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AUTOTROPHIC LEACHING

CRITICAL REVIEWS™ in MICROBIOLOGY

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CRC CRITICAL REVIEWS in MICROBIOLOGY

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Leaching of copper has been practiced since 1700; however, the involvement of bacteria in extraction of metals was not recognized until the late 1940s, when an unidentified bacterium (*Thiobacillus ferrooxidans*) was isolated from mine waters. Subsequently, intense study of bacterial leaching during the 1950s and 1960s elucidated the role of *T. ferrooxidans* as the oxidation of ferrous iron and metallic sulfide minerals and defined conditions necessary for activity of the organism. Impending depletion of mineral reserves has stimulated interest in hydrometallurgical techniques, including microbial leaching processes, for extraction of metals from materials which cannot be processed by conventional methods. However, solution mining technology has primarily been approached independently through the chemical, physical, and biological fields; but for development of economically and technically workable operations, the disciplines must be integrated. In this perspective, processes for bacterial extraction of metals will be reviewed with relationship to geochemical and hydrometallurgical aspects of metal leaching.

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Polyoma is a small DNA-containing virus with oncogenic potential. In the past few years, significant new information on the molecular biology of polyoma has been obtained. Studies on the virus particle have provided new insight into the genetic relationship of the viral-specified structural proteins. The chromosome of the virus has been dissected with the use of restriction nucleases, and a physical map locating markers of biological significance has been prepared. A partial genetic map has also been constructed by marker rescue experiments. Studies on the early events during a polyoma infection have provided new ideas on the factors required for transporting the virus to the nucleus, where uncoating occurs. Transcriptional events occurring after polyoma infection have been clarified, and models to account for "early" and "late" strand switching and messenger selection have been proposed. Studies on the replication of polyoma DNA have provided information on the origin of initiation, the overall direction of synthesis, and the general nature of replicative intermediates. The polyoma T antigen, a protein involved in viral DNA synthesis and establishment of transformation, has been partially purified. The intracellular polyoma DNA has been found to exist as a nucleoprotein complex in which histones are arranged along the DNA as nucleosomes in a manner similar to the organization of histones on cellular chromatin. The polyoma mouse system has played a vital role as a model for studying the events involved in lytic infection as well as the complex molecular events leading to cellular transformation. The knowledge obtained from the polyoma and simian virus systems is now being used to study other members of the papovavirus group, namely, JC and BK viruses, which have been implicated in human disease.

BACTERIAL LEACHING

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INTRODUCTION

Hydrometallurgical processes, of which bacterial leaching is a significant part, are playing an increasingly important role in the extraction of metals from low-grade ore minerals. The growing interest in such processes is attributed to the following: the relative absence of land and water pollution in the process; the need to mine increasingly lower grade ores, which cannot be economically processed by conventional mining, smelting, and refining operations; the ease with which hydrometallurgical operations can be implemented; and the lower capital costs necessary for hydrometallurgical operations as compared with conventional processing.

Hydrometallurgy consists of the dissolution of metals from minerals and the recovery of the desired elements. The major emphasis of this review will be on the leaching of elements into solution, particularly on bacterial mechanisms which enhance the dissolution of metals from economically valuable minerals. An exhaustive review is not presented, but a critical examination of recent developments in bacterial leaching most relevant to industrial application is included. Special attention is placed on the importance and relationship of bacterial leaching to hydrometallurgical aspects of metal extraction and geochemical aspects of metal mobilization.

At present, the leaching of low-grade copper waste material in the western states produces about 11.5% of the total copper production in the U.S.[1] The extraction of copper by hydrometallurgical processes has a long history. Although the first large-scale development was probably Rio Tinto, Spain, about 1750, it is likely that the Romans recovered copper from sites of natural leaching. The operation at Rio Tinto, as detailed by Taylor and Whelan[2] in 1943, remains the technique used by most copper leaching companies in the world today. Researchers in many disciplines, and the practical experience of leaching, have contributed to the vast amount of knowledge now available on the subject. The "art" of leaching encompasses many areas, including physical, chemical, and biological aspects.

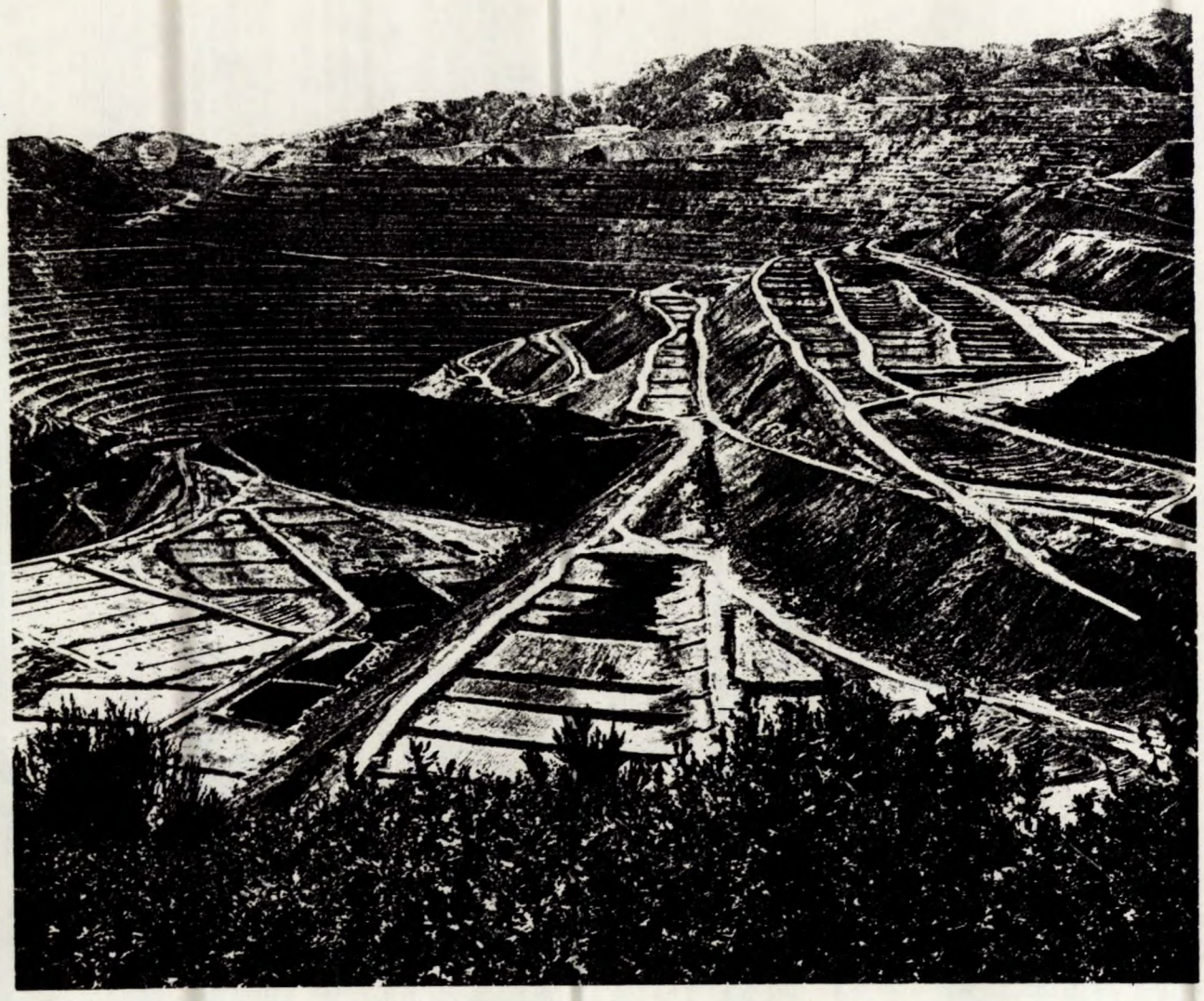

FIGURE 1. Copper leach dumps at Bingham Canyon, Utah, showing lifts for aeration. (Courtesy of Don Green, Staff Photographer and the Kennecott Copper Corporation).

LEACHING METHODS

The principal physical methods of leaching are dump, heap, vat, and *in situ* leaching. Not all leaching is accomplished by bacterial participation. Leaching of oxide ores by dump, heap, or vat methods is purely chemical.

Dump leaching is used to extract copper from low-grade oxide and sulfide minerals and waste materials removed from open-pit mining operations. Such materials contain less than 0.4% copper and remain uncrushed. The leach dumps are usually located in valleys to use natural slopes for stability and recovery of solutions. Ideally, localities should be selected to give an impermeable base, although impervious pads have been constructed at some dump sites to insure that leach solutions are not lost. Run-of-the-mine material hauled to the dump includes boulders weighing many tons and material of fine particle size, but most is less than 2 ft in diameter. Some dumps in the western U.S. are constructed on 50- or 100-ft lifts, up to 1200 ft high. The largest dump, located at Bingham Canyon, Utah, contains 4 billion tons of material (see Figure 1).

Leach solutions are usually introduced on the top surfaces of dumps by flooding or sprinkling. The solutions then percolate through the dumps and are collected in catch basins at the

base of the dumps. Copper is recovered from the "pregnant liquor" by either precipitation by metallic iron, electrowinning, or solvent extraction. The barren solution, or tailings solution, is then returned to the dump surfaces for reapplication. The leach cycle for dumps is measured in years. Copper dump leaching practices in the western U.S. have been reviewed by Sheffer and Evans.[3]

Heap leaching is primarily used to extract copper and uranium from crushed or uncrushed oxide ores of a somewhat higher grade than ores considered for dump leaching. The material to be leached is placed on a specially prepared surface. The volume of material in heaps for copper leaching varies from 100,000 to 500,000 tons. Metals from these ores are generally soluble in sulfuric acid, and the leach cycle is completed in months.[1,3] Gold can also be heap leached, usually with cyanide.

Vat leaching, as currently employed on oxide ores, is the sulfuric acid dissolution of crushed materials in a confined tank. Leaching is completed in days.[1,3]

Underground *in situ* leaching as a process is applicable to oxide and sulfide minerals of copper and uranium. Wadsworth[1] describes three types of deposits amenable to *in situ* leaching. Type I is a fractured ore body near the surface. This includes worked-out regions of mines and deposits fractured by explosives or hydrofracturing. Type I deposits are leached with the same techniques currently applied to dump leaching. Deposits that exist at depths less than 1000 ft and are under the water table constitute Type II deposits. These deposits require fracturing and de-watering. Type II deposits may be leached by alternating the oxidation and leach cycles. During the oxidation phase, water is removed, processed, and stored. During the leach cycle, the water is injected into the deposit. In a percolation leach system, the solutions are injected into the deposit, and the pregnant liquors are collected at recovery wells. The hydrology of such deposits must be well characterized to avoid contamination of ground water. Deposits below the water table and at a depth greater than 1000 feet constitute Type III deposits. After fracturing of these ore bodies, leach solutions are introduced by injection wells. The hydrostatic pressure increases oxygen solubility so that oxidation of sulfide minerals occurs without the presence of additional oxidizing agents. As in Type II deposits, the hydrology of such ore bodies must be well known. *In situ* mining has the advantage of reducing surface disturbance over the deposit, thus eliminating much of the problem of solid waste disposal and acid drainage from sulfide-bearing rocks which otherwise would be brought to the surface. On the other hand, with the present state of knowledge of ground-water hydrology in fractured crystalline rocks, it is difficult to predict and control the path of leach solutions. Another problem is that the handling and storage of large volumes of leach solutions is risky. Losses of solution have the potential of contaminating ground and surface waters.[4]

It is necessary that leach solutions reach all metal-bearing minerals in the dump, heap, or *in situ* deposit for recovery of the metals. However, the distribution of solutions is subject to channeling, and formation of impermeable layers results in lateral solution flow. One very important aspect in leaching is the necessity for an adequate amount of air for the leaching process. The problem of lack of air currently exists in dump leaching and will undoubtedly be a serious problem in some *in situ* applications. The temperature of the leach solutions is also an important factor in the chemistry and biology of leaching. Winter temperatures of leach solutions can be as low as 3 to 4°C, and summer temperatures as high as 35°C are encountered.[3] Temperatures of 80°C have been recorded within leach dumps,[5] due to the exothermic reaction of pyrite oxidation. Elevated temperatures increase the rate of leaching but are detrimental to the activity of most bacteria.

Leaching of uranium deposits has successfully been carried out but not to the extent of copper leaching. Leaching technology is attractive for uranium recovery because it decreases the environmental impact of mining, increases the feasibility of recovering ore from deeper deposits, and allows the economic working of low-value mineral reserves.[6] The future of uranium production is directly related to the use of nuclear energy for electrical generation. Production of uranium from New Mexico resources, which supplies 43% of the national uranium, will fall short of anticipated demands by 1980.[7] Development and utilization of technology for recovery of deep and low-grade ura-

nium resources would significantly ease the anticipated shortfall.

THE CHEMISTRY OF LEACHING

The chemistry of leaching is exceedingly complex, but substantial information is now available. It is beyond the scope of this article to examine in-depth reactions which are thought to occur; however, to critically evaluate contributions in bacterial leaching, it is necessary to have a basic understanding of the chemistry involved.

The leaching of elements, silicates, oxides, and sulfides can be effected by oxidizing, reducing, and neutral conditions. This review will be confined to the solubilization of metal sulfide and uranium oxide minerals, and only the chemistry of leaching by oxidation will be described. Unlike the base metal oxides, sulfide metals and tetravalent uranium require an oxidizing agent to effect dissolution of the metal. One of the least expensive and most effective oxidizing agents is ferric iron. An excellent review by Dutrizac and MacDonald[8] summarizes the preparation, regeneration, and properties of this leaching medium and the kinetics of leaching a variety of minerals with ferric iron.

Copper

The mineralogy of the ore minerals and the associated gangue are extremely important in establishing the feasibility of leaching. American porphyry-type deposits range between 0.5 and 1.0% copper.[9] The average grade of copper is about 0.8%. Other metals of economic value found in porphyry copper deposits besides copper include molybdenum, rhenium, gold, and silver.[10] Copper porphyries supply 17% of the world's molybdenum reserves. The principal copper sulfide minerals in porphyry deposits are chalcopyrite ($CuFeS_2$) and chalcocite (Cu_2S). The latter is quite easily leached by oxidation with ferric iron; however, chalcopyrite is more resistant to attack by this oxidizing agent.

Table 1 summarizes the order of resistance of some common sulfide minerals.[11]

The dissolution of ore minerals is best illustrated by Pourbaix (Eh-pH) diagrams, although such diagrams do not consider the kinetics of the reaction. An Eh-pH diagram for the $Cu\text{-}H_2O\text{-}O_2\text{-}S\text{-}CO_2$ system at 25°C shows that covellite (CuS) and chalcocite (Cu_2S) are soluble at acid pH values and 0.4 volts (see Figure 2). Sulfate is stable at this potential in an acidic environment.[12] If oxidation is required for copper solubilization, effective dissolution occurs with ferric iron concentrations of about 4 m*M* (0.25g/l). Ferric iron in excess of 20 m*M* (1 g/l) does not increase the rate of leaching. Acid consumption by gangue material greatly affects metal dissolution. Although copper porphyries are found in igneous host rocks, acid-consuming carbonates are often present. Also present, but less obvious, are clays which can consume vast quantities of acid. Excessive acid causes increased dissolution of gangue and increased acid consumption. If the acid concentration is too low, iron salts precipitate. In general, as the pH decreases, the solubility of ferric iron increases.

TABLE 1

Minerals of Increasing Recalcitrance[11]

Pyrrhotite
Chalcocite
Covellite
Tetrahedrite
Bornite
Galena
Arsenopyrite
Sphalerite
Pyrite
Enargite
Marcasite
Chalcopyrite
Molybdenite

The general equation for the dissolution of the metal sulfides (MS) in an acidic ferric iron solution is

$$MS + 2Fe^{3+} \longrightarrow M^{2+} + 2Fe^{2+} + S^0 \qquad (1)$$

The elemental sulfur is stable over a variety of conditions. However, sulfate is undoubtedly formed by some base metal sulfide oxidation

$$MS + 8Fe^{3+} + 4H_2O \longrightarrow M^{2+} + SO_4^{2-} + 8H^+ + 8Fe^{2+} \qquad (2)$$

as well as by the bacterial oxidation of elemental sulfur formed in Equation 1

$$S^0 + 3/2O_2 + H_2O \longrightarrow H_2SO_4 \qquad (3)$$

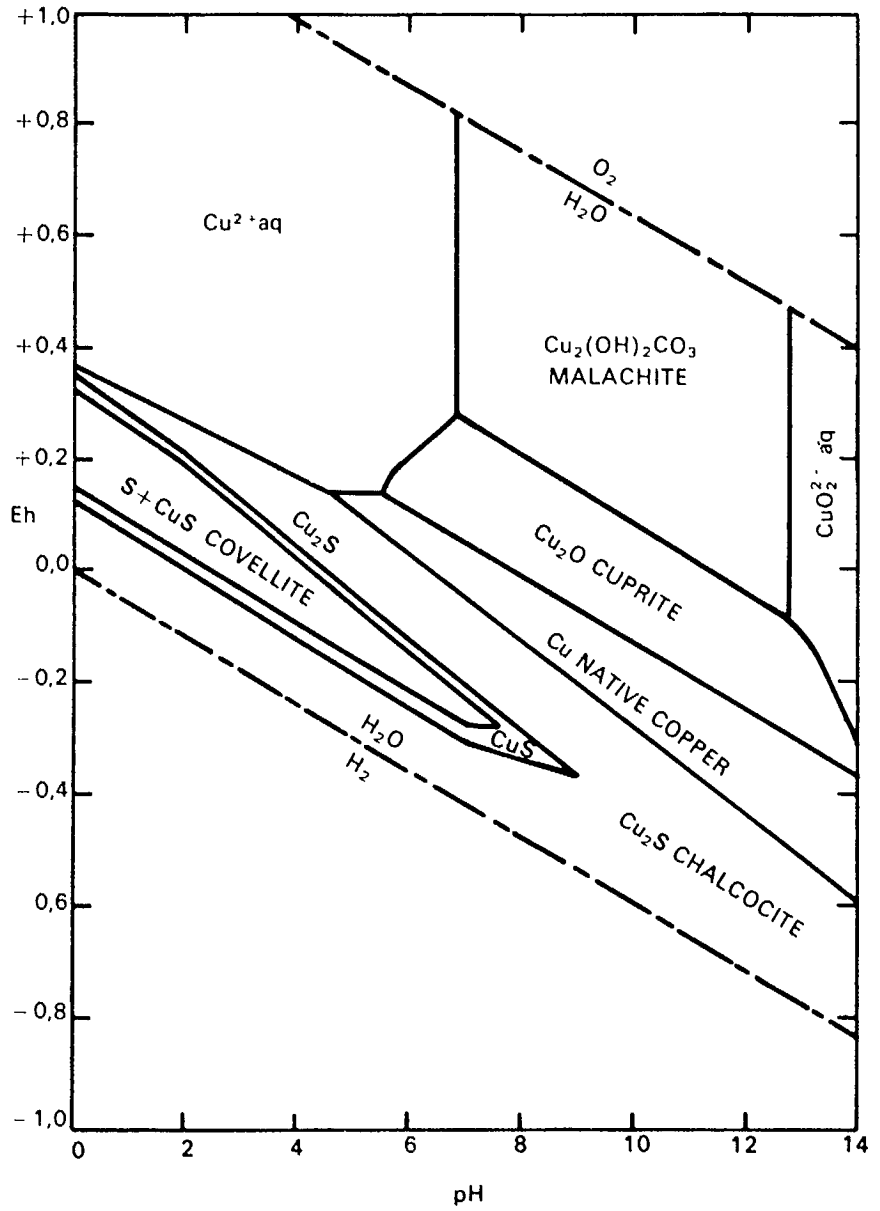

FIGURE 2. Stability relations among some copper compounds in the system Cu-H_2O-O_2-S-CO_2 at 25°C and 1 atm total pressure. $P_{CO_2} = 10^{-3.5}$, total dissolved sulfur species = 10^{-1}. (From Garrels, R. M. and Christ, C. L., *Solution, Minerals, and Equilibria*, Freeman, Cooper, and Co., San Francisco, Calif, 1965. Illustration courtesy of J. Anderson).

The kinetics of the chemical attack of ferric ion on copper sulfide minerals are dependent upon a number of factors: temperature, ferric iron concentration, and the inhibiting effect of the sulfur layer. A number of lesser-known copper sulfide minerals can be leached by ferric iron, including cubanite ($CuFe_2S_3$), enargite (Cu_3AsS_4), and tetrahedrite ($Cu_{12}Sb_4S_{13}$). The dissolution of other sulfide minerals can be effected using ferric iron as an oxidizing agent. These include millerite (NiS), pentlandite ($(Fe,Ni)_9S_8$), sphalerite (ZnS), galena (PbS), and pyrrhotite ($Fe_{1-x}S$). Pyrite (FeS_2) is ubiquitous and is the prime source of ferric ion in dump leaching. Molybdenite (MoS_2) can be solubilized by ferric iron, but the dissolution is complicated by the formation of insoluble leach products.

One of the biggest problems in dump leaching is the precipitation of iron salts in pipe lines, on the surface of dumps, and within dumps. The build-up in concentrations of calcium, fer-

ric iron, potassium, and sodium results in the precipitation of hydrolysis products, such as jarosites, X $Fe_3(SO_4)_2(OH)_6$ where X = H^+, Na^+, K^+, or NH^+_4. Jarosite forms more readily at higher temperatures.[14] Iron phosphates can also form, producing impermeable slimes on mineral grains. Surfaces of dumps are periodically furrowed or scraped to remove the precipitate, but formation of jarosites within dumps creates severe impermeability to solution flow. Strict control of pH and iron content in the leach solution does alleviate the problem somewhat.

Uranium

Uranium occurs in the tetravalent and hexavalent states in the natural environment. Uranium (IV) is not very soluble, but when oxidized to uranium (VI), it is very mobile, giving rise to many secondary minerals. Uranium (VI) transported by surface or ground water into reducing environments such as lignite beds or black shale, can precipitate as coffinite ($USiO_4 \cdot nH_2O$) or pitch-blende (uraninite) (UO_2).[15] More complex mineral assemblages can result by complexing uranium with vanadium.[16] Uranium deposits of the Colorado Plateau and central Wyoming are of this nature. A variety of oxidizing agents can be used to solubilize uranium (IV) including ferric iron, manganese dioxide, hydrogen peroxide, sodium and potassium chlorate, and nitric acid. Hydrogen peroxide is exceedingly expensive, and excessive concentrations result in the precipitation of uranium.[17] If iron is necessary, oxidizing agents are added to maintain the iron in the ferric state. Bacterial activity also generates ferric iron. The dissolution reaction for tetravalent uranium by ferric iron is

$$UO_2 + Fe_2(SO_4)_3 \longrightarrow UO_2SO_4 + 2FeSO_4 \quad (4)$$

Acid comsumption for uranium leaching can be considerable, but this is primarily due to the presence of acid-consuming gangue associated with the ore. The choice for use of acid or alkali for leaching is dependent upon the presence of clays, organic compounds, and soluble impurities. Alkaline leaching is usually selected when there is a high percentage of calcite, limestone, and other acid-consuming carbonates. The consumption of sodium carbonates can be excessive if the ore is high in gypsum or sulfide. The dissolution of uranium in sodium carbonate can be expressed as

$$2UO_2 + O_2 + 6Na_2CO_3 + 2H_2O \longrightarrow 2Na_4UO_2(CO_3)_3 + 4NaOH \quad (5)$$

The sodium hydroxide generated will attack the uranium-carbonate complex and dissolve it, so sodium bicarbonate must be added to neutralize the sodium hydroxide as it forms.[18,19]

$$NaHCO_3 + NaOH \longrightarrow Na_2CO_3 + H_2O \quad (6)$$

BACTERIAL LEACHING

The iron-oxidizing bacterium, *Thiobacillus ferrooxidans,* was isolated from a coal mine drainage, and its discovery was reported by Colmer and Hinkle[20] in 1947. This bacterium and the sulfur-oxidizing *T. thiooxidans* were soon associated with the dissolution of metals from ores.[140]Both of these organisms are aerobic and require an acid environment between pH 2 and 3.5. They are rod-shaped bacteria about 0.5 × 1.0μm in size and can utilize carbon dioxide as their sole source of carbon. They require a nitrogen source, usually ammonium, although it has been shown that nitrogen can be fixed by a strain of *T. ferrooxidans.*[21] Urea can serve as a nitrogen source for some strains of *T. thiooxidans.*[22,23] Additional requirements are phosphate and some trace elements usually present in their environment. These organisms exhibit different temperature optima for different strains and substrates, but optimum is often considered 25°C, with a maximum at 35°C. The organisms are slow-growing, and division time is variable with conditions. *T. ferrooxidans* bacteria derive energy for growth from the oxidation of ferrous iron

$$2FeSO_4 + \tfrac{1}{2}O_2 + H_2SO_4 \longrightarrow Fe_2(SO_4)_3 + H_2O \quad (7)$$

Chemically, this reaction occurs in air but is very slow. *T. ferrooxidans* are able to oxidize ferrous iron at a rate 500,000 times as fast as would occur in their absence.[24] In the leaching environment, ferrous sulfate, produced by the chemical oxidation of pyrite

$$FeS_2 + 7/2\,O_2 + H_2O \longrightarrow FeSO_4 + H_2SO_4 \quad (8)$$

or available from the cementation of copper

$$Cu^{2+} + Fe^0 \longrightarrow Cu^0 + Fe^{2+} \quad (9)$$

is oxidized to ferric sulfate by *T. ferrooxidans* (see Equation 7). The ferric sulfate then reacts with metallic sulfide minerals according to Equation 1 or 2. The resulting reduced iron is then reoxidized biologically by *T. ferrooxidans*.

Another bacterium often found in leaching environments is *T. thiooxidans*. This organism oxidizes sulfur according to Equation 3. Elemental sulfur formed during the reaction of ferric sulfate with metallic sulfides (Equation 1) can be oxidized by *T. thiooxidans* or *T. ferrooxidans* in the absence of an iron substrate.

The reaction of ferric sulfate dissolution of mineral sulfides is considered an indirect method of bio-leaching. Considerable research effort has been expended to determine if metallic sulfides can be attacked directly by the thiobacilli.[25] Such studies are often confusing since even museum-grade specimens of copper sulfide minerals contain trace amounts of iron which participate in leaching. Evidence supporting the direct bacterial leaching mechanism was reviewed by Tuovinen and Kelly.[162]

The thiobacilli are surprisingly tolerant of most heavy metals; however, mercury, silver, and molybdenum[26] are inhibitory. Uranium toxicity to unadapted strains of *T. ferrooxidans* has been observed. The addition of 5 to 100 m*M* (1.2 to 24 g/l) uranium to *T. ferrooxidans* results in cessation of carbon dioxide fixation, loss of viability, and depression of ferrous iron oxidation. *T. ferrooxidans* does not readily bind uranium, but toxicity is probably due to the loose binding of uranium at iron-oxidation and metal-transport sites on the cell membrane. Bivalent cations and EDTA (ethylenediaminetetraacetate) alleviate the toxicity of uranyl sulfate to *T. ferrooxidans*, and tolerance to uranium can be developed by successive subculturing of *T. ferrooxidans* in increasing uranium concentrations.[27,28] Adapted strains are used successfully in the leaching of uranium.[29] It has been suggested that the energy produced by the oxidation of tetravalent uranium to hexavalent uranium is sufficient to support the growth of the chemoautotrophic bacteria; there is no evidence yet that enzymes catalyzing this reaction exist.[30] The role of *T. ferrooxidans* in solubilization of uranium appears to be indirect and confined to the generation of the chemical oxidant, ferric sulfate, and the production of the solvent, sulfuric acid. In mineral belts such as the Elliot Lake area of Ontario, uranium occurs in the mineralized form, brannerite ($(U, Ca, Fe, Th, Y)_3 Ti_5 O_{16}$) and uraninite ($UO_2$), with pyrite present.[31] The bacteria probably oxidize pyrite by the reaction

$$2FeS_2 + 15/2O_2 + H_2O \xrightarrow{\text{bacteria}} Fe_2(SO_4)_3 + H_2SO_4 \quad (10)$$

producing both the oxidant and solvent. Pyrite in air yields ferrous sulfate according to Equation 8; the ferrous sulfate is oxidized by *T. ferrooxidans* (Equation 7). The oxidation and solubilization of uranium occur according to Equation 4. The ferric iron is regenerated biologically by *T. ferrooxidans*. Like the leaching of copper, uranium dissolution in which bacterial activity is present occurs in an acid environment.[31] The oxidation of tetravalent uranium in oxide ore such as uraninite requires a potential of +410 mv, which is easily attained at low ferric iron concentrations.[32] This potential is usually maintained in laboratory suspensions of *T. ferrooxidans* growing on ferrous iron.[33] If considerable acid-consuming gangue rock is present in the leaching environment, acid and iron concentrations in solution will be weakened by reaction of these reagents with the gangue. Therefore, leaching of economic minerals will be diminished. The rate of dissolution of uranium is directly dependent on the ferric iron concentration.[8] In practice, however, the amount of oxidation required for dissolution of the uranium is a function of the ore minerals and complexing agents, such as the carbonaceous materials, which bind uranium in some secondary deposits. In general, bacterial leaching of uranium is feasible in mineralogical belts where the ore is in the tetravalent state and is associated with reduced sulfur and iron minerals, which provide a suitable environment and energy source for the bacteria.

Other articles reviewing bacterial leaching by Tuovinen and Kelly,[34] Trudinger,[35] and Corrans et al.[25] have been published.

COMMERCIAL LEACHING OPERATIONS USING BACTERIA

Copper

A review by Sheffer and Evans[3] on copper leaching in the western U.S. contains data on heap and dump leaching and precipitation of

copper. Although some of these operations leach oxide ores, many involve the leaching of sulfide minerals. Microbiological data are unavailable on most of these leach endeavors, but the chemoautotrophic thiobacilli play an active role in the generation of ferric iron and sulfuric acid in the sulfide mineral operations. Most investigations on the contribution of *Thiobacillus ferrooxidans* to the extraction of copper from low-grade ore have been conducted in the laboratory under simulated field conditions. One study[13] suggested that oxygen availability, temperature, moisture content, and toxicity to the leach solution controlled the distribution and activity of *T. ferrooxidans* in leach dumps. Bruynesteyn and Cooper[36] obtained scaled-up data by correlating shake-flask and column tests with studies from a test dump. The rate of copper released among the three systems appeared to be directly related to particle size. This was investigated further by Bruynesteyn and Duncan.[37] Most of the problems associated with dump leaching result from poor dump construction and inadequate knowledge of the internal conditions or reactions occurring within dumps.[38]

In recent years, leaching has been initiated on worked-out mines of mixed oxide-sulfide copper minerals.[39-41] To obtain adequate permeability, the old workings are explosively fractured. The ore is leached with a ferric iron-sulfuric acid solution. In industrial situations, the effluent solutions from the leach operations are monitored for ferric and ferrous iron content. High Fe^{3+}/Fe^{2+} ratios are indicative of good bacterial activity, but counts and other direct measurement of bacterial activity are not made. To date, most *in situ* leaching of copper has been from worked-out mines. Project Sloop at Safford, Arizona, between Kennecott Copper Corporation and the Energy Research and Development Administration was to have leached on ore body after fracturing with nuclear explosives; however, this project was abandoned. Recently, a copper-oxide-ore body in New Mexico was fractured by conventional explosives, and *in situ* leaching will be used to recover copper values. St. Peter[42] has summarized the successes and problems of several *in situ* mining operations. The role of bacteria in these types of *in situ* operations is not known, because studies have not been conducted at field sites.

Vat leaching of oxide or mixed oxide-sulfide minerals is conducted at several western U.S. copper operations.[3] Bacteria are not used in vat leaching, although a feasible process has been described for concentrate leaching.[43,44] The most important factor in the use of bacteria in vat leaching is the leach rate. Slow rates mean long retention times and large inventories of material. Both can make the process uneconomical.[45]

Uranium

Uranium leaching on a commercial scale has experienced success for a number of years. Uranium ores are generally considered low grade if they contain an average of less than 0.05% U_3O_8. However, this can vary from one operation to another. Leaching of mined-out areas and low-grade heaps at the Stanrock Uranium Mines, Ltd., Elliot Lake, Ontario, began in the early 1960s. High-pressure washing of stopes with both fresh water and recirculated acid water is followed by a 3 to 4 month rest period and then rewashing. This has proven most effective for uranium extraction. The lixiviant containing uranium flowed to sumps in the mine where it was pumped to the surface and uranium values recovered. Stope washing in combination with intermittent sprinkling of low-grade heaps has produced 10,000 to 12,000 lb U_3O_8 per month.[46] Uranium recovery was attributed to production of ferric sulfate and sulfuric acid by thiobacilli. The uranium in the Elliot Lake area is associated with pyritic material which provides the bacteria with reduced iron and sulfur.[47] Similar procedures for uranium recovery were initiated in mined-out stopes of the Milliken Mine of Rio Algom Mines, Ltd., Elliot Lake,[48] Ontario.

