface.

Organic acid production may play an important role in the corrosion of metals submerged in the sea. In addition, organic acids produced by fermentative members of a microbial corrosion community may be utilized, subsequent to their release, as energy sources by other members of the community.

Our laboratory acquired a Varian High Pressure Liquid Chromatograph (HPLC) recently, and we have been involved during the past few months in adapting and modifying techniques for the detection and quantitation of organic acids produced by microorganisms in marine microhabitats. The measurement of organic acids in seawater presents problems due mainly to the high salt concentrations and the volatility of the short chain organic acids of interest. In addition, our intent to measure these acids in very small samples (ca. 100-1000 microliters) added another level of difficulty to the development of appropriate techniques. We wish to sample the very small microhabitats, crack and crevice corrosion sites, where the fermentative production of organic acids most probably occurs.

During the past three months we have been evaluating several analytical methods for measuring organic acids by HPLC techniques (Barcelona et al., 1980; Bush et al., 1979; Durst, 1974; Durst et al., 1975; Jordi, 1978). Most of the significant technical problems have been solved and we are now ready to begin quantifying organic acid production by specific marine bacteria growing in association with known metal surfaces. A test chamber has been designed to allow the simultaneous measurement of dissolved oxygen concentration, bacterial cell numbers, and hydrogen ion activity within experimental micro-scale, marine crevice corrosion sites. In addition, corrosion rates will be monitored using

the potentiostat which we obtained recently (See Hydrogen Embrittlement Section).

C. The Role of Microorganisms in Marine Hydrogen Embrittlement Processes

Hydrogen embrittlement is mechanical damage to, or 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. Sources of hydrogen which cause such failures can be hydrogen gas ( $H_2$ ), if dissociation into atomic hydrogen ( $H^0$ ) occurs, or electrolytic hydrogen, if recombination of  $H^0$  into  $H_2$  is prevented (as by sulfides or other hydrogen evolution poisons). The latter may result from corrosion reactions, pretreatment processes such as pickling, and plating processes, etc. In many cases, the critical hydrogen concentration which can lead to the catastrophic failure of sensitive materials is fairly low.

The importance of hydrogen-induced failure of metals has been widely recognized. The role of bacteria in this corrosion process is not understood. We have hypothesized several mechanisms by which microorganisms could be involved in the hydrogen embrittlement of metals:

(1) Since hydrogen production is a major factor in embrittlement it can be assumed that microbial transformations involving the production of atomic hydrogen might be relevant. A wide range of bacteria produce molecular hydrogen as a normal metabolic product. In the presence of microbially produced  $H_2$ , some noble metals (hydrogen dissociation catalysts) that tend to segregate within the grain boundaries of stainless steels may stimulate  $H^0$  absorption and subsequent cracking of the metal (Latanision et al., 1977). Alternatively, reduction of microbially produced  $H^+$  (from organic or mineral acids) may be coupled to metal oxidation, resulting in the formation of atomic

hydrogen. Many different bacteria can also consume hydrogen during the fixation of  $\text{CO}_2$ . These organisms may suppress hydrogen absorption by recycling the hydrogen within the microbial community before it is absorbed into the metal. Whether atomic hydrogen itself is an intermediate in any of these metabolic pathways of microorganisms remains unclear.

(2) Sulfides are known to be effective poisons of the hydrogen evolution reaction



Thus,  $\text{H}_2\text{S}$  production by bacteria may stimulate the absorption of  $\text{H}^{\circ}$  into metals by preventing its recombination into  $\text{H}_2$ . The presence of  $\text{H}_2\text{S}$  in aqueous environments does, in fact, limit the use of hardened, high-strength steels in oil and gas equipment. Embrittlement and failure of offshore oil platforms frequently occurs at the sediment-water interface, where  $\text{H}_2\text{S}$  production by sulfate-reducing bacteria is greatest.

(3) Metal oxide films have been shown either to suppress or to stimulate atomic hydrogen absorption, depending upon a variety of conditions. Bacterial destabilization of these films, then, may be expected to influence hydrogen embrittlement.

During 1981-1982, we have has been involved in the development of methods for investigating microbial involvement in hydrogen embrittlement of stainless steels in seawater. The following has been accomplished:

(1) A laboratory potentiostat/galvanostat has been purchased from AIS, Division of Floyd Bell Associates, Inc. (Columbus, OH). This instrument will be used to measure corrosion rates in all of our experiments and will also become part of a hydrogen-permeation measurement system which is now being developed (see next paragraph). The AIS potentiostat is equipped with a built-in logarithmic converter and digital meters for more accurate reading of

output values. To complete our corrosion measurement system we intend to purchase an X-Y recorder from another company and to have a corrosion cell custom-made to our specifications. Although complete, prepackaged corrosion measurement systems are available from companies such as Princeton Applied Research Co. (Princeton, NJ), the equipment that we are purchasing will give us a system tailored more to our experimental requirements at nearly half the cost.

(2) We intend to measure the production of atomic and molecular hydrogen by bacteria at metal/liquid interfaces in seawater. We now are developing a modification of a sensitive electrochemical technique used to measure diffusion coefficients and permeation rates of hydrogen through metallic membranes (Devanathan et al., 1963). This method is being developed in cooperation with Prof. Ronald Latanision of the MIT corrosion laboratory. Our modification of this technique should allow us to compare the amounts of hydrogen produced by films of various individual strains and consortia of bacteria. The main advantage of this method is that it will tell us how much hydrogen accumulates at the immediate surface of the metal; this is important because only the hydrogen that is actually adsorbed to the metal surface can initiate embrittlement. We expect to have this device working to begin experimentation with bacterial cultures this spring.

(3) We are examining various types of devices which can be used to measure the hydrogen content of different metals. Such a device is necessary to perform laboratory and field tests of the effect of microbial films on the amount of hydrogen absorbed by various materials. Systems being considered include hydrogen determinators such as those marketed by the Leco Corporation (St. Joseph, MI) and a "barnacle electrode" device, which consists of a cell with magnetic attachment to steel surfaces coupled to an electronic measurement

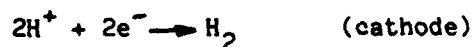
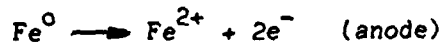
system (Mansfield et al., 1982). We have not yet determined which type of system would be most appropriate for our use.

(4) Much of our research on hydrogen embrittlement will involve the use of anaerobic bacteria known to produce and/or consume hydrogen. We have begun to acquire cultures of bacteria from the national type culture collection in Germany, which offers the most complete selection of such strains. We also intend to sample natural anaerobic environments for such bacteria, and to this end we have spent the past several months learning and refining techniques for isolating and maintaining pure cultures of anaerobic bacteria. An anaerobic culture chamber purchased this year now gives us the ability to grow and work with all of these microorganisms.

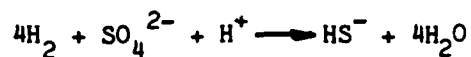
#### D. The Role of Anaerobic Bacteria in Marine Corrosion Processes

The most popular theory of microbially mediated anaerobic iron corrosion is that of cathodic depolarization. The theory suggests that the process is enhanced by the removal of hydrogen by hydrogen-oxidizing, sulfate-reducing bacteria, such as Desulfovibrio species.

Metal surface:



Sulfate reducing bacteria:

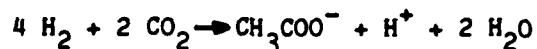


Overall process:



Other theories propose roles for sulfide, acids, and other bacterial products in controlling the rates of marine corrosion processes. For example, Iverson (1981) proposes that corrosion is actually caused by a highly corrosive extracellular product rather than by the removal of hydrogen.

Recent advances in techniques for culturing strictly anaerobic bacteria will allow us to study under carefully controlled conditions their role in marine corrosion processes. The different theoretical mechanisms that have been proposed to explain anaerobic corrosion can be tested individually by utilizing bacteria that mediate specific biochemical transformations. For example:



If the removal of molecular hydrogen is the key factor in the oxidation of

iron, exposure of the metal to bacteria carrying out the reactions shown above should yield rates of corrosion that are comparable to the ones observed in the presence of sulfate reducers.

In anaerobic freshwater habitats, methane producing bacteria play a much more important role in removing hydrogen than sulfate reducers. We are studying the role of methanogens in anaerobic corrosion. Our experiments in this area are directed at uncovering the main mechanism by which iron-containing metals are oxidized in anaerobic environments. We are utilizing pure cultures of specific anaerobic bacteria as biochemical tools to explain particular transformations in the corrosion process. We intend to assess the involvement of anaerobic bacteria other than sulfate-reducers in these corrosion processes, methanogens, for example. The results of these experiments should allow us to determine the overall importance of the hydrogenase enzymes in anaerobic marine corrosion processes.

We acquired an anaerobic chamber (Coy Laboratory Products Inc.) in February 1982. The chamber is a glove box type incubator, with an air-lock entry, which allows maintenance of an internal anaerobic atmosphere with less than 1 ppm  $O_2$ . The chamber has been installed and run through the initial start-up phase to develop the appropriate working atmosphere. The chamber functions with continual re-circulation of the internal atmosphere through palladium catalyst packs to remove residual oxygen constantly. We are now initiating the first of our experiments with pure cultures of anaerobes.

A Hewlett Packard gas chromatograph, with a flame ionization detector in our laboratory has been re-fitted to analyze for microbial metabolic products such as alcohols and volatile fatty acids. A gassing system has been designed and constructed to allow control of the atmosphere composition in the anaerobe culture vessels. During the next two months we expect to gain access to a gas

chromatograph fitted with a thermal conductivity detector which we will use to measure hydrogen and methane in the experiments described above. We are in the process of upgrading and re-fitting a Perkin-Elmer atomic absorption spectrophotometer to allow the measurement of various dissolved metal species.

We intend to obtain pure cultures of newly isolated anaerobic bacteria from the German Collection of Microorganisms. Additional anaerobes will be isolated from natural anaerobic habitats. We will begin our experiments with these organisms at the end of May.

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