FINAL REPORT
of Project

THE MICROBIAL CORROSION OF IRON
November 1958 to December 1964

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THE MICROBIAL CORROSION OF IRON

Introduction

This is a final report for the proposal from the Institute of Marine Science, University of Texas Nonr 375(10), University of Miami, Institute of Marine Science Nonr 840(21), November 1958 to December 1964 from a research grant to study the fundamental principles of microbial corrosion of iron in marine environments. The grant was used to supplement University funds for personnel, equipment, supplies and other direct expenses necessary to complete the proposed research plan. The microbial activities to be studied in the field and laboratory were: 1. bacterial consumption of oxygen and subsequent production of oxygen differential corrosion cells, 2. microbial hydrogenase enzyme activity and it's effect on the depolarization of metallic iron surfaces.

Scientific Background

Microorganisms are known to cause corrosion of iron by several mechanisms (Beerstecher, 1954 and Updegraff, 1955). However, the significance of the corrosion mechanisms in natural environments has been little studied. Only a few of the published papers on microbial corrosion attempt to relate laboratory experiments and theoretical reactions to field conditions.

The process of chemically removing iron from a metallic surface is called corrosion. The term microbiological corrosion is generally used to describe the corrosion of metallic iron caused by the direct or indirect action of living microorganisms. The following information about microbial corrosion has been selected and modified from published data (Butlin, 1949; Beerstecher, 1954; Updegraff, 1955; Oppenheimer, 1957).

The role of bacteria in corrosion problems has been known since 1910 when Gains proposed that bacteria might be responsible for the corrosion of iron in moist soils. However, very little recognition was given to Gains' report until the classical paper on anaerobic corrosion by sulfate-reducing bacteria was published by Von Wolzogen Kühr and Van der Vlugt in 1934. Since 1934 many reports on bacterial corrosion have been published.
Summary of Results to January 1959

The project was initiated at the University of Texas on November 11, 1958. The first two months of the project was devoted to the purchase of special equipment and the organization of the research program. Mr. Roscoe Lamplugh was hired as a technician to assist in the initial stages of the program. He was later replaced by an advanced graduate student, Miss Volkman, who was working for the MA degree in bacteriology, had research problem on the bacterial degradation of organic matter in sediments, intimately related to this project. Miss Volkman's work is included as part of this report, although she is paid from other contract funds. The basic program of the Microbiology Section of the Institute of Marine Science consists of a study of the ecological relationships between microorganisms and the sedimentary environments of the shallow marine bays of Texas. Thus it is expected that much of the work conducted will be interrelated to provide a composite effort to study the many activities of the bacteria in sediments; one of which is the corrosion of iron.

The first series of experiments developed were concerned with the production of oxygen differential cells. The amount of organic matter in natural sediments and in artificial environments was measured to determine relationships between organic matter in sediments and consumption of oxygen. Oxygen consumption in marine sediments was related to the distribution of microorganisms, type of sediment and the amount of organic matter. Experiments were set up to measure the consumption of oxygen in controlled artificial sedimentary environments. The term "microcosm" is used here to describe a small volume of artificial or natural sedimentary environment where bacterial activity and the type of sediment appears to be uniform. In natural sediments a microcosm may consist of a few cubic millimeters or many square meters. In the shallow bays near the Institute the distribution of sediments is influenced mainly by fresh water runoff, wind and tidal currents. Artificial microcosms may be made by mixing various amounts of sediment of different types organic matter and the natural microbial Flora of some sedimentary type. Preliminary experiments on the direct microscopic examination of sediments have shown that each sedimentary type of environment (sand, shell, silt, clay, etc.) is populated by a microflora of specific morphological size. The finer particle sediments (down to 1 micron) with small interstitial spaces provide a limited living room for only the smaller bacteria. The finer particle sediments thus appear to contain fewer bacterial morphological types of microorganisms than coarse sediments. It is assumed that the
total number of bacterial species will be somewhat related to the morphological types and one may expect that the different sized sediments may support different bacteria species and related activities. Each microcosm will thus be different with respect to total bacterial activity.