Solution mining for uranium was initiated at Shirley Basin, Wyoming, by Utah Construction and Mining Company. This deposit occurred below the natural water table. The leach solution was introduced into the deposit through inflow wells; pumping at a production well was started simultaneously with injection of the fluid. The solvent was sulfuric acid, and sodium chlorate was used to convert ferrous to ferric iron. The ferric iron was the agent which oxidized tetravalent uranium. This was a chemical rather than a biological process.[49,50] The leaching operation was abandoned in 1970.

Underground leaching and heap leaching

have been used to recover uranium from low-grade ores in Russia. Sulfuric acid was used as the solvent.[51]

A number of *in situ* uranium leaching operations have been attemped or are in developmental stages.[52] These include

1. The Atlantic Richfield project at George West, Texas[53]
2. Union Carbide and Wyoming Minerals (a Westinghouse subsidiary) in Texas
3. Rocky Mountain Energy Company (a Union Pacific subsidiary) and Exxon in Wyoming
4. Anaconda Company's basic study now underway

In Texas, leaching of uranium is usually by alkaline means using a mixture of ammonium carbonate and ammonium bicarbonate. Sulfuric acid with sodium chlorate as the oxidizing agent is generally used in Wyoming. These leaching operations are similar to the Utah Construction and Mining Company operation[49,50] in that the leach solutions are brought to the ore body through injection wells, and uranium-bearing solutions are removed through production wells. The major problem at this time appears to be the permeability of the ore body, with resultant loss of the leach solution.[53] Other important factors are the responses of the minerals and associated gangue to the leach solution, the kinetics of the extraction, and the processes of solution recovery.[6]

Subsequent sections will be concerned with developments in microbial leaching as it relates to the commercial extraction of metals.

MICROBIAL/SUBSTRATE INTERACTIONS

The close association between the chemoautotrophic bacteria and mineral particles was observed early in the study of these organisms,[54] and experiments were initiated to determine the nature and reasons for the association and observed attachment. [55,56] Many of these studies resulted from interest in the mechanisms of metabolism and attachment of the organisms to inorganic substrates,[57] but others were initiated as a result of the problem of enumerating the chemoautotrophic bacteria.

Enumeration

A number of techniques are used to directly and indirectly enumerate or measure the activity of the chemoautotrophic bacteria. Often, the activity of *Thiobacillus ferrooxidans* and the iron-oxidizing thermophilic bacteria is measured as the oxidation of ferrous iron.[58] For the sulfur-oxidizing bacteria, a decrease in pH or increase in titratable acidity is a measure of activity. The manometric technique[13,59,216] of measuring the uptake of oxygen as the bacteria oxidize the substrate is frequently used. Other methods of measuring bacterial activity include the monitoring of metal dissolution and analyzing for protein[60,216] or total cellular nitrogen.[216] The latter two procedures are affected by interference from inorganic ions, especially if colometric techniques are used. Turbidity is not often used to measure bacterial growth, as most substrates for the growth of chemoautotrophic bacteria are themselves particulate, or growth results in the formation of inorganic precipitates. Bacterial dry weight is not used for the same reasons. The direct counting of the chemoautotrophic bacteria is extremely difficult, because these bacteria are frequently attached to the inorganic substrates, making counting difficult at best, with resulting inaccurate results. Since the chemoautotrophs are difficult to grow with reproducible results on solid agar plates, the most probable number (MPN) method[13,61,168] must be done using serial dilutions of liquid medium. A severe limitation of this technique results when working with bacteria attached to solid substrates or entrapped in ferric iron precipitates. Representative samples cannot be made of the mineral particles or the microorganisms.

Enumeration of thiobacilli and the more recently discovered acidophilic, thermophilic *Sulfolobus*[62] and *Sulfolobus*-like[63] organisms is complicated by the attachment of these organisms to the sides of culture vessels and mineral substrates and the entrapment of these organisms in ferric iron precipitates.[64] Techniques for desorption from surfaces and release from entrapment have been attemped with limited success. [216,242] The culturing of mesophilic and thermophilic chemoautotrophs on solid agar plates has not been entirely successful. Although the thiobacilli can be cultured on agar, growth is sporadic and certainly undependable as a counting technique. *Sulfolobus* has been

cultured on yeast extract agar, but reproducible growth has not been obtained;[62] growth of other *Sulfolobus*-like organisms on solid agar has not been achieved.[232] It is certain that the inability to measure cellular numbers of the chemoautotrophic organisms has impeded research in this field. Several investigators have advanced the techniques for enumeration of these organisms.

A technique by Smith et al.[65,66] was adapted from methods used in aquatic systems for studying autotrophic carbon dioxide fixation. This adaptation allows examination of $^{14}CO_2$ uptake in soils by photosynthetic or nonphotosynthetic organisms. Gaseous $^{14}CO_2$ is added to soils, time is allowed for cellular incorporation of the labeled carbon, and incubations are stopped by adding perchloric acid. This technique is easily adaptable for field studies. Wet oxidations of the ^{14}C-labeled organic material are made; the $^{14}CO_2$ produced is transferred to a phenethylamine-liquid scintillation counting system. The technique has been one of the best methods for evaluating the activity of microorganisms in soil habitats. It has the advantage of ease of use in the field, and addition of gaseous $^{14}CO_2$ preserves the original sample moisture and ionic strength of the sample. The authors tested the procedure in coal-mine regions to evaluate the applicability of the technique for measuring the activity of iron-oxidizing autotrophs and in geothermal habitats to measure the activity of algae. However, results obtained from these studies were not compared with results using other techniques. Fliermans and Brock[67] used the isotope technique to study the ecology of sulfur-oxidizing bacteria in hot acid soils. A close correlation existed between viable counts of sulfur-oxidizing bacteria and the $^{14}CO_2$ incorporation. Soils with less than 300 of these bacteria per gram yielded $^{14}CO_2$-uptake values of less than 200 cpm/g with no correlation between $^{14}CO_2$-fixation and viable counts. The $^{14}CO_2$ test was applied to the determination of activity of the chemoautotrophic sulfur-and iron-oxidizing bacteria in column tests to study the leachability of low-grade chalcopyrite ($CuFeS_2$) ores.[68] In this instance, the technique was suitable for measurement of the activity of the mesophilic chemoautotrophs associated with the ore in the columns. There was general agreement between $^{14}CO_2$-uptake and *Thiobacillus ferrooxidans* counts, as determined by the most probable number (MPN) method using serial dilution in liquid medium. However, there was no correlation between the $^{14}CO_2$ uptake and *Sulfolobus* counts ascertained by MPN in liquid medium, since no $^{14}CO_2$ uptake was detected. These data contradicted tests run by Smith et al.[65] on sterile soils inoculated with *Sulfolobus*. The numbers of *Sulfolobus* encountered in the leach columns were considerably less than the 8×10^5 *Sulfolobus* which Smith et al.[65] used to inoculate their soil. The technique may simply not be sensitive enough to detect small numbers of thermophilic chemoautotrophs. Other factors may enter into this as well. It is known that organic material is used by these organisms, and it may well be that available organic carbon is assimilated in preference to carbon dioxide in some environments. The $^{14}CO_2$ technique also has the disadvantage of not differentiating between organisms which take up carbon dioxide;[65] however, it is one of the few suitable techniques available for determination of bacterial activity associated with solid substrates.

Tuovinen and Kelly[69] examined the development of *Thiobacillus ferrooxidans* colonies using membrane filters on ferrous sulfate agar. Membrane filters suitable for the technique included Sartorius ® and Millipore,® manufactured in Göttingen, Germany and London, respectively. Iron deposition occurred as *T. ferrooxidans* colonies developed; however, this was eliminated by maintaining the medium pH at 1.3. Adaptation was necessary for *T. ferroxidans* to resume normal growth rates at this pH. Toxicity of agar to *T. ferrooxidans* has been observed.[64,70] In the Tuovinen and Kelly[69] studies, *T. ferrooxidans* were separated from the agar by the membranes, but toxicity of some diffusible soluble agar component was noted. The authors[69] suggest that galactose produced by acid hydrolysis of agar is responsible. This technique offers a reproducible procedure for determination of viable numbers of *T. ferrooxidans*; only one strain of *T. ferrooxidans* was tested, and it is likely than other strains may have slightly different requirements. It will probably be necessary to establish optimum conditions for each strain of *T. ferrooxidans* used. This procedure will be of immense value in laboratory studies; however, it is likely that

not all strains found in leaching operations or other ecological habitats will grow on the membranes under a single set of conditions.

Determination of bacterial nitrogen has been used as a method of estimation of *T. ferrooxidans* concentrations.[64] This method cannot discern between bacterial nitrogen and nitrogen which precipitates as ammoniojarosite ($NH_4F_3(SO_4)_2(OH_6)$). Gormely and Duncan[71] reported a method whereby bacterial nitrogen is estimated as the difference between total nitrogen and inorganic nitrogen. Total nitrogen is determined by Kjeldahl digestion; inorganic nitrogen concentration (distillable nitrogen) is determined by steam-distilling a sample with caustic. The authors[71] correlated the nondistillable nitrogen with organic carbon (determined by organic carbon analyzer) and cell number (extimated with a Petroff-Hausser counting chamber). The nondistillable nitrogen content of 0.157×10^{-10} mg per cell agrees with that reported by Silverman and Lundren.[72] The cellular carbon concentration of 0.767×10^{-10} mg per cell corresponds to values obtained by Tuovinen and Kelly.[69,161] Gormely and Duncan[71] used their bacterial nitrogen procedure to determine that 65% of the *T. ferrooxidans* population is attached to a sphalerite concentrate during leaching. A similar study with chalcopyrite concentrate predicts a 95.6 attachment of the total *T. ferrooxidans* population. The obvious disadvantage to the bacterial nitrogen technique is its use in leaching environments, where it will measure the organic nitrogen of all organisms present. The method is also indirect and does not give a cell count. Another disadvantage is that this technique will also measure nitrogen-containing, organic by-products of microbial growth.

Schuler and Tsuchiya[73] used a Coulter Counter® for determination of cell number and size of *Beijerinckia lactocogenes* and three strains of *Thiobacillus ferrooxidans.* The ferric iron salts which precipitated as a result of *T. ferrooxidans* growth at pH values greater than 2.0 were dissolved with a mixture of 0.6% NaCl and 0.02% EDTA (disodium ethylenediaminetetraacetate dihydrate). Cell counts of the diluted cultures were made using Coulter Counters® with 30 μm apertures. Although no data were presented to support the supposition, the authors claim that mixed cell cultures can be enumerated, since each species would have its own cell size. The obvious disadvantage in using this device for enumeration of bacterial populations in natural environments is that species such as *T. thiooxidans* and *T. ferrooxidans* will not be differentiated because their cell sizes are similar. The authors[73] point out that the Coulter Counter cannot be used to determine cell numbers attached to mineral particles, as EDTA will not attack the particles as it does the ferric iron precipitate. A potential problem that the authors[73] encountered is that background pulse height on the Coulter Counter is nearly equal to the pulse height encountered for very small bacterial cells such as *T. ferrooxidans.* The technique will be of limited value in controlled laboratory situations and will probably have no application for field studies, since particles of similar size to bacteria must be absent. The cost of the apparatus is substantial, which will also discourage its use.

The fluorescent antibody (FA) staining technique was adapted for detection of *T. ferrooxidans.*[74] Anti-*T. ferrooxidans* immunoglobulin G(IgG) was prepared in rabbits and tested against 23 bacterial isolates. Fluorescence was observed with iron-grown and sulfur-grown *T. ferrooxidans,* and slight cross-reaction was noted for two unrelated bacterial species; the FA stain did not react with *T. thiooxidans.* To test the applicability of the procedure for environmental samples, coal refuse was FA-stained. Nonspecific fluorescence (absorption of fluorescent-conjugated compounds by mechanisms other than immunological reactions) and autofluorescence (natural fluorescence of compounds by exposure to UV light) were problems in these natural specimens. This fluorescence could be suppressed by rhodamine isothiocynate (RITC)-conjugated bovine serum albumin (BSA). When the FA stain was applied directly to coal refuse particles, no *T. ferrooxidans* were observed. The authors[74] deduced that the cell density would have to be greater than 10^8 cells per gram to observe one cell per microscopic field. To alleviate the problem, refuse samples were washed with pH 3 sulfuric acid solution, and cells in the supernatant were concentrated by centrifugation. The washed refuse was ground, sonicated to remove attached organisms, and washed with acidified, distilled water to release attached *T. ferrooxidans.* This

liquid was then centrifuged to concentrate the organisms. Smears were prepared from the resuspended pellets of each washing, and the cells were stained and enumerated. *T. ferrooxidans* were observed in the surface washings of the refuse, but none were detected in the washings from the disrupted and sonicated refuse samples. Scanning electron microscope (SEM) observation indicated that the samples were irregular, with pores large enough to accomodate *T. ferrooxidans*. The authors[74] suggested that the paucity of *T. ferrooxidans* in washings of the ground and sonicated samples resulted from bacteria unable to be washed from pores. The immunoflorescence technique has been successfully applied to the study of *Sulfolobus* in hot acid springs.[75] Microscope slides that had been immersed in hot springs were FA stained; distinct serologic types of *Sulfolobus* were identified, and doubling times of these organisms were established.

Studies on the attachment of *Thiobacillus ferrooxidans* to mineral samples indicate that the association is quite tenacious[64,76] and that bacteria are found in pitted and eroded surfaces of mineral particles.[77] The FA technique appears to have considerable applicability in the study of chemoautotrophic bacteria and mineral particles. A limitation seems to be the autofluorescence and nonspecific fluorescence resulting when the FA stain is applied directly to mineral samples. Although *T. ferrooxidans* were easily removed by washing the refuse samples with acidified distilled water,[74] it cannot be assumed that other chemoautotrophs can be so readily removed from all mineral specimens. Apel et al.[74] tested the FA stain on only one strain of *T. ferrooxidans;* further experiments should be conducted to confirm the cross-reactivity among a number of *T. ferrooxidans* strains.

In 1947, McElroy[78] discovered that the luminescence of the firefly had an absolute requirement for adenosine triphosphate (ATP). From this initial discovery, it was reported that ATP could be used as a reliable indicator of biomass. In vitro light production by firefly lantern extracts has been shown to be dependent upon luciferin (an oxidizable substrate), the enzyme luciferase, oxygen, magnesium, and ATP. For each molecule of ATP hydrolyzed, one photon of light is emitted allowing for quantification of sample ATP by spectrophotometric means. To obtain the amount of ATP present, the amount of light per unit time is measured, and this value is compared with values obtained when ATP standards are injected into the enzyme preparation. ATP photometers are available which automatically integrate the light emitted over a certain period of time and produce a direct read-out of the integrated values. It is possible to detect 10^{-10} mg ATP. ATP measurement of biomass has been applied to aquatic and terrestrial samples, activated sludge, and sediment samples.[79]

The ATP photometer method is currently being developed for enumeration of the chemolithotrophic bacteria.[233] It is obvious that the limitation of this technique is its inability to differentiate among the bacterial species encountered in the natural environment.

Attachment

Controversy has long existed regarding the role of the chemolithotrophic bacteria in minerals' dissolution. Many investigators support the notion that the bacteria only indirectly aid in oxidation of sulfide minerals through the generation of the oxidant, ferric iron. Others suggest that the bacteria are more directly involved in attack of the mineral lattice. Studies to solve this difference of opinion have generally been impeded by the difficulties in observing the chemolithotrophic bacteria. Much of the information is obtained by oxygen uptake, iron oxidation, and metal release. It has only been in recent years that researchers have looked at the minerals directly and studied the attachment of bacteria to mineral particles.

Early investigators noted the direct contact between *Thiobacillus thiooxidans* and elemental sulfur[55] and the tenacity with which *T. thiooxidans* was attached to sulfur.[80] This association was considered a requisite for sulfur oxidation.[81] Schaeffer et al.[57] prepared carbon replicas of sulfur crystals before and after attack by *T. thiooxidans* and showed that the organism eroded the sulfur crystal in the vicinity adjacent to the bacteria. There was no direct evidence to explain the stability of the attachment, and the authors suggested that the association was chemical rather than physical. Others [82,83] related attachment to a variety of

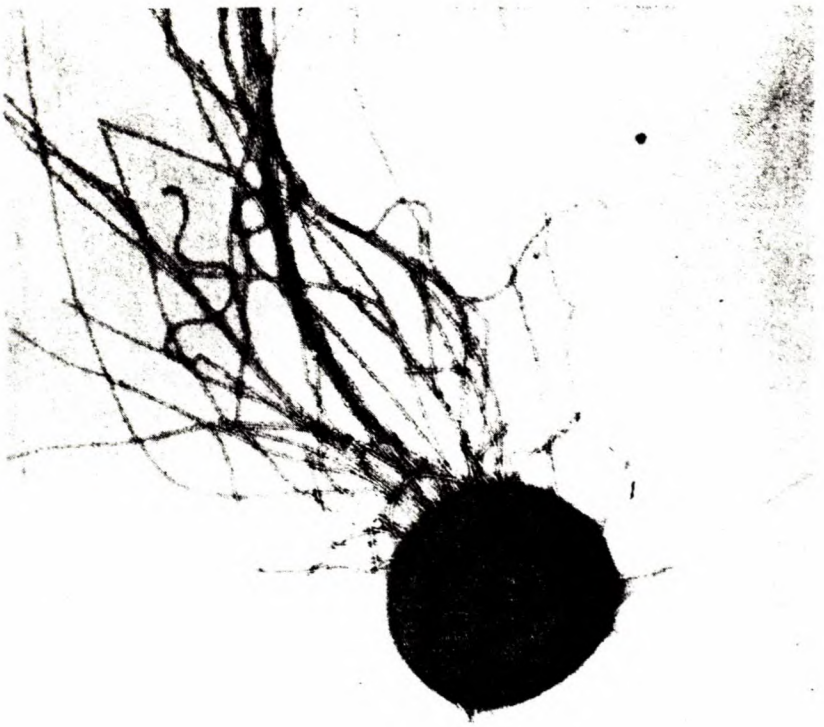

FIGURE 3. Pili of *Sulfolobus* on bacteria attached to sulfur in a flowing acid hot spring, pH 2.3; 75°C. (From Weiss, R. L., Attachment of bacteria to sulphur in extreme environments, *J. Gen. Microbiol.*, 77, 501, 1973. With permission of Cambridge University Press.)

holdfasts which bacteria, although not specifically *Thiobacillus*, possess.

The advent of and sophistication in instrumentation has opened the way for new methods of studying microbial/mineral interaction. In recent years, the transmission electron microscope (TEM) and scanning electron microscope (SEM) have experienced increased use in this study. The following section examines some of these recent studies. It is obvious that only cursory studies have been conducted, but it is anticipated that future work will see a greater and better correlation between the biological observations made by instrumentation and the chemistry of leaching.

Weiss[76] examined the attachment of *Sulfolobus*[62] to sulfur. He characterized the attachment and noted its occurrence in laboratory and field environments. *Sulfolobus* attached to elemental sulfur by means of pili which were adhesive, acid- and heat-stable, and generally irregular in shape (see Figure 3).

Observations of pili were made using a TEM. Although the bacteria could be observed by using the SEM, the pili could not be seen because these filaments are below the resolution of this instrument. When *Sulfolobus* was initially grown in the laboratory on a basal medium with 0.1% yeast extract, an abundance of pili was noted upon attachment to sulfur. However, transfers of the organisms in basal medium with sulfur and trace elements without yeast extract resulted in a reduced number of pili and an accompanying inability to attach to sulfur. Subsquent addition of 0.01% yeast extract to the culture did not enable the cells to attach to sulfur; however, culturing of *Sulfolobus* in basal salts with 0.01% yeast extract resulted in the organism's ability to once again attach to sulfur. Weiss[76] correlated the ability and inability of bacteria to attach to sulfur with the numbers and lengths of the pili. When attached to sulfur, most of the bacteria possessed one to three pili which were 1 to 2 μm long; bacteria unable to attach to sulfur had one to two pili usually less than 0.25 μm long. Shivvers and Brock[84] observed that during the first 3 to 6 days of growth of *Sulfolobus* on elemental sulfur the number of unattached organisms exceeded the number of attached organisms.

After 6 days of growth, the number of unattached organisms decreased, and after 17 days, the sulfur crystals were covered with *Sulfolobus*. There was also a direct correlation between unattached organisms and sulfur oxidation; as attachment increased, the sulfur oxidation increased. Weiss[76] noted that in bubbling pools bacteria rarely attached to sulfur, and pili were not observed on these cells. In flowing springs, sulfur crystals had large numbers of cells attached, often several layers thick, and these bacteria possessed many pili. Interestingly, the attached bacteria were often separated from the sulfur particle by a short distance. Upon examining the attachment to glass slides of organisms in flowing springs, Weiss[76] concluded that the bacteria attached by adhesion rather than by pili. The most important conclusion which Weiss made was that attachment to sulfur by *Sulfolobus* is not requisite for sulfur oxidation. It has been suggested that *T. thiooxidans* must attach to sulfur before oxidation occurs and that wetting of the sulfur results.[57] In the low-pH and high-temperature habitats of *Sulfolobus*, wetting occurs naturally.[76]

While pursuing ultrastructure studies on the extremely acidophilic, thermophilic microorganisms, Millonig et at.[85] reported the observation of pili on organisms isolated from volcanic hot springs near Naples, Italy. These pili were observed on cells which were heterotrophically grown on yeast extract in either agitated or stationary cultures. The mode of attachment to a solid substrate was not studied.

Duncan and Drummond[86] investigated direct bacterial attack on sulfide minerals by examining bacterial action on metallic and silicate phases of column-leached, copper sulfide ore. Pyrite grains leached in several leach liquors were observed for bacterial degradation. In the column-leaching experiment, chalcopyrite ($CuFeS_2$) was weakly leached and molybdenite (MoS_2) appeared unleached. These data are in agreement with published data on the resistance of these minerals to leaching.[11] Pyrite was deeply leached, particularly along crystallographic directions and fractures. The authors[86] suggested that chemical attack on pyrite may occur "selectively" at such locations but that microbial attack was direct and not necessarily limited to crystal direction nor imperfections. Pyrite grains were subjected to several leach liquors in petri dishes to model results obtained in the large leach columns. In the modeling experiments, erosion of pyrite grains occurred only in the presence of *T. ferrooxidans*. Although ferric iron was added to several of the experiments, pitting of the pyrite did not occur unless *T. ferrooxidans* were present. The authors[86] suggested that ferric iron did not erode the pyrite and that pitting of the surfaces was manifested by *T. ferrooxidans* only. It was noted that not all portions of the pyrite grain were subjected to the bacterial attack and that preferential attack may be associated with bacterial production of surface-active agents which allow bacterial attachment. The authors[86] did not elaborate on the techniques used for sample preparation; however, it is unusual that attached bacteria were not observed when pyrite grains were examined with the SEM. Studies such as these have considerable value in establishing the role of bacteria in leaching, but credence would be added if these studies were supported with chemical and biological data. For example, analyses of iron species would indicate whether pyrite was actually solubilized, and an estimation of bacterial numbers would clearly indicate the development of organisms. The authors[86] suggested that *T. ferrooxidans* may not have developed in pH 2 distilled water due to a nitrogen deficiency; however, there is now evidence that some *T. ferrooxidans* are able to fix atmospheric nitrogen.[21]

The SEM was used to examine the attachment of *T. thiooxidans* and *T. denitrificans* to elemental sulfur.[87] Membrane filters were coated with colloidal sulfur prepared by acidifying a sodium thiosulfate solution. The organisms were filtered onto the membranes. The filters were placed on agar plates for growth of *T. thiooxidans* and in test tubes to obtain growth of *T. denitrificans*. After adequate growth, the filters were fixed for SEM observation and affixed to specimen stubs. *T. thiooxidans* readily attached to the sulfur crystals, and the investigators[87] observed the attachment to be very tenacious, because a mortar and pestle were required to remove the organisms. Baldensperger et al.[87] did not speculate on the nature of the attachment but noted that the association of *T. denitrificans* with sulfur was less tenacious than that of *T. thiooxidans* with sulfur. There also appeared to be some

separation between *T. denitrificans* and the sulfur. Although the cells were sunk into the sulfur, they appeared to be bridged to the energy source. This latter phenomenon was also observed by Weiss[76] in his study of the attachment of thermophilic, chemoautotrophic microorganisms in flowing sulfur springs.

It was observed by Baldensperger et al.[87] that the sulfur layer on the membrane filter which had supported the growth of *T. denitrificans* often flaked off. This was attributed to nitrogen production by *T. denitrificans,* but no studies were conducted to confirm this supposition. The membrane filters upon which *T. denitrificans* had grown were treated with lead acetate to determine if sulfide was an intermediate in the oxidation of sulfur. Although the evidence of lead sulfide production was not conclusive, the investigators[87] did suggest that sulfur oxidation by *T. denitrificans* proceeded by a sulfide pathway.

A direct observation of a thermophilic, *Sulfolobus*-like[63] organism on molybdenite fines was made using the SEM.[88,89] These specific observations did not clearly indicate attachment, but did suggest the formation of microcolonies by the organism. Using a nondispersive, X-ray, microanalysis attachment, it was determined that molybdenum was not accumulated by the organisms grown on molybdenite fines. The attachment of *Sulfolobus*[62,63] has been further explored. One study by Berry and Murr[90] attempted to correlate associated mineral properties of molybdenite with the attachment of *Sulfolobus*. The question was asked whether the attachment of the organism was random or whether it occurred with relation to ionic character, chemistry of the mineral surface, or crystal energetics. However, this specific study only dealt with a related factor, crystal dislocations. Chemical and biological attack along mineral fractures and crystallographic planes had earlier been proposed by Duncan and Drummond.[86] Berry and Murr[90] prepared thin sections of molybdenite which were incubated in a medium with *Sulfolobus,* and observations of the attached organisms were made with a TEM. The investigators attempted to correlate the attachment of the thermophilic organism on the cleaved section of molybdenite with the emergence of dislocation lines on the crystal surface. The authors[90] said that although there appeared to be some correlation between bacterial attachment and the density of dislocations, there was not a strong enough correlation to implicate this as the controlling factor. Dislocations, or structural defects in a crystal, are associated with sites of activity such as crystal formation and lattice vacancies. Stress and strain energy is stored in these regions, and energy changes can and do occur.[91] Although the authors[90] suggest the possibility that energetics of dislocation play a role in selective attachment of bacteria to these regions, it is unlikely that the bacteria are able to harness the stress and strain energy of such defects. Since dislocations are the active site of crystal changes, it does seem possible that chemical and/or biological attack on the crystalline structure may be more effective at these defect points. The thin sections of molybdenite used in this work were taken from "mineral samples." It is unfortunate that the samples were not chemically and mineralogically defined, because such information could shed considerable light on some of the observations. If the samples contained impurities, some of the dislocation lines observed by the workers may be related to the gangue phases rather than the ore-mineral phases. It is also likely, in preparing thin sections of molybdenite, that defects could be introduced or existing dislocation altered. These factors may explain the inability to correlate the defect density and attachment density. The authors[90] claimed that biogenic oxidation of molybdenite resulted in an end product of pentavalent molybdenum. There are no experiments described by the authors to support this claim. It is unlikely that pentavalent molybdenum is the end product, since subjecting spent medium to a reducing agent after oxidation of molybdenite by *Sulfolobus* yields pentavalent molybdenum, suggesting that much of the molybdenum end product is the more oxidized, hexavalent molybdenum species.[232]

Murr and Berry[77] expanded on earlier observations[88,89] of the attachment of the thermophilic, acidophilic microbes to chalcopyrite. The organisms, *Sulfolobus* strains,[62,63] were maintained in basal salt solutions with 0.02% yeast extract and colloidal sulfur. Low-grade chalcopyrite ore, varying in size from 2 to 6 mm, polished on two sides and cleansed thoroughly, was incubated with cells in basal salts

media with 0.02% yeast extract. After varying incubation periods, ore specimens were removed from the media, rinsed lightly with distilled water, air-dried, and sputter-coated in preparation for SEM observation. From the results of this study, the authors[77] drew the following conclusions:

1. The observations strongly support the direct contact mechanism for bacterial leaching.
2. There is evidence of preferential attachment of bacteria to phases containing reduced iron and sulfur.
3. Attachment corresponds with the dissolution of iron and copper.
4. Surface area is important in bacterial attachment, since increased surface exposure provides greater area for attachment.
5. There is evidence for the production of biomatter at bacterial attachment sites.

The question of preferential attachment to mineral phases containing energy substrates is very interesting and very difficult to substantiate. The authors'[77] use of the phrase "selective attachment" implies that the organisms are attracted only to mineral phases containing oxidizable substrates. This would indicate that chemotaxis is operable, and this was not demonstrated. Indeed, the organisms may randomly attach to all phases of the sample, but because of the lack of oxidizable substrate in siliceous regions, the organisms die and shed from the sample. Murr and Berry[77] claim that after 18 days of incubation the bacteria are exclusively attached to phases containing reduced sulfur and iron and further suggest that the organisms derive their energy from these solid substrates. Weiss,[76] however, was able to demonstrate attachment of *Sulfolobus* to glass slides (siliceous phase) in flowing spring environments. It would be most beneficial to determine if *Sulfolobus* strains in Murr and Berry's experiments would attach to a glass slide if immersed in the basal salts with the yeast extract and the mineral particles. Weiss[76] demonstrated that while bacterial attachment was not essential for sulfur oxidation, direct contact between the bacteria and the particle did enhance sulfur oxidation. On the other hand, Murr and Berry concluded that contact between the sulfide mineral and the bacteria was probably essential for biogenic oxidation. The medium used for these investigations[77] contained yeast extract, which can also serve as an energy source for *Sulfolobus*.[62] The addition of yeast extract could seriously confuse the results, as this provides the organisms with a soluble energy source. All observations were made of ore minerals which had been incubated with bacteria. In view of problems encountered during drying and vacuum sputtering of samples which yielded distortions of the organism (see Figure 4), it is suggested that micrographs of uninoculated ore samples should have been observed for comparison. The authors[77] indicated that the bacteria were attached to the ore-mineral phases by adsorption. Weiss[76] noted that *Sulfolobus* in flowing springs and under certain laboratory conditions were attached to sulfur by pili and explained that pili were not within the resolution of the SEM. From this information, it is suggested that attachment should be determined by TEM as conducted by Weiss.[76] It is likely that Murr and Berry simply couldn't observe the pili using the SEM. It is evident from the work of Weiss[76] that growth conditions, i.e., the presence or absence of yeast extract, greatly affects the production of pili by *Sulfolobus* and the organism's ability to attach to solid surfaces. Weiss[76] found the greatest piliation among cells cultured in basal salts and sulfur supplemented with yeast extract. Murr and Berry[77] also cultured their organisms with yeast extract, and it is therefore quite possible that their bacteria would possess pili. The authors[77] concluded that attachment corresponds to the dissolution of iron and copper. This conclusion was not supported with any data on the total number of bacteria present in the culture flask, the number of bacteria attached to the mineral, and the amount of iron and copper solubilized from the ore. An interesting experiment to establish the necessity of attachment for metal dissolution would be to isolate a mineral sample from bacteria by a semipermeable membrane and quantify the rate of metal dissolution.

It cannot be denied that increased surface area increases the amount of area available for bacterial attachment; however, increased surface area also provides greater area for chemical attack of the mineral. Therefore, it cannot be unequivocally stated that leach rates are en-

FIGURE 4. Scanning electron micrograph of pyrite phase with attached *Sulfolobus*-like bacteria.(Magnification 11,000. Each organism ca. 1 μm in diameter). From Murr, L. E. and Berry, V. K., *Hydrometallurgy*, 2, 11, 1976. With permission.)

hanced merely by providing greater area for biogenic attack. It must be proven by extraction data.

Murr and Berry examined a turquoise specimen with the SEM. It is unclear from the publication[77] whether this specimen was selected from nature or whether it was subjected to bacteria in the laboratory. The attachment of a microbe, described by the authors as being similar to *Thiobacillus ferrooxidans,* was characterized as adsorptive with the production of bio-matter. This work appears highly speculative, as there was no identification of the organism, nor is the nature of the specimen clear from the text. It is possible that the material could be an inorganic precipitate, such as jarosite.

Murr and Berry[92] examined thin sections of chalcopyrite using the TEM as they had for molybdenite.[90] Problems in obtaining very thin sections of the ore were encountered, so no correlations were made regarding the emergence of dislocation sites and the attachment of *Sulfolobus* to these sites. The authors[92] supplemented the work with SEM micrographs of *Sulfolobus* attached to chalcopyrite. It was concluded that the organisms were attached adsorptively. The investigators did not define their method of growth of the organism, but culture conditions are likely to affect attachment mode. It is also probable that shaking vs. stationary culture flasks will affect attachment, and the use of percolation columns may also influence the association of microorganisms to mineral particles.

