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Marine Microbiology

THE MICROBIOLOGICAL CORROSION OF IRON

Office of Naval Research

"Contract Nonr-375 (10)

Project NR 103-433"

Institute of Marine Science

University of Texas

Port Aransas, Texas

Annual Progress Report
January 1, 1960 to December 1, 1960

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Contents

A. Objectives

To study the properties of bacteria which are pertinent to the microbiological corrosion of iron in natural marine environments: (1) bacterial consumption of oxygen and subsequent production of oxygen differential corrosion cells, (2) the production of corrosive acids, and (3) microbial hydrogenase enzyme activity and its effect on the depolarization of metallic iron surfaces.

B. Summary of Results Since the Start of the Project

Bacterial Corrosion

It has been shown that enrichment cultures of marine sulfate-reducing bacteria and a pure culture of a hydrogenase positive Pseudomonas can actively increase the rate at which iron is removed from the metallic state. Cell-free extract from sonicated Pseudomonas cells is apparently as corrosive as the intact growing cells, suggesting an enzyme process. The parallel reduction of methyl viologen suggests hydrogenase activity. Corrosion caused by hydrogen sulfide is considerably less than that caused by the bacterial contact. The iron sulfide produced from the activities of sulfate-reducing bacteria is found to occur in a diffuse state surrounding test coupons and apparently does not protect the metal as suggested in the past. Current flow through iron in oxygen differential cells produced by bacteria has been measured.

Distribution of Organic Matter and Oxygen Consumption

Organic matter is not evenly distributed laterally or horizontally over the surface sediments in the shallow marine bays studied. The

organic matter may vary from 0.03 to 4.2 percent carbon in the various environments. Organic matter is deposited in layers during sedimentation processes with the result that abrupt differences in concentration are found. The organic matter is available for bacterial activity which indicates that active oxygen differential corrosion of iron may exist in sediments.

C. Corrosion Experiments

Procedures

All corrosion tests were conducted with two types of bacteria: a purified culture of a marine sulfate-reducing bacterium which has been found to be corrosive, and a pure culture of a hydroganase-positive marine Pseudomonas provided by Dr. F. D. Sisler of the United States Geological Survey.

The iron coupons (approximately 6 x 65 x 1 mm with a small hole at one end) used in weight loss experiments were cut from one piece of cold rolled sheet iron. A thin piece (50 mm long) of the same iron was attached to the coupon by means of the hole. The purpose of the second iron piece was to provide a lead to the medium-gas interface and thus provide conditions for the establishment of a cathode. The coupon can thus act as an anode and weight loss can be measured. When a piece of metal is completely covered by the medium, a corrosion cell may only be produced where a differential is produced along the iron.

The coupons were cleaned with a mild abrasive to prepare the coupons for use and to remove the corrosion products. This procedure produced a clean metal surface with no appreciable weight loss.

Weight measurements to 0.0001 grams were made. Peptone broth medium contained 0.5 percent peptone in 75 percent aged sea water at a pH of approximately 7.6.

Oxygen Differential Corrosion Cell

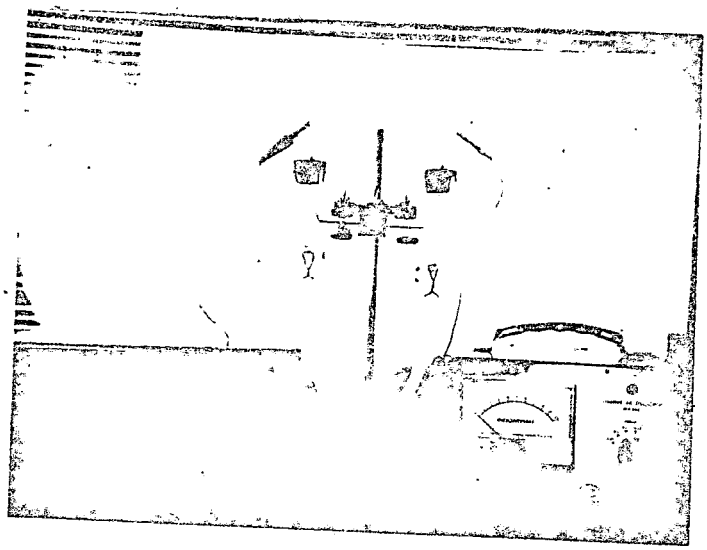
A corrosion cell is being developed which may permit the study of the production and variations of current through iron coupons resulting from bacterial activity. The cell shown in Figure 1 is coupled with an ultrasensitive microammeter and single stage transistor amplifier. The cell consists of two separate glass chambers between which an iron coupon extends. The chambers are connected by a salt bridge through a plastic tube. Sea water peptone medium is placed in the chambers and one side is used as a sterile control while the other is inoculated with a culture of sulfate-reducing or any other bacteria. A current develops (Figure 1) as a result of bacterial activity and the reducing effect of hydrogen sulfide produced by the bacteria. It was also observed that a current develops when both cells are made anaerobic and one side inoculated.