Summary of Results from January to December 1959

Experiments were continued to determine the season changes, oxygen consumption and organic matter in sediments of Redfish Bay, shallow marine bay near the Institute of Marine Science. Monthly samples were taken from along the shores and in the center of the Bay. Distinct sediments layers were sampled to a total depth of 20 cm from the surface of the sediment.

Data show that sediments from a single marked area show differences in texture and placement of oxidized and reduced zones. Sediments separated by a few centimeters in depth or latorial area, differ appreciably in their organic matter content. The organic matter in the sediments does not decrease consistently with depth. Where an increase in carbon with depth is found, the increase is usually associated with either a sea grass sediment layer or a high shell content.

The bay sediments contain a large amount of refractive material which is being oxidized slowly.

The organic matter in the coarser sediments appears to be more easily oxidized, and therefore such sediments must have a higher oxygen demand. The complex matter usually associated with clay minerals, the low permeability, and the greater absorptive power may explain why the rate of organic matter decomposition is slower in clays.

Bacterial populations are larger in coarse sand than in the clays, and the bacterial numbers do not decrease consistently with depth, but appear to be associated with different sedimentary layers.

Seasonal changes in the initial organic carbon have been noted. Larger amounts of organic matter are found in October and the smaller amounts in February. This indicates that the rate of oxygen consumption within the sediments will vary with the season.

There is no correlation with the seasonal changes and the numbers of aerobic bacteria determined on nutrient sea water agar plate counts.
Microcosm Experiments

In the laboratory, experiments have been organized to measure the rate of oxygen consumption, the rate of organic matter decomposition, and the production of organic acids in sediments. The experiments are in progress and no conclusive data has been obtained to date.

Enrichment Cultures

Bacterial corrosion experiments conducted with enrichment cultures of sulfate-reducing bacteria showed that the presence of the bacteria accelerated the loss of iron from test coupons. The rate of iron loss was proportional to the number of bacteria during the logarithmic growth phase. The soluble iron diffuses away from the metallic surface during bacterial growth.

The amount of organic matter in surface sediments fluctuates during the year due to primary production by indigenous photosynthetic microorganisms and the accumulation of organic matter during sedimentation processes. The numbers of bacteria and organic carbon were highest during October and November and decreased during the winter. The total organic matter in the sediments and organic matter available to the bacteria was not uniformly distributed. Thus bacterial activities are not uniform with the resulting production of layers of aerobic and anaerobic sediments. The varying consumption of oxygen in sedimentary layers 10 to 30 mm wide may result in the production of differential corrosion cells in the presence of iron.

Summary of Results from January to December 1960

Bacterial Corrosion

It has been shown that enrichment cultures of marine sulfate-reducing bacteria and a pure culture of a hydrogenase positive \textit{Pseudomonas} can actively increase the rate at which iron is removed from the metallic state. Cell-free extract from sonicated \textit{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.

Origin of Sulfide in Sediments

Research is being conducted to measure the amount of sulfide in sediment derived from bacterial sulfate reduction and from organic sulfur during decomposition. The data are in the process of being collected and have not been analyzed to date.

Summary of Results to March 1961 - Change of locality to University of Miami

Simple tests using iron coupons have been developed which will measure bacterial corrosion of iron. Experiments to date have indicated that both sulfate-reducing and non-sulfate-reducing bacteria are corrosive, and that the corrosive effect can be traced to an enzyme fraction of the cells, believed to be hydrogenase. Hydrogen sulfide corrosion accounts for only 25 percent of the total corrosion possible by the sulfate-reducing bacteria tested. Not all sulfate reducing bacteria are corrosive. Results of measurement of organic carbon distribution and decomposition rates in the sediments of the shallow marine bays near the Institute of Marine Science show that environments exist which are favorable for the production of oxygen differential cells and anaerobic corrosion. Up to 40 percent of the corrosive sulfides produced in sediments studied may be produced by activities of heterotrophic bacteria and organic sulfur.

Corrosion Cells

During experiments designed to determine the nature of the polarity of non test coupons during corrosion tests, it was found that the iron coupon, completely submerged in sea water under anaerobic conditions, was anodic. A similar piece of iron in the same system with only its tip touching at the air-medium interface was cathodic. When a mixture of sulfate-reducing microorganisms was grown in anaerobic sea water medium contained in such cells as above, an average potential of 0.6 V
and a current of 10 μA was recorded. Control cells containing the sterile
growth medium produced only 0.1 V and approximately 1 μA.