The advent of the transmission and scanning electron microscopes has increased the ease with which microbial attachment studies can be made. Initial interest in bacterial/mineral interaction stems from the desire to understand the metabolism of these organisms, i.e., to explain their ability to oxidize solid substrates. The tenacity with which these organisms attach to solid surfaces also sparks interest in deciphering the mechanism of attachment. The difficulty of

enumeration of the organisms due to the attachment to solid surfaces has undoubtedly led to innovative approaches in both determination of cell numbers and identification of organisms, but recent interest in bacterial attachment is probably more economically related. A better understanding of the mechanism of bacterial action on metallic sulfides may lead to better and more effective methods for dissolution of metals. The study of microbial interaction at solid surfaces has not been confined to the study of the acidophilic, chemoautotrophic bacteria.[93,94] Informative studies on heterotrophic bacteria have been conducted on the orientations of bacteria at the interfaces of two-phase systems, electrostatic phenomenon, cellular hydrophobicity, and the nature of cellular attachment. Some of the techniques and results of these studies should be considered in elucidating the microbial/mineral interaction.

The attachment of marine *Pseudomonas* to Millipore filters in laboratory culture indicates that interaction is mediated by polymers.[95] A primary polysaccharide is apparently responsible for initial attachment, with the formation of a secondary polysaccharide later which secures the attachment (see Figure 5). Studies with the marine *Pseudomonas*[96] have indicated that this bacterium prefers attachment to surfaces of low energy and a low negative charge. Clumping of cells with low attachment suggests a cell-to-cell attraction rather than a cell-to-surface affinity. This might suggest that observation of *Sulfolobus* microcolonies noted on MoS_2[88] were demonstrative of clumping as a result of cell-cell attraction rather than propagation. An exception to the attachment of *Pseudomonas* to surfaces of low energy and low negativity was its attachment to platinum, which is positively charged with high surface energy.[97] Fletcher and Loeb[96] contrasted this preference of *Pseudomonas* to that of cultured tissue cells, which show enhanced growth on negatively charged surfaces of high energy.

Fletcher[98] questioned whether bacteria attach to substrates randomly or whether a physiological response by the bacterium is required. Attachment of *Pseudomonas* to polystyrene was influenced by the cell concentration, the length of time allowed for contact to the substrate, temperature, and age of the organism. Since Fletcher found that bacterial adhesion could be modeled similarly to molecular adsorption, she suggested that initial bacterial attachment may be controlled by nonbiological phenomena.

Marshall and Cruickshank[94] observed the orientation of several microorganisms in solid-water systems. *Flexibacter auranticus* does not contact the solid surface but becomes anchored by extracellular adhesive material. Figure 6 is an electron micrograph of a thin section showing the attachment mode of the organism to an araldite block and the presence of holdfast material. It is probable that the techniques described by Marshall and Cruickshank[94] could be applied toward observing attachment phenomenon between microbes and solid mineral substrates. Studies on the relationship between the chemolithotrophic bacteria and mineral particles have relied heavily on the SEM and TEM. It is essential to correlate the micrograph observations with chemical data on the dissolution of metals and the ferrous and ferric iron production. With improved techniques in enumeration of the chemoautotrophs, it is now possible to obtain with a better degree of accuracy the number of organisms attached to the mineral particles vs. the concentration of unattached organisms. There have definitely been strides in observing attachment, but little has been resolved in understanding the attachment mechanism or its significance to metal dissolution.

MICROBIAL LEACHING OF URANIUM

In the past 4 years, there has been a four fold increase in uranium prices. Commensurate with the demand has been increased interest in hydrometallurgical recovery of uranium, particularly from low-grade deposits.[99]

Some deposits, particularly those in sands which have controllable permeability, are most amenable to solution mining. These include uranium deposits in Texas and Wyoming.[6] The use of *Thiobacillus ferrooxidans* to produce ferric iron and sulfuric acid for dissolution of uranium has been studied primarily for the Elliot Lake (Ontario) ores or other uranium ores containing pyrite.[31,32,100] This bacterial process has been used successfully to recover uranium from mined-out areas as a scavenger operation.[29,46-48] Agnew Lake Mines Ltd. in northern

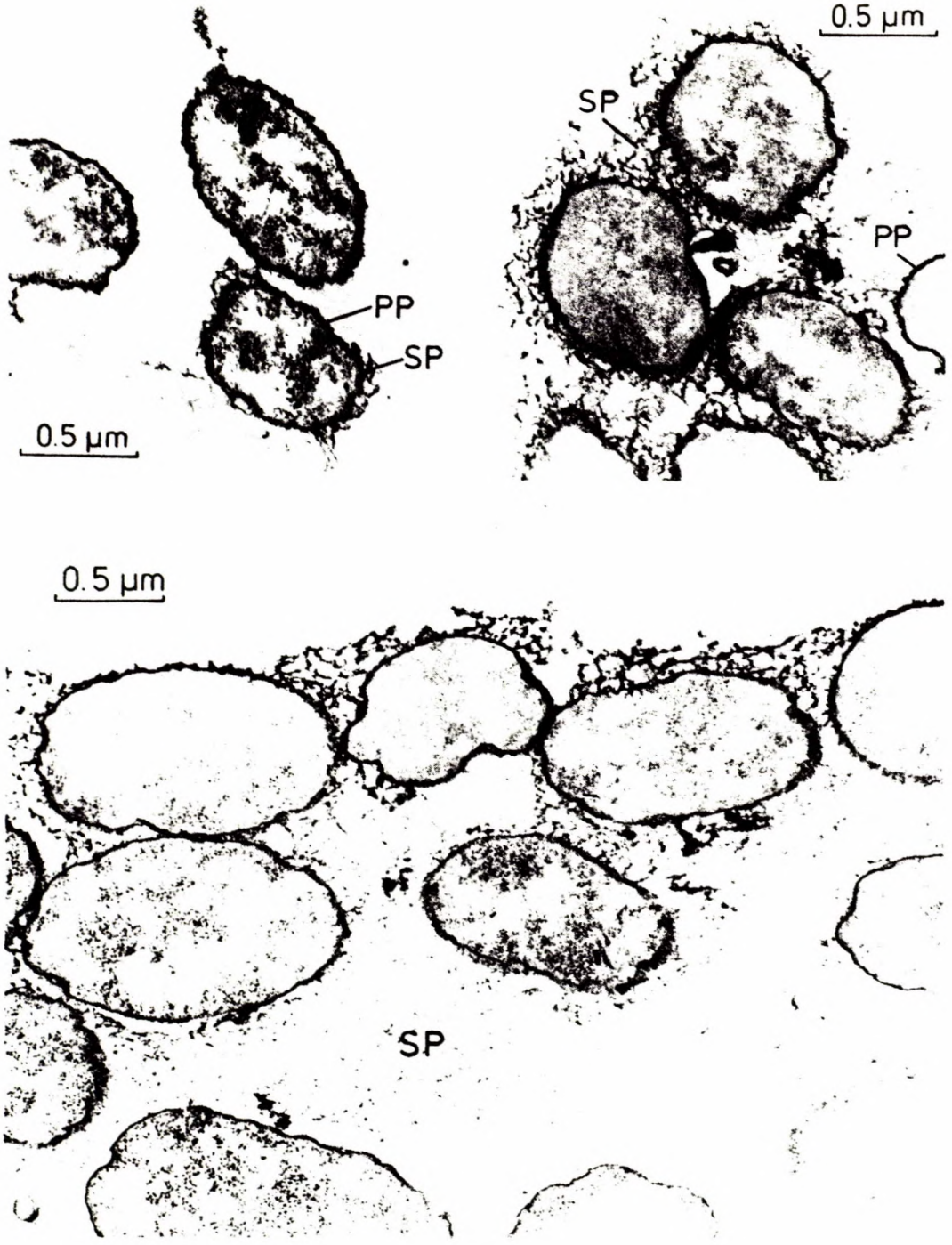

FIGURE 5. Sections from bacterial films showing *Pseudomonas* and their associated primary (PP) and secondary (SP5 polysaccharides. Top: The increase in the amount of secondary polysaccharide is apparently related to an increase in cell number. Bottom: Primary polysaccharide is eventually replaced by secondary polysaccharide, which forms an intercellular matrix. (From Fletcher, M. and Floodgate, G. D., in *Microbial Ultra-structure — the Use of the Electron Microscope*, Fuller, R. and Lovelock, D. W., Eds., Academic Press, London, 1976, 101. With permission.)

FIGURE 6. Electron micrograph of a thin section of an embedded araldite block showing the extracellular fibrous material anchoring *Flexibacter aurantiacus* to the solid surface. (Magnification × 130,000.) (From Marshall, K. C. and Cruickshank, R. H., *Arch. Mikrobiol.*, 91, 29, 1973. With permission.)

Ontario announced that it will be the first to use bacteria as the principal means of extracting uranium.[100] This operation was expected to be on-stream in early 1977. The Agnew Lake Mines ore is amenable to bacterial leaching, with the primary uranium ore being uranothorite ($(Th,U)SiO_4$) which is readily soluble in weak acid. It is anticipated that a million pounds of U_3O_8 per year will be produced. The operation consists of blasting underground stopes to break the ore. The "swell" due to the explosion will be leached on the surface with acidic ferric sulfate generated by *T. ferrooxidans*. The underground ore will be leached by percolating the solutions through the ore. Preparatory to the operations, bench-scale studies and field tests were conducted to establish the economics. The process will be viable if 70% of the uranium is recovered in 1 year from run-of-the-mine ore. Simulated tests indicated this was possible with ore less than 8 in. in diameter. The effects of the organic compounds used in the solvent extraction circuit for uranium recovery were tested on *T. ferrooxidans*. Although Alamine 336, a tertiary amine, adversely affected ferrous iron oxidation by the bacteria, it was suggested that dilution of the solvent encountered in the overall circuit was enough to minimize the effects.

An in-plant process which uses bacterial leaching to treat ores has been described by Derry et al.[101] In this process, pyritic uranium ores of less than 3 mm particle size are placed in beds 5 m long and 23 cm in diameter and heated to 50°C. An externally-generated lixiviant, pumped into the base of one bed, is allowed to overflow into a surge pot and is pumped to the inlet at the base of the next bed. The plant operates with five beds of ore leaching at one time. The flow rate of the leach liquor through each bed is 180 l/day. The leached beds are washed with a three-stage countercurrent wash procedure, and a portion of the wash liquor is used for leaching. The water balance is maintained. The uranium is removed by ion exchange, and the barren liquor passes to the lixiviant generator at a rate of 140 l/day. Iron-oxidizing bacteria regenerate ferric iron at 30°C in an aerated generator. After 2 days, 95% of the ferrous iron is oxidized and the Eh is + 450 mv. The lixiviant contains 0.22 *M* (12 g/l) ferric iron, but acid must be added to the regenerated lixiviant to avoid precipitation of jarosite. Additional iron is also generated by the reaction of ferric iron with pyrite, but some iron is lost due to jarosite precipitation. At a pilot scale level, 95% of the uranium can be extracted from ore containing 0.12% U_3O_8. The process is economical because the lixiviant is bacterially generated and acid consumption is low. The ore can be treated while having a fairly coarse particle size, thus avoiding fine grinding. The separation of leach liquor from ore is facilitated because of the coarse ore size, which avoids the conventional filtration or sedimentation step. Derry et al.[101] estimate a cost savings of $2/kg as compared with conventional processes. The process does require con-

siderably more space, and the most obvious cost is the heating of the ore and the leach liquor. With discoveries of thermophilic bacteria[62,63,102] which oxidize iron, it may be possible to generate ferric iron at 50°C and avoid reheating the leach solution.

The bacterial leaching of pyritic uranium ores is both feasible and apparently economical. Further aspects of uranium leaching by the iron-oxidizing bacteria were reviewed by Tuovinen[32] in 1972. He examined the toxicity of uranium, the effect of organic solvents on *T. ferrooxidans,* and the role of pyrite in the leaching of uranium.

Uranium ores found in Texas, Wyoming, and New Mexico are less amenable to bacterial leaching.[103] The mineralogy of the Grants uranium belt in New Mexico, which presently supplies 40% of the U.S. uranium requirements, is quite diverse. The uranium in the reduced form is usually associated with organic matter and is either coffinite ($U(SiO_4)_{1-x}(OH)_{4x}$) or uraninite (UO_2). The oxidation of these minerals forms uranium silicates or phosphates. In the presence of vanadium, the uranium forms uranium vanadate minerals. When mining commences, local oxidation of uranium occurs, and evaporite minerals form.[16] Minor elements associated with uranium include molybdenum, vanadium, and selenium. Often, the host rocks are acid-consuming. Little research has been conducted in the area of bacterial leaching of these uranium minerals, and the mineralogy of the ore is not compatible with current bacterial leaching technology. Much of the uranium ore in these areas is deposited in the roll front configuration, i.e., ground waters become reduced, and uranium precipitates out. Uranium mineralization is localized at the interface between the two zones.[6] In the Grants uranium belt, deposits occur in close association with organic material.[16] The organic material associated with the uranium ore was assumed to be humic acids, and few studies have closely examined its nature. The material is highly insoluble and nearly opaque to visible and infrared spectra.[104] The uranium is associated with highly substituted, polyaromatic compounds, and dimethylsulfoxide-extractable matter has a spectra resembling coal.[234] When the host rock contains acid-consuming gangue, uranium is leached by ammonium carbonate-ammonium bicarbonate solution, using either oxygen or hydrogen peroxide as an oxidizing agent. Otherwise, sulfuric or nitric acid is used, to which sodium chlorate is added to oxidize the ferrous iron which in turn converts tetravalent uranium to hexavalent uranium.[50,52] It is likely that much of the oxidizing agent is used to oxidize the associated organic matter. Studies of bacterial leaching of Grants uranium ore[103] indicate that neither the thiobacilli nor the thermophilic, chemoautotrophic bacteria enhance uranium dissolution. It is probable that insufficient energy sources are available for the organisms. Even when the ores are supplemented with oxidizable, inorganic substrates, the organisms appear to lose viability after a 10-day period in contact with the ore. This suggests that components of the ore are toxic to the microbes or environmental conditions within the ore are unsuitable for bacterial development.[103]

Leaching of uranium ores such as those found in Texas, Wyoming, and New Mexico will require bacterial leaching technology of a nature different than is presently available. The use of organisms other than *T. ferrooxidans* is one of the more promising prospects. In the following sections, work that has been published on organisms found in uranium deposits is reported, and other recently studied microbes which may eventually play a role in the extraction of uranium are examined.

In 1972, Updegraff and Douros[105] examined uranium ores and associated sediments from Grants, New Mexico, Gas Hills, Wyoming, and Uravan, Colorado for microorganisms. The purpose of the study was to establish a correlation between uranium distribution and the microbes present to determine if microorganisms could be used as a geomicrobiological prospecting tool. High-grade uranium ore samples and barren background samples were collected aseptically. Qualitative broad spectrum analyses were completed for the New Mexico and Wyoming samples. Using stationary and agitated culturing techniques, 13 different media at four different temperatures were used. Anaerobic cultures were also prepared. Quantitative counts were made on samples from Uravan. No obligate or facultative anaerobes were isolated, and thiobacilli were not present in the samples. Fungi were found only occasionally, with *Penicillium* being the most frequent isolate. Bacte-

rial isolates included *Arthrobacter, Bacillus,* and *Streptomyces.*

The authors[105] concluded that the uranium ore samples had very few organisms present, and there appeared to be little difference between samples high in uranium and barren samples in terms of the numbers of types of organisms present. The authors made no decision as to the source of microbes, whether they were the result of percolating ground water or were introduced by recent mining operations. There appeared to be no correlation between the numbers and types of microbes and the presence of uranium.

Although this particular study[105] was primarily aimed at examining biogeochemical prospecting tools, research may be of value to the extraction of uranium. *Arthrobacter,* the most abundant microbe found in the study, has some species which have the capability of decomposing phenolic compounds, lignin, and cellulose. Likewise, *Penicillium,* the predominant fungus, degrades humic acids. It is possible that these organisms could be used to break down the organic matter in uranium deposits to release uranium, or at least make the uranium more easily oxidized.

Magne et al.[106] studied the solubilization of uranium by microorganisms other than thiobacilli. The authors studied ores containing up to 5% uranium, nonweathered granite with 0.08 m*M* uranium, and weathered granitic sand containing 0.04 m*M* uranium. Five times as much uranium could be solubilized from ore containing 5% uranium using complex, heterotrophic microflora compared with sterile controls. However, the actual percentage extracted from the ore was very low for the 28-day leach period. According to the authors,[107] it is not likely that heterotrophic organisms can be used on an industrial scale to leach uranium, since uranium is toxic to these organisms. The uranium extraction rates are low, and the economics are simply not favorable.

It has been proposed that *Thiobacillus denitrificans* may be useful in uranium leaching.[108] *T. denitrificans* grows chemolithotrophically at neutral pH values on thiosulfate or tetrathionates using either oxygen or nitrate as an electron acceptor. When nitrate is used as the electron acceptor, diatomic nitrogen is the end product, and it has been suggested that gas production may facilitate the movement of solutions in the leaching environment. The oxidation of reduced sulfur compounds by *T. denitrificans* may create an environment suitable for the development of *T. ferrooxidans* and *T. thiooxidans.*

A group of organisms which has not previously been considered in metals extraction is the iron bacteria. *Gallionella* and *Leptothrix* have been extensively studied, but *Metallogenium* species which oxidize iron and manganese are not as well characterized. An acid-tolerant, iron-oxidizing *Metallogenium* was isolated by Walsh and Mitchell[109] from coal and zinc mine drainages and streams. The organism has a wide pH range (3.5 to 6.8) for growth. However, a ferrous iron concentration of 1.8 m*M* (100 mg/l) is toxic unless phthalate is present to complex the iron. The autooxidation of iron in the pH range 3.5 to 6.8 is substantial; but studies by the authors[109] show that the iron oxidation rate is 24 times greater when *Metallogenium* is present. The metabolism of the organism has not been defined. Although either ferrous iron or organic matter can serve as an energy source, ferrous iron must be present for growth.

Further work by Walsh and Mitchell[110] suggests that *Metallogenium* may be involved in a succession of microorganisms which contributes to the production of acid in coal mine waters. The abiotic oxidation rate of iron in the mesoacidic range (pH 2.5 to 4.5) is not enough to produce sufficient ferric iron for reaction with pyrite to yield pH values as low as those usually encountered in coal mine waters. The authors[110] suggest that *Metallogenium* is responsible for oxidizing iron and creating an environment suitable for *Thiobacillus ferrooxidans.* Very few studies have been conducted to establish the ecological niche of this organism, its metabolic functions, and its tolerance to metals. In the leaching of uranium, organisms like *Metallogenium* may be used to produce an oxidizing agent for uranium dissolution in the mesoacidic range.

It has been demonstrated that *Thiobacillus*-like organisms metabolize dimethylsulfide (CH_3SCH_3) and dimethyldisulfide (CH_3SSCH_3).[111] The organisms were isolated from a biofilter of fertilized pine bark which was tested for odor removal from a sulfate cel-

lulose mill. Since very little research has been conducted on the oxidation of the organo-sulfur compounds, particularly the volatile compounds, it is likely that bacteria do exist which play an important role in this part of the biogeochemical sulfur cycle. Within uranium deposits of Wyoming, Colorado, New Mexico, and Texas, considerable quantities of sulfur are tied up as organic sulfur. It may be feasible to use bacteria to degrade the organic sulfur. This may make the uranium more available for release and produce an environment suitable for uranium extraction.

Numerous thiobacilli have been isolated and studied recently which are not obligately autotrophic. Whether these organisms play important roles in the biogeochemical cycle or are merely "curiosities" remains to be determined.

Thiobacillus organoparus was isolated by Markosyan[112] from acid mine water and oxidized sulfide deposits. It is capable of reverting from heterotrophic to autotrophic growth without preadaption. Autotrophically, this bacterium oxidizes elemental sulfur at pH 1.5 to 5.0, and heterotrophically, it grows on simple compounds. It is aerobic and mesophilic.

Thiobacillus perometabolis was described by London and Rittenberg[113] in 1967. This organism will grow with yeast extract or casein hydrolysate, but growth is greatly enhanced by adding thiosulfate. *T. perometabolis* oxidizes sulfur, thiosulfate, and tetrathionate to sulfate but will not grow on the inorganic compounds without the presence of an organic substrate. It will grow at pH 7 and 30°C.

Myers and Millar,[114] studying the ecology of acid coal mine drainage waters, isolated heterotrophic bacteria that showed enhanced growth when supplemented with inorganic sulfur. Thiosulfate was oxidized to sulfate; enhanced growth was observed with elemental sulfur, sulfate, and sulfide. Elemental sulfur was not deposited. The authors examined the growth capabilities of these organisms and concluded that the organisms were closely related to *T. perometabolis*. The organisms were present in acid drainage water at a level of 10^1 to 10^2 cells/ml; these numbers were far lower than the number of heterotrophic and iron-oxidizing, chemolithotrophic bacteria reported for this ecosystem.

Although it has yet to be studied in a natural ecosystem, the new organism *Thiobacillus acidophilus*[115] has been characterized in the laboratory. It was isolated from a culture of *T. ferrooxidans* by using increased concentration of glucose with concomitant decrease of ferrous iron concentration. *T. acidophilus* can grow autotrophically on elemental sulfur and then be transferred to glucose without an adaptation period. *T. acidophilus* is acidophilic (pH 1.5 to 6) with an optimum pH at 3.0, and it is mesophilic (25 to 30°C). The organism does not grow on ferrous iron, sulfite, sulfide, thiosulfate, or metal sulfides. *T. ferrooxidans* have been isolated from a supposed "pure" culture of *T. acidophilus*.[245,246] The studies of this organism have been primarily physiological. It has not yet been isolated in nature, so nothing is known of its role in the environment. It may simply be a laboratory phenomenon. There appears to be very little difference between this organism and *T. organoparus*.[112]

Guay et al.[243] reported that variations exist in the DNA base composition of *T. ferrooxidans* grown on different substrates. Although these researchers did not specifically explain the phenomenon, they indicated that adaptation of the microbe to the specific substrate would not adequately explain the observation. They suggested that mutation or cohabitation may be involved. Tuovinen et al.[244] also reported on variations in DNA composition during work with acidophilic thiobacilli. The variations in DNA composition noted in pure cultures of acidophilic thiobacilli indicate that cultures once considered to be pure strains may actually be mixed cultures.

The question has arisen whether these nonautotrophic thiobacilli may be mutants of *T. ferrooxidans* or *T. thiooxidans*. In-depth studies of the role of these organisms in the environment have not been undertaken, nor has it been established how densely populated with such organisms ecosystems may be. It is likely that these bacteria may contribute to the chemistry of the environment by alteration of sulfur compounds or organo-sulfur complexes.

THE EXTREMELY THERMOPHILIC, ACIDOPHILIC BACTERIA

Operators of dump-leaching operations have noted that areas within dumps often become

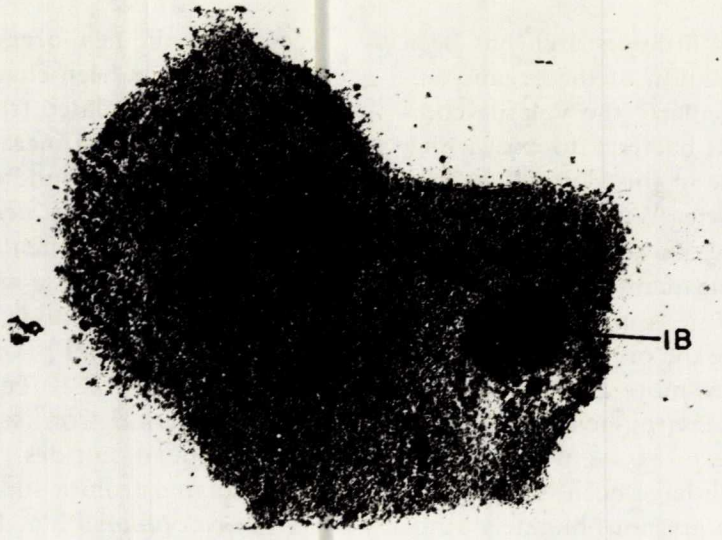

FIGURE 7. Thin section of *Sulfolobus*-like organism with intracellular body (IB). (Reproduced by permission of the National Research Council of Canada from Brierley, C. L. and Brierley, J. A., *Can. J. Microbiol.* 19, 183, 1973.)

hot. Beck[5] observed temperatures of 80°C. Although high temperatures do enhance the kinetics of metals dissolution, they are a detriment to the activity of the mesophilic thiobacilli. The discovery of thermophilic microorganisms with the ability to oxidize reduced sulfur and iron compounds was hailed with excitement, because these organisms may indeed enhance metals extraction at elevated temperatures often encountered in leaching operations.

Spherical, Thermophilic Microbes

The ecological studies[116,117] of hot springs in Yellowstone National Park, Wyoming, proved the existence of microorganisms capable of growing at temperatures of 75 to 80°C and pH values of 2 to 3. The first of the extremely thermophilic, acidophilic bacteria was isolated from an acid hot spring of Yellowstone National Park by J. A. Brierley.[116] This microbe, characterized by Brierley and Brierley,[63] oxidizes reduced iron and sulfur in a manner similar to the thiobacilli but is an obligate thermophile requiring a temperature range of 45 to 70°C. A supplement of 0.02% yeast extract enhances growth of the organism on sulfur and iron. Later studies[232] showed that the organism is mixotrophic, i.e., able to use both inorganic substrates and simple organic material as energy sources. The mechanism for yeast extract utilization may be fermentative, since oxygen is not reduced during yeast extract oxidation. Growth of the organism on solid medium has not been accomplished; however, a pure culture was obtained by end-point dilution.[232] Morphologically, the organism is unlike the thiobacilli in that it lacks a rigid cell wall and is spherical. The DNA composition is 57 ±3% guanine plus cytosine. The cells possess a highly refractile intracellular body (see Figure 7). It is likely that this organism is a strain of the acidophilic thermophile, *Sulfolobus acidocaldarius*, described by Brock et al.[62] *Sulfolobus* oxidizes sulfur at temperatures of 55-80°C and a pH of 0.9 to 5.8. *Sulfolobus* can be isolated both autotrophically on sulfur and heterotrophically on 0.1% yeast from thermal, acid soils, and acid hot springs. *Sulfolobus* is slightly irregular, with distinct lobes. Brock et al.[62] did not observe a distinct intracellular body in organisms studied. The DNA composition of *Sulfolobus* is 60 to 68% guanine plus cytosine.

Observation by transmission electron microscope indicates an unusual cell envelope, and chemical studies show that *Sulfolobus* lacks a cell wall and a peptidoglycan layer, typically associated with cell walls of Gram-negative organisms.[62] The cell envelope is a protein-lipid complex having a high proportion of charged amino acids and an excess of acidic amino

acids. Weiss[118] speculates that survival of the organism in the extreme environment may depend on the charged surface, the stabilization of lipoprotein by divalent cations, a special stabilizing interaction between the cell envelope and cell membrane, and the absence of peptidoglycan. This last factor is indeed interesting, since the presence of this compound is usually considered as a cell-wall stabilizer. Several of the peptides of *Sulfolobus* are unique,[119] and only inositol-containing phospholipids are present. It is proposed that thermophily is related to the long chain isopranols, and acidophily is correlated with ether lipids.[119] DeRosa et al.[120] found that membrane lipids in *Sulfolobus* and other acidophilic thermophiles are derived from cyclic, glycerol diethers and that the presence of such "cholesterol lipids" promotes fluidity under conditions which would otherwise cause lipid crystallization. Langworthy[121] reported that the complex lipid composition of *Sulfolobus*[62] and *Sulfolobus*-like[63] organisms is nearly identical, but the lipid character of these organisms is radically different from that of other microbes found in hot, acid environments.

Millonig et al.[85] characterized two strains of acidophilic, thermophilic microorganisms[122] from volcanic hot springs near Naples, Italy. Using the transmission electron microscope, these organisms and *Sulfolobus*[62,63] were compared and characterized. It was noted by these workers[85] that centrifugation of these cells at high speeds tends to produce the bizarre pleomorphic forms of the organism noted in transmission electron micrographs by Brock et al.[62] and Brierley and Brierley[63] (see Figure 7). This apparently results because of the extreme plasticity of the organisms. Millonig et al.[85] observed dark granular material and considered it to be ribosomes. They also observed the intracellular body reported by Brierley and Brierley[63] (see Figure 7). Fibrils of DNA sere seen, and observations of dividing cells indicated replication to be by binary fission rather than by septation, as described by Brock et al.[62] Pili were seen in cultures obtained from the Naples volcanic area. These correspond to pili observed on *Sulfolobus* by Weiss.[76] It did not seem to matter whether the cultures were agitated or stationary, provided yeast extract was a component of the medium.[85]

DeRosa et al.[122] studied six microbial strains, designated MT, similar to *Sulfolobus*.[62] These bacteria, isolated from pools with a temperature range of 74 to 89°C and a pH of 1.4 to 2.6, were cultured in spring water amended with 0.1% yeast extract. Heterotrophic growth is attainable with yeast extract, tryptone, and casamino acids, provided the concentrations do not exceed 0.1%. Autotrophic growth by the organisms is achieved on sulfur after gradual passage through yeast extract-sulfur medium. Growth is also attainable on iron. These organisms, like *Sulfolobus,* also exhibit resistance to antibiotics that inhibit cell wall synthesis. Studies indicated that lipids are primarily ether-linked, and the authors[122] suggest that this may be advantageous in stabilizing the organisms against environmental extremes.

The major difference noted by DeRosa et al[122] between their isolates and *Sulfolobus* is the extremes in DNA base composition. The percentages of guanine and cytosine for MT strains are 42% and 39%, whereas Brock et al.[62] report 60 to 68%. Despite the radical differences in DNA base composition between the MT organisms and *Sulfolobus,* DeRosa et al.[122] concluded that the stringent environmental conditions withstood by *Sulfolobus* and the MT strains are enough to class these organisms in a single "form/habitat" group, which the authors suggested be called *Caldariella.* In addition to the MT organisms and *Sulfolobus,*[62,63] the group would include the heterotrophic, acidophilic, and thermophilic microbe, *Thermoplasma acidophilia.*[123] An interesting observation made by DeRosa et al.[122] is that if the MT strains are cooled to room temperature in acid medium, viability is quickly lost. Viability can be maintained longer at temperatures below the growth limit if the cells are stored in a medium with a pH of 6. The authors conclude that acidophily is probably dependent on a mechanism driven by active cells which keeps the hydrogen ions out of the cell, This concept has also been expressed by Brock.[124] Research by Noguchi et al.[125] on the acidostability of *Thiobacillus ferrooxidans* spheroplasts may be applicable to the stability of acidophilic thermophiles. Spheroplasts lack the peptidoglycan layer but remain acid stable. If spheroplasts are subjected to proteolytic enzyme, they lose their acidostability. It is suggested by Noguchi et al.[125] that acid stability is energy dependent and related to the re-

pulsion of hydrogen ions. This mechanism is inferred to be proteinaceous and located in the membrane fraction.

The extremely acidophilic, thermophilic microbes are truly amazing organisms and seem to exist at the very limits at which physicochemical factors will allow life. Since the isolation of these organisms, the research has been multifaceted and includes physiological and ecological aspects as well as examination of these organisms in industrial application.

Rod-Shaped, Thermophilic Microbes

During his ecological studies of Yellowstone National Park hot springs, Brierley[116] observed rod-shaped organisms which grew at temperatures up to 55°C. Observations of similar organisms have been made by Kaplan,[126]Schoen and Erlich [127] and Schwartz and Schwartz.[128] In the intense studies of Yellowstone National Park which followed the isolation of *Sulfolobus,*[62,63] thermophilic, acidophilic, rod-shaped bacteria were noted. In an ecological study of hot, acid soils, Fliermans and Brock[67] observed *Thiobacillus* to be present at temperatures of 55°C. They concluded that mesophilic thiobacilli are mainly responsible for acid production in solfatara soils. Weiss,[129] in a study of bacterial survival at high temperatures and low pH, observed rod-shaped bacteria at concentrations of 10^7 to 10^8 ml^{-1} in environments at pH 2 to 3 and 75 to 80°C. On slides immersed in springs, rods form colonies rapidly at temperatures up to 78°C. In springs with temperatures between 70°C and 75°C, equal numbers of rods and *Sulfolobus* are observed, and in flowing springs, rods develop at 75°C to the exclusion of *Sulfolobus.* Mosser et al.[130] noted that some hot springs in Yellowstone National Park contain thermophilic thiobacilli. Counts of these organisms in one spring indicated their presence at 6.4×10^6/ml, and they are present in springs similar to those inhabited by *Sulfolobus;* however, the temperatures of these springs are somewhat lower. Bohlool[131] observed rod-shaped bacteria in New Zealand hot springs ranging from 43 to 84°C. *Sulfolobus* and the rod-shaped bacteria coexist in some springs, but generally where both organisms are found, the rods predominate.