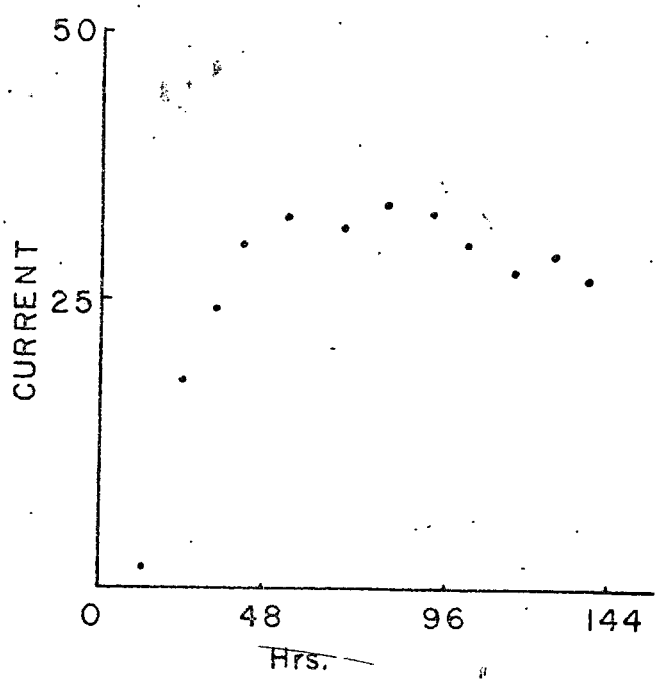
No further work has been performed with the apparatus because of its temperature sensitivity and lack of facilities for its stabilization. The procedure may have some value as a screening test for the ability of some corrosive waters to produce corrosion at an air interface. The usual half-cell arrangement has been avoided because the potential applied to the system may have some significant influence on the bacterial activities; however, no data have been accumulated to prove the point.

Hydrogen Sulfide Corrosion

A series of tests were conducted to compare hydrogen sulfide

Figure 1. Apparatus used to measure current flow by oxygen concentration gradient produced by a sulfate-reducing bacterium. Current flow in arbitrary units (less than μA).




50
25
CURRENT
0
48
96
144
Hrs.

corrosion with direct contact corrosion caused by hydrogen sulfide-producing cultures of bacteria. Each experiment was conducted in three tubes. Tube 1 contained 20 ml of iron-free inoculated peptone broth medium into which a smaller tube with a fritted glass (ultra-fine) filter was placed with the top of the smaller tube extending out of the medium. A clean, sterile test coupon with a lead was placed in the inside tube. Tube 2 contained twenty ml of iron-free peptone broth medium and a test coupon with a lead. Tube 3 was prepared identically to tube 2 except that it was uninoculated. Tubes 1 and 2 were inoculated with 0.1 ml of stock culture of the sulfate-reducing bacteria and all tubes were flushed with sterile nitrogen and sealed. The tubes were incubated at 21 C for 21 days allowing the hydrogen sulfide to come to equilibrium in tube 1. The test coupons were removed, cleaned, dried, and weighed. The weight losses compared from six tests were averaged as shown in Figure 2.

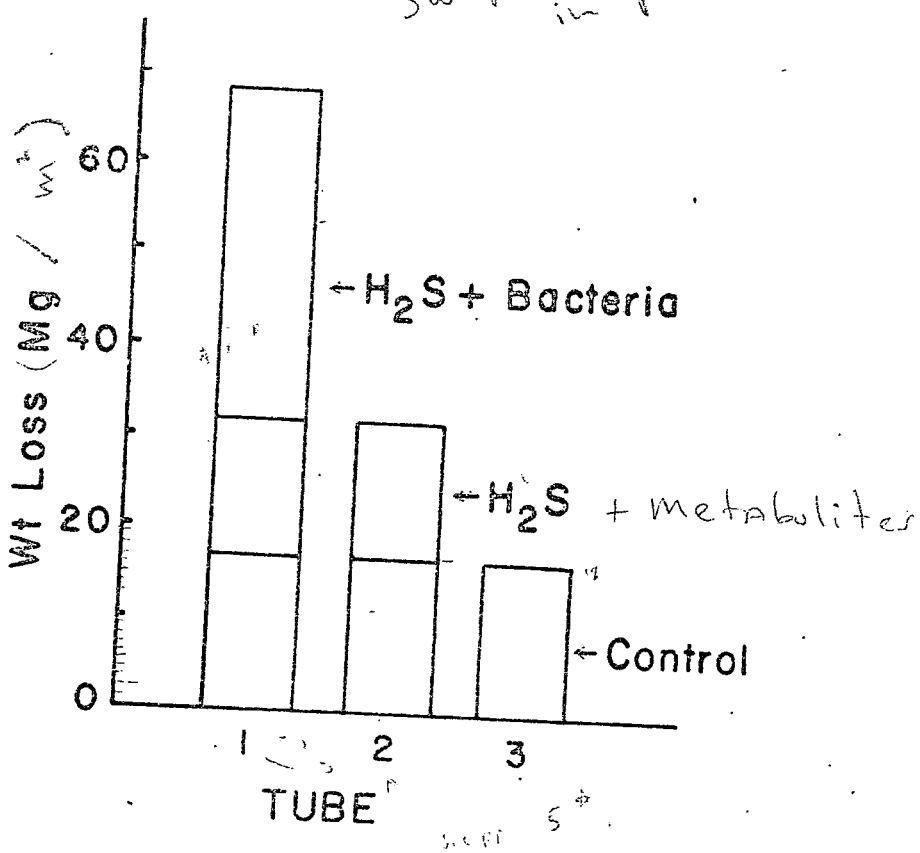
When the correction is made for the sterile controls (tube 3) the ratio becomes 14.5 mg in tube 1 to 36.6 mg in tube 2. The corrosion in tube 2 was 60.4 percent more than in tube 1.

The results with this sulfate-reducing culture indicate that hydrogen sulfide corrosion is not as great as direct contact corrosion in the presence of bacteria.

It is evident that some mechanism other than hydrogen sulfide corrosion is responsible for the major part of the corrosion. The most probable explanation for the increased "contact-corrosion" is the removal of hydrogen from localized areas on the iron surface by

Figure 2. Weight loss of iron coupons by sulfate-reducing bacteria: Tube 1 Direct contact between bacteria and coupon, Tube 2 Coupon separated from bacteria but not H_2S by ultrafine glass filter, Tube 3 Control no bacteria.

Swap 1 and 2 Tube graph in position



the bacteria thus producing galvanic microcells. The hydrogen sulfide would thus act as an acceptor for the iron removed by galvanic corrosion and would become cathodic to the corrosion site.

The formation of FeS nodules and underlying pits shown in Figure 3 are characteristic of the type of corrosion caused by the sulfate-reducing bacterium. One nodule was removed to show the underlying pit.

Anaerobic Diffusion Chamber

Experiments were continued in the study of the diffusion of iron from a test coupon in an anaerobic diffusion chamber. The apparatus consisted of a chamber formed by two lucite plates separated by a 1/4" lucite divider. The chamber was sterilized with 50% EtOH for one hour. Iron free semisolid sea water peptone agar was placed in the chamber directly from the autoclave and a layer of oil was floated over it to prevent oxygen uptake. Qualitative data on the actions of corrosion bacteria can be visually obtained. Figure 4A, similar to that given in the January, 1959-60 Progress Report, shows the action of hydrogen-sulfide producing bacteria when the inoculation is made directly against the iron test coupon. Blackening occurs against the coupon and then spreads slowly by diffusion.

When the inoculation site was moved 4 cm away from the coupon (Figure 4B), the blackening appeared to result from diffusible products of the bacterial metabolism. The medium at the inoculum site was turbid whereas the turbidity did not spread to the coupon area. A broad, even area of blackening appeared around the coupon. The ungraded shade of blackening from the coupon to near the edge of the "halo" indicates a uniform iron concentration. In this way,

Figure 3. Photomicrographs of the pitting action of sulfate-reducing bacteria. Pits and nodules are approximately 1/2 mm in diameter.

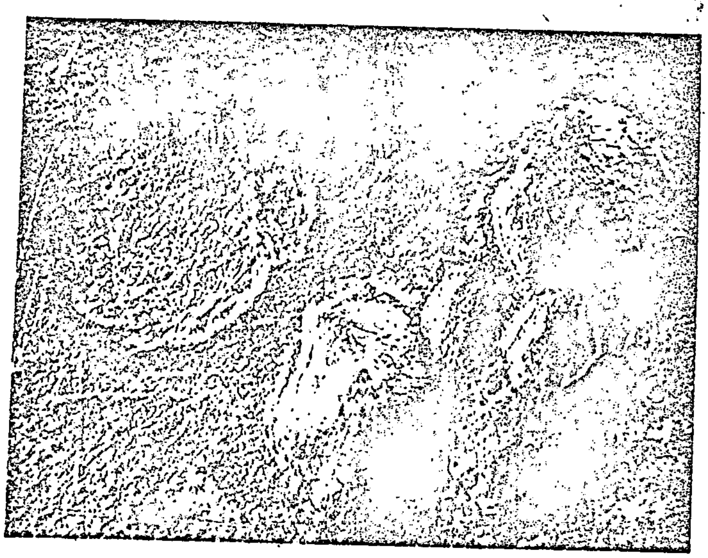


Figure 4. Diffusion of iron during corrosion by sulfate-reducing bacteria. A. Inoculum direct on iron surface. B. Inoculum 4 cm from iron surface.

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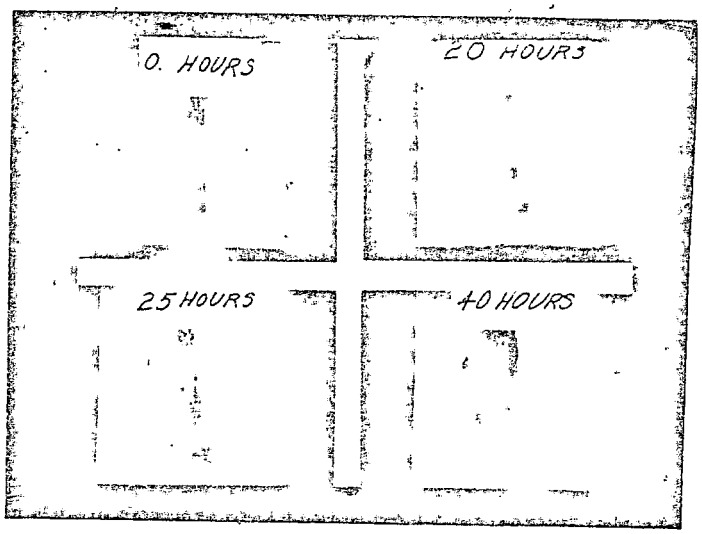
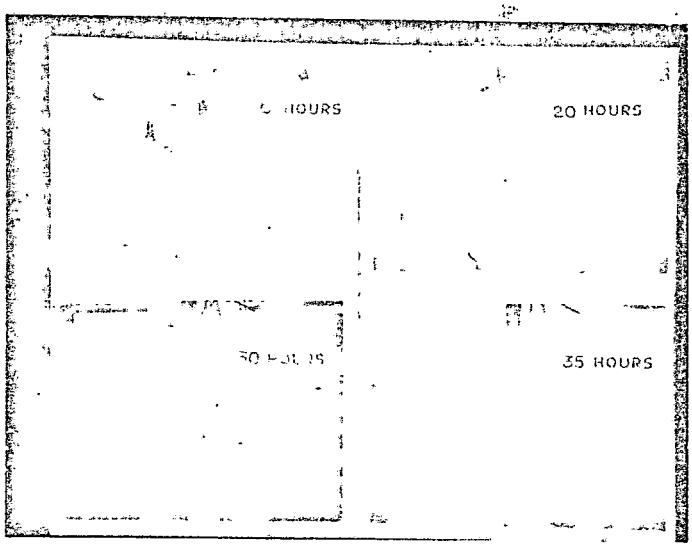




the reaction resembles a saturated reaction where the amount of iron diffusion is slow and perhaps constant.

Bacterial Contact Corrosion

A marine Pseudomonas species with high hydrogenase activity was tested for corrosive ability. Tubes with iron coupons and sea water peptone medium were prepared. The organisms were inoculated in one series and a second series was used as a control. The tubes were incubated in a nitrogen atmosphere in an anaerobic chamber. At time intervals the coupons in three replicas were tested for weight loss. Representative results are given in Figure 5. The data clearly show that the organism is corrosive. No hydrogen sulfide was produced.

The initial corrosion found in the first 12 hours of these tests and previously reported tests with sulfate-reducing bacteria was studied. The manipulation required during the addition of the sterile test coupons and inoculum allowed considerable oxygen to diffuse into the medium where it remained for some time even in the anaerobic chamber. Therefore, it was thought that the initial corrosion was due to oxygen. Precautions were taken to eliminate as much of the oxygen as possible in repeated experiments. Figure 6 gives weight loss data for the initial corrosion in sterile controls already shown in Figure 4 (5?) as compared to weight loss in experiments designed to minimize oxygen content. There appears to be a relationship between residual oxygen content and corrosion.

Corrosion by Cell-Free Preparations of Sonicated Pseudomonas Cells

A culture of Pseudomonas was cultivated in a 10L container, agitated, and inoculated under anaerobic conditions. The cells were

Figure 5. Corrosion measured by weight loss of iron coupons in presence of Pseudomonas species. A. With Bacteria B. Control.

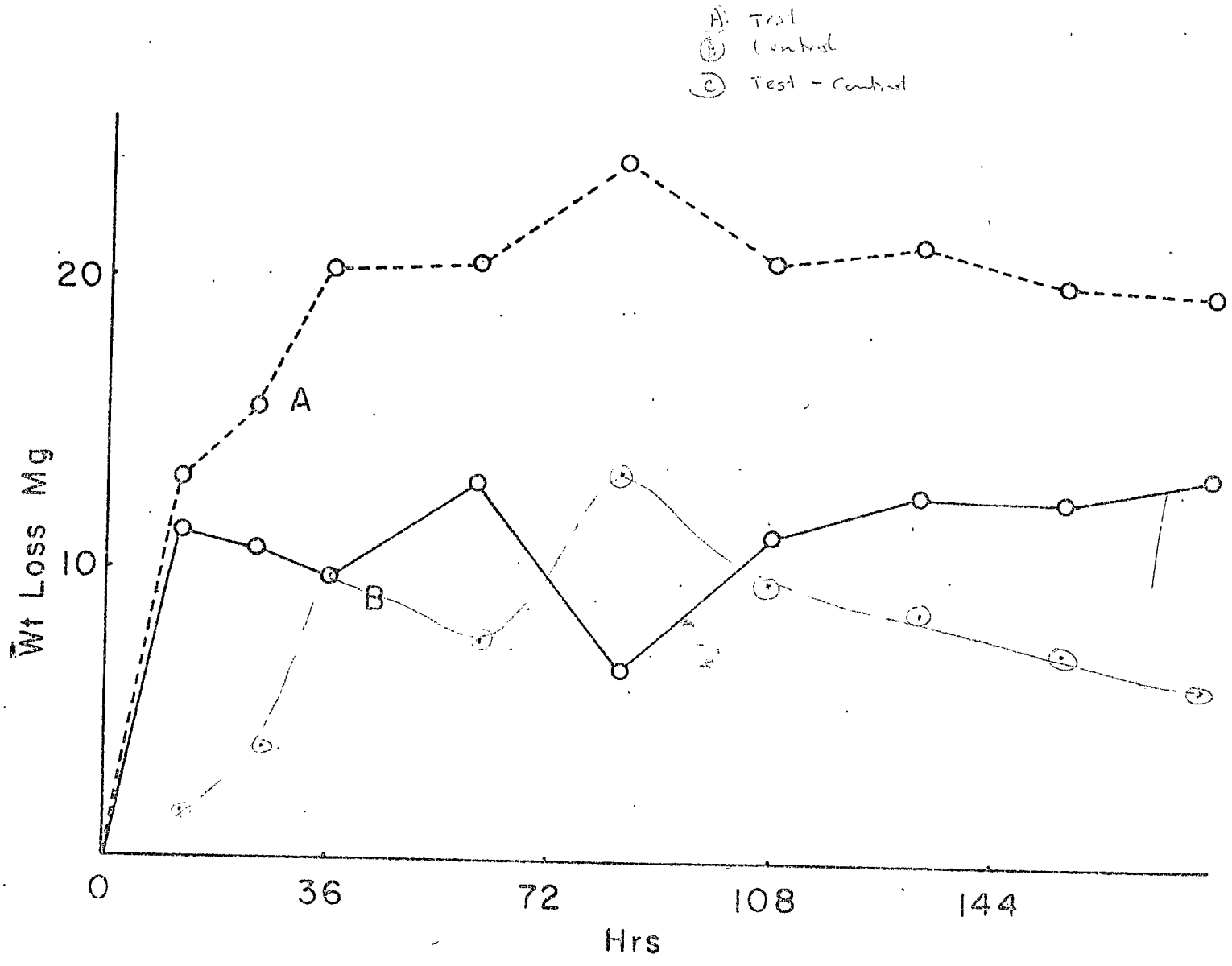
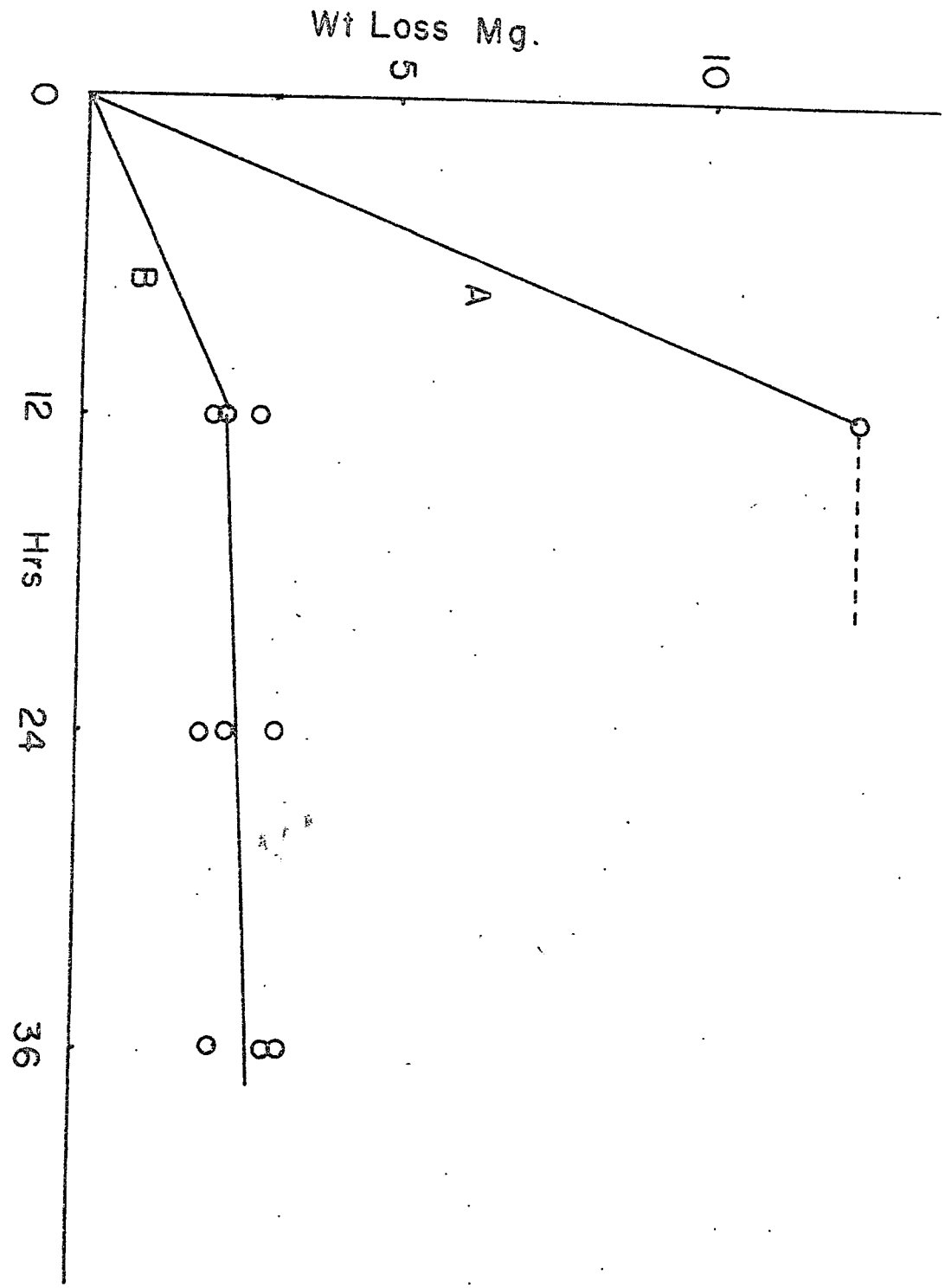

A Test
B Control
C Test - Control
20
10
Wt Loss Mg
A
B
0
36
72
108
144
Hrs

Figure 6. Weight loss of iron. A. Data from Figure 4. B. When extra precautions were taken to remove residual oxygen.



harvested in a Sharples continuous flow centrifuge and resuspended in 100 ml of sterile 50% sea water. The cells were broken up by sonication at 10KC for 30 minutes. The cell material was removed by centrifugation at 10,000 x g for 15 minutes and the supernatent further cleared by centrifugation at 24,000 x g for one hour. The procedure was carried out under refrigeration. Tests showed no viable cells in the final preparation.

Preliminary tests for corrosion were performed by placing concentrated cell extract and iron test coupon in a Thunberg tube. A solution of methyl viologen was placed in the arm of the tube. A second tube containing basic pyrogalol to absorb residual oxygen was connected to the reaction tube and the apparatus flushed with sterile nitrogen.

At the end of 38 days incubation at 20°C the corrosion in five replicates was averaged and compared with controls containing heat inactivated enzyme and no enzyme. The corrosion caused by the cell extract averaged 42.25 mg/cm^2 per coupon more than the controls. The methyl viologen turned blue, indicating hydrogenase activity. The corrosion occurred evenly over the entire coupon without the formation of pits.

After the preliminary experiments showed positive results, dilution tests were conducted. Several tests failed because the enzyme system was evidently inactivated by heat and residual detergents on the glassware and coupons. Figure 7 gives data for the corrosive nature of the cell extracts. The initial high corrosion possibly may

Figure 7. Corrosion measured by weight loss of iron coupons in presence of cell free extract of sonicated Pseudomonas species

OI FSU OI

be explained by a differential concentration of enzyme and cofactor where one is diluted faster than the other. The methyl viologen was reduced in the tubes containing the extract suggesting further that hydrogenase activity was present. These experiments will be continued to test media from cell growth for extracellular activity unsonicated cell extracts and the effect of dialysis on the enzyme in sonicated extracts.

D. Distribution of Organic Matter in Sediments

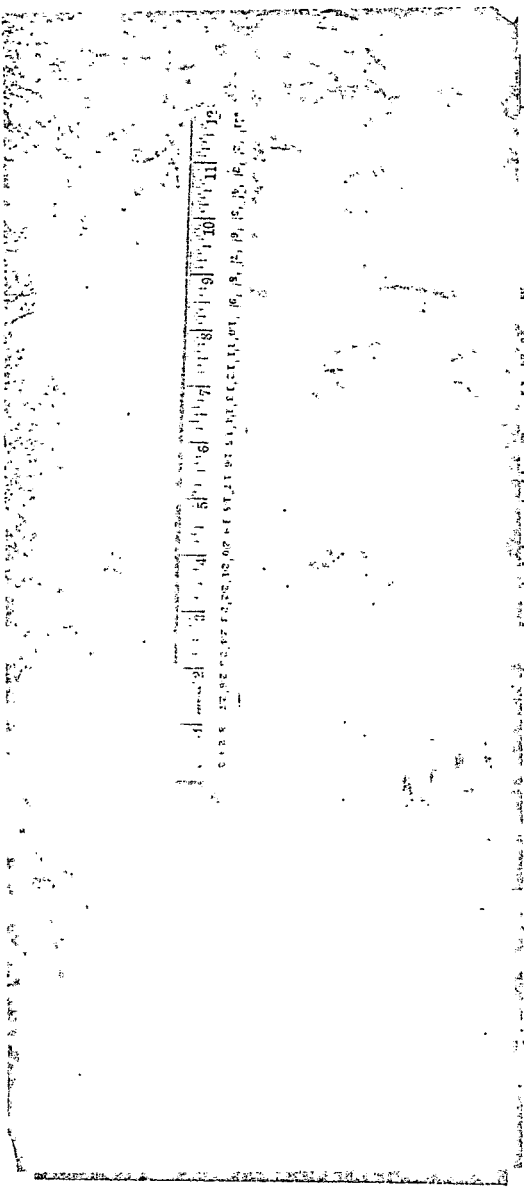
The organic matter in the surface layers of the sediments from shallow bays varies according to location. The following table lists the average organic carbon concentration of several localities in the bays near the Institute of Marine Science.

Location	Percent Carbon Dry Wt.
Beach sand, Gulf	0.03 - 0.09
Bay sand	0.5 - 3.5
Bay clay	0.7 - 4.2
Redfish Bay A	1.2 - 3.9
Redfish Bay B	0.3 - 1.2
Bluf green algae mat	0.6 - 1.5

Variations of organic carbon and redox potential with depth are shown in Figure 8. The abrupt transitions of redox potential from positive to negative is explained by differences in porosity and organic matter within the sedimentary layers. The intensity of the black color is somewhat indicative of the amount of organic matter or energy available to the sulfate-reducing bacteria. This type of sediment structure is common along the edges of the bays. In the centers of the bays more uniform conditions are found. (Data was presented in the Annual Report of 1959/60.)

Figure 8. Sediment profile at edge of bay near the Institute of Marine Science showing layers and redox potential.

SEDIMENT PROFILE



SURFACE

BANDING		Eh(Mv)
$Fe(OH)_3$ / Fe S / $Fe(OH)_3$	OXIDIZED	+350
Fe S	REDUCED	−250
SHELL LAYER	OXIDIZED	+150
APPROX WATER LEVEL		
Fe S	REDUCED	−325
$Fe(OH)_3$	OXIDIZED	+100
	REDUCED	−300

The data on carbon distribution and availability within the sedimentary layers of the shallow bays provide oxygen gradients and sites for oxygen differential corrosion cells on iron situated in the sediments. Such gradients could be responsible for the pitting of iron pipes buried in the sediments.

E. Origin of Hydrogen Sulfide in Sediments

Dr. Wilfred Gunkel from the Biological Laboratory, Helgoland, Germany, was employed for a period of five months to study the origin of sulfide in sediments. The sulfide can occur from either sulfate-reduction or from organic sulfur. The determination of the origin of sulfide may be significant to the understanding of the fundamental processes of microbial corrosion.

Experiments were organized in which the total sulfur, sulfate, sulfide, pyrite sulfur, iron, organic carbon, anaerobic bacteria, aerobic bacteria, sulfate reducing bacteria, and bacteria producing sulfide from organic matter were measured in natural and artificial sediments and algae. The analysis of the data is not complete to date, and will not be added to this report. However, preliminary results indicate that more sulfide is produced from organic sulfur than expected from past speculations, and that the algae contain an appreciable amount of organic sulfur.

F. Plans for the Future

To continue studies to characterize the enzymatic corrosion of iron and to test different bacteria for their corrosive properties. To continue the measurement of organic matter in surface sediments.

G. Publications

Bacterial activity in sediments of shallow marine bays. Geochimica et Cosmochimica Acta, 19:244-260 (1960).

Microbial corrosion of iron by marine sulfate reducing bacteria. With W. G. Blanton (Abstract) Bact. Proceedings, p. 36 (1960).

Organic matter in shallow marine bays (Abstract), Program 1960 Annual GSA Meeting, p. 170-171 (1960).