Corrosion cells of this nature were investigated in regard
to their use as a simple and rapid method for determining the corrosive
nature of microorganisms isolated from the marine environment.

A light-independent fuel cell, similar in construction to the
cell to be used for measuring microbial corrosion, was presently under
considerations.

Enzyme Studies

Data from corrosion experiments involving the corrosive nature
of DPN, well known for its hydrogen-activating properties, have shown the
enzyme to be corrosive. Thus, the process of iron corrosion is shown
to involve removal of hydrogen from and the depolarization of the iron
surface. As a result an anodic area is produced on an otherwise cathodic
surface.

A cell-free extract of an organism which had lost its corrosive
capacity has been found to cause a weight increase in iron test coupons
during corrosion tests. Assuming a parallel loss of hydrogenase activity
with loss of corrosive capacity, the test coupons would remain polarized
and cathodic. Under these circumstances, cations would be attracted,
thus causing an increase in weight of the coupons. Spectrochemical
analysis of the cell-free extract has shown concentration of several
cation, including copper (250 ppm). It is quite possible that at a potential
of 0.6 V the copper was plated out on the surface of the cathodic iron
coupon (weight relationships would permit this). Further considera-
tion is being given this effect as a means for concentrating cations in sea waters.

Attempts have been made to activate cell-free extracts of corrosive
microorganisms which during preparation may have been exposed to oxygen
and therefore inactivated. Deoxygenation of these extracts with sodium
hydrosulfite in vacuo has not produced increased corrosive activity.
Since the step in the procedure where oxygenation and/or oxidation is most
likely to occur is during the usual membrane filtration, an effective and
unique mechanism for anaerobic filtration was developed.

A glycine-trypsin extraction method is now being used for
preparation of cell-free extracts of sulfate-reducing microorganisms.
This method allows cellular disintegration under completely anaerobic
conditions. Previous methods used for extraction preparations have not had this feature. Tests to determine the corrosive capacity of extracts prepared in this manner are currently underway.

Summary of results July to December 1982

Microorganisms which have been assayed and found to possess hydrogenase activity (i.e., activate molecular hydrogen) cause corrosion of metallic iron in pure culture. In addition, experiments have shown that iron is also corroded in an anaerobic system in the presence of various reducible molecules but not in their absence (e.g., methylviologen). Further experimentation has shown that metallic iron, in the absence of reducible molecules (or corrosive bacteria), becomes polarized with molecular hydrogen. This polarizing layer of hydrogen is believed to be removed (hence, depolarized and anodic) in the presence of microorganisms possessing the hydrogen-activating enzyme, hydrogenase.

The fact that corrosion of iron does occur in a system containing metallic iron and reducible molecules (with a simultaneous reduction of the molecules) leads to the conclusion that there is an initial formation of hydrogen atoms at the iron surface as follows.

Iron in water has a constant solution pressure which is in equilibrium with polarization by hydrogen. The electrons liberated by the solution, Fe⁰ → Fe⁺⁺ + 2e⁻, are accepted by the hydrogen ions (from the dissociation of water) to produce atomic hydrogen, H⁺ + e⁻ → H. In the event that there is no acceptor in the system for the atomic hydrogen formed, molecular hydrogen will be formed (H + H → H₂) on the iron surface, and the iron will become polarized. However, reducible molecules in the system will rapidly accept the atomic hydrogen and be reduced if initially present. As a result of this removal of atomic hydrogen as it is formed, the reaction, Fe⁰ → Fe⁺⁺ + 2e⁻, will continue and corrosion will proceed. The hydroxyl ion "excess" produced by the removal of hydrogen ions in H formation will associate with the ferrous iron liberated at the anode and produce a slight increase in the pH of the system.

If this hypothesis is correct, any material which will accept the atomic hydrogen formed on the iron surface will produce a depolarization of the iron creating anodic conditions adjacent to the polarized cathode. Corrosion will continue as long as the hydrogen is being taken up.
Such a phenomenon could explain why we find that not all sulfate-reducing bacteria are corrosive. Inasmuch as sulfate is not necessarily an acceptor of atomic hydrogen (it can also be reduced by hydrogen from organic molecules during metabolism), an iron surface in the presence of such sulfate-reducers need not be depolarized. Of course, hydrogen sulfide produced during sulfate reduction is within itself corrosive (as stated in our annual report for 1961 which separated corrosion caused by the sulfate-reducing bacteria *per se* and hydrogen sulfide).

The direct results of our interpretation show how complex microbial corrosion can be. If we assume that hydrogen acceptors as intermediate products of metabolism can cause corrosion, the substrate and type of microorganism becomes an important feature of the corrosion system. An organism growing in one environment may not be corrosive, whereas in another environment with different nutritional sources it may cause corrosion. The rate of corrosion will be directly related to the concentration of the hydrogen acceptor or the activity of the organisms. This has been shown in our results where DPN decreases in corrosive activity with dilution. Apparently at the higher concentration the DPN retarded corrosion (at present this is unexplainable).

As most enzyme systems require a hydrogen acceptor, it may be impossible to separate the corrosive effects of cell free extracts. However, in entire cell suspensions, we may assume corrosion to be due to enzyme activity or to metabolic products, inasmuch as metabolic hydrogen acceptors are usually within the cell and therefore may not be directly active as depolarizers in the medium.

**Summary of Results January to December 1953**

Metal coupons contained in completely anaerobic and sterile aqueous environment are believed to become polarized. In the absence of a depolarizer, such as oxygen, the weight loss is held to a minimum, dependent upon the characteristics of the metal. Should similar coupons be kept under identical conditions (as controls), weight loss would be expected to be uniform. Only when the hydrogen, polarizing the metal, is removed by the action of a depolarizing agent, does further weight loss occur. Exactly what constitutes a depolarizer under biological anaerobic conditions such as those with which this work is involved has not yet been conclusively determined.
Suspected depolarizers in the systems worked with here are the sulfate reducing bacteria, hydrogen utilizing anaerobes, reducible organic and, in several cases, inorganic compounds. Further work will continue to single out various of these possible depolarizing agents for closer consideration.

One other point which can now be examined is the frequency of high weight loss by any of the 24 gauge iron coupons used thus far. In three tests where the same coupons were used, of the 40 coupons tested, only one experienced a high weight loss in two different experiments. The remaining coupons which had shown high weight loss in a single instance failed to do so in any of the other two tests.

**Anaerobic corrosion theory**

**Nature of polarizing hydrogen on metallic surfaces**

In our previous work the assumption had been made that the hydrogen, which polarizes the surface of metals in aqueous, anaerobic solutions was essentially a thin film in the molecular form. The origin of this monomolecular layer of hydrogen was believed to originate as a result of the reactions

\[
\begin{align*}
\text{Fe} & \rightarrow \text{Fe}^{++} + 2e^- \quad \text{(anode)} \\
2 \text{HOH} & \rightarrow 2 \text{H}^+ + 2 \text{OH}^- \quad \text{(2)} \\
2 \text{H}^+ + 2e^- & \rightarrow \text{H}_2 \quad \text{(cathode)} 
\end{align*}
\]

in which reactions (1) and (3) occur at the anodic and cathodic areas of the metal, respectively.

As it is necessary for a metal to be depolarized before it can corrode, mechanisms other than, or perhaps common with, bacteria were sought which might fulfill this requisite. One such mechanism investigated in regard to depolarization of metals under anaerobic conditions was that of enzymatic activity.

That the enzyme, hydrogenase found in certain anaerobic bacteria might effect depolarization of metallic surfaces has been investigated and reported in other progress reports. In the process of developing experiments to test the theory of enzymatic depolarization, the terminal anaerobic hydrogen acceptor chosen was the redox indicator dye, methyl viologen (colorless when oxidized, blue when reduced).
Various hydrogenase preparations were used in corrosion tests (made under nitrogen) in which methyl viologen was used as the hydrogen acceptor. The results of these tests proved to be somewhat doubtful. It was noticed, however, that in many (but not all) cases, the liquid in the tests would appear as various intensities of blue (reduced indicator).

It was not until methyl viologen was used as the hydrogen acceptor in a corrosion test using the coenzyme, DPN (earlier report), that it became obvious that the color intensity of methyl viologen was not indicative of enzymatic depolarization alone. Tests made using clean iron coupons in a sea water solution of 0.002 molar methyl viologen affirmed the belief that the hydrogen acceptor (methyl viologen), independent of the enzyme, could cause weight loss of the iron specimen with a consequent reduction of the dye.

From the above, it was theorized that the mechanism responsible for the weight loss of iron coupons in anaerobic solutions of methyl viologen was essentially depolarization of the metal surface by the indicator dye. If, according to some previously held assumptions, the hydrogen of polarization is in the molecular form, the methyl viologen must react in some way with molecular hydrogen to convert it to the atomic state before it can be taken up and thus effect depolarization.

As a means of checking the above supposition, experiments were designed which attempted reduction of methyl viologen with molecular hydrogen in various ways. Of significance here is the fact that only in the presence of an inorganic catalyst (such as platinum oxide or bacterial hydrogenase) could molecular hydrogen reduce this, as well as similar dyes.

Molecular hydrogen is known to be converted to atomic hydrogen in the presence of metallic iron, and current theory holds that hydrogenase is capable of the same. One could, then, from the foregoing facts, conclude that the hydrogen (capable of dye reduction) initially produced on a clean iron surface under anaerobic conditions is not in the molecular, but atomic, form. Under these terms, the reactions given earlier should now show the cathode reaction as

\[ 2 \text{H}^+ + 2e^- \rightarrow 2 \text{H} \text{ (atomic)} \]

in the absence of an anaerobic hydrogen acceptor, this atomic hydrogen would be expected to proceed to the molecular form by the combination of two hydrogen atoms. This would then result in sufficient accumulation of molecular hydrogen to polarize the cathodic areas and therefore prevent...
further dissolution of iron (corrosion) from the anodic areas.

The significance of the theory of intermediate, atomic hydrogen formation of iron surfaces should be noted. The discussion has thus far been exclusively in terms of anaerobic conditions. However, it is interesting to note that solutions of redox indicators (methyl viologen, methylene blue, triphenyltetrazolium chloride, as well as others) which show a color change upon reduction in the absence of oxygen, can also be observed to do so under aerobic conditions. This reduction (as indicated by the color change) occurs, however, only in the immediate area of the iron coupon. Since the absence of molecular oxygen is a requisite for the reduced color to remain and be observed, it can be assumed that the oxygen in the vicinity of the coupon has been removed or inactivated.

It is possible that the inactivation of oxygen in the area of the coupon is effected by the dye itself. That is, as the atomic hydrogen is produced it immediately reduces a layer of the dye near the coupon. This is followed by rapid oxidation of the reduced dye by the dissolved oxygen in the reaction area (coupon surface). After a short time the oxygen in the area could be completely removed, thereby effectively rendering the area around the metal anaerobic. With the latter condition established, the color of any dye further reduced could then be observed.

In the theory above, the metal is assumed to be stripped of the atomic hydrogen produced as its surface as a result of the dye acting as an atomic hydrogen acceptor and a "mediator" between the iron and dissolved oxygen. The basic nature of such a hydrogen mediator would be the relative ease with which it, itself, is reduced and oxidized. That compounds exist in natural environments which are similar in nature to a theoretical hydrogen mediator proposed here is known --- those most noted being various enzymes and co-enzymes. Should such compounds be available to serve either as hydrogen mediators (as in aerobic situations mentioned above) or simply as hydrogen acceptors (in either aerobic or anaerobic conditions), formation of polarizing hydrogen would be inhibited and corrosion would proceed.

In cases where no such depolarizing agents are available and a metal becomes polarized with molecular hydrogen, those microorganisms capable of utilizing molecular hydrogen may play the key role in initiating, and perhaps continuing, metallic corrosion.

Corrosion cells

It was mentioned earlier in this report that corrosion of iron is believed to be accelerated when the hydrogen of polarization is removed
from the metal surface. It was pointed out that origin of this hydrogen was from the combination of electrons (leaving the anodic surfaces of a specimen and migrating to the more cathodic areas) with the hydrogen ions being derived from the dissociation of water.

It has also been pointed out that hydrogen-utilizing microorganisms (notably the sulfate reducers) may be active as biological depolarizers of cathodic hydrogen. That the hydrogen polarizing metallic iron surfaces is essentially at the cathode is shown by the reactions on page 9.

In corrosion tests made thus far, tared iron coupons have been routinely exposed to cultures being examined for their ability to serve as depolarizers. However, when one considers the principle of oxygen differential corrosion and then examines the methods used in our endeavors, a question arises as to the accuracy of our measurements of microbiological corrosion.

If oxygen has access to certain parts of a metal, the polarizing hydrogen will be removed more readily from these areas and the potential will be more negative here than on the other parts. As a result, the latter parts will tend to dissolve because an E.M.P. has been set up. "It seems strange, at first, that the parts of the metal which dissolve are those where the oxygen has not access, whereas the more highly oxygenated areas do not dissolve." (Glasstone, S. 1940. Textbook of Physical Chemistry, 2nd Ed. D. Van Nostrand Co., Inc. N.Y. p. 1035.)

If in an anaerobic bacterial corrosion test, the bacteria are to serve as the acceptor of cathodic hydrogen, it becomes important to examine the location of said cultures in relation to the metal specimens to be measured for weight loss.

**Summary of Results to November 1964**

Previous work has shown that the anaerobic corrosion of iron is accelerated by the presence of enrichment cultures of sulfate-reducing bacteria in sea-water media. Of 51 cultures tested to date, two have shown extensive corrosive activity and have been used in much of the research during the past year.

Various corrosion cells have been constructed in which bacteriological and chemical corrosion of iron have been compared. Results seem to indicate no basic differences between the two types of corrosion; e.g., they are both galvanic but through separate mechanism.
Methods for effectively testing microbial corrosion have been refined and simplified. The new methods have been tested and found to produce statistically significant results and have been particularly useful in conducting in situ and laboratory tests for marine sediment aggressiveness.

All enrichment cultures have been screened for both mesophilic and thermophilic sulfate reduction using a recently modified anaerobic medium and dispenser. All but one culture were found to be mesophilic in their requirement for the reduction of sulfate.

The highly corrosive enrichment culture, MC 28, has been used to test the susceptibility of two high-strength alloys and two each of the Group V and VIB metals, respectively. Similar tests using aluminum and iron were made for comparative purposes.

The rate of uptake of molecular hydrogen by a metabolizing culture of MC 28 has been examined using two methods. That which permitted the absorption of metabolic CO₂ during the determination (Warburg method) proved to be most useful. Uptake rates indicated a pure hydrogen-utilizing component of the mixed culture by virtue of the curve produced.

Corrosion rates of both MC 28 and 30 have been made over periods extending through 60 days. Weight loss data in both cases gave curves resembling expected growth curves for sulfate-reducing bacteria.

An attempt has been made to isolate bacterial strains occurring in the enrichment cultures, MC 28 and 30. However, the procedures used have not yet been effective.
CONCLUSIONS

The investigation has shown the potential and magnitude for microbial corrosive effects on iron in marine environments.

Aerobic and anaerobic corrosion cells can be developed by a wide variety of microorganisms. Aerobic corrosion is developed through metabolic oxygen consumption by bacteria on localized iron surfaces where organic food is present. Alternating bands of aerobic-anaerobic zones are formed in sediments by layering effects of deposition involving different amounts of organic materials. The layering does produce oxygen differential cells that are very corrosive.

Anaerobic corrosion cells are produced by depolarization of the iron due to proton or hydrogen uptake. The activity is proportional to hydrogenase activity or to the presence of hydrogen acceptors in the area.

Suitable tests involving weight loss of iron test coupons can be employed to show the corrosive nature of microorganisms in the environment.
CURRENT REPORTS AND PUBLICATIONS


THESSES


PUBLICATIONS

The following accumulative list of publications was entirely or partly from research resulting from the contract.