Of these studies,[130,131] little emphasis was placed on the presence, characterization, or role of these rod-shaped, acidophilic microorganisms in the environment. Le Roux et al.[132] isolated several thermophilic, rod-shaped bacteria on ferrous iron and thiosulfate media from hot springs in southwest Iceland which range from 58 to 86°C and a pH of 4.1 to 8.9. Since these isolates oxidize sulfur, reduced sulfur compounds, metal sulfides, and ferrous iron over a range of pH values, and since their morphological characteristics are similar to those of the mesophilic thiobacilli, Le Roux et al.[132] suggested classification of these organisms as thermophilic *Thiobacillus.* One of the thermophilic, alkaline isolates slowly oxidizes chalcopyrite under basic conditions at 60°C; this temperature is much higher than has been reported for alkaline leaching of mineral sulfides. Another acidothermophile isolated from the Icelandic hot springs at 64°C and pH 4.3 grows well on pyrite, but growth on ferrous iron is poor. Supplementing the medium with 0.02% yeast extract enhances growth, but 0.1% yeast extract is inhibitory. Le Roux and colleagues[132] speculated that since the Icelandic isolates are found over a range of pH values, they perhaps form a bacterial succession in which reduced compounds could be oxidized from alkaline conditions to eventually produce sulfuric acid.

The thermophilic *Thiobacillus,* isolated by Le Roux et al.,[132] was the subject of study by Brierley and Le Roux.[102] This work examines the effect of various physiochemical factors on the oxidation of ferrous iron and pyrite by the acidothermophilic *Thiobacillus.* This organism grows on ferrous iron at 30 to 50°C, but 0.02% yeast extract is required. Growth does not occur at higher temperatures. Oxygen uptake is not enhanced when the bacterium oxidizes iron in the presence of yeast extract, and oxygen uptake increases with increasing iron concentrations to 81 m*M* (4.9 g/l). The growth of the acidothermophilic *Thiobacillus* on pyrite requires yeast extract, and growth is observed at 40 to 55°C but not at 30°C and 60°C; pyrite oxidation occurs from pH 1.1 to 2.6. Increasing pyrite concentrations enhance oxygen uptake. The authors[102]measured growth on pyrite by pH decline, which infers oxidation of the sulfide moiety; iron dissolution was not measured, so it is not known whether the iron moiety is biogenically oxidized. When the organism is

supplied with ferrous iron, pyrite, and yeast extract as substrates, ferrous iron is not entirely oxidized. Brierley and Le Roux[102] suggest that this may be due to the microbe having a two-enzyme system — one for pyrite oxidation and the second for ferrous iron oxidation. The authors[102] suggest that these systems function independently. The pyrite oxidation system may be the same as required for sulfur oxidation. Oxygen uptake is greater when the ferrous iron and pyrite substrates are both present than when either substrate is provided alone. The organism is able to grow on elemental sulfur; but respiration of the organism on sulfur is not measurable. The test cells were initially grown on pyrite, and this may affect respiration on sulfur. Although a requirement for growth on iron and pyrite, yeast extract serves as a sole energy source. No oxygen uptake is observed during manometric studies when yeast extract is the sole substrate. This work[102] is the first detailed study of the acidophilic, thermophilic, rod-shaped bacteria which have so often been observed in acid, thermal environments. There is need to study the ecology of thermophilic thiobacilli to determine their geochemical role and possible use in metals extraction.

One only needs to make a cursory examination of the nutritional requirements of the acidothermophilic bacteria to note that yeast extract in concentrations less than 0.1% is needed to initiate growth on some inorganic substrates[102] or to enhance growth.[62,63] By itself, yeast extract will serve as a sole energy source for these organisms but in manometric studies, oxygen is not used by the bacteria when yeast extract is the only substrate. Most investigators have given this need for yeast extract only a superficial examination, but Shivers and Brock[84] reported that supplementing inorganic substrates with yeast extract has a complex effect on chemoautotrophic metabolism. They propose that yeast extract affects both carbon assimilation and energy generation. Sulfur oxidation is greatly inhibited by yeast extract, but because of increased cellular production, the total sulfur oxidized is only reduced by approximately one third. Possibly, enzymes responsible for inorganic substrate oxidation are repressed, and carbon dioxide assimilation is likewise suppressed. The implications of yeast extract addition and the presence of other organic matter in metals dissolution by the acidothermophilic microorganisms have not been fully realized.

Environments of the Thermophilic Microorganisms

Since the acidothermophilic microorganisms were isolated from acid hot springs, the organisms' relationships to these environments have been extensively studied. Since we do not yet know if these organisms occur naturally in leach dump environments, we must examine their natural habitat to learn of their abilities to exist in harsh environments and oxidize inorganic substrates.

In 1970, Brock and Darland[117] investigated the environmental extremes for microorganisms. They discovered that the added environmental stress of high temperature and low pH places limitations on the numbers and kinds of organisms present. There indeed seems to be a physicochemical limitation beyond which life cannot exist. The discovery of the extremely thermophilic and acidophilic bacterium *Sulfolobus acidocaldarious*[62] and the related strains[63,122] initiated many studies on the ecology of these extremely tolerant microbes. A correlation between the numbers of *Sulfolobus* and *Thiobacillus*, the pH, and soil temperature of acid thermal areas was made.[67] Thiobacilli were found at temperatures less than 55°C and *Sulfolobus* at temperatures of 50 to 85°C. Thiobacilli were found in greater numbers in soil than were *Sulfolobus*. It was suggested by the authors[67] that *Thiobacillus* species are primarily responsible for the production of sulfuric acid in thermal, solfatara soils. It was concluded that *Sulfolobus* is less adapted to life in soils, since they are far more abundant in acid hot springs. Mosser et al.[133] reported that *Sulfolobus* oxidizes elemental sulfur up to 85°C, and the amount of sulfur oxidized by *Sulfolobus* in several thermal pools is calculated to be 1/15 to 1/23 of the total amount of elemental sulfur in the pools. Other bacteria were not observed to be present, so biogenic sulfur oxidation is attributed to *Sulfolobus*.

Weiss[129] concluded that the highest temperature at which *Sulfolobus* is found in nature is 93°C. Above 89°C, the cell walls are disturbed, and the cells appear as irregular lobed spheres. Studies using immersed slides show that both

Sulfolobus and rod-shaped thermophilic microbes become attached. Two distinct colony types can be observed for *Sulfolobus* — one being a dense aggregation and the second a dispersed colony type. *Sulfolobus* do not readily attach to slides in flowing springs, and Weiss[129] suggested that physicochemical characteristics other than temperature influence the habitation of *Sulfolobus*. In laboratory culture, *Sulfolobus* will initiate sulfur oxidation between the pH range of 2.0 and 3.8; the final pH after sulfur oxidation is 1.1 to 1.5[84] Continuous aeration of *Sulfolobus* cultures with air supplemented with 5% carbon dioxide is deterimental to sulfur oxidation, but periodic bubbling enhances growth. Doubling time on sulfur is reported as 36.8 to 55.3 hr. Most strains of *Sulfolobus* oxidize sulfur between 67°C and 75°C; however, one strain has a broader range (55 to 84°C), with maximum sulfur oxidation occurring at 70 to 75°C.

Mosser et al.[130]examined the growth rates of *Sulfolobus* in acid hot springs. To perform this study, the turnover time for water passing through a spring is determined by adding sodium chloride to the spring and following the dilution time. This is a rather innovative and simple approach to ecological studies. This dilution rate and other physiochemical characteristics are correlated with bacterial numbers in the spring. In addition, killed cells of a distinct serological strain are added to the spring, and the rate of disappearance of these cells is correlated with the water dilution rate. From this information, it can be concluded that the springs act as natural chemostats; the bacteria divide once or twice a day to maintain a steady-state number. This is slightly faster than the doubling time of *Sulfolobus* in the laboratory, when the organism is using elemental sulfur as an energy source.[84] Mosser et al.[130] speculated that perhaps nutrient limitation or build-up of toxic products may limit growth. For several small springs, the authors completely drained the pools. The incoming water, which was derived from underground seepage, was free of bacteria. An attempt was made to remove attached bacterial populations from the sides and bottoms of the pool by scraping. Natural filling of the pool was followed with bacterial counts, and it was determined that bacterial growth is exponential — doubling time is only a few hours. It is therefore possible for bacterial growth rates in nature to greatly exceed laboratory growth rates, thus indicating that laboratory culturing is being conducted under optimum conditions. Flow rates from large pools are far greater than rates from small springs. But growth rates of *Sulfolobus* are apparently dependent not on the water flow rate but on the average dilution rate of the cells. Mosser et al.[130] explained that they scraped the walls of small pools free of attached bacteria; therefore, this study seems valid only for unattached bacteria and makes no compensation for the attachment of bacteria to the sides of the pool. There is no description of what percentage of the total bacterial population might be so attached. A study by Mehta and Le Roux[134] on the effect of wall growth on the continuous oxidation of ferrous iron by *Thiobacillus ferrooxidans* indicates that the production of bacterial films influences iron oxidation more than the unattached population, i.e., iron oxidation is greater by attached bacterial populations because washout of bacteria from the reactor is avoided, and the dilution rate of bacteria is negligible compared with the bacterial accumulation rate. It is therefore important that future studies of population dynamics in ecological systems consider the attached bacterial population. Information obtained from the study of natural chemostats can be applied to the use of fixed film bacterial populations in the production of lixiviants for leaching purposes.

Mosser et al.[135]correlated temperature optima of *Sulfolobus* isolates with their habitat temperatures. This study showed that few *Sulfolobus* strains are adapted to their environmental temperature. In most instances, the bacterial temperature optima are higher than the pool tmeperature. The authors[135]speculated that perhaps bacterial development occurs near the steam jets which heat the pools. These authors[135] did not find *Sulfolobus* growing at temperatures greater than 80°C; however, Weiss[129] reportedly found one strain at 93°C. Brock[235] found *Sulfolobus* populations in Iceland at 90 to 95°C.

Immunofluorescence and immunodiffusion techniques were applied to establish the diversity of *Sulfolobus* strains in natural habitats.[75] Microscope slides immersed in springs were found to have a large and diverse population of

Sulfolobus, but this technique is ineffective in flowing springs, as *Sulfolobus* will not readily attach. In nonflowing hot springs, water harbors larger *Sulfolobus* populations than in flowing springs; in flowing springs, the majority of the *Sulfolobus* population is found attached to the sediment. It has been noted by Mosser et al.[135] that different temperature strains exist in different springs, and the present study[75] confirms that many different serological types of *Sulfolobus* exist in the hot springs. These serological types may correspond with the different temperature strains. With immunofluorescence techniques, a diversity of serologic types of *Sulfolobus* in hot springs of New Zealand was found.[131] Bohlool speculated on the evolutionary significance of finding *Sulfolobus* in the relative isolation of New Zealand. The low viability of this organism outside its habitat may preclude its inoculation from other sources, but its similarity to other *Sulfolobus* strains does suggest a very close relationship which may preclude *de novo* evolution. The frequency with which *Sulfolobus* is observed in hot spring environments around the world suggests that it may inhabit suitable leach dump environments. A superficial examination of leach dump core samples, leach solutions, blast hole samples, and pyritic tailings was made for microbial growth at 45°C, 60°C, and 80°C on iron and sulfur media supplemented with yeast extract.[232] Growth of rod-shaped bacteria was noted at 45°C, but this probably represented the uppermost temperature limit for mesophilic thiobacilli; no thermophilic microbes were observed. None of the samples was collected from areas observed to be at temperatures greater than ambient. Perhaps a more thorough search in leach dump regions known to have high temperatures may yield thermophilic microbes. Brock et al.[136] examined biogenic iron oxidation in acid hot springs. Iron concentrations are variable, ranging from 0.05 to 3.6 m*M*. The iron is assumed to enter the pools by underground water seepage, and most is assumed to be oxidized, although some iron is probably reduced by hydrogen sulfide. A survey indicates that the amount of iron oxidized varies from negligible to nearly 100% in 24 hr. The temperature optima for iron oxidation varies just as it does for sulfur oxidation, but iron oxidiation occurs from 80 to 85°C. The upper limit is probably 85 to 90°C. Some autooxidation of iron occurs at high temperatures even when the pH is low. Since the rate of iron oxidation is more rapid than the flow rate, Brock et al.[136] concluded that the iron oxidation rate is directly related to the inflow of iron from ground water and the rate of iron reduction by the sediments.

An interesting observation made by Brock and colleagues[136] was that the sediment of the pool possesses reducing capacity. Brock and Gustafson[137] present data which provides an explanation for the reductive capacity of the sediments of the acid hot springs of Yellowstone National Park. In the laboratory under microaerophilic conditions, *Sulfolobus* oxidizes sulfur and reduces ferric iron. This may indeed be the reaction which is occurring in the sediments. However, in the pools, the organisms are most likely oxidizing available ferrous iron, because it would be a more readily available energy source since it is in solution. Brock and Gustafson[137] studied the reductive ability of *Thiobacillus thiooxidans* and *T. ferrooxidans*. It was found that if *T. ferrooxidans* is conditioned to grow on sulfur in the absence of oxygen, it will use ferric iron as an oxidant, i.e., sulfur is oxidized and ferric iron reduced. Likewise, *T. thiooxidans* aerobically reduces ferric iron. The authors[137] question why these organisms substitute ferric iron as a reductant in the presence of oxygen, since more energy is available when oxygen is used as an electron acceptor. It is known that *T. denitrificans* uses nitrate as an electron acceptor in the absence of oxygen, and the energy available from this reaction is only slightly higher than that available from the reduction of iron. These studies have considerable implication in bacterial leaching. In the oxidation of some sulfide minerals, sulfur can theoretically exist as a solid phase (see Equation 1). It has long been thought that *T. thiooxidans* may play an important role in the dissolution of this sulfur layer (see Equation 3) to produce sulfuric acid. Brock and Gustafson[137] suggest that such sulfur-layer oxidation may occur at the expense of ferric iron, which would be present in the leach solution. This reaction would, of course, occur in dumps where oxygen demand is great and anaerobic or microaerophilic conditions prevail. The authors claim this would be a beneficial reaction in that

it would allow the unveiling of unleached surfaces for direct microbial attack.

The reduction of metals by *Sulfolobus*-like bacteria has been observed by Brierley and Brierley.[138] In an aerobic or microaerophilic environment, *Sulfolobus* reduces hexavalent molybdenum when elemental sulfur is provided as an energy source. Molybdenum, supplied as $Na_2MoO_4 \cdot 2H_2O$, is reduced to "moly blue," the pentavalent state. Adding yeast extract as a supplement to these cultures does not affect the organism's ability to reduce the molybdenum. It is thought that hexavalent molybdenum serves as an electron acceptor rather than oxygen. It can also be suggested that pentavalent molybdenum is produced by the interaction between an intermediate in the oxidation of sulfur, such as thiosulfate, and hexavalent molybdenum. This would indicate that *Sulfolobus* and the hexavalent molybdenum may be in competition for the intermediary product, as it is known that *Sulfolobus* oxidizes sulfur to sulfuric acid. However, growth is nearly equal in cultures with and without hexavalent molybdenum, suggesting that competition for the substrate has not occurred. It is possible that molybdenum cycling may be biogenically mediated as has been reported for the cyclic, biogenic oxidation and reduction of iron.[137]

Leaching Applications of the Thermophilic Microorganisms

The ability of the acido-thermophilic bacteria to oxidize inorganic substrates makes them potential microbes for use in the leaching of metallic sulfides; the study of their capabilities has just begun. The leaching of molybdenite (MoS_2) by *Sulfolobus* was first reported in 1973[88] and described more thoroughly by Brierley in 1974.[139] The leaching of molybdenite by the chemoautotrophic thiobacilli is limited because of their inability to tolerate high concentrations of soluble molybdenum.[26,70,140] Molybdenum toxicity is not a problem with *Sulfolobus* as these organisms can tolerate 21 m*M* hexavalent molybdenum and grow in a concentration of 7.8 m*M* hexavalent molybdenum. In batch reactors, maximum yield of molybdenum leached from a molybdenite concentrate (98.5% MoS_2; 12 to 65 μm) at 60°C and pH 2.5 is 13.3% in 30 days. This was achieved by using a basal salts medium supplemented with 0.02% yeast extract and 1% ferrous sulfate.[88,139] This is compared with a yield of 0.1% for uninoculated control reactors. The end products of molybdenite leaching are hexavalent molybdenum and sulfuric acid. Elemental sulfur is inhibitory to molybdenite leaching, and pyrite in excess of 50% of the total solids suppresses molybdenum extraction. High-grade molybdenite ore is similarily leached by *Sulfolobus*, but molybdenite in waste and tailings is not successfully leached because of the presence of acid-consuming material that prohibits biogenic molybdenum extraction.[139] During the column leaching of 100-lb aliquots of chalcopyrite ores containing economic quantities of molybdenite, the concentration of molybdenum in solution varies with the iron content, suggesting the formation of insoluble iron-molybdenum complexes that are dependent on the concentration of the iron and molybdenum present.[68]

Several copper sulfide ores and concentrates were leached in stationary batch reactors using *Sulfolobus*.[141] The ability to leach these substrates is dependent on supplements added to the media, such as ferrous iron and yeast extract, and the mineralogy of samples. If chalcocite is leached, the contribution of the thermophile to the dissolution of copper is not significant owing to the extreme solubility of the mineral in a hot, acid solution. Yeast extract does not greatly affect copper leaching by *Sulfolobus*, but ferrous iron enhances copper extraction. Chalcopyrite is the most resistant of the copper sulfide minerals to leach. Some chalcopyrite ores have not been satisfactorily leached with either ferric iron or thiobacilli. Preliminary studies with *Sulfolobus* indicate that 51% of the copper can be leached in 60 days at 60°C from a chalcopyrite concentrate (29% Cu; less than 212 μm); from sterile controls 8% of the copper is leached.[141] Although these studies were conducted with pulp densities ranging from 1 to 10%, the data are encouraging enough to indicate that *Sulfolobus* may enhance copper extraction from dump environments.

Leach columns, containing ca. 45 kg of ore and heated to 60°C, were designed to simulate leach dump conditions. Low-grade chalcopyrite ores (0.31% Cu; particle size between 150 μm and 6.7 mm) were leached for 160 days.[68] From

the inoculated ore, 38% of the copper can be leached, while 4% of the copper is extracted from the sterile control. Zinc and nickel extractions from sterile and inoculated columns are nearly equal; lead is not solubilized. Analysis of the ore from the inoculated column after the completion of the leach tests showed an active and abundant number of *Sulfolobus* present in the ore. This suggests attachment and colonization of the ore particles by the bacteria.[68] Fliermans and Brock[67] suggest that *Sulfolobus* may be an aquatic organism, because they were unable to detect *Sulfolobus* activity in solfatara soils. This does not appear to be the case with the *Sulfolobus* organism in its leaching of ores, and the data collected definitely suggest that the presence of *Sulfolobus* in the ore greatly enhances the extraction of copper.[68]

Batch-reactor leaching of uranium ore from Grants, New Mexico, by *Sulfolobus* indicates that uranium is not readily solubilized by the organism, and in fact, conditions manifested by the ore are not conducive to the viability of the organisms.[103] The acidothermophilic *Thiobacillus*-like organism reported by Le Roux et al.[132] and Brierley and Le Roux[102] grows well on pyrite. It also oxidizes pentlandite ($(NiFe)_9S_8$) and chalcopyrite ($CuFeS_2$).[236] The ability of this organism to extract metals has not been examined in depth; however, these preliminary experiments suggest that this organism may be important in dump leaching where temperatures exceed 40°C because of exothermic oxidation of pyrite.

Dump leaching has been modeled in a large leach tank containing 1.7×10^5 kg of low-grade chalcopyrite ore.[142] This tank measures 12 m high and 3 m in diameter. Sampling and data collection are made at four ports. In 300 days of leaching, the temperature rose from a uniform 10°C to 59°C at the bottom of the column. Culturing of ore samples from all four portals in ferrous iron media resulted in biogenic iron oxidation at 50°C.[143] Growth of subcultures could be obtained at 50°C and 55°C, but only by supplementing iron medium with 0.02% yeast extract. The cultures are mixed populations of rods. These are the first data obtained on the development of thermophilic bacteria in a large, simulated leach system. Since the columns are not inoculated with thermophilic organisms, the microbes must be indigenous to the run-of-the-mine ore or the acid lixiviant used to leach the ore[143]

BIOGENIC IRON OXIDATION

Bacterial ferric iron generation is the key to solubilization of metal sulfide and reduced uranium minerals. Bacteria that oxidize ferrous iron on a geochemical scale include *Thiobacillus ferrooxidans,* the extremely thermophilic, acidophilic *Sulfolobus,*[62,63] and the thermophilic *Thiobacillus*-like organisms.[102,132] Another less well-studied organism which oxidizes iron in the mesoacidic pH range (2.5 to 4.5) is *Metallogenium.*[109,110] The iron-oxidation reactions mediated by *Sulfolobus,* the thermophilic thiobacilli, and *Metallogenium* have not been critically examined, as these organisms have only recently been described. The iron-oxidation reaction mediated by *Thiobacillus ferrooxidans* has been extensively studied and can be described by Equation 7. The biogenic reaction usually occurs at pH values less than 3.5. At pH 4.0, abiotic iron oxidation rates substantially increase,[144] and above pH 3, the precipitation of ferric iron is appreciable. [145] At pH 2.2 and 31°C, *T. ferrooxidans* reportedly increases the rate of iron oxidation 500,000 times over the abiotic oxidation rate.[24] Lacey and Lawson[24] formulated the following equation describing the rate of oxidation:

$$\frac{dS}{dt} = \frac{\mu_m SX}{Y(K+S)}$$

In this equation, t = time (hr), S = concentration of Fe^{2+}(g/l), μ_m = maximum specific growth rate of bacteria per hour, Y = mass of bacteria produced per gram of Fe^{2+} oxidized (g/g), K = saturation constant (g/l Fe^{2+}), and X = concentration of bacteria (g/l). From this equation, it was established that at 20°C and an initial ferrous iron concentration of 36 m*M* (2g/l), the maximum oxidation rate occurs when the ferrous iron concentration is 20 m*M* (1.15 g/l). From the low activation energy of 8000 cal/g-mol for the reaction $Fe^{2+} \rightarrow Fe^{3+} + e^-$, it can be concluded that the reaction is diffusion controlled. Lacey and Lawson[24] suggested that the rate-limiting step in iron oxidation may be the transport of iron across the cell membrane.

The growth of *T. ferrooxidans* on iron was

further studied by MacDonald and Clark.[146] They found that growth rate is dependent upon time and pH. The optimum temperature is also pH dependent. The specific growth rate of this particular *T. ferrooxidans* strain is dependent on ferrous iron concentration, if present at less than 9 m*M* (0.5 g·l^{-1}), but at higher concentrations (above 36 m*M*), the dependency decreases and growth becomes zero order with respect to iron concentration.[146]

Schaitman et al.[147] performed kinetic studies on *T. ferrooxidans* cells and found iron oxidation follows zero order kinetics. The amount of iron used was equivalent to 9 m*M* (0.5 g/l). They found that the pH optimum for the cells extends from 2.4 to 3.6, with a rapid decline in iron oxidation noted above pH 3.6 and below 2.4. The pH affects the V_{max} but not the K_m of Michaelis-Menten kinetics. The authors[147] suggest that this may be due to the pH ionizing the active iron oxidation sites or the deterioration of iron-binding groups.

Inhibition by ferric iron of *T. ferrooxidans* iron oxidation has been studied. Wong et al.[148] applied a noncompetitive inhibition model to the phenomenon. They found that ferric iron inhibition of *T. ferrooxidans* occurs at 30°C, pH 2, a cell concentration equal to 2.5 mg/l and a ferric iron concentration greater than 2.58 m*M* (0.14 g/l) and less than 15.0 m*M* (0.84 g/l).

In a more recent study with *T. ferrooxidans* Kelly et al.[149] found that

1. Low ferric iron concentrations enhance oxygen uptake of 5 to 80 m*M* $FeSO_4$ (0.3 to 4.5 g/ l), but as the ferric iron concentration increases, oxidation decelerates, with ferric iron acting as a competitive inhibitor.
2. The presence of 200 m*M* (11 g/l) ferric iron results in deceleration in substrate oxidation, thus indicating substrate inhibition by ferric iron.

Data obtained by these researchers indicate that two or more binding or transport sites for ferrous iron exist, and that the competitive inhibition demonstrated by ferric iron reduces the organism's affinity for ferrous iron.

Variations in ferric iron concentrations which produce inhibitions in *T. ferrooxidans* are most likely the result of strain differences. Bodo and Lundgren[150] found that the rate of ferrous iron oxidation by *T. ferrooxidans* cells is dependent upon the cell concentration. Although iron oxidation occurs over the pH range 1 to 4.5, the pH optimum is 2.0. The enzymes for iron oxidation are located in the cell envelope, and the optimum pH for iron oxidation by cell envelopes is 3.5.

Imai et al.[151] also determined that the iron oxidation mechanism of *T. ferrooxidans* is located in the cell membrane system. Like previous workers,[150] Imai and colleagues[151] found cell-free extracts of *T. ferrooxidans* to optimally oxidize iron at a higher pH (3.5 to 4.0) than whole cells.

In 1963, Lazaroff[152] elaborated upon the requirement for sulfate ions by *T. ferrooxidans* for iron oxidation. He postulated that sulfate controls the entrance of ferrous iron into the cell or that perhaps sulfate is required in energy transfer for the iron oxidase system. It was determined that chloride cannot totally replace sulfate; some sulfate is necessary in the medium for iron to be oxidized. This finding was substantiated by Lees et al. in 1969.[145]

Schnaitman et al.[147] found that adding sulfate in concentrations of 3.6 to 50 m*M* (0.2 to 2.8 g/l) doubled the iron oxidation rate. The velocity of the reaction is increased but not the K_m. These workers found chloride to be stimulatory at low concentrations, but they too found that iron cannot be oxidized if sulfate is absent. Since the K_m of the reaction is not altered by adding different sulfate concentrations, these workers did not conform to Dugan and Lundgren's[153] theory that binding of iron requires sulfate. They[147] did not exclude the possibility that sulfate exposes more enzymatic sites. It was discovered that dibasic phosphate (HPO_4^{2-}) and diabasic arsenate ($HAsO_4^{2-}$) can substitute for sulfate. Since these anions precipitate with ferric iron, they cannot totally replace sulfate in the reaction. Borate (BO_3^-) is ineffective, and nitrate (NO_3^-) and molybdate (MoO_4^{2-}) are inhibitory. It was hypothesized by Schnaitman and co-workers[147] that the divalent anions, such as dibasic phosphate and dibasic arsenate, may be able to replace sulfate, whereas chloride and nitrate cannot, owing to an unsuitable charge. The divalent anions may depolarize positively charged sites and enable ferrous iron to approach the cell. The toxicity

of molybdate to *T. ferrooxidans* has not been explained. It is notable that iron oxidation by the *Sulfolobus*-like organisms is unaffected by high molybdate concentrations. [139] Imai et al.[151] determined that nitrate prevents growth of *Thiobacillus ferrooxidans* at a concentration of 0.1 *M* (6.2 g/l) but that iron oxidation is not inhibited. These authors concluded that iron oxidase is not affected. Further work by these researchers[151] on iron oxidation showed that divalent cations (cobalt, nickel, manganese, magnesium) competitive to ferrous iron do not inhibit iron oxidation. The authors postulated that ferrous iron must form some complex of low potential which can be oxidized by the iron oxidase system, since the ferrous/ferric potential is quite high. Using a technique whereby ferric iron is directly and continuously monitored by measuring the absorbance of ferric iron formation in a cuvette, Steiner and Lazaroff[154] found that ferrous iron oxidation by *T. ferrooxidans* decreases at high sulfate levels. This inhibition occurs at greater levels than those inhibiting oxygen uptake as measured manometrically. This indicates that biogenic oxidation of iron is not paralleled by oxygen reduction. The work of others[147,152] was confirmed by Steiner and Lazaroff[154] when they showed that sulfate enhances iron oxidation to a point (0.216 *M*) (12 g/l). Selenate (SeO_4^{2-}) will substitute for sulfate as an anionic requirement for ferrous iron oxidation, although selenate is inhibitory to growth of *T. ferrooxidans*. Lazaroff[155] contends that either sulfate or selenate serves as ligands for iron complex formation to facilitate oxidation of ferrous iron. If sulfate or selenate are present, tellurate (TeO_4^{2-}), tungstate (WO_4^{2-}), arsenate (AsO_4^{3-}), or phosphate (PO_4^{3-}) enhance iron oxidation. Enhancement by these anions is regarded as a nonspecific requirement.[155]

The absolute requirement for sulfate is of interest with regard to leaching of copper sulfide minerals with ferric chloride solutions.[156] Ferric chloride is more effective than ferric sulfate in the leaching of chalcopyrite. The reaction is

$$CuFeS_2 + 3FeCl_3 \longrightarrow 4FeCl_2 + CuCl + 2S^0 \quad (11)$$

Unlike ferric sulfate, ferric chloride uniformly attacks the entire surface of the mineral. The ferrous chloride generated by the reaction is chemically oxidized. The finding that sulfate is a requirement for iron oxidation by *T. ferrooxidans* precludes the use of this organism for regeneration of ferric iron in chloride systems, unless the necessary sulfate ions could be generated by biogenic oxidation of the elemental sulfur (see Equation 3).

Tuovinen and Kelly,[28] investigating the effect of metals on the iron oxidation mechanism of *T. ferrooxidans*, suggested that the uranyl cation (UO_2^{2+}) may inhibit iron oxidation by competing with ferrous iron for binding sites on the iron oxidase system. Since adding divalent cations of nickel, zinc, magnesium, and manganese relieve uranium toxicity,[27] it may be that each of these metals competes for iron binding sites, but that the latter metals are less toxic than the uranyl cation. The toxicity of molybdenum to *T. ferrooxidans*[26,70,140,147] may be due to the entrance of the molybdate ion into the cell and its interference with the postulated iron-sulfate complex.[28]

Now that optimum conditions for biological oxidation of iron have been established, the chemical and physical aspects of iron as a leaching medium should be considered. As one examines the Pourbaix (Eh-pH) diagram (see Figure 8) for iron, it can be seen that the aqueous ferrous iron species is stable from pH 0 to nearly 8.0 and over a wide Eh zone. Ferric iron is very sensitive to pH changes, especially if temperatures above ambient are encountered. Recent studies[157] indicate that concentrations greater than 0.02 *M* (1 g/l) ferric iron do not increase the rate of leaching. At approximately pH 3, ferric iron is theoretically insoluble at concentractions greater than 0.01 *M* (0.5 g/l). Higher concentrations result in precipitation of ferric salts, which may blind the minerals and decelerate leaching. Substrate inhibition occurs at 0.2 *M* (11 g/l) ferric iron, according to Kelly et al.[149] and 0.003 to 0.015 *M* (0.17 to 0.84 g/l) ferric iron, according to Wong et al.[148] This would suggest that should ferric iron concentrations exceed 0.2 *M* (11 g/l), *T. ferrooxidans* may not function optimally in the conversion of ferrous iron. Sheffer and Evans[3] reported that ferric values in the influents to leach dumps range from 0 to 5 m*M* (0 to 0.3 g/l) and ferric concentrations in effluents from the leach dumps are between 1 to 50 m*M* (0.06 to 2.8 g/

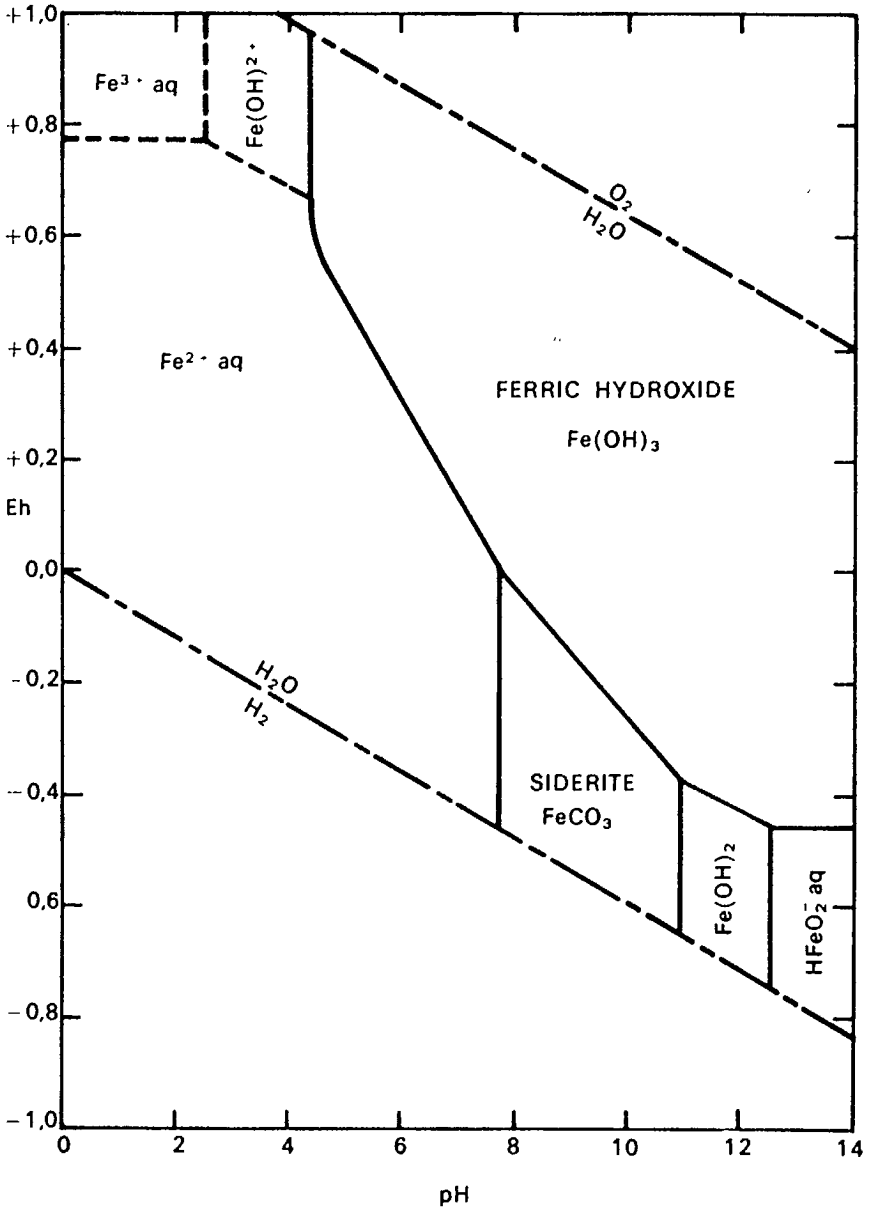

FIGURE 8. Diagram showing the relations among the metastable iron hydroxides and siderite at 25°C and 1 atm total pressure. Boundary between solids and ions at total activity of dissolved species = 10^{-6}. Total dissolved carbonate species = 10^{-2}. Dashed lines are boundaries between fields dominated by the labeled ion. (From Garrels, R. M. and Christ, C. L., *Solutions, Minerals, and Equilibria*, Freeman, Cooper, and Co., San Francisco, Calif. 1965. With permission.)

l)[3] suggesting that existing ferric iron concentrations are near optimum for biogenic iron conversion.

Leaching systems are generally self-buffering, i.e., some reactions occurring within the leaching environment are acid-consuming, while others are acid-producing. Among the acid-consuming reactions is the dissolution of gangue

$$CaCO_3 + 2H^+ + SO_4^{2-} + H_2O \longrightarrow CaSO$$

$$2H_2O + CO_2 \qquad (12)$$

and the oxidation of iron (Equation 7). The air oxidation of pyrite

$$FeS_2 + 3/4\ O_2 + 3H^+ \longrightarrow Fe^{3+} + 2S^0 + 3/2\ H_2O \qquad (13)$$

and the air oxidation of chalcopyrite

$$CuFeS_2 + 5/4\ O_2 + 5H^+ \longrightarrow Cu^{2+} + Fe^{3+} + 2S^0 + 5/2\ H_2O \quad (14)$$

have also been proposed as acid-consuming reactions,[1] if they stop at this point. However, the oxidation of pyrite and chalcopyrite is acid-producing if elemental sulfur is subsequently oxidized (see Equation 3) and ferric iron is hydrolyzed as in Equation 15

$$Fe^{3+} + 3/2H_2O \longrightarrow 3H^+ + \tfrac{1}{2}Fe_2O_3 \quad (15)$$

Also acid-generating are the reactions of metallic sulfides with ferric iron (Equation 2).[1] Acid-generating reactions which buffer the leach system are the precipitation of basic ferric sulfate. A number of reactions[1,15,159] have been proposed including

$$Fe^{3+} + 7/3H_2O + 2/3SO_4^{2-} \longrightarrow 5/3\ H^+ + 1/3\ Fe_3(SO_4)_2(OH)_5 \cdot 2H_2O \quad (16)$$

$$Fe_2(SO_4)_3 + 2H_2O \longrightarrow 2Fe(OH)(SO_4) + H_2SO_4 \quad (17)$$

$$3Fe_2(SO_4)_3 + 12H_2O \longrightarrow 2HFe_3(SO_4)_2(OH)_6 + 5H_2SO_4 \quad (18)$$

The buffering capacity is determined by the change in sulfate concentration. Lacey and Lawson[24] simply stated the acid-producing reactions as

$$Fe^{3+} + H_2O \longrightarrow Fe(OH)^{2+} + H^+ \quad (19)$$

$$Fe^{3+} + 2H_2O \longrightarrow Fe(OH)_2^+ + 2H^+ \quad (20)$$

$$Fe^{3+} + 3H_2O \longrightarrow Fe(OH)_3 + 3H^+ \quad (21)$$

MacDonald and Clark[146] noted that the continuous culture method cannot be used to measure specific growth rate unless the surfaces of the vessel are free of bacterial growth. Reactors with an area/volume ratio equal to 0.5 cm^{-1} have an attached *T. ferrooxidans* population four times greater than the liquid phase population. The attached cells are bound to the vessel surfaces and build up layers coincident with the precipitation of basic ferric sulfates. It had been observed earlier[64] that *T. ferrooxidans* attach to basic ferric sulfate and hydrated ferric oxide. These authors[146] stated that this factor may greatly affect the results of leaching studies. This work was followed up by Mehta and Le Roux.[134] In a stirred tank reactor in which *T. ferrooxidans* oxidize iron, steady-state conditions cannot be attained because of the build-up of bacteria on the surfaces of the vessel. This attached population influences iron oxidation more than the bacteria in the medium. It is only conjecture whether attached *T. ferrooxidans* have a different growth rate than unattached *T. ferrooxidans* populations. It is known that attachment of these organisms increases the cell numbers per reactor volume; this increased population exerts more influence on iron oxidation than the lesser numbers of organisms in solution.

Ferric iron generation by bacteria is basic to the leaching of base metal sulfides and uranium oxides. This section has examined some factors which control effective bacterial oxidation of ferrous iron and compared optimum conditions with factors in leaching operations.

BACTERIAL METALS EXTRACTION

The industrial application of the chemolithotrophic bacteria for the economic recovery of metal sulfide and uranium oxides ores is a reality. Although there is much known about the bacterial process, the prospects of using microorganisms more intensively as a mechanism for recovery of metals from both low-grade ores and concentrates have provoked many studies in the practical aspects of bio-leaching. In this regard, much consideration has been given to factors which influence metals extraction by bacteria.

Factors Affecting Bacterial Leaching

Metal Tolerance

The iron-oxidizing bacterium, *Thiobacillus ferrooxidans,* has a notably high tolerance to metals compared with most other microorganisms. It must be emphasized that heavy metal tolerance varies with the strains of *T. ferrooxidans,* and universal data for the many strains are sparse. The subject was first reviewed in 1967[160] and was reconsidered in review by Tuovinen and Kelly.[161] *T. ferrooxidans* tolerates 0.37 *M* aluminum, 0.15 *M* zinc, 0.17 *M* cobalt, 0.17 *M* nickel, 0.18 *M* manganese, and

0.16 *M* copper (10 g/l). Silver and the anions of selenium, tellurium, and arsenic are inhibitory at 0.2 to 9 m*M* (50 to 100 mg/l). Molybdate is inhibitory at concentrations above 0.03 m*M* (5 mg/l).[24] Mercuric ion at a concentration of 5×10^{-4} m*M* can inhibit the activity of *T. ferrooxidans,* but attempts to sterilize pyritic ore columns with a single dose of mercuric ion at a concentration of 0.5 m*M* has been only partially successful.[236] It has been observed that 2 to 3 weeks after addition of mercury to ore columns, the mercury is no longer detectable in solution but is associated with the ore.[236] It can be suggested that soluble mercury probably is toxic to *T. ferrooxidans,* but mercury complexed to ore is not toxic. The toxicity of mercury to *T. ferrooxidans* needs further study, since many ores contain small amounts of mercury and mercuric ion is frequently used as a sterilant.

Tuovinen et al.[26] noted that in the presence of metals *T. ferrooxidans* exhibits a lag period before iron oxidation proceeds. This is presumably an adaptation period where the cell material is undergoing changes. The authors[26] also considered this lag period to be a selection phase in which only tolerant cells survive. The authors also noted in their study that *T. ferrooxidans* are far more tolerant to heavy metals during iron oxidation than during thiosulfate oxidation, suggesting that enzymatic differences contribute to metal susceptibility.

Tuovinen and Kelly[28] found that copper, nickel, and uranyl ion in concentrations of 0.1 to 1.0 *M* are inhibitory to both iron oxidation and carbon dioxide fixation, therby suggesting that metals affect enzymes and/or production of ATP and NADH. Heavy metals appear less toxic in acidic environments, and it is thought that binding and uptake of some metals is decreased as hydrogen ion concentration increases. The extreme toxicity of molybdate may be due to its competition with sulfate which is known to be required by *T. ferrooxidans* for growth.[147,152,154]

Uranium toxicity to *T. ferrooxidans* was further explored by Tuovinen and Kelly,[162] who reported that inhibition of growth to *T. ferrooxidans* in the presence of 0.7 g/l uranyl sulfate is partially alleviated by 200 m*M* potassium (35 g/l), sodium (28 g/l), lithium (22 g/l), or ammonium (26 g/l) as sulfates. Since the toxicity of uranium is not understood, the relief noted by monovalent cations cannot be fully explained. The authors suggested that uranium toxicity may result from alterations of sites on the cell membrane or interference with potssium transport. Both divalent[27] and monovalent cations[162] reverse the toxic effects by having a greater affinity than uranium for these sites. Unlike some metals, uranium toxicity is enhanced by increased acidity, so it cannot be concluded that increasing the positive ion concentration ameliorates the uranium toxicity.[162]

It is unkown whether metal toxicity is a factor in environmental systems. It is likely that, over the long term, adaptation or selection has yielded microbial strains that are tolerant to the conditions they encounter in such environments as leach dumps and acid mine drainages. Ashida[163] discussed the adaptation of fungi to metals, and much of this study is relevant to the bacteria. The resistance of microbes to metals is the subject of a paper by Griffiths et al.[164] If metal toxicities in nature exist, the intraction among the bacteria and environmental factors will be important. Babich and Stotzky[165,166] have reported that the clay minerals montmorillonite and kaolinite afford protection for microbes from the inhibitory effects of cadmium. The protective features can be correlated with the cation exchange capacity of the clays. Not only are clays responsible for cation removal,[165,166] but ores also absorb cations including ammonium, hydrogen, copper, and ferrous and ferric iron.[167] The formation of insoluble ferric salts is also responsible for removal of cations.[160,161]

pH and Eh

In general, *Thiobacillus ferrooxidans* are unable to initiate growth on ferrous iron at a pH greater than 3, but once growth is initiated, the pH can increase to 3.4 without inhibiting *T. ferrooxidans* development. Erlich and Fox[167] suggest that high pH may affect cell attachment by alteration of the cell surface. This hypothesis has not been pursued.

The chemoautotrophic bacteria are restricted to intermediate oxidation-reduction conditions.[167]

Nutrients

Ammonium-nitrogen, phosphorus, sulfate,

and magnesium are essential for growth of *Thiobacillus ferrooxidans*.[168] Magnesium is necessary for carbon dioxide fixation, and phosphorus is required for energy metabolism and for the first steps of iron oxidation.[168] Sulfur is important as a component of sulfur-containing amino acids. The important role of sulfate in iron oxidation was elaborated upon earlier.[147,152,154,155]

Nitrogen appears to be the most important nutrient for the thiobacilli, and although it has never been shown to be deficient in leach dumps, the potential exists. Nitrogen has been reported to be fixed by *T. ferrooxidans*,[21] and it is possible that heterotrophs[169,170] which inhabit dump environments may provide nitrogen for the thiobacilli. Erlich and Fox[167] suggested that these heterotrophs may also devour the thiobacilli and interfere with leaching by physically blocking sulfide surfaces and obstructing leach solution flow. The latter occurrence has been observed in laboratory leach columns.[232] Several investigators have attempted to establish a mutual relationship between the thiobacilli and the acid-tolerant, nitrogen-fixing bacterium, *Beijerinckia lacticogenes*. It is proposed that thiobacilli could provide organic carbon for *Beijerinckia*, and *Beijerinckia* could provide nitrogenous compounds for thiobacilli. Tsuchiya et al.[171] adapted *Beijerinckia* to grow under acid conditions and high metal content and then compared the growth of *Thiobacillus ferrooxidans* in mixed culture with growth of *T. ferrooxidans* in nitrogen-free medium. A fivefold improvement was noted. The investigators were unable to show that *T. ferrooxidans* fixed nitorgen,[21] and therefore they attributed enhanced growth to the activity of *Beijerinckia*. However, the authors[171] did not perform qualitiative or quantitative tests on the *Beijerinckia* during mixed population studies, so it is not known whether viable *Beijerinckia* actually contributed to the *Thiobacillus* development. It should also be shown that viable *Beijerinckia* fix nitrogen by either[15] nitrogen or acetylene reduction tests. The thiobacilli may simply have used nitrogen compounds of the inoculum for growth. Trivedi and Tsuchiya[172,173] leached a copper-nickel sulfide concentrate with the *Thiobacillus-Beijerinckia* consortium and compared the leach rates with chemical controls, *T. ferrooxidans* only, and *Beijerinckia* only. Copper and nickel extraction was greatly enhanced, using mixed cultures with nickel being more effectively leached. A Coulter Counter was used to obtain an approximate count of the bacteria. It was of limited success because of the precipitates and particles present. A size difference between *Thiobacillus ferrooxidans* and *Beijerinckia* was used to distinguish the two strains. No direct culturing was used to ascertain the viability of either strain. The mutualistic or symbiotic relationship between *Beijerinckia* and *Thiobacillus ferrooxidans* is not known to occur naturally. Becking[174] suggested that the main habitat of *Beijerinckia* may be the lateritic soils of the tropics; Trivedi and Tuschiya[172] indicated that any natural relationship between thiobacilli and *Beijerinckia* may be limited to the acid mine waters of Cuba or the Philippines. The authors[172] proposed that the use of *Beijerinckia* in leaching operations would probably limit the undesirable formation of ammonia-jarosite, since ammonium would not be intentionally added if *Beijerinckia* were used. It is possible, however, that naturally occurring ammonium ion in the leach operation may inhibit the nitrogen-fixing capability of *Beijerinckia*.[175] Upon lysing, *Beijerinckia* release complex polysaccharides,[176] and Trivedi and Tsuchiya[172] hypothesized that these compounds may act as surfactants to facilitate the attack of *Thiobacillus* on the mineral. Research in the area of bacterial mutualism to enhance leaching is still in its infancy. In addition to the studies reported here, research in mutualism between thiobacilli and *Beijerinckia* is being conducted at Warren Spring Laboratory[237] and the Departamento Nacional da Producao Mineral,[238] and by H. M. Tsuchiya.[239]

Water Potential and Surface Tension

According to Brock,[177] water potential, the free energy difference between the system understudy and pure water, is important in controlling the activity of *Thiobacillus ferrooxidans* in nature. Brock found that *T. ferrooxidans* are able to grow and oxidize iron at water potentials of -1.5×10^6 Pa to -2×10^6 Pa (−15 to −20 bars), but that some environments that are otherwise suitable for the growth of *T. ferrooxidans* have water potential values too low for the organisms. It should be noted that concentrations of 0 to 1.7 *M* NaCl were

used to obtain water potentials of ca. -3×10^5 to -8×10^6 Pa (−3 to −85 bars). Growth of *T. ferrooxidans* was inhibited at 0.3 *M* NaCl, which yielded a water potential of between -2×10^6 and -8×10^6 Pa (−18 and −85 bars). Glycerol in concentrations of 0 to 2 *M* were used to obtain water potentials between -3×10^5 and -6×10^6 Pa (−3 and −61 bars). Growth of *T. ferrooxidans* was inhibited by between 0.1 and 0.2 *M* glycerol, a water potential of -6×10^5 and -9×10^5 Pa (−6 and −9 bars), depending on the strain. Using concentrations of glycerol and sodium chloride to control water potential raises a question whether these reagents may themselves inhibit *T. ferrooxidans.* Brock,[177] however, reported that *T. ferrooxidans* were inhibited by water potentials of -7×10^5 to -2×10^6 Pa (−7 to −23 bars) when water potential was controlled by ferrous sulfate and relative humidity. These latter results suggest that water potential may be a valid parameter but does not preclude the toxicity of sodium chloride and glycerol. The significance of water potential on *T. ferrooxidans* development is novel and deserves more intense study.

Brock attempted to correlate the bacterial activity data of Bhappu et al.[13] from the Chino Mines Division, Kennecott Copper Corporation, Santa Rita, New Mexico with the water potential data of Kennedy and Stahl.[178] He found that where *T. ferrooxidans* are active, water potential values are greater than -1.5×10^6 Pa (−15 bars), but where *T. ferrooxidans* are absent or their cell concentration is low, water potential values are -5×10^6 Pa to 10^7 Pa (−50 to −100 bars). Brock[177] observed that by oxidizing pyrite and producing sulfuric acid, the bacteria are generating conditions detrimental to their own development, since sulfuric acid promotes the formation of clays which bind water.

Metals can be removed from leach solutions by cementation, ion exchange, chemical precipitation, and solvent extraction. In recent years, solvent extraction has increased in popularity. An organic solvent is added to the leach solution, and after the aqueous and organic phases separate, the barren, aqueous phase is recycled to the leach operation, and the metal is recovered from the organic phase. Some entrainment of the organic solvent in the leach liquor is inevitable. The effect of the organic material on *T. ferrooxidans* has been examined. Torma and Itzkovitch[179] showed that organic solvents decrease the surface tension of the leach liquor and diminish the ability of *T. ferrooxidans* to leach chalcopyrite. The 19 solvents tested demonstrated differing degrees in inhibiting oxygen uptake by *T. ferrooxidans* when chalcopyrite was the substrate. It should be pointed out that the concentrations of solvent extraction reagents used far exceed those found in field operations. Torma and Itzkovitch[179] contend that organic solvents interfere with nutrient uptake and growth by adsorbing to the organism or altering the environment. Alterations of the environment were not described by the authors.[179] They also suggest that by wetting the mineral surface the solvents discourage attachment of the bacteria to the mineral surface.[179] This hypothesis was also reported by Erlich and Fox.[167] Torma[180] and Torma et al.[181] examined the effect of the Tweens ® (surface active agents) on the activity of *T. ferrooxidans* during leaching of chalcopyrite and found that bacterial activity decreased as the concentration of Tween increased. This was in disagreement with earlier studies.[182] Torma[180] and Torma and colleagues[181] feel that the inhibition of activity results from the lowering of surface tension of the leach solution and cite data which indicate that bacterial growth is curtailed below 28 to 30 dynes/cm. The Tween compounds are detergents, and small amounts may indeed enhance leaching activity, but excessive concentrations could easily be detrimental to bacterial activity.

Oxygen and Carbon Dioxide

The availability of oxygen in leach dumps is undoubtedly one factor which controls bacterial metals extraction. Recent studies indicate that *Thiobacillus ferrooxidans, T. thiooxidans,* and *Sulfolobus* may be able to use ferric iron as an oxidant,[137] and *Sulfolobus* may also use molybdate as an electron acceptor.[138] It is not known how extensive is the use by microorganisms of oxidants other than oxygen in leaching environments.

Carbon dioxide solubility is low in acid solutions and therefore may be a limiting factor in growth. In natural situations, heterotrophic growth occurs with the chemoautotrophic bacteria and probably furnishes some carbon dioxide. Carbonate gangue is commonly found with

ore minerals, and this also provides necessary carbon dioxide. Torma et al.[183] demonstrated that the availability of carbon dioxide is a limiting factor in zinc sulfide concentrate leaching. Increasing the carbon dioxide concentration increases leaching. Torma[240] indicates that the limitation of carbon dioxide is due to the mass transfer of the gas through the mineral particles. Kelly[149] found that in the absence of carbon dioxide *Thiobacillus ferrooxidans* can oxidize iron; he suggested[246] that carbon dioxide becomes rate controlling in zinc sulfide concentrate leaching because it controls the number of bacteria that grow. This implies that *T. ferrooxidans* could be used in fixed films to regenerate leach solutions without encountering carbon dioxide deficiency.

Temperature

Leach dumps are known to contain hot areas.[5] These have been attributed to the exothermic reaction of the oxidation of pyrite. Although Lyalikova[184] contends that *Thiobacillus ferrooxidans* participate in the initial heating of dumps, there is no substantial evidence to support this. High temperatures certainly limit the distribution of the mesophilic thiobacilli, but the thermophilic, chemolithotrophic microorganisms may be important in metals dissolution at temperatures in excess of 45°C.[68,88,102,139]

Light

Both visible and unfiltered light have an inhibitory effect on some species of thiobacilli, with the blue end of the spectrum demonstrating the greatest inhibition. Particulates and ferric iron offer some protection from visible rays.[185]

Pressure

Pressure has not been considered an environmental factor in leaching to date, but if bacterial methods are to be used in *in situ* leaching, the pressure factor must be examined. Few studies have examined the ability of thiobacilli to develop and function under high pressure. Torma[186] reported that the chemoautotrophic bacteria are barotolerant and can withstand hydrostatic pressures as high as 1.5×10^7 Pa (2200 psia). It has recently been reported that bacteria which oxidize hydrogen sulfide[187] have been found in the ocean at a depth of 2743 m — a pressure of 2.65×10^7 Pa (3900 psia).[188]

Conditions for nonbiological *in situ* leaching of chalcopyrite have been described.[189] Optimum extraction occurs at a self-buffered pH of 2 and 90°C. At higher temperatures, gangue materials decompose and minerals are excessively altered. Braithwaite and Wadsworth[190] oxidized chalcopyrite under simulated, deep solution mining using a temperature range of 30 to 150°C, an initial pH of 0.86 to 5.9, and an oxygen pressure of 2.7×10^5 Pa to 1.1×10^7 Pa (40 to 1620 psia).

Whether bacterial leaching can be used to enhance metals extraction under the optimum leaching conditions described for *in situ* mining has not been determined.

Mineralogy

Ore minerals can be categorized according to their resistance to leaching.[11] For example, the recalcitrance of chalcopyrite to dissolution is known by all who attempt to leach the ore. Many other minerals are not only resistant to dissolution by microbes, but upon solubilization the metals are toxic to the organisms or form insoluble products.

Torma and Subramanian[191] described the leaching of galena (PbS) concentrate with adapted *Thiobacillus ferrooxidans.* The leaching was characterized by a lag phase in which the pH rose. When leached, lead sulfate was the insoluble end product. This, and ferric salts produced by the biooxidation of iron present in the concentrate, precipitate on the ore and block the substrate surface from further oxidation. Regrinding is necessary for leaching to proceed. Torma and Subramanian[191] proposed a scheme in which a galena concentrate, containing low metal values of other sulfide minerals including zinc, copper, and cadmium, could be leached by *T. ferrooxidans.* The soluble metals are effectively separated from the lead, which forms an insoluble product. Tomizuka[192] evaluated the biodegradation of galena with consideration for particle size, pH, pulp density, and inoculum size. Studies were conducted in shake-flasks using *T. ferrooxidans.* Tomizuka reached the following conclusions:

1. *T. ferrooxidans* use galena as an energy source.
2. Oxidation rates are dependent on inoculum size, but the final percent galena oxidized is independent of inoculum size.

3. The percent galena oxidized is dependent on particle size.
4. The percent galena oxidized decreases with increasing pulp density over 4%.
5. The optimum pH for galena oxidation is 2.0.
6. Ferrous iron in amounts corresponding to 10% of the galena on a molar basis yields optimum oxidation, but all of the iron is not oxidized until galena oxidation has ceased.
7. The maximum percent galena extracted is about 84%.

Like Torma and Subramanian,[191] Tomizuka[192] also observed a pH rise during initiation of galena leaching. Although this can be attributed to the presence of acid-consuming gangue, Tomizuka attributed the acid consumption to the reaction

$$PbS + H_2SO_4 + \tfrac{1}{2}O_2 \longrightarrow PbSO_4 + H_2O + S^0 \quad (22)$$

$$PbS + H_2SO_4 \longrightarrow PbSO_4 + H_2S\uparrow \quad (23)$$

H_2S production was noted. Blockage of reactive surfaces was observed and attributed to the formation of lead sulfate and elemental sulfur. The role of the bacteria is thought to be the oxidation of sulfur, according to Equation 3. The significance of galena leaching rests primarily with the extraction of soluble metal values, as described by Torma and Subramanian,[191] and with the release of precious metals from the galena lattice.

The bio-leaching of arsenopyrite (FeAsS) was described by Pinches.[193] Earlier work on the bacterial degradation of this mineral was conducted by Ehrlich.[194,195] The proposed reactions for the solubilization are

$$2FeAsS + 13/2O_2 + 3H_2O \xrightarrow{\text{bacteria}} 2H_3AsO_4 + 2FeSO_4 \quad (24)$$

$$2FeAsS + Fe_2(SO_4)_3 + 4H_2O + 6O_2 \xrightarrow{\text{chemical}} 2H_3AsO_4 + 4FeSO_4 + H_2SO_4 \quad (25)$$

Arsenic acid (H_3AsO_4) will react with ferric sulfate to form insoluble iron arsenate, accordingly

$$2H_3AsO_4 + Fe_2(SO_4)_3 \rightleftharpoons 2FeAsO_4 + 3H_2SO_4 \quad (26)$$

The use of bacteria to leach arsenopyrite would have the advantage of recovering arsenic as arsenate — a less toxic compound than arsenic trioxide, which is presently formed through roasting processes. The leaching of arsenopyrite could be used to free gold associated with the mineral and other metal values which could be recovered in a fashion discussed by Torma and Subramanian.[191]

Torma et al.,[196] using *T. ferrooxidans* adapted to the substrates, examined the oxidation of synthetically pure cadmium, cobalt, nickel, and zinc sulfides. They attempted to correlate the solubility product of the sulfide metal with the extraction rate. Although their work indicates that metals with a low solubility product have the fastest metal extraction rate, further studies are needed to substantiate this because pure sulfide minerals were used for this study, and no consideration was given for interactions among multisulfide systems.

In further work, Silver and Torma[197] grew *T. ferrooxidans* on either ferrous sulfate, galena, or chalcopyrite and demonstrated oxygen uptake and carbon dioxide fixation when using nickel sulfide (NiS), ferrous sulfate, chalcopyrite, pyrite, stibnite (Sb_2S_3), and cobalt sulfide. The *T. ferrooxidans* were not a single strain. They found that ferrous sulfate- and chalcopyrite-grown cells readily consume oxygen in the presence of pyrite and chalcopyrite with lesser utilization of nickel sulfide and stibnite. Oxygen consumption is minimal for covellite, cobalt sulfide, chalcocite, lead sulfide concentrate, and galena. If *T. ferrooxidans* were initially grown on lead sulfide concentrate, the oxygen uptake pattern differs. *T. ferrooxidans* then exhibits oxygen uptake for chalcopyrite, galena, lead sulfide concentrate, cobalt sulfide, covellite, and chalcocite. The workers reported that oxygen consumption and metal solubilization are not well correlated; however, this may be because the presence of iron would consume oxygen during oxidation as well as solubilize metal. In all instances, the oxygen uptake by *T. ferrooxidans* when subjected to chalcocite and covellite was slight, but the authors[197] attributed this to the increase in pH of the reaction

solution over the experimental period. In the oxidation of nickel sulfide, oxygen is readily assimilated by *T. ferrooxidans*, but carbon dioxide fixation is low. Silver and Torma[197] suggested that adaptation to nickel sulfide may be necessary before carbon dioxide assimilation is normal. They reported that lead sulfide is oxidized to anglesite ($PbSO_4$) and suggested that *T. ferrooxidans* derive energy from the oxidation of the sulfide moiety. The authors[197] observed oxygen uptake and carbon dioxide fixation with *T. ferrooxidans* when red stibnite served as a substrate.

Rossi[198] reported that stibnite can be oxidized by *T. ferrooxidans* as follows:

$$Sb_2S_3 + 6O_2 \longrightarrow Sb_2(SO_4)_3 \quad (27)$$

and the product is partially oxidized to produce antimony (III) oxide sulfate

$$Sb_2(SO_4)_3 + 2H_2O \rightleftharpoons (SbO)_2SO_4 + 2H_2SO_4 \quad (28)$$

Torma[199] further proposed that *T. ferrooxidans* can oxidize the product of this reaction to antimony (V) sulfate

$$Sb_2(SO_4)_3 + O_2 + 2H_2SO_4 \longrightarrow Sb_2(SO_4)_5 + 2H_2O \quad (29)$$

The antimony (V) sulfate is hydrolyzed to antimony (V) bioxide sulfate ($(SbO_2)_2SO_4$), which is insoluble. There does not appear to be any direct oxidation of antimony (III) to antimony (V) by *T. ferrooxidans*.[199]

The oxidation of copper (II) selenide by *T. ferrooxidans* was studied by Torma and Habashi.[200] They established that the bacteria grew with the dissolution of copper and the production of red amorphous selenium. The proposed reaction is

$$CuSe + 2H^+ + \tfrac{1}{2}O_2 \xrightarrow{\text{bacteria}} Cu^{2+} + Se^0 + H_2O \quad (30)$$

The authors[200] noted that despite the similarity between sulfur and selenium, selenium is not oxidized by *T. ferrooxidans* in the same manner as sulfur, since neither selenite nor selenate was detected.

It is evident that ore mineralogy greatly affects leaching, but the mineralogy of associated gangue can also influence leaching by exhibiting a buffering capacity, acting as a cation or anion absorbent, and affecting water potential.[167] Gangue mineralogy is one of the most important parameters in field leaching operations.

Particle Size and Surface Area

The size of the particles to be leached is critical. Large lumps may require years to decrepitate before leaching of internally located sulfide minerals can be contacted[167] The effect of particle size on leaching has been extensively studied for chalcopyrite and sphalerite. Torma et al.[183] examined the effect of carbon dioxide and particle surface area on sphalerite bioleaching. They found zinc extraction dependent upon the amount of particle surface area available per unit volume and upon the carbon dioxide availability. In shake-flask tests, the highest zinc extraction rates (17.6 M*M*/hr) were obtained with the finest particle size and 1% carbon dioxide. If the total available surface area of the particle is increased, the rate of zinc extraction is increased to a point. Torma and Legault[201] extended the work on the effect of total surface area by reporting on the bio-leaching of other sulfide minerals. They found that when the pulp density is calculated as surface area per unit volume of leach solution that the order of resistance of metal sulfides to leaching is as follows:

nickel sulfide > cobalt sulfide > zinc sulfide > cadmium sulfide

Finding that zinc extraction is dependent on particle surface area available, Gormeley et al.[202] modelled the kinetics of a bio-leached sphalerite concentrate in a continuous stirred tank reactor. It was found that leach rates are first order with respect to surface area. Although it has been found that wall growth of *Thiobacillus ferrooxidans* influences steady-state conditions in continuous oxidation of ferrous iron,[134,146] Gormeley et al.[202] reported that this would not be a factor in the continuous oxidation of a solid substrate, since no soluble energy source would be available for the organisms.

Torma and Guay[203] applied the parameters of substrate concentration, specific surface area, total surface area, and particle size to the

Monod equation to obtain information on the biodegradation of sphalerite concentrate by *T. ferrooxidans*. It was found that the highest extraction rates of zinc are obtained using the smallest size fraction. Using Monod's equation, the predicted value for zinc extraction was 9 m*M*/hr. The experimental value was 8 m*M*/hr. When the Monod equation is applied, it is assumed that the following reaction occurs:

$$ZnS + 2O_2 \xrightarrow{T.\ ferrooxidans} ZnSO_4 \qquad (31)$$

However, chemical analysis of the concentrate indicates nearly 2% iron present, and it is assumed that *T. ferrooxidans* would oxidize this iron which would then be present to react with the sphalerite.

Pinches et al.[204] concluded that the most important factor affecting the extraction of copper from a concentrate is the size of the mineral particle. Chalcopyrite with a known surface area was inoculated with *T. ferrooxidans* adapted to copper sulfide minerals. The number of *T. ferrooxidans* in the inoculum was just less than the number required to cover the mineral surface with a monolayer of bacteria. These workers found that at the highest pulp densities copper extraction is less dependent on the solid concentration and is attributed to particle sedimentation, limited mixing, and build-up of precipitates on substrate surfaces. Pinches et al.[204] discovered, as did Torma,[180] that regrinding allows additional copper to be solubilized. Pinches et al.[204] found that the copper yield is proportional to the external surface area for particles larger than 7 μm and that leach depths are constant for particles of this size. If the particle is smaller than 7 μm, the leach depth is more shallow. When the leach rates vs. initial particle size were examined, Pinches et al.[204] found that the rates increase linearly with decreasing particle size but are less dependent when the particle sizes are very small. Torma et al.[183] also observed this when working with a sphalerite concentrate, and they attributed the phenomenon to a dependency on carbon dioxide. However, Pinches et al.[204] indicated that the particle size phenomenon may be a geometrical effect. The authors also suggested that the smaller particle size may affect microbial/mineral interaction.

Jones and Peters[156] found, when leaching chalcopyrite abiotically with ferric sulfate, that if particle sizes are reduced below −300 + 150 μm (−50 + 100 mesh), copper extraction does not improve. Using the SEM, they observed that attack of ferric sulfate on the chalcopyrite crystal is very selective, in that leaching occurs along grain boundaries and fissures. From this, Jones and Peters[156] concluded that the rate of leaching of chalcopyrite by ferric sulfate is dependent upon the area of grain boundaries exposed and that reducing the particle size no longer exposes any more grain boundaries. In contrast to the work of Jones and Peters,[156] Beckstead et al.[205] found that 90% of the copper from chalcopyrite can be extracted in 3 hr using ferric sulfate to leach attritor-ground particles (median size 0.5 μm). These workers[205] attributed enhanced leaching entirely to the increased surface area obtained by particle reduction. Pinches et al.[204] were working with particle sizes ca. 4 to 20 μm. When surface area was increased, either by decreasing the particle size or by increasing the solid concentration, each had similar effects on the leach rate. Therefore, surface area concentration is a real variable in leach rates. These findings correspond to studies on sphalerite[206] and arsenopyrite[193] concentrates. It was shown[204] that as the extraction of metal increases exponentially, the growth of bacteria also increases exponentially. It was suggested that as the particle surface area decreases because of bacterial attachment and reaction products, leaching decreases. It was further suggested that particle-particle collision results in bacterial attrition and reduces the effective number of bacteria taking place in the reaction. These studies strongly indicate that mineral particle size and distribution influence the bacterial growth rate and hence leaching of the chalcopyrite mineral.

Bruynesteyn and Duncan[37] proposed an "active leaching volume." In column tests using an ore containing chalcopyrite, sphalerite, and pyrite, they found that the relationship between the extraction rate and the particle size is a hyperbolic function. The "active leaching volume" is the surface area multiplied by the depth of penetration by bacteria and the lixiviant. The authors[37] disagree with the shrinking core model,[1] because it has been shown that minerals near the surface are not always leached before minerals deeper into the parti-

cles have been oxidized.[86] Bruynesteyn and Duncan[37] found that sphalerite is leached faster than chalcopyrite in particles less than 3.8 cm.

The importance of specific and total surface area has been well documented;[42,183,193,196,201,203] however, it is worth noting that Erlich and Fox[167] observed that the particle size of the host rock can significantly influence the leaching process. Reduction of the particle size will not only increase the available sulfide mineralization, but will increase the gangue surface.

Secondary Mineral Formation

Burkin[207] described three solid-state transformations which can occur during leaching:

1. A solid may solubilize and then precipitate from solution as some other compound.
2. An element may solubilize and remain in solution.
3. An ion or molecule in solution may enter into a crystal lattice to form a new solid.

Iron, if present in high concentrations in solution, will often precipitate and excessive precipitates cause blockage or blinding, i.e., coating of the ore particle, which severely limits leaching[208,209] Other secondary mineral formations also diminish leach rates. During percolator leaching experiments with chalcopyrite, Torma[180] noted copper extractions from 54 to 65% in 8 to 14 months. X-ray analyses of residues showed the formation of jarosite and basic copper sulfate (antherite). The latter is formed by the process

$$3CuSO_4 + 4H_2O \rightleftharpoons CuSO_4 \cdot 2Cu(OH)_2 + 2H_2SO_4 \quad (32)$$

It was noted that jarosite, precipitating on the surface of the mineral, inhibited the leaching process. In tank experiments (30 liters of leach solution and 20% pulp density), it was in fact necessary to regrind the chalcopyrite after leaching to remove the precipitate. After two regrindings and the third leach, 97.6% of the copper was extracted using *T. ferrooxidans*. Each stage of leaching required about 180 hr.

Mossbauer spectra[24] of the $Fe(OH)_3$, produced by the action of *T. ferrooxidans* on ferrous iron, and the $Fe(OH)_3$, produced chemically, showed that spatial patterns of the charges around the ferric ions in the two hydroxides are not the same. This suggests that some precipitates may be biologically generated. Jarosite-type minerals can be formed and are represented as $AFe(SO_4)_2(OH)_6$, where A is one of a number of cations.[208] Ivarson[209] attempted to correlate the formation of basic ferric sulfates with the presence of iron-oxidizing bacteria. Although basic ferric sulfates are found in laboratory experiments when *Thiobacillus ferrooxidans* are present, jarosites can be formed chemically at ambient pressure and temperature.[210] When growing *T. ferrooxidans* on 9K [72] agar medium, ammoniojarosite ($NH_4Fe_3(SO_4)_2(OH)_6$) is formed, as opposed to the formation of jarosite ($KFe_3(SO_4)_2(OH)_6$) when *T. ferrooxidans* are grown in 9K liquid medium.[209] Ivarson concluded that *T. ferrooxidans* play a role in basic ferric sulfate formation under natural conditions. It has been noted that the precipitation of jarosite often depletes the leach solution of cations such as potassium, sodium, and ammonium necessary for the growth of chemoautotrophic bacteria.[209,210]

Chen et al.[211] investigated the blockage problem associated with the leaching of chalcopyrite ores that had been leached with *T. ferrooxidans*. They determined that the blockage problem is associated with the ore particles themselves rather than with the leach solution. This blockage was noted with 45 μm (+325 mesh) ore before copper extraction was complete. Electron microprobe and X-ray diffraction analyses of blocked ore showed the presence of calcium sulfate and elemental sulfur. Mossbauer spectrographic analyses revealed three types of iron in blocked ore, and Auger spectroscopic analyses showed large amounts of oxygen, compared with only small amounts of oxygen present in fresh ore. The oxygen is probably present as insoluble sulfate salts and iron oxides. Although jarosite is found during the leach process, there is no evidence that this salt precipitated on the ore particles. The barrier appears to be physical. Treatments to remove the barrier included solvent extraction, caustic, acid, wet heat, acid plus heat plus oxygenation, reducing agents, and oxidizing agents; none was effective. Of several physical methods used for unblocking, only wet ball milling and dry heat above 300°C were effective. Unblocked ore leached similarly to fresh

ore; however, blockage reoccurs. The authors[211] pointed out that the regrinding operation is of limited practicality at the industrial scale.

When leaching arsenopyrite, Pinches[193] established that the arsenic leach rate is proportional to pulp density at low substrate concentrations; increasing the pulp density decreases the percent arsenic extracted. Pinches[193] attributed the decrease in leach rate to bacterial growth limitations resulting from diminished diffusion and blockage by leach products. As the surface area of arsenopyrite particles increases, leaching rates became constant, suggesting some other rate-limiting factor. However, if the smallest fraction of a pyrite-arsenopyrite concentrate is leached by *T. ferrooxidans*, the extraction of arsenic is low. Pinches[193] proposed that this is due either to flocculation of mineral particles or an interference of microbial/mineral interaction because the particle size is approaching the bacterial size. Leaching must be conducted under acid conditions (pH 1.5), since this suppresses precipitation reactions.

Silver and Torma[197] examined secondary mineral formation after leaching chalcocite and covellite. X-ray diffraction analysis of the residue indicated that mineralogical changes in the minerals had occurred. Products included digenite ($Cu_{9-x}S_5$), antlerite ($Cu_3SO_4(OH_4)$), and metallic copper. Such structure changes have been thoroughly examined by King et al.[212]

Only superficial studies have been made regarding the role of bacteria in the formation and degradation of secondary minerals during leaching. Bacteria can oxidize elemental sulfur formed during the oxidation of some sulfide minerals[207,213] but alternatively bacteria may be responsible for the formation of jarosite[209] and other secondary minerals.

Direct Bacterial Attack of Minerals

The ability of acidified, ferric iron to leach copper from sulfide minerals has been thoroughly reviewed by Dutrizac and MacDonald.[8] The question remains regarding the contribution of the chemolithotrophic bacteria, i.e., do the bacteria directly attack reduced inorganic elements in the mineral structure, or are the bacteria relegated to the production of ferric iron and the oxidation of elemental sulfur formed during metal sulfide reaction with ferric sulfate?

Duncan and Walden[159] addressed this question in the study of the influence of ferric iron on the leaching of chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S), and marmatic zinc sulfide ((ZnFe)S) by *Thiobacillus ferrooxidans*. These workers discovered that adding ferrous iron to bacterial leach systems did not enhance the extraction of copper. It was noted that iron was released from chalcopyrite during the bacterial leaching, but the amount did not correlate with expected iron dissolution. It is unclear from their text whether or not an iron precipitate was observed, but at the conditions used (pH 2.0), precipitation of ferric iron could be expected. The contribution of this released and unaccounted for iron to the extraction of copper cannot be determined. Adding ferric iron in concentrations of 0.27 *M*, 0.54 *M*, and 0.81 *M*, (15, 30, or 45 g/l) to inoculated chalcopyrite actually suppressed copper extraction. It may be that this concentration of ferric iron inhibited *T. ferrooxidans* or that basic ferric sulfate formation sealed the chalcopyrite surface to further leaching. It was also shown that added iron had no effect on the leaching of chalcocite. This would indicate that *T. ferrooxidans* are deriving energy from the oxidation of copper (I) or sulfide moiety. There was no analysis of this concentrate provided; however, even museum-grade specimens ofter contain iron in concentrations high enough that when oxidized could leach chalcocite. Similar experimentation was conducted by Nielsen and Beck[214] in which pure chalcocite was leached biogenically. This specimen however, contained 0.17% iron which is probably sufficient to provide energy for the bacteria and leach the chalcocite with ferric iron. Corrans et al.[25] investigated the leaching of synthetic chalcocite and found ferric iron necessary for the dissolution of copper. Sakaguchi et al.[215] showed that synthetic chalcocite was optimally leached with ferric iron present in concentrations from 4 to 10 m*M* (0.2 to 0.6 g/l.) In the absence of ferric iron or at low concentrations, copper dissolution was greatly diminished. When iron-rich zinc sulfide was leached with *T. ferrooxidans*, the extraction curve was the same with or without added ferric iron.[208] The authors[208] concluded from these data that the extraction of zinc is not mediated

by ferric iron, but it is likely that the iron already present in the sample is sufficient to extract the zinc, and that addition of 0.4 *M* (22.4 g/l) ferric iron did not significantly increase the rate of zinc dissolution. Although the experiments of Duncan and Walden[159] were interpreted to suggest that ferric iron has no effect on the leaching of chalcopyrite, chalcocite, and marmatic zinc sulfide, the fact that these minerals contain iron or at least traces of iron (as in the case of chalcocite) preclude stating that iron is not involved in the leaching of the metals. Alternatively, these experiments do not rule out *T. ferrooxidans* directly attacking the mineral structure.

Duncan and Drummond[86] leached pyrite grains in the presence and absence of *T. ferrooxidans* using micrographic data from the SEM. They concluded that pyrite is leached only by *T. ferrooxidans* and that added ferric iron has no effect on pyrite which had been inoculated. It is possible that the added ferric iron precipitated on the pyrite grains and blocked the pyrite from further leaching.

Le Roux et al.[216] also investigated the leaching of pyrite by *T. ferrooxidans*. Using shake flasks, they concluded that dissolution of pyrite occurs primarily by the reaction

$$FeS_2 + Fe_2(SO_4)_3 \longrightarrow 3FeSO_4 + 2S^0 \qquad (33)$$

Iron is reoxidized by *T. ferrooxidans* according to Equation 7, and sulfur is oxidized biologically by reaction 3. Elemental sulfur slows down reaction 33. After sulfur is oxidized by *T. ferrooxidans* according to reaction 3, reaction 33, will proceed. When sufficient ferrous sulfate is produced by reaction 33, iron will be oxidized by *T. ferrooxidans* according to Equation 7. The authors[216] suggest that this pattern of reactions repeats until 99.8% of the pyrite is leached which requires 12 weeks. Some dissolution (1% over 8 weeks) occurs in sterile controls, and this is attributed to reaction 33 and

$$FeS_2 + 7Fe_2(SO_4)_3 + 8H_2O \longrightarrow 15FeSO_4 + 8H_2SO_4 \qquad (34)$$

An 84-l leaching vessel was used to continuously oxidize pyrite with *T. ferrooxidans*. With tap water as a medium, the generator operated for several years. Data collected indicate about 10^9 bacteria per milliliter were in solution and about 6×10^9 bacteria per milliliter of slurry were associated with the pyritic material, but the oxygen uptake of the bacteria associated with the two phases was equivalent on a cell-to-cell basis.

Pinches[193] found that during the growth and leaching phases, *T. ferrooxidans* are associated with arsenopyrite particles. Leaching is not determined to be by direct attack, and the author[193] emphasized that the bacteria associated with the mineral particle were in effect separated from the true mineral surface by the layer of reaction products. Electron microprobe analyses of leached arsenopyrite particles showed an unleached core and a surficial layer much reduced in iron, arsenic, and sulfur content. Murr and Berry[77] attempted to correlate the attachment of thermophilic, chemolithotrophic organisms to copper and iron dissolution from chalcopyrite. This study was designed to illustrate that attachment signifies direct attack by the bacteria of the mineral substrate. Data provided were not in support of the claim.

The direct attack of pyrite,[70,247] pyrrhotite ($Fe_{n-1}S_n$), and marcosite (FeS_2)[44,248] by *T. ferrooxidans* has been shown. The leaching by these organisms of sulfide minerals containing no iron has also been convincingly demonstrated.[206,249,250]

The contribution of direct bacterial attack to metals dissolution is not known. The vast amount of published data on the role of ferric iron in the oxidation of metal sulfides is irrefutable, but experiments to elucidate the role of bacteria in direct attack of mineral structure are few and contradictory. It is, therefore, too early to disregard the mechanism of direct bacterial attack.

Bacterial Leaching of Mineral Concentrates

A number of investigations have been made on the bacterial leaching of sulfide concentrates, and feasibility studies indicate that biohydrometallurgical techniques are an alternative to smelting operations. Bruynesteyn and Duncan have actively researched the field of bacterial leaching of sulfide concentrates and fast-leaching techniques. In 1971,[43] they reported on the fast-leach technique conducted in stirred tanks with volumes between 5 and 50 l. Sphalerite leaches well, and extractions near 100% can be reached. Solutions are produced with

zinc concentrations up to 1.8 *M* (120 g/l) which approach the zinc concentration necessary for electrowinning. However, when chalcopyrite is leached, blockage problems result, and regrinding of the concentrate is necessary before further copper extraction can result. These investigators[43] contend that bacteria penetrate the chalcopyrite particles about 1 μm, but when particle size increases, the extraction declines.

McElroy and Bruynesteyn[217] reported on a 30-l, single-stage reactor in which chalcopyrite can be bio-leached to obtain leach solution grades of 0.3 *M* to 0.8 *M* (20 to 50 g/ l) copper in 50 hr. By regrinding the chalcopyrite in leach residues and releaching, greater than 96% extraction of copper can be realized.

Sakaguchi et al.[218] investigated the leaching of a chalcopyrite concentrate by *Thiobacillus ferrooxidans* with regard to pH, temperature, and pulp density. They found that at a pH of 2.3, 35°C, and a pulp density of 22%, the maximum rate of copper extraction is obtained. A copper concentration of 0.87 *M* (55 g/l), a value high enough for electrowinning processes, can be obtained with no detrimental effects on *T. ferrooxidans*. The formation of jarosite is a problem, but regrinding of the concentrate to expose new surfaces followed by leaching yields about 80% extraction of copper. An industrial scale leaching process is described by the authors[218] whereby *T. ferrooxidans* can be used to obtain high rates of copper dissolution with good yields.

Engineering Assessment and Dump Leaching

The study of physical and chemical events in dump leaching is difficult due to the massive nature of dumps. Therefore, most studies are conducted in the laboratory using small columns or simulated in large-scale leach tests. Most of the early bio-leaching studies were done in shake flasks and small airlift percolators.[30] The percolator columns usually operate with about 100 g of ore and several hundred milliliters of leach solution. Leaching assessment tests are best conducted in larger columns holding 45 to 454 kg of ore.[37,219] Such tests often require several years to obtain data. These leach columns do, however, offer a great degree of flexibility in controlling conditions, such as heat control for establishing optimum conditions for leach assessment tests on the thermophilic bacteria.[68]

Small-column leach tests were used to ascertain that acid-ferric sulfate leaching of ores containing high percentages of potassium-aluminum silicate is accompanied by a drastic increase in pH with lessening capacity to transport copper. Acid consumption can be minimized by adjusting the leach solutions to near equilibrium with respect to the siliceous material.[220] Leach tests of chalcopyrite ore containing potassium-aluminum silicates produced results which suggest that adding potassium and ferrous chlorides to the lixiviant stabilized acid consumption at a value calculable from mineral stability relationships. Stability can be attained in a pH range suitable for copper transport and bacterial activity.

Column studies have shown that copper release from low-grade porphyry ore is directly proportional to the quantity of oxygen reacting with the ore.[221] Optimum oxidation occurs if the ore is first wetted then drained. The pellicular water (nongravitational) that remains allows for the storage of solubilized salts. The length of the rest period, or time in which the pellicular water remains in association with the ore, is dependent on changes in the pH of the water film resulting from contact with the minerals. If the pH of the pellicular water decreases too much, bacterial activity ceases; conversely, if the pH increases, iron will precipitate, and minerals will be altered. Such pH changes are governed by sulfide and sulfur oxidation or gangue material. Brimhall and Wadsworth[221] cautioned that bubbling oxygen through solution-inundated deposits would be of limited effectiveness, since the diffusion paths are too long. It is really only the pellicular water which is important in leaching.

The injection of compressed air into a copper leach dump yielded a 25% increase in copper extraction, as compared with extraction rates before air injection.[222] It is unclear whether air was applied during the inundation phase or during the rest period. Although the reasoning for the increased extraction rates cannot be determined at this scale, it may be a result of increased bacterial activity and increased permeability. Column tests on the leaching characteristics of ore from the Anaconda Company Berkeley Pit at Butte, Montana[223,251] showed that oxygen would impove bacterial activity and enhance copper extraction. The U.S. Bureau of Mines has conducted leach tests us-

ing 4500- to 9000-kg lots of chalcocite ore.[224] It was found that leaching was greatly enhanced when fines were removed. This improvement was attributed to increased permeability and greater air circulation, which enhanced bacterial activity. Column tests in which the fines were not removed resulted in low leach rates and ultimate dormancy of the leach. Bacteria were absent in the leach solution. The fines apparently interfere with solution and air distribution. The injection of oxygen into the dormant leach system yielded an increase in leaching. It was theorized[224] that initial leaching of chalcocite yielded covellite. When covellite began leaching, elemental sulfur was formed.

$$CuS + Fe_2(SO_4)_3 \longrightarrow CuSO_4 + S^0 + 2FeSO_4 \quad (35)$$

In the absence of bacteria, this sulfur formed a layer prohibiting further leaching. Activation of the leach by air resulted in oxidation of the sulfur (Equation 3) and resumption of leaching.[224] Data from large-scale leaching of copper sulfide ores were compared with calculated results from a mixed kinetics reaction model.[225] Predictions of copper recovery with differing particle size distribution were in agreement with laboratory leaching results. Present column leaching studies at the Salt Lake City Metallurgy Research Center (U.S. Bureau of Mines) include the leaching of 7260-kg lots of low-grade porphyry chalcopyrite with the thermophilic bacteria, *Sulfolobus*,[62,63] at 60°C.[241]

The largest instrumented and contained leaching facility ever to be constructed is the 1.7×10^5-kg (190-ton) test columns at New Mexico Institute of Mining and Technology.[142] The dump leaching of chalcocite and chalcopyrite is being simulated in these tanks, and the chemical, biological, and metallurgical results are correlated with computer models.

Bio-leaching of Oil Shale

The use of thiobacilli is not restricted to the dissolution of metal sulfides and uranium oxide. Findley et al.[226] reported on the leaching of oil shale by thiobacilli to effect matrix dissolution. Presently the release of kerogen from oil shale is by retorting — an energy-consuming process. Development of less energy-costly methods for dissolution of the carbonaceous and siliceous matrix to release kerogen is desirable. Oil shale at 2% pulp density was leached by *Thiobacillus thiooxidans*, using elemental sulfur as an energy source. After 30 days, and ca. 12% weight loss, the oil shale was realized. This represented the dissolution of dolomite ($MgCa(CO_3)_2$). Findley et al.[226] presented no quantitative data on bacterial populations, carbonate extraction, or acid consumption. In further studies, an attempt was made to establish a coupling between *Desulfovibrio* and *Thiobacillus* species.[227] Thiobacilli, using sulfur as a substrate, were grown in the presence of oil shale. Growth was terminated by sterilization, and the spent medium was supplied with lactate and inoculated with *Desulfovibrio*. The medium was sparged with nitrogen to obtain anaerobiosis. Growth of *Desulforvibrio* was evidenced by iron sulfide (FeS) production. However, there were no quantitative data presented by Meyer and Yen[227] as to the actual growth of either organism. It is likely that if the thiobacilli were readily oxidizing sulfur, the pH would be too low for *Desulfovibrio* to initiate growth. Likewise, the medium in which *Desulfovibrio* had grown may be too alkaline for initiation of thiobacilli growth. These factors will be dependent upon the concentration of acid-consuming matrix in the oil shale and the pulp density used in the experiments. In other leach tests,[227] *Thiobacillus* were cultured on 14 l of sulfur medium, and the entire 14 l of spent medium were percolated through 50 g of crushed oil shale. No determination was made of the concentration of sulfuric acid in the spent medium. Dissolution of carbonate was indicated by carbon dioxide production and weight loss of material. At an industrial scale, it probably would be impractical to use such a system unless sulfur could be generated by *Desulfovibrio* using some inexpensive and readily available substrate.

CONCLUDING REMARKS

Much research in geomicrobiology has been conducted in the Soviet Union, Eastern Europe, and Japan. A recent English edition book by Karaivko et al.[252] extensively reviews this literature.

I have examined what I consider to be the most exciting and significant western world de-

velopments in the feild of bacterial leaching, but from this examination, questions can be asked. What accomplishments have really been made? Have these achievements contributed to the development of bio-leaching as a viable industrial process? What future developments would be most contributory to biohydrometallurgy?

We have made substantial progress in describing the physiology and laboratory behavior of the thiobacilli, and we can assess bacterial leaching activity in laboratory leach columns; but we know relatively little about the activity of these organisms in the dump-leaching environment. Nothing is known of the interaction between the thiobacilli and the large heterotrophic population which also inhabits leach dumps. We are likely to discover that complex, mutualistic relationships exist which contribute (or inhibit) to the overall metals dissolution process. This is a fruitful and necessary area of study which could lead to better methods in dump leaching.

The discovery and characterization of the acidothermophilic organisms are first steps in finding new microorganisms which may be used to more effectively and efficiently extract metals from recalcitrant ores. Organisms which oxidize reduced sulfur and iron compounds successfully increase uranium solubilization from pyrite-bearing ores, but ores impoverished in pyrite are unsuitable for leaching by the chemolithotrophic bacteria.[103] Other microorganisms should be carefully examined for their abilities to release uranium. Some fungi are notable for their ability to withstand harsh environmental conditions[228] and produce products which enhance metals dissolution.[229,230] A comprehensive study of these microbes for their bio-leaching characteristics is long overdue.

Attachment of the chemolithotrophic organisms to mineral particles may not in itself be significant, but the understanding of microbial/mineral interactions may be an important disclosure. Elucidation of the mechanisms which are used by bacteria to directly attack the mineral lattice and perhaps utilize the energy from the oxidation of reduced elements other than iron and sulfur would indeed be a consequential step toward further understanding of the organisms. From that discovery, we might learn to further encourage and enhance the industrial activity of microorganisms.

The present trends in economic conditions and increasing environmental restrictions dictate that biohydrometallurgical processes will be competitive with current metal recovery methods. Engineering assessment studies do indicate that bacteria will be able to effectively leach metal concentrates. Vat leaching of both base metal sulfides and uranium oxides is a predictable recovery technique for the near future. Not yet seriously considered, but a probable development, is the use of fixed-film, chemolithotrophic bacterial populations for generation of leach liquors in situations where direct bacterial contact with the ore reduces microbial activity or viability.

Genetic manipulation of chemolithotrophic bacteria has been suggested as a means to increase productivity in leaching.[231] It is known that metal resistance of some organisms is a plasmid-borne characteristic, and the mechanism of resistance usually entails a metal transformation. This field of study is particularly lucrative for development of bacterial strains which could specifically be used in fast-leach operations for dissolution of metals from mineral concentrates and in the leaching of particularly recalcitrant ores or ores containing toxic metals.

Developments in optimizing bacterial leaching have been correlated with studies in hydrometallurgy and geochemistry, and this corroboration has led to such significant advancements in dump leaching as the construction of finger dumps[1] to increase natural aeration and artificial aeration with compressed air;[222] both have enhanced copper extraction. However, the optimum dimensions and configurations for dump construction in a given situation is still not known. Much work is needed to develop reliable mathematical models to predict recovery rate and yields from biological, and indeed other, leaching systems. The increased dialogue among reasearchers of the three disciplines will undoubtedly lead to increased technology in the many research areas described in this paper.

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REFERENCES

1. **Wadsworth, M. E.**, Physico-chemical aspects of solution mining, in *Symp. Innlace. Leaching and Solution Mining*, Kim, Y. S., Ed., Mackay School of Mines, University of Nevada, Reno, November 10 to 14, 1975, chap. 8.
2. **Taylor, J. H. and Whelan, P.F.**, The leaching of cupreous pyrites and the precipitation of copper at Rio Tinto, Spain, *Inst. Min. Metall. Trans.*, 52, 35, 1943.
3. **Sheffer, H. W. and Evans, L. G.**, Copper Leaching Practices in the Western United States, U.S. Bureau of Mines, Information circular 8341, 1968; see also Roman, R. J. and Sheffer, H. W., Copper Leaching Practices in the Western United States, U. S. Bureau of Mines, in preparation.
4. **Rouse, J. V.**, Environmental aspects of *in-situ* mining and dump leaching, in *Solution Mining Symposium 1974*, Aplan, F. F., McKinney, W. A., and Pernichele, A.D., Eds., The American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1974, 3.
5. **Beck, J. V.**, The role of bacteria in copper mining operations, *Biotechnol. Bioeng.*, 9, 487, 1967.
6. **Shock, D. A. and Conley, F. R.**, Solution mining — its promise and its problems, in *Solution Mining Symposium 1974*, Aplan, F. F., McKinney, W. A., and Pernichele, A.D., Eds., The American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1974, 79.
7. **Arnold, C. H., Foster, R. W., Hill, J. M., Kottlowski, F. E., Page, G. B., Reiter, M. A., and Stone, W. J.**, *New Mexico's Energy Resources 1975*, Bull. 107, New Mexico Bureau of Mines and Mineral Resources, Socorro, 1976.
8. **Dutrizac, J. E. and MacDonald, R. J. C.**, Ferric ion as a leaching medium, *Miner. Sci. Eng.*, 6, 59, 1974.
9. **McKinstry, H. E.**, *Mining Geology*, Prentice-Hall, Englewood Cliffs, N.J. 1948.
10. **Sutulov, A.**, *Copper Porphyries*, University of Utah Printing Service, Salt Lake City, 1974.
11. **Malouf, E. E.**, Bioextractive Mining, in *Society of Mining Engineers Short Course*, American Institute of Mining Engineers, Denver, Colo., 1970.
12. **Garrels, R. M. and Christ, C. L.**, *Solutions, Minerals, and Equilibria*, Harper & Row, New York, 1965.
13. **Bhappu, R. B., Johnson, P. H., Brierley, J. A., and Reynolds, D. H.**, Theoretical and practical studies on dump leaching, *AIME Trans.*, 244, 307, 1969.
14. **Fletcher, A. W.**, Metal extraction from waste materials, *Chem. Ind.* (New York), 28, 776, 1971.
15. **Krauskopf, K. B.**, *Introduction to Geochemistry*, McGraw-Hill, New York, 1967.
16. **Granger, H. C.**,Mineralogy, in Geology and Technology of the Grants Uranium Region, Memoir 15, New Mexico Bureau of Mines and Mineral Resources, Socorro, 1963, 21.
17. **Shabbir, M. and Tame, K. E.**, Hydrogen peroxide precipitation of uranium, Bureau of Mines Report of Investigations RI-7931, Washington, D. C., 1974.
18. **Anon.**, Hydrometallurgy is key to winning U_3O_8, *Min. Eng.*, 26, 26, 1974.
19. **Merritt, R. C.**,*The Extractive Metallurgy of Uranium*, Colorado School of Mines Research Institute, Golden, Colo., 1971.
20. **Colmer, A. R. and Hinkle, M. E.**, The role of microorganisms in acid mine drainage: a preliminary report, *Science*, 106, 253, 1947.
21. **Mackintosh, M. E.**, Fixation of $^{15}N_2$ by *Thiobacillus ferrooxidans*, *Proc. Soc. Gen. Microbiol.*, 4, 23, 1976.
22. **Brierley, J. A. and Brierley, C. L.**, Urea as a nitrogen source for thiobacilli, *J. Bacteriol.*, 96, 573, 1968.
23. **O'Conner, L. T., Brierley, J. A., and Bhappu, R. B.**, Bacterial Leaching of Metallic Constituents from Metallurgical Materials, U. S. Patent 3,679,397, 1972.
24. **Lacey, D. T. and Lawson, F.**, Kinetics of the liquid-phase oxidation of acid ferrous sulfate by the bacterium *Thiobacillus ferrooxidans*, *Biotechnol. Bioeng.*, 12, 29, 1970.
25. **Corrans, I. J., Harris, B., and Ralph, B. J.**, Bacterial leaching: an introduction to its application and theory and a study on its mechanism of operation, *J. S. Afr. Inst. Min. Metall.*, 72, 221, 1972.
26. **Tuovinen, O. H., Niemela, S. I., and Gyllenberg, H. G.**, Tolerance of *Thiobacillus ferrooxidans* to some metals, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 37, 489, 1971.
27. **Tuovinen, O. H. and Kelly, D. P.**, Studies on the growth of *Thiobacillus ferrooxidans*. II. Toxicity of uranium to growing cultures and tolerance conferred by mutation, other metal cations, and EDTA, *Arch. Microbiol.*, 95, 153, 1974.
28. **Tuovinen, O. H. and Kelly, D. P.**, Studies on the growth of *Thiobacillus ferrooxidans*. III. Influence of uranium, other metal ions, and 2:4-dinitrophenol on ferrous iron oxidation and carbon dioxide fixation by cell suspensions, *Arch. Microbiol.*, 95, 165, 1974.
29. **MacGregor, R. A.**, Uranium dividends from bacterial leaching, *Min. Eng.*, 21, 54, 1969.
30. **Zaijic, J. E.**, *Microbial Biogeochemistry*, Academic Press, New York and London, 1969.
31. **Duncan, D. W. and Bruynesteyn, A.**, Microbiological Leaching of Uranium, paper presented at 1970 Uranium Symp Socorro, N. M., May 6 to 8, 1970.
32. **Tuovinen, O. H.**, Microbiological aspects in the leaching of uranium by *Thiobacillus ferrooxidans*, *At. Energy Rev.* 10, 251, 1972.
33. **Mrost, M. and Lloyd, P. J.**, The recovery of uranium, in *Symp. Sao Paulo, Proc.*, International Atomic Energy Agency, Vienna, 1970, 79.
34. **Tuovinen, O. H. and Kelly, D. P.**, Use of microorganisms for the recovery of metals, *Int. Metall. Rev.*, 19, 21, 1974.

35. **Trudinger, P. A.**, Microbes, metals, and minerals, *Minerals Sci. Eng.*, 3, 13, 1971.
36. **Bruynesteyn, A. and Cooper, J. R.**, Leaching of Cananea ore in test dumps, in *Solution Mining Symposium 1974*, Aplan, F. F., McKinney, W.A., and Pernichele, A.D., Eds., The American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1974, 268.
37. **Bruynesteyn, A. and Duncan, D. W.**, Effect of particle size on the microbiological leaching of chalcopyrite bearing ore, in *Solution Mining Symposium 1974*, Aplan, F. F., McKinney, W.A., and Pernichele, A.D., Eds., The American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1974, 324.
38. **Le Roux, N. W.**, Bacterial Leaching and its Environmental Implications, paper presented at conf. entitled Introduction to Hydrometallurgy, Hatfield Polytechnic, England, September 13 to 16, 1976.
39. **Ward, M. H.**, Surface blasting followed by *in-situ* leaching in the Big Mike Mine, *Solution Mining Symposium 1974*, Aplan, F. F., McKinney, W. A., and Pernichele, A. D., Eds., The American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1974, 243.
40. **Rudershausen, C. G.**, Copper Solution Mining at Old Reliable, paper presented at conf. Amer. Inst. Chem. Eng., Salt Lake City, Utah, August 18 to 21, 1974.
41. **Catanach, C. B.**, Development and in place leaching of Mountain City chalcocite ore body, in *Extractive Metallurgy of Copper*, Vol. 2, Yannopoulos, J.C. and Agarwal, J.C. Eds., The American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1976, 849.
42. **St. Peter, A. L.**, *In-situ* Mining: A Design for Positive Results, Preprint No. 76-AS-36, presented at AIME Annu. Meet., Las Vegas, February 22 to 26, 1976.
43. **Bruynesteyn, A. and Duncan, D. W.**, Microbiological leaching of sulphide concentrates, *Can. Metall. Q.*, 10, 57, 1971.
44. **Duncan, D. W., Walden, C. C., and Trussell, P. C.**, Biological leaching of mill products, *Can. Min. Metall. Bull.*, 59, 1075, 1966.
45. **Roman, R. J. and Benner, B. R.**, The dissolution of copper concentrates, *Miner. Sci. Eng.*, 5, 3, 1973.
46. **MacGregor, R. A.**, Recovery of U_3O_8 by underground leaching, *Can. Min. Metall. Bull.*, 59, 583, 1966.
47. **MacGregor, R. A.**, The bacterial leaching of uranium, *Nucl. Appl.*, 6, 68, 1969.
48. **Fisher, J. R.**, Bacterial leaching of Elliot Lake uranium ore, *Can. Min. Metall. Bull.*, 59, 588, 1966.
49. **Anderson, J. S. and Ritchie, M. I.**, Solution mining of uranium, *Min. Congr. J.*, 54, 20, 1968.
50. **Anon.**, New method suggested for leaching uranium ore in place, *Eng. Min. J.*, 168, 106, 1967.
51. **Becherkin, S. G., Bakhurov, V. G., and Lutsenko, I. K.**, Underground leaching of uranium from low-grade *in-situ* ores, *Sov. At. Energy*, 24, 154, 1968.
52. **Anon.**, New solution for those who dig uranium, *Chem. Week*, 29, 1975.
53. **Narayan, K. and Rand, P. J.**, Environmental Considerations in Uranium Solution Mining, Preprint No. 76-AS-70, presented at AIME Ann. Meet., Las Vegas, February 22 to 26, 1976.
54. **Temple, K. L. and Koehler, W. A.**, Drainage from bituminous coal mines, *W. Va. Univ. Bull.*, Ser. SY, Number 4, 1954.
55. **Vogler, K. G. and Umbreit, W. W.**, The necessity for direct contact in sulfur oxidation by *Thiobacillus thiooxidans*, *Soil Sci.*, 51, 331, 1941.
56. **Umbreit, W. W., Vogel, H. R., and Vogler, K. G.**, The significance of fat in sulfur oxidation by *Thiobacillus thiooxidans*, *J. Bacteriol.*, 43, 141, 1942.
57. **Schaeffer, W. I., Holbert, P. E., and Umbreit, W. W.**, Attachment of *Thiobacillus thiooxidans* to sulfur crystals, *J. Bacteriol.*, 85, 137, 1963.
58. **Beck, J. V.**, A ferrous-iron-oxidizing bacterium. I. Isolation and some general physiological characteristics, *J. Bacteriol.*, 79, 502, 1960.
59. **Umbreit, W. W., Burris, R. H., and Stauffer, J. F.**, *Manometric and Biochemical Techniques*, 5th ed., Burgess Publishing, Minneapolis, 1972.
60. **Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.**, Protein measurement with the Folin phenol reagent, *J. Biol. Chem.*, 193, 265, 1951.
61. **Collins, C. H.**, *Microbiological Methods*, 2nd ed., Plenum, New York, 1967.
62. **Brock, T. D., Brock, K. M., Belly, R. T., and Weiss, R. L.**, *Sulfolobus:* a new genus of sulfur-oxidizing bacteria living at low pH and high temperatures, *Arch. Microbiol.*, 84, 54, 1972.
63. **Brierley, C. L. and Brierley, J. A.**, A chemoautotrophic and thermophilic microorganism isolated from an acid hot spring, *Can. J. Microbiol.*, 19, 183, 1973.
64. **McGoran, C. J. M., Duncan, D. W., and Walden, C. C.**, Growth of *Thiobacillus ferrooxidans* on various substrates, *Can. J. Microbiol.*, 15, 135, 1969.
65. **Smith, D. W., Fliermans, C. B., and Brock, T. D.**, Technique for measuring $^{14}CO_2$ uptake by soil microorganisms *in-situ*, *Appl. Microbiol.*, 23, 595, 1972.
66. **Smith, D. W., Fliermans, C. B., and Brock, T. D.**, An isotopic technique for measuring the autotrophic activity of soil microorganisms *in-situ*, *Ecol. Res. Comm. Bull.*, 17, 243, 1973.
67. **Fliermans, C. B. and Brock, T. D.**, Ecology of sulfur-oxidizing bacteria in hot acid soils, *J. Bacteriol.*, 111, 343, 1972.
68. **Brierley, C. L.**, Thermophilic microorganisms in extraction of metals from ores, *Dev. Ind. Microbiol.*, 18, 273, 1977.

69. **Tuovinen, O. H. and Kelly, D. P.,** Studies on the growth of *Thiobacillus ferrooxidans*. I. Use of membrane filters and ferrous iron agar to determine viable numbers, and comparison with $^{14}CO_2$-fixation and iron oxidation as measures of growth, *Arch. Microbiol.*, 88, 285, 1973.
70. **Bryner, L. C. and Jameson, A. K.,** Microorganisms in leaching sulfide minerals, *Appl. Microbiol.*, 6, 281, 1958.
71. **Gormely, L. S. and Duncan, D. W.,** Estimation of *Thiobacillus ferrooxidans* concentrations, *Can. J. Microbiol.*, 20, 1453, 1974.
72. **Silverman, M. P. and Lundgren, D. G.,** Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An impoved medium and a harvesting procedure for securing high cell yields, *J. Bacteriol.*, 77, 642, 1959.
73. **Shuler, M. L. and Tsuchiya, H. M.,** Determination of cell numbers and cell size for *Thiobacillus ferrooxidans* by the electrical resistance method, *Biotechnol. Bioeng.*, 17, 621, 1975.
74. **Apel, W. A., Dugan, P. R., Filppi, J. A., and Rheins, M. S.,** Detection of *Thiobacillus ferrooxidans* in acid mine environments by indirect fluorescent antibody staining, *Appl. Environ. Microbiol.*, 32, 159, 1976.
75. **Bohlool, B. B. and Brock, T. D.,** Population ecology of *Sulfolobus acidocaldarius*. II. Immunoecological studies, *Arch. Microbiol.*, 97, 181, 1974.
76. **Weiss, R. L.,** Attachment of bacteria to sulphur in extreme environments, *J. Gen. Microbiol.*, 77, 501, 1973.
77. **Murr, L. E. and Berry, V. K.,** Direct observations of selective attachment of bacteria on low-grade sulfide ores and other mineral surfaces, *Hydrometallurgy*, 2, 11, 1976.
78. **McElroy, W. D.,** The energy source for bioluminescence in an isolated system, *Proc. Nat. Acad. Sci. U.S.A.*, 33, 342, 1947.
79. **Holm-Hansen, O.,** Determination of total microbial biomass by measurement of adenosine triphosphate, in *Estuarine Microbial Ecology*, Stevenson, L. H., and Colwess, R. R., Eds., University of South Carolina Press, Columbia, 1973, 73.
80. **Starkey, R. L., Jones, G. E., and Frederick, L. R.,** Effects of medium agitation and wetting agents on oxidation of sulfur by *Thiobacillus thiooxidans J. Gen. Microbiol.*, 15, 329, 1956.
81. **Schaeffer, W. I., Holbert, P. E., and Umbreit, W. W.,** Attachment of *Thiobacillus thiooxidans* to sulfur crystals, *J. Bacteriol.*, 85, 137, 1963.
82. **Hirsch, P. and Pankratz, S. H.,** Study of bacterial populations in natural environments by use of submerged electron microscope grids, *Z. Allg. Mikrobiol.*, 10, 589, 1970.
83. **Dugiud, J. P.,** Fimbriae and adhesive properties in *Klebsiella* strains, *J. Gen. Microbiol.*, 21, 271, 1959.
84. **Shivvers, D. W. and Brock, T. D.,** Oxidation of elemental sulfur by *Sulfolobus acidocaldarius, J. Bacteriol.*, 114, 706, 1973.
85. **Millonig, G., De Rosa, M., Gambacorta, A., and Bu'lock, J. D.,** Ultrastructure of an extremely thermophilic acidophilic micro-organism, *J. Gen. Microbiol.*, 86, 165, 1975.
86. **Duncan, D. W. and Drummond, A. D.,** Microbiological leaching of prophyry copper type mineralization: post-leaching observations, *Can. J. Earth Sci.*, 10, 476, 1973.
87. **Baldensperger, J., Guarraia, L. J., and Humphreys, W. J.,** Scanning electron microscopy of thiobacilli grown on collodial sulfur, *Arch. Microbiol.*, 99, 323, 1974.
88. **Brierley, C. L. and Murr, L. E.,** Leaching: use of a thermophilic and chemoautotrophic microbe, *Science*, 179, 488, 1973.
89. **Brierley, C. L., Brierley, J. A., and Murr, L. E.,** Using the SEM in mining research, *Res. Dev.*, 24, 24, 1973.
90. **Berry, V. K. and Murr, L. E.,** Bacterial attachment to molybdenite: an electron microscope study, *Metall. Trans.*, B6, 488, 1975.
91. **Weertman, J. and Weertman, J. R.,** Forces on a dislocation, in *Elementary Dislocation Theory*, Macmillan, New York, 1964, 45.
92. **Murr, L. E. and Berry, V. K.,** An electron microscope study of bacterial attachment to chalcopyrite: microstructural aspects of leaching, in *Extractive Metallurgy of Copper*, Vol. 2, Yannopoulos, J. C. and Agarwal, J. C., Eds., The American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1976, 670.
93. **Marshall, K. C., Stout, R., and Mitchell, R.** , Selective sorption of bacteria from seawater, *Can. J. Microbiol.*, 17, 1413, 1971.
94. **Marshall, K. C. and Cruickshank, R. H.,** Cell surface hydrophobicity and the orientation of certain bacteria at interfaces, *Arch. Microbiol.*, 91, 29, 1973.
95. **Fletcher, M. and Floodgate, G. D.,** The adhesion of bacteria to solid surfaces, in *Microbial Ultrastructure-The Use of the Electron Microscope*, Fuller, R. and Lovelock, D. W., Eds., Academic Press, London, 1976, 101.
96. **Fletcher, M. and Loeb, G. I.,** The influence of substratum surface properties on the attachment of a marine bacterium, *Colloid Sci.*, 3, 459, 1976.
97. **Fletcher, M. and Loeb, G. I.,** The Importance of Substratum Surface Charge Density and Surface Free Energy in Marine Bacterial Attachment, paper presented at 3rd Int. Symp. Environ. Biogeochem., Wolfenbüttel, Germany, March 27 to April 2, 1977.
98. **Fletcher, M.,** The effects of culture concentration and age, time, and temperature on bacterial attachment to polystyrene, *Can. J. Microbiol.*, 23, 1, 1977.
99. **Wadsworth, M. E.,** Annual review, metallurgical processes: hydrometallurgy, *J. Met.*, 29, 8, 1977.

100. **McCreedy, H. H.,** Bacterial Leaching to be Applied to a New Canadian Uranium Mine, paper presented at Coordinated Research Committee Meeting of International Atomic Energy Agency, Coventry, England, December 13 to 15, 1976.
101. **Derry, R., Garrett, K. H., Le Roux, N. W., and Smith, S. E.,** A bacterially assisted plant process for leaching uranium ores, *Inst. Min. Metall. Trans.*, in press, 1977.
102. **Brierley, J. A. and Le Roux, N. W.,** A facultative thermophilic *Thiobacillus*-like bacterium: oxidation of iron and pyrite, in *Conference Bacterial Leaching 1977*, Schwartz, W., Ed., Verlag Chemie, New York, 1977, 55.
103. **Brierley, C. L., Biogenic Extraction of Uranium from Ores of the Grants Region, paper presented at Int. Symp. Metallurg. Applic. Bacterial Leaching Related Microbiol. Phenomena, Proc., Socorro, N. M., August 3 to 5, 1977, in press.**
104. **Jacobs, M. L., Warren, C. G., and Granger, H. C.,** Chemical Extraction of an Organic Material from a Uranium Ore, U.S. Geological Survey, Prof. paper 700-B, B184, 1970.
105. **Updegraff, D. M. and Douros, J. D.,** The relationship of microorganisms to uranium deposits, *Dev. Ind. Microbiol.*, 13, 76, 1972.
106. **Magne, R. A., Berthelin, J. R., and Dommergues, Y. R.,** Microbial Solubilization of Uranium from Ores, Granite, and Granite Sand, paper presented at Symp. Envir. Biogeochem., Utah State University, Logan, March 22 to 24, 1973.
107. **Berthelin, J. R. and Magne, R.,** Some aspects of the mechanisms of solubilization and insolubilization of uranium from granitic rocks by heterotrophic microorganisms, in *Conference Bacterial Leaching 1977*, Schwartz, W., Ed., Verlag Chemie, New York, 1977, 251.
108. **Kelly, D. P., Justin, P. M. G. S., Eccleston, M., and Jones, C. A., Growth and Substrate Metabolism of *Thiobacillus ferrooxidans* and *Thiobacillus denitrificans* Studied Using Continuous Flow Culture Techniques, paper presented at International Atomic Energy Agency Coordination Meeting, University of Warwick, England, December 13 to 15, 1976.**
109. **Walsh, F. and Mitchell, R.,** An acid-tolerant iron-oxidizing *Metallogenium*, *J. Gen. Microbiol.*, 72, 369, 1972.
110. **Walsh, F. and Mitchell, R.,** A pH-dependent succession of iron bacteria, *Environ. Sci. Technol.*, 6, 809, 1972.
111. **Sivelä, S. and Sundman, V.,** Demonstration of *Thiobacillus*-type bacteria, which utilize methyl sulphides, *Arch. Microbiol.*, 103, 303, 1975.
112. **Markosyan, G. E.,** A new mixotrophic sulfur bacterium developing in acid media, *Thiobacillus organoparus*, sp. N., *Dokl. Akad. Nauk SSSR*, 211, 1205, 1973.
113. **London, J. and Rittenberg, S. C.,** *Thiobacillus perometabolis* nov. sp. , a non-autotrophic thiobacillus, *Archiv. Mikrobiol.*, 59, 218, 1967.
114. **Myers, P. A. and Millar, W. N.,** Nonautotrophic *Thiobacillus* in acid mine water, *Appl. Microbiol.*, 30, 884, 1975.
115. **Guay, R. and Silver, M.,** *Thiobacillus acidophilus* sp. nov.; isolation and some physiological characteristics, *Can. J. Microbiol.*, 21, 281, 1975.
116. **Brierley, J. A., Contribution of Chemoautotrophic Bacteria to the Acid Thermal Waters of the Geyser Spring Group in Yellowstone National Park, Ph.D. thesis, Montana State University, Bozeman, 1966.**
117. **Brock, T. D. and Darland, G. K.,** Limits of microbial existence: temperature and pH, *Science*, 169, 1316, 1970.
118. **Weiss, R. L.,** Subunit cell wall of *Sulfolobus acidocaldarius*, *J. Bacteriol.*, 118, 275, 1974.
119. **Langworthy, T. A., Mayberry, W. R., and Smith, P. F.,** Longchain glycerol diether and polyol dialkyl glycerol triether lipids of *Sulfolobus acidocaldarius*, *J. Bacteriol.*, 119, 106, 1974.
120. **De Rosa, M., Gambacorta, A., Minale, L., and Bu'Lock, J. D., Cyclic diether lipids from very thermophilic acidophilic bacteria, *J. Chem. Soc. Chem. Commun.*, 1974, 543, 1974.**
121. **Langworthy, T. A.,** Comparative lipid composition of heterotrophically and autotrophically grown *Sulfolobus acidocaldarius*, *J. Bacteriol.*, 130, 1326, 1977.
122. **De Rosa, M., Gambacorta, A., and Bu'lock, J. D.,** Extremely thermophilic acidophilic bacteria convergent with *Sulfolobus acidocaldarius*, *J. Gen. Microbiol.*, 86, 156, 1975.
123. **Darland, G., Brock, T. D., Samsonoff, W., and Conti, S. F.,** A thermophilic, acidophilic mycoplasma isolated from a coal refuse pile, *Science*, 170, 1416, 1970.
124. **Brock, T. D.,** Microbial growth under extreme conditions, *Symp. Soc. Gen. Microbiol.*, 19, 15, 1969.
125. **Noguchi, A., Takama, M., Sekiguchi, T., Koyama, N., and Nosoh, Y.,** Acidostability of spheroplasts prepared from *Thiobacillus thiooxidans*, *Arch. Microbiol.*, 112, 163, 1977.
126. **Kaplan, I. R.,** Evidence of microbiological activity in some of the geothermal regions of New Zealand, *N.Z.J. Sci. Technol.*, 37, 639, 1956.
127. **Schoen, R. and Ehrlich, G. G.,** Bacterial origin of sulfuric acid and sulfurous hot springs, *Twenty-third Int. Geol. Cong. , (Prague)*, Vol. 17, 1968, 171.
128. **Schwartz, A. and Schwartz, W.,** Geomikrobiologische untersuchungen. VII. Ueber des vorkommen von mikroorganismen in solfataran und heissen quellen, *Z. Allg. Mikrobiol.*, 5, 395, 1965.
129. **Weiss, R. L.,** Survival of bacteria at low pH and high temperature, *Limnol. Oceanogr.*, 18, 877, 1973.
130. **Mosser, J. L., Bohool, B. B., and Brock, T. D.,** Growth rates of *Sulfolobus acidocaldarius* in nature, *J. Bacteriol.*, 118, 1075, 1974.
131. **Bohlool, B. B.,** Occurrence of *Sulfolobus acidocaldarius*, an extremely thermophilic acidophilic bacterium, in New Zealand hot springs. Isolation and immunofluorescence characterization, *Arch. Microbiol.*, 106, 171, 1975.

132. **Le Roux, N. W., Wakerley, D. S., and Hunt, S. D.,** Thermophilic *Thiobacillus*-type bacteria from Icelandic thermal areas, *J. Gen. Microbiol.*, 100, 197, 1977.
133. **Mosser, J. L., Mosser, A. G., and Brock, T. D.,** Bacterial origin of sulfuric acid in geothermal habitats, *Science*, 179, 1323, 1973.
134. **Mehta, K. B. and Le Roux, N. W.** Effect of wall growth on continuous biological oxidation of ferrous iron, *Biotechnol. Bioeng.*, 16, 559, 1974.
135. **Mosser, J. L., Mosser, A. G., and Brock, T. D.,** Population ecology of *Sulfolobus acidocaldarius*. I. Temperature strains, *Arch. Microbiol.*, 97, 169, 1974.
136. **Brock, T. D., Cook, S., Petersen, S., and Mosser, J. L.,** Biogeochemistry and bacteriology of ferrous iron oxidation in geothermal habitats, *Geochim. Cosmochim. Acta*, 40, 493, 1976.
137. **Brock, T. D. and Gustafson, J.,** Ferric iron reduction by sulfur- and iron-oxidizing bacteria, *Appl. Environ. Microbiol.*, 32, 567, 1976.
138. **Brierley, C. L. and Brierley, J. A.,** Reduction of Molybdenum by a Thermophilic Bacterium, paper presented at Int. Conf. Heavy Metals in the Environment, Toronto, October 27 to 31, 1975, 211.
139. **Brierley, C. L.,** Molybdenite-leaching: use of a high-temperature microbe, *J. Less-Common Met.*, 36, 237, 1974.
140. **Bryner, L. C. and Anderson, R.,** Microorganisms in leaching sulfide minerals, *Ind. Eng. Chem.*, 49, 1721, 1957.
141. **Brierley, J. A. and Brierley, C. L.,** Microbial Leaching of Copper at Ambient and Elevated Temperature, paper presented at Int. Symp. Metallurg. Applic. Bacterial Leaching and Related Microbiol. Phenomena, Proc., Socorro, N. M., August 3 to 5, 1977, in press.
142. **Murr, L. E., Cathles, L. M., Reese, D. A., Hiskey, J. B., Popp, C. J., Brierley, J. A., Bloss, D., Berry, V. K., Schlitt, W. J., and Hsu, P. C.,** Chemical, Biological and Metallurgical Aspects of Large-scale Column Leaching Experiments for Solution Mining and *In-situ* Leaching, paper presented at Amer. Nucl. Soc. Topical Meeting, Golden, Colo., April 11–14, 1977.
143. **Brierley, J. A. and Lockwood, S. J.,** The occurrence of thermophilic iron-oxidizing bacteria in a copper leaching system, *FEMS Microbiol. Letters*, 2, 163, 1977.
144. **Singer, P. C. and Stumm, W.,** Acidic mine drainage: the rate-determining step, *Science*, 167, 1121, 1970.
145. **Lees, H., Kwok, S. C., and Suzuki, I.,** The thermodynamics of iron oxidation by the ferrobacilli, *Can. J. Microbiol.*, 15, 43, 1969.
146. **MacDonald, D. G. and Clark, R. H.,** The oxidation of aqueous ferrous sulphate by *Thiobacillus ferrooxidans*, *Can. J. Chem. Eng.*, 48, 669, 1970.
147. **Schnaitman, C. A., Korczyniski, M. S., and Lundgren, D. G.,** Kinetic studies of iron oxidation by whole cells of *Ferrobacillus ferrooxidans*, *J. Bacteriol.*, 99, 552, 1969.
148. **Wong, C. W., Scharer, J. M., and Reilly, P. M.,** A Discrimination among Microbial Iron Oxidation Mechanisms, paper presented at 4th Joint American Institute of Chemical Engineering-Canadian Society of Chemical Engineering Conf., Vancouver, Canada, September 9 to 12, 1973.
149. **Kelly, D. P., Jones, C. A., and Green, J. S.,** Factors Affecting Metabolism and Ferrous Iron Oxidation in Suspensions and Batch Cultures of *Thiobacillus ferrooxidans*, paper presented at Int. Symp. Metallurg. Applic. Bacterial Leaching and Related Microbiol. Phenomena, Proc., Socorro, N. M., August 3 to 5, 1977, in press.
150. **Bodo, C. and Lundgren, D. G.,** Iron oxidation by cell envelopes of *Thiobacillus ferrooxidans*, *Can. J. Microbiol.*, 20, 1647, 1974.
151. **Imai, K., Sugio, T., Yasuhara, T., and Tano, T.,** Iron Oxidation by Bacteria, paper presented at Joint Meet. Mining and Metallurgical Institute of Japan and American Institue of Mining, Metallurgical, and Petroleum Engineers, Tokyo, Japan, May 24 to 27, 1972.
152. **Lazaroff, N.,** Sulfate requirement for iron oxidation by *Thiobacillus ferrooxidans*, *J. Bacteriol.*, 85, 78, 1963.
153. **Dugan, P. R. and Lundgren, D. G.,** Energy supply for the chemoautotroph *Ferrobacillus ferrooxidans*, *J. Bacteriol.*, 89, 825, 1965.
154. **Steiner, M. and Lazaroff, N.** Direct method for continuous determination of iron oxidation by autotrophic bacteria, *Appl. Microbiol.*, 28, 872, 1974.
155. **Lazaroff, N.,** The specificity of the anionic requirement for iron oxidation by *Thiobacillus ferrooxidans*, *J. Gen. Microbiol.*, 101, 85, 1977.
156. **Jones, D. L. and Peters, E.,** The leaching of chalcopyrite with ferric sulfate and ferric chloride, in *Extractive Metallurgy of Copper*, Vol. 2, Yannopoulos, J.C. and Agarwal, J. D., Eds., The American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1974, 633.
157. **Dutrizac, J. E., MacDonald, R. J. C., and Ingraham, T. R.,** The kinetics of dissolution of synthetic chalcopyrite in aqueous acidic ferric sulfate solution, *Trans. Metall. Soc. AIME*, 245, 955, 1969.
158. **Leathen, W. W., Braley, S. A., and McIntyre, L. D.,** The role of bacteria in the formation of acid from certain sulfuritic constituents associated with bituminous coal. I. *Thiobacillus thiooxidans*, *Appl. Microbiol.*, 1, 61, 1953.
159. **Duncan, D. W. and Walden, C. C.,** Microbiological leaching in the presence of ferric iron, *Dev. Ind. Microbiol.*, 13, 66, 1972.
160. **Sadler, W. R. and Trudinger, P. A.,** The inhibition of microorganisms by heavy metals, *Miner. Deposita*, 2, 158, 1967.
161. **Tuovinen, O. H. and Kelly, D. P.,** Biology of *Thiobacillus ferrooxidans* in relation to the microbiological leaching of sulphide ores, *Z. Allg. Mikrobiol.*, 12, 311, 1972.

162. **Tuovinen, O. H. and Kelly, D. P.,** Studies on the growth of *Thiobacillus ferrooxidans.* IV. Influence of monovalent metal cations on ferrous iron oxidation and uranium toxicity in growing cultures, *Arch. Microbiol.*, 98, 167, 1974.
163. **Ashida, J.,** Adaptation of fungi to metal toxicant, *Annu. Rev. Phytopathol.*, 3, 153, 1965.
164. **Griffiths, A. J., Hughes, D. E., and Thomas, D.,** Some Aspects of Microbial Resistance to Metal Pollution, paper presented at Conf. Minerals and the Environment, Paper Number 24, The Institution of Mining and Metallurgy, London, June 4 to 7, 1974.
165. **Babich, H. and Stotzky, G.,** Reductions in the toxicity of cadmium to microorganisms by clay minerals, *Appl. Envir. Microbiol.*, 33, 696, 1977.
166. **Babich, H. and Stotzky, G.,** Effect of cadmium on fungi and on interactions between fungi and bacteria in soil: influence of clay minerals and pH, *Appl. Envir. Microbiol.*, 33, 1059, 1977.
167. **Ehrlich, H. L. and Fox, S. I.,** Environmental effects on bacterial copper extraction from low-grade copper sulfide ores, *Biotechnol. Bioeng.*, 9, 471, 1967.
168. **Tuovinen, O. H., Niemela, S. I., and Gyllenberg, H. G.,** Effect of mineral nutrients and organic substances on the devlopment of *Thiobacillus ferrooxidans, Biotechnol. Bioeng.*, 13, 517, 1971.
169. **Dugan, P. R., MacMillan, C. B., and Pfister, R. M.,** Aerobic heterotrophic bacteria indigenous to pH 2.8 acid mine water: predominant slime-producing bacteria in acid streamers, *J. Bacteriol.*, 101, 982, 1970.
170. **Ehrlich, H. L.,** Microorganisms in acid drainage from a copper mine, *J. Bacteriol.*, 86, 350, 1963.
171. **Tsuchiya, H. M., Trivedi, N. C., and Schuler, M. L.,** Microbial mutualism in ore leaching, *Biotechnol. Bioeng.*, 16, 991, 1974.
172. **Trivedi, N. C. and Tsuchiya, H. M.,** Microbial mutualism in leaching of Cu-Ni sulfide concentrate, *Int. J. Miner. Process.*, 2, 1, 1975.
173. **Tsuchiya, H. M.,** Microbial Leaching of Cu-Ni from Ore from the Duluth Gabro, paper presented at the Round Table Conf. on Leaching, Braunschweig, Germany, March 27 to April 2, 1977, 101.
174. **Becking, J. H.,** Studies on the nitrogen-fixing bacteria of the genus *Beijerinckia.* I. Geographical and ecological distribution in soils, *Plant Soil*, 14, 49, 1961.
175. **Alexander, M.,** *Introduction to Soil Microbiology*, John Wiley & Sons, New York and London, 1961.
176. **Becking, J. H.,** Studies on the nitrogen-fixing bacteria of the genus *Beijerinckia.* II. Mineral nutrition and resistance to high levels of certain elements in relation to soil type, *Plant Soil*, 14, 297, 1961.
177. **Brock, T. D.,** Effect of water potential on growth and iron oxidation by *Thiobacillus ferrooxidans, Appl. Microbiol.*, 29, 499, 1975.
178. **Kennedy, W. A. and Stahl, J. R.,** Fluid retention in leach dumps by capillary action, in *Solution Mining Symposium 1974*, Aplan, F. F., McKinney, W.A., and Pernichele, A.D., Eds., The American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1974, 99.
179. **Torma, A. E. and Itzkovitch, I. J.,** Influence of organic solvents on chalcopyrite oxidation ability of *Thiobacillus ferrooxidans, Appl. Envir. Microbiol.*, 32, 102, 1976.
180. **Torma, A. E.,** Biodegradation of chalcopyrite, in *3rd Int. Biodegradation Symposium, Proc.*, Sharpley, J. M. and Kaplan, A. M., Eds., Applied Science Publishers, London, 1976, 937.
181. **Torma, A. E., Gabra, G. G., Guay, R., and Silver, M.,** Effects of surface active agents on the oxidation of chalcopyrite by *Thiobacillus ferrooxidans, Hydrometall.*, 1, 301, 1976.
182. **Duncan, D. W. and Trussell, P. C.,** Advances in the microbiological leaching of sulphide ores, *Can. Metall. Q.*, 3. 43, 1964.
183. **Torma, A. E., Walden, C. C., Duncan, D. W., and Branion, R. M. R.,** The effect of carbon dioxide and particle surface area on the microbiological leaching of a zinc sulphide concentrate, *Biotechnol. Bioeng.*, 14, 777, 1972.
184. **Lyalikova, N. N.,** Participation of *Thiobacillus ferrooxidans* in the oxidation of sulfide ores in pyrite beds of the Middle Ural, *Mikrobiologya*, 29, 281, 1960.
185. **Le Roux, N. W. and Marshall, V. M.,** Effect of Light on Thiobacilli, paper presented at the Round Table Conf. on Leaching, Braunschweig, Germany, March 27 to April 2, 1977, 21.
186. **Torma, A. E.,** Microbiological Extraction of Cobalt and Nickel from Sulphide Ores and Concentrates, Canadian Patent 1382357, 1972.
187. **Jannasch, H. W. and Wirsen, C. O.,** Microbial life in the deep sea, *Sci. Am.*, 236, 42, 1977.
188. **Anon.,** Life in the depths, *Time*, 59, May 2, 1977.
189. **Leach, D. L. And Braun, R. L.,** Leaching of Primary Sulfide Ores in Sulfuric Acid Solutions at Elevated Temperatures and Pressures, paper presented at the American Institute Mining Engineers Meet., New York, February 16 to 20, 1975, Preprint Number 75-B-68.
190. **Braithwaite, J. W. and Wadsworth, M. E.,** Oxidation of chalcopyrite under simulated conditions of deep solution mining, in *Extractive Metallurgy of Copper*, Vol. 2, Yannopoulos, J.C. and Agarwal, J.C., Eds., The American Institute Mining, Metallurgical, and Petroleum Engineers, New York, 1976, 752.
191. **Torma, A. E. and Subramanian, K. N.,** Selective bacterial leaching of a lead sulphide concentrate, *Int. J. Miner. Process.*, 1, 125, 1973.
192. **Tomizuka, N.,** Microbiological oxidation of a lead sulphide, *Fermentation Res. Inst. Report*, 48, 51, 1976.
193. **Pinches, A.,** Bacterial leaching of an arsenic-bearing sulphide concentrate, in *Leaching and Reduction in Hydrometallurgy*, Burkin, A. R., Ed. Institution of Mining and Metallurgy, London, 1975, 28.

194. **Ehrlich, H. L.**, Bacterial action of orpiment, *Econ. Geol.*, 58, 991, 1963.
195. **Ehrlich, H. L.**, Bacterial oxidation of arsenopyrite and enargite, *Econ. Geol.*, 59, 1306, 1964.
196. **Torma, A. E., Legault, G., and Kougioumoutzakis, D.**,Kinetics of bio-oxidation of metal sulphides, Paper Number 39d, presented at the 4th Joint American Institute of Chemical Engineering-Canadian Society of Chemical Engineering Conf., Vancouver, Canada, September 9 to 12, 1973.
197. **Silver, M. and Torma, A. E.**, Oxidation of metal sulfides by *Thiobacillus ferrooxidans* grown on different substrates, *Can. J. Microbiol.*, 20 141, 1974.
198. **Rossi, G.**, The microbiological leaching of ore minerals. III. The action of stibnite ores on microorganisms found in acid drainage waters of Italian mines, *Resoconti Assoc. Min. Sarda*, 76, 1, 1971.
199. **Torma, A. E. and Garbra, G. G.**, Oxidation of stibnite by *Thiobacillus ferroxidans, Antonie van Leuwenhoek J. Microbiol. Serol.*, 43, 1, 1977.
200. **Torma, A. E. and Habashi, F.**, Oxidation of copper (II) selenide by *Thiobacillus ferrooxidans, Can. J. Microbiol.*, 18, 1780, 1972.
201. **Torma, A. E. and Legault, G.**, Rôle de la surface des minerais sulfurés lors de leur biodegradation par *Thiobacillus ferrodoxidans, Ann. Microbiol., (Inst. Pasteur)*, 124A, 111, 1973.
202. **Gormely, L. S., Duncan, D. W., Branion, R.M.R., and Pinder, K. L.**, Continuous culture of *Thiobacillus ferrooxidans* on a zinc sulfide concentrate, *Biotechnol. Bioeng.*, 17, 31, 1975.
203. **Torma, A. E. and Guay, R.**, Effect of particle size on the biodegradation of a sphaferite concentrate, *Nat. Can.* 103, 133, 1976.
204. **Pinches, A., Al-Jaid, F. O., and William, D.J.A.**, Leaching of chalcopyrite concentrates with *Thiobacillus ferrooxidans* in batch culture, *Hydrometall.*, 2, 87, 1976.
205. **Beckstead, L. W., Munoz, P. B., Sepulveda, J. L., Herbst J. A., Miller, J. D., Olsen, F. A., And Wadsworth, M. E.**, Acid ferric sulfate leaching of attritor-ground chalco-pyrite concentrates, in *Extractive Metallurgy of Copper*, Vol. 2, Yannopoulos, J. C. and Agarwal, J. C., Eds., American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1976, 611.
206. **Torma, A. E., Walden, C. C., Duncan, D. W., and Branion, R.M.R.**, Microbiological leaching of a zinc sulfide concentrate, *Biotechnol. Bioeng.*, 12, 501, 1970.
207. **Burkin, A. R.**, Solid-state transformations during leaching, *Miner. Sci. Eng.*, 1, 4, 1969.
208. **Duncan, D. W. and Bruynesteyn, A.**, Enhancing bacterial activity in a uranium mine, *Can. Min. Metall. Bull.*, 64, 32, 1971.
209. **Ivarson, K. C.**, Microbiological formation of basic ferric sulfates, *Can. J. Soil Sci.*, 53, 315, 1973.
210. **Brown, J. B.**, A chemical study of some synthetic potassium-hydronium jarosites, *Can. Mineral.*, 10, 696, 1970.
211. **Chen, C. S., Huddleston, R. L., and Johnson, M. A.**, Bioleaching of Chalcopyrite Concentrate, Paper Number 39c, presented at the 4th Joint American Institute of Chemical Engineering-Canadian Society of Chemical Engineering Conf., Vancouver, Canada, September 9 to 12, 1973.
212. **King, J. A., Burkin, A. R., and Ferreira, R. C. H.**, Leaching of chalcocite by acidic chloride solutions, in *Leaching and Reduction in Hydrometallurgy*, Burkin, A. R., Ed., Institution of Mining and Metallurgy, London, 1975, 36.
213. **Mishra, R. K. and Wadsworth, M. E.**, Low Temperature Surface Reactions in the Leaching of Chalcopyrite. Paper Number 39f, presented at the 4th Joint A. I. Ch. E. - C. S. Ch. E. Chemical Engineering Conference, Vancouver, Canada, September 9 to 12, 1973.
214. **Nielsen, A. M. and Beck, J. V.** Chalcocite oxidation and coupled carbon dioxide fixation by *Thiobacillus ferrooxidans, Science*, 175, 1124, 1972.
215. **Sakaguchi, H., Torma, A. E., and Silver, M.**, Microbiological oxidation of synthetic chalcocite and covellite by *Thiobacillus ferrooxidans, Appl. Envir. Microbiol.*, 31, 7, 1976.
216. **Le Roux, N. W., North, A. A., and Wilson, J. C.**, Bacterial Oxidation of Pyrite, Paper Number 45, paper presented at the 10th Intl. Mineral Processing Cong., Institution of Mining and Metallurgy, London, April 2 to 14, 1973.
217. **McElroy, R. O. and Bruynesteyn, A.**, Continuous Biological Leaching of Chalcopyrite Concentrates: Demonstration and Economic Analysis, paper presented at the Intl. Symp. Metallurg. Applic. Bacterial Leaching and Related Microbiol. Phenomena, Socorro, N. M., August 3 to 5, 1977, in press.
218. **Sakaguchi, H., Silver, M. and Torma, A. E.**, Microbiological leaching of a chalcopyrite concentrate by *Thiobacillus ferrooxidans, Biotechnol. Bioeng.*, 18, 1091, 1976.
219. **Bruynesteyn, A. and Duncan, D. W.**, Biological Leaching of Sulphide Ores, paper presented at the meeting entitled Means of Exploiting Low Grade Ores, United Nations Headquarters, New York, April 3 to 7, 1972.
220. **Beane, R. E. and Popp, C. J.**, The Application of Solution-Mineral Equilibrium Chemistry to the Leaching of Low-grade Chalcocite, paper presented at the Society of Mining Engineers — American Institute of Mining Engineers Fall Meeting and Exhibit, St. Louis, Mo., October 19 to 21, 1977.
221. **Brimhall, D. B. and Wadsworth, M. E.**, Oxygen consumption in dump leaching, *Am. Inst. Min. Eng. Trans.*, 254, 68, 1973.
222. **Moodry, R. P.**, Compressed Air Injection into a Sulphide Leach Dump, paper presented at the 105th American Institute of Mining Engineers Meeting, Las Vegas, February 22 to 26, 1976.
223. **Bruynesteyn, A., Duncan, D. W., and Ballard, J. K.**, An Evaluation of the Leaching Characteristics of Butte Leaching Ore, 1975, Preprint Number 75-AS-335,paper presented at the Society of Mining Engineers Meeting, Salt Lake City, Utah, September 10 to 12, 1975.

194. **Ehrlich, H. L.**, Bacterial action of orpiment, *Econ. Geol.*, 58, 991, 1963.
195. **Ehrlich, H. L.**, Bacterial oxidation of arsenopyrite and enargite, *Econ. Geol.*, 59, 1306, 1964.
196. **Torma, A. E., Legault, G., and Kougioumoutzakis, D.**,Kinetics of bio-oxidation of metal sulphides, Paper Number 39d, presented at the 4th Joint American Institute of Chemical Engineering-Canadian Society of Chemical Engineering Conf., Vancouver, Canada, September 9 to 12, 1973.
197. **Silver, M. and Torma, A. E.**, Oxidation of metal sulfides by *Thiobacillus ferrooxidans* grown on different substrates, *Can. J. Microbiol.*, 20 141, 1974.
198. **Rossi, G.**, The microbiological leaching of ore minerals. III. The action of stibnite ores on microorganisms found in acid drainage waters of Italian mines, *Resoconti Assoc. Min. Sarda*, 76, 1, 1971.
199. Torma, A. E. and Garbra, G. G., Oxidation of stibnite by *Thiobacillus ferroxidans, Antonie van Leuwenhoek J. Microbiol. Serol.*, 43, 1, 1977.
200. Torma, A. E. and Habashi, F., Oxidation of copper (II) selenide by *Thiobacillus ferrooxidans, Can. J. Microbiol.*, 18, 1780, 1972.
201. Torma, A. E. and Legault, G., Rôle de la surface des minerais sulfurés lors de leur biodegradation par *Thiobacillus ferrodoxidans, Ann. Microbiol., (Inst. Pasteur)*, 124A, 111, 1973.
202. Gormely, L. S., Duncan, D. W., Branion, R.M.R., and Pinder, K. L., Continuous culture of *Thiobacillus ferrooxidans* on a zinc sulfide concentrate, *Biotechnol. Bioeng.*, 17, 31, 1975.
203. Torma, A. E. and Guay, R., Effect of particle size on the biodegradation of a sphalerite concentrate, *Nat. Can.* 103, 133, 1976.
204. Pinches, A., Al-Jaid, F. O., and William, D.J.A., Leaching of chalcopyrite concentrates with *Thiobacillus ferrooxidans* in batch culture, *Hydrometall.*, 2, 87, 1976.
205. Beckstead, L. W., Munoz, P. B., Sepulveda, J. L., Herbst J. A., Miller, J. D., Olsen, F. A., And Wadsworth, M. E., Acid ferric sulfate leaching of attritor-ground chalco-pyrite concentrates, in *Extractive Metallurgy of Copper*, Vol. 2, Yannopoulos, J. C. and Agarwal, J. C., Eds., American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1976, 611.
206. Torma, A. E., Walden, C. C., Duncan, D. W., and Branion, R.M.R., Microbiological leaching of a zinc sulfide concentrate, *Biotechnol. Bioeng.*, 12, 501, 1970.
207. Burkin, A. R., Solid-state transformations during leaching, *Miner. Sci. Eng.*, 1, 4, 1969.
208. Duncan, D. W. and Bruynesteyn, A., Enhancing bacterial activity in a uranium mine, *Can. Min. Metall. Bull.*, 64, 32, 1971.
209. Ivarson, K. C., Microbiological formation of basic ferric sulfates, *Can. J. Soil Sci.*, 53, 315, 1973.
210. Brown, J. B., A chemical study of some synthetic potassium-hydronium jarosites, *Can. Mineral.*, 10, 696, 1970.
211. Chen, C. S., Huddleston, R. L., and Johnson, M. A., Bioleaching of Chalcopyrite Concentrate, Paper Number 39c, presented at the 4th Joint American Institute of Chemical Engineering-Canadian Society of Chemical Engineering Conf., Vancouver, Canada, September 9 to 12, 1973.
212. King, J. A., Burkin, A. R., and Ferreira, R. C. H., Leaching of chalcocite by acidic chloride solutions, in *Leaching and Reduction in Hydrometallurgy*, Burkin, A. R., Ed., Institution of Mining and Metallurgy, London, 1975, 36.
213. Mishra, R. K. and Wadsworth, M. E., Low Temperature Surface Reactions in the Leaching of Chalcopyrite. Paper Number 39f, presented at the 4th Joint A. I. Ch. E. - C. S. Ch. E. Chemical Engineering Conference, Vancouver, Canada, September 9 to 12, 1973.
214. Nielsen, A. M. and Beck, J. V. Chalcocite oxidation and coupled carbon dioxide fixation by *Thiobacillus ferrooxidans, Science*, 175, 1124, 1972.
215. Sakaguchi, H., Torma, A. E., and Silver, M., Microbiological oxidation of synthetic chalcocite and covellite by *Thiobacillus ferrooxidans, Appl. Envir. Microbiol.*, 31, 7, 1976.
216. Le Roux, N. W., North, A. A., and Wilson, J. C., Bacterial Oxidation of Pyrite, Paper Number 45, paper presented at the 10th Intl. Mineral Processing Cong., Institution of Mining and Metallurgy, London, April 2 to 14, 1973.
217. McElroy, R. O. and Bruynesteyn, A., Continuous Biological Leaching of Chalcopyrite Concentrates: Demonstration and Economic Analysis, paper presented at the Intl. Symp. Metallurg. Applic. Bacterial Leaching and Related Microbiol. Phenomena, Socorro, N. M., August 3 to 5, 1977, in press.
218. Sakaguchi, H., Silver, M. and Torma, A. E., Microbiological leaching of a chalcopyrite concentrate by *Thiobacillus ferrooxidans, Biotechnol. Bioeng.*, 18, 1091, 1976.
219. Bruynesteyn, A. and Duncan, D. W., Biological Leaching of Sulphide Ores, paper presented at the meeting entitled Means of Exploiting Low Grade Ores, United Nations Headquarters, New York, April 3 to 7, 1972.
220. Beane, R. E. and Popp, C. J., The Application of Solution-Mineral Equilibrium Chemistry to the Leaching of Low-grade Chalcocite, paper presented at the Society of Mining Engineers — American Institute of Mining Engineers Fall Meeting and Exhibit, St. Louis, Mo., October 19 to 21, 1977.
221. Brimhall, D. B. and Wadsworth, M. E., Oxygen consumption in dump leaching, *Am. Inst. Min. Eng. Trans.*, 254, 68, 1973.
222. Moodry, R. P., Compressed Air Injection into a Sulphide Leach Dump, paper presented at the 105th American Institute of Mining Engineers Meeting, Las Vegas, February 22 to 26, 1976.
223. Bruynesteyn, A., Duncan, D. W., and Ballard, J. K., An Evaluation of the Leaching Characteristics of Butte Leaching Ore, 1975, Preprint Number 75-AS-335,paper presented at the Society of Mining Engineers Meeting, Salt Lake City, Utah, September 10 to 12, 1975.

224. **Madsen, B. W. and Groves, R. D.**, Leaching a low- grade copper sulfide ore, in *Extractive Metallurgy of Copper*, Vol. 2, Yannopoulos, J. C. and Agarwal, J. D., Eds. The American Institute of Mining, Metallurgical, and Petroleum Engineers, New York, 1976, 926.
225. **Madsen, B. W., Wadsworth, M. E., and Groves, R. D.**, Application of a mixed kinetics model to the leaching of low-grade copper sulfide ores, *Am. Inst. Min. Eng. Trans.*, 258, 69, 1975.
226. **Findley, J., Appleman, M. D., and Yen, T. F.**, Degradation of oil shale by sulfur-oxidizing bacteria, *Appl. Microbiol.*, 28, 460, 1974.
227. **Meyer, W. C. and Yen, T. F.**, Enhanced dissolution of oil shale by bioleaching with thiobacilli, *Appl. Environ. Microbiol.*, 32, 610, 1976.
228. **Starkey, R. L.**, Effect of pH on toxicity of copper to *Scytalidium* sp., a copper-tolerant fungus, and some other fungi, *J. Gen. Microbiol.*, 78, 217, 1973.
229. **Silverman, M. P. and Munoz, E. F.**, Fungal attack on rock: solubilization and altered infrared spectra, *Science*, 169, 985, 1970.
230. **Silverman, M. P. and Munoz, E. F.**, Fungal leaching of titanium from rock, *Appl. Microbiol.*, 22, 923, 1971.
231. **Chakrabarty, A. M.**, Genetic Basis of Resistance, Concentration and Recovery of Metal Ions by Microorganisms, paper presented at the Int. Symp. Metallurg. Applic. Bacterial Leaching and Related Microbiol. Phenomena, Socorro, N. M., August 3 to 5, 1977, in press.
232. **Brierley, C. L.**, unpublished data.
233. **Brierley, J. A.**, personal communication.
234. **Blankenship, W. L.**, unpublished data.
235. **Brock, T. D.**, unpublished data.
236. **Le Roux, N. W.**, unpublished data.
237. **Le Roux, N. W.**, personal communication.
238. **de Melo Guedes, P.**, personal communication.
239. **Tsuchiyo, H. M.**, personal communication.
240. **Torma, A. E.**, unpublished data.
241. **Groves, R. D. and Madsen, B. W.**, personal communication.
242. **Pinches, A.**, The Use of Microorganisms for the Recovery of Metals from Mineral Materials, Ph.D. thesis, University of Wales, Cardiff.
243. **Guay, R., Silver, M., and Torma A. E.**, Base competition of DNA isolated from *Thiobacillus ferrooxidans* grown on different substrates, *Rev. Can. Biol.*, 35, 61, 1976.
244. **Tuovinen, O. H., Kelly, D. P., Dow, C. S., and Eccleston, M.**, Metabolic Transitions in Cultures of Acidophilic Thiobacilli, paper presented at Intl. Symp. Metallurg. Applic. Bacterial Leaching and Related Microbiol. Phenomena, Proc., Socorro, N. Mex., August 3 to 5, 1977, in press.
245. **Silver, M.**, personal communication.
246. **Kelly, D.**, personal communication.
247. **Silverman, M. P.**, Mechanism of bacterial pyrite oxidation, *J. Bacteriol.*, 94, 1046, 1967.
248. **Silverman, M. P., Rogoff, M. H., and Wender, I.**, Bacterial oxidation of pyrite materials in coal, *Appl. Microbiol.*, 9, 491, 1961.
249. **Silver, M.**, Oxidation of elemental sulfur and sulfur compounds and CO_2 fixation by *Ferrobacillus ferrooxidans (Thiobacillus ferrooxidans). Can. J. Microbiol.*, 16, 845, 1970.
250. **Razzell, W. E., and Trussell, P. C.**, Microbiological leaching of metallic sulphides, *Appl. Microbiol.*, 11, 105, 1963.
251. **Bruynesteyn, A., Duncan, D. W., and Ballard, J. K.**, An evaluation of the leaching characteristics of Butte leaching ore, *Hydrometallurgy*, 2, 235, 1976.
252. **Karaivko, G. I., Kuznetsov, S. I., and Golonizik,** *The Bacterial Leaching of Metals from Ores*, Technicopy Limited, Stonehouse, Gloucester, England, 1977.

UNITED NATIONS ENVIRONMENT PROGRAMME
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MICROBIOLOGICAL PROCESSES FOR THE LEACHING OF METALS FROM ORES

State-of-the-Art Review

by Dr. *G. I. Karavaiko* (USSR)

Edited by Prof. *A. E. Torma* (USA)

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Review *Karavaiko, G. I.*

The role of microorganisms in the oxidation of sulfide minerals and leaching of metals from ores and concentrates, and the factors underlying the kinetics of the above processes, as well as technical, technological, economic, and environmental aspects of the biogeotechnology of metals, are reviewed.

This Review is intended for microbiologists, geochemists, hydrometallurgists, and biogeotechnologists.

This Review has been prepared by the Centre of International Projects of the USSR State Committee for Science and Technology in accordance with the programme of the International UNEP/CMEA/USSR/Bulgaria Project on Microbiological Leaching of Metals from Ores.

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CONTENTS

PREFACE

Bacterial leaching deals with the extraction of metals from ores, concentrates and rocks under the influence of bacteria or their metabolites at atmospheric pressure and temperatures ranging from 5 to 80° C. For the most part, the leach solution contains sulphuric acid (which can be of bacterial or chemical origin) as well as organic acids, proteins, polysaccharides and other products of the microbial synthesis. Therefore, it is probably more correct to denote this leach technique as a mixed bacterial and chemical process. There are at least three different microbiological processes which play an important role in hydrometallurgy:

1. Oxidation of sulphide minerals, sulphur and ferrous iron;

2. Production of organic compounds, peroxides and so on, by heterotrophic microorganisms. These products dissolve minerals through complexation and oxidation; and

3. Precipitation of metals in order to purify industrial effluents or to extract non-ferrous and precious metals from solutions.

Recently, a number of reviews have appeared concerning the leaching of non-ferrous and other metals from ores [1—7]. This *Review* considers mostly the microbiological aspects of dump, underground and tank leaching of metals from ores and concentrates, and is related to the International Training Course on Microbiological Leaching of Metals from Ores. The technological problems of bacterial leaching of metals are considered in general since they have been covered in detail by other authors [1—5].

The section on Economic Aspects includes the few data available in the literature. It is not possible to estimate the application of this technology in different countries and geographical zones in economic terms since such information is not available. Obviously, the economic aspects of leaching will be determined by many factors typical of each country.

This *Review* does not consider the role of heterotrophic microorganisms in hydrometallurgy since this problem is still under study.

Chapter I

LITHOTROPHIC BACTERIA IN THE OXIDATION AND LEACHING OF SULPHIDE MINERALS

Thionic bacteria and a number of thermophilic microorganisms belonging to new genera are responsible for the oxidation of sulphide minerals, elemental sulphur and ferrous iron in ore deposits. Oxidation of reduced compounds of sulphur and iron is the only energy source for chemolithoautotrophic bacteria. The atmospheric CO_2 is used as a carbon source in the constructive processes.

1. Mesophilic Bacteria

Thiobacillus ferrooxidans [8, 9] (Fig. 1a) oxidizes virtually all known sulphide minerals and a number of elements with variable valency (Fe^{2+}, Cu^{+}, Sn^{2+}, Se^{2+}, Sb^{3+} (?) and U^{4+}) at temperatures ranging from 2 to 40° C (the optimum from 28 to 35° C) and pH from 1.0 to 4.8 (the optimum from 2.0 to 3.0) [3, 10—19]. Sulphide sulphur is oxidized while in such minerals as pyrite, chalcopyrite, arsenopyrite and so on, all reduced valency sulphur and iron compounds.

Leptospirillium ferrooxidans (Fig. 1b) isolated by Markosyan [20] oxidizes only Fe^{2+}. However, in the binary culture with *Thiobacillus organoparus* [21] (syn. *T. acidophilus* [22]) or *Thiobacillus thiooxidans*, they oxidize pyrite and chalcopyrite [23, 24].

T. thiooxidans (Fig. 1c) oxidizes elemental sulphur, $S_2O_3^{2-}$, SO_3^{2-}, $S_4O_6^{2-}$ and Sb_2S_3 and sphalerite (ZnS) at temperatures from 2 to 40° C (the optimum from 28 to 30° C) and pH from 0.5 to 5.0 [25—27].

T. organoparus oxidizes only elemental sulphur and grows at pH 1.5 to 5.0 (the optimum from 2.5 to 3.0) [21].

T. thioparus oxidizes S^0, $S_2O_3^{2-}$ and a number of sulphide minerals (PbS, Bi_2S_3, Sb_2S_3) at pH 8.0 [26, 28]. Khalid and Ralph [27] showed that *T. thioparus* also oxidizes zinc sulphides.

Abdrashitova et al. [29] proved that *Pseudomonas putida* and *Alcaligenes eutrophus* isolated from aurous arsenite ores oxidize As^{3+} to As^{5+} at pH 6 to 9. Oxidation of As^{3+} is accompanied by a decrease in pH of the medium.

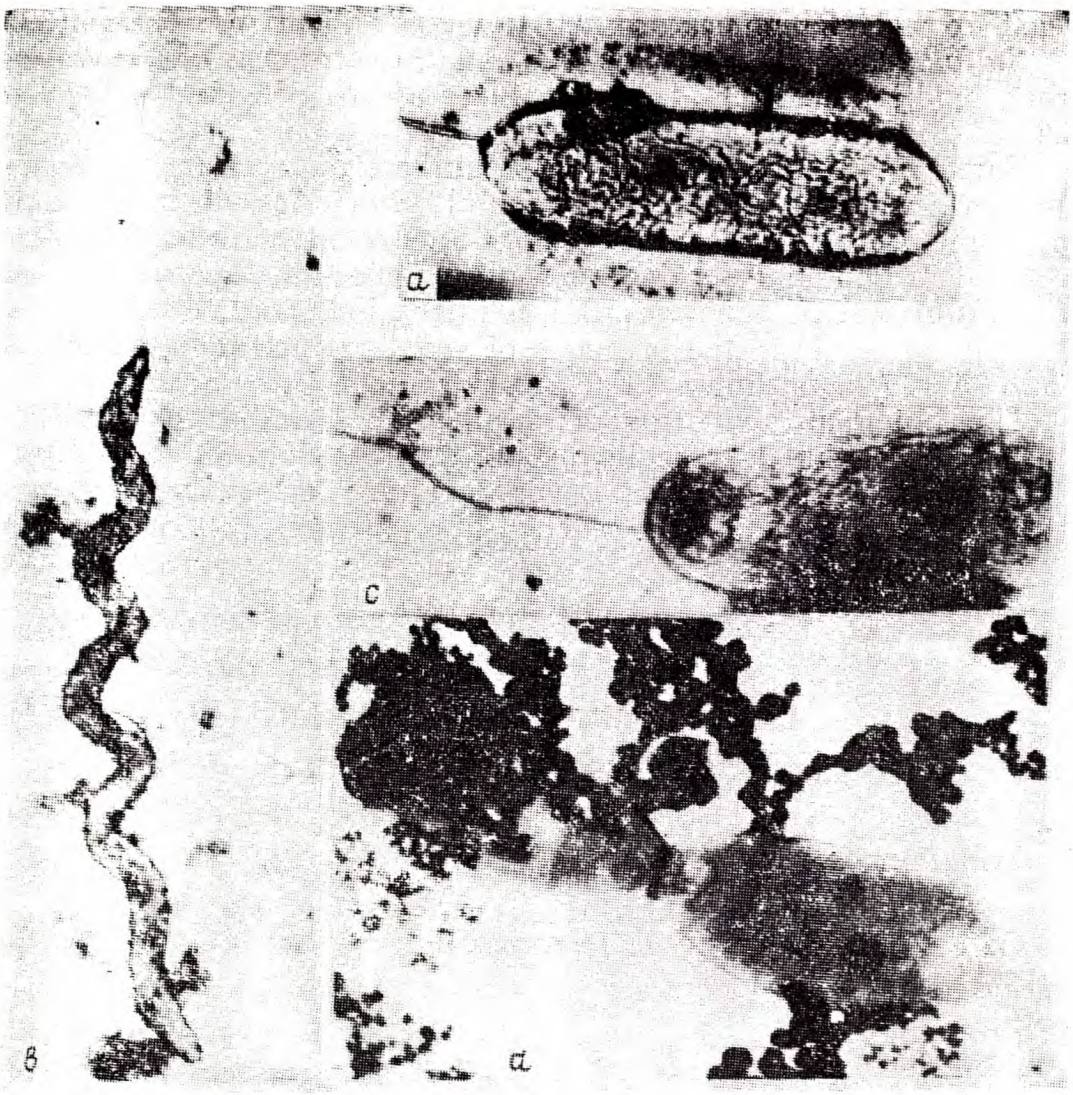

Fig. 1. Cells of microorganisms important for hydrometallurgical processes: (a) — *T. ferrooxidans;* (b) — *L. ferrooxidans;* (c) — *T. thiooxidans;* (d) — *S. thermosulfidooxidans*

2. Thermophilic Bacteria

Sulphide minerals, sulphur and ferrous iron are also oxidized by thermophilic bacteria similar to thionic bacteria, such as isolated by Brierley and Le Roux from thermal springs and ore [30—32]. The oxidation of non-organic substrates occurs at temperatures 50 to 55° C and pH 1.6 to 2.2. Addition of 0.02% yeast extract accelerates the bacterial growth and the oxidation processes.

Sulfobacillus thermosulfidooxidans (Fig. 1 d) was isolated from hot spots in the leach dump of sulphide-bearing copper ores. This organism oxidizes sulphur, Fe^{2+} and sulphide minerals (FeS_2, $CuFeS_2$, FeAsS, PbS, copper-zinc ore) in the presence of 0.02%

yeast extract at temperatures 20 to 60° C and pH 1.9 to 3.0 [33].

Sulfolobus acidocaldarius [34], in the presence of yeast extract, oxidizes sulphur at temperatures 80 to 85° C and pH 0.9 to 5.8.

Furthermore, Brierley and other authors isolated from thermal springs a number of other thermophilic bacteria which are similar to the *Sulfolobus* species [7, 35, 36]. Recently, these organisms were identified and received specific names of *S. brierleyi* and *S. solfataricus*. *S. brierleyi* [37] oxidizes Fe^{2+} and S^0 as well as sulphide minerals in the presence of yeast extract at temperatures 45 to 75° C (the optimum 70° C at pH 1.5 to 2.0).

Thus, biogenic oxidation of sulphur, Fe^{2+} and sulphide minerals occurs within a wide temperature (from 5 to 80° C) and pH (from 0.5 to 8.0) range. At low pH values, the majority of nonferrous and rare metals are leached from the ores [3].

Chapter II

BACTERIO-CHEMICAL OXIDATION AND LEACHING PROCESSES

1. Oxidation of Ferrous Iron

Oxidation of Fe^{2+} by *T. ferrooxidans* can be described by the following equations:

$$Fe^{2+} \rightarrow Fe^{3+} + \bar{e}; \quad (1)$$

$$2\bar{e} + {}^1/_2O_2 + 2H^+ \rightarrow H_2O; \quad (2)$$

$$2Fe^{2+} + {}^1/_2O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O; \quad (3)$$

$$Fe^{3+} + 3H_2O \rightleftarrows Fe(OH)_3 + 3H^+. \quad (4)$$

The rate of Fe^{2+} oxidation is determined by the rate of bacterial growth which in its turn depends on the method and conditions of cultivation. The doubling time of bacteria cultured on the media containing Fe^{2+} at temperatures 28 to 35° C varies from 3.6 to 10 hours [38—41].

When *T. ferrooxidans* is cultured in a reactor with electrochemical reduction of Fe^{3+}, one can obtain high concentrations of cells (up to 4.5 $g \cdot l^{-1}$ of dry biomass weight) at low concentrations of total iron (4 to 6 $g \cdot l^{-1}$) [42, 43]. The rate of oxidation of Fe^{2+} in this reactor can be as high as 50 $g \cdot l^{-1} \cdot h^{-1}$. Without bacteria at low pH values, the chemical oxidation of Fe^{2+} progresses relatively slowly. In this reactor, the ferric iron is constantly reduced to ferrous iron whereby favourable conditions for bacterial activity are created. As such, both the rate of bacterial growth

and the rate of Fe^{2+} oxidation are enhanced. The ferric iron solutions containing large number of bacteria can be used for intensive leaching of metals from ores and concentrates.

2. Oxidation of Pyrite

Pyrite is a rather widely spread sulphide mineral, and can be found in association with many other ore minerals. Its oxidation produces H_2SO_4 and sulphates of the trivalent iron. Pyrite, like other sulphide minerals, is a semiconductor. It can possess either electron (*n*-type) or hole (*p*-type) conductivity [44]. Differences are observed in the chemistry of pyrite oxidation for samples possessing different conductivities. According to Yakhontova and Nesterovich [45], pyrite of the *n*-type at pH<4.0 is oxidized according to the scheme:
Diluted solutions:

$$FeS_2+3O_2+8H_2O\rightarrow Fe(H_2O)_6^{2+}+2HSO_4^-+2H^++2\bar{e}; \quad (5)$$

Concentrated solutions:

$$FeS_2+3^1/_2O_2+H_2O\rightarrow[FeHSO_4]^{2+}+SO_4^{2-}+H^++\bar{e}. \quad (6)$$

At pH>4.0 FeS_2 is oxidized according to the scheme:

$$FeS_2+2^1/_2O_2+9H_2O\rightarrow Fe(H_2O)_6^{3+}+2H_2SO_4+2H^++4\bar{e}. \quad (7)$$

Pyrite of the *p*-type is oxidized according to equation (7) within a wide range of pH (2 to 9). In addition, the trivalent iron readily interacts with the pyrite:

$$FeS_2+2Fe^{3+}\rightarrow 3Fe^{2+}+S^0. \quad (8)$$

Elemental sulphur produced during the chemical oxidation of FeS_2 is further oxidized both by bacteria (*T. ferrooxidans, T. thiooxidans, T. organoparus*) and chemically by Fe^{3+}, according to the following forms:

$$S^0+H_2O+1^1/_2O_2\rightarrow H_2SO_4; \quad (9)$$

$$S^0+6Fe^{3+}+4H_2O\rightarrow 6Fe^{2+}+SO_4^{2-}+8H^+. \quad (10)$$

Calculations by Arkesteyn [46] showed that a large part of the elemental sulphur formed according to equation (8) at low pH values is oxidized not bacterially but chemically according to equation (10). Therefore, indirect chemical oxidation of FeS_2 can be expressed by an overall equation:

$$14Fe^{3+}+FeS_2+8H_2O\rightarrow 15Fe^{2+}+2SO_4^{2-}+16H^+. \quad (11)$$

Many researchers investigated the kinetics of pyrite oxidation under the influence of *T. ferrooxidans* and found that the rate of oxidation increased 20 to 1000-fold as compared to the purely chemical process. The chemistry of reactions in case of direct bacterial oxidation of FeS_2 is, apparently, different and the end products of oxidation are Fe^{3+} and H_2SO_4.

The extent of bacterio-chemical oxidation of pyrite in natural conditions can be illustrated by the following examples: a) the mine waters of the Degtyarsky copper-pyrite deposit with the daily drainage rate of about 3,000 m^3 had a pH of 2.5; b) according to Davis, the coal mine drainage waters carried daily about 9,000 tons of sulphuric acid into the rivers of the Pittsburg area [in ref. 47].

3. Oxidation of Sulphide Copper Minerals

The most widely spread copper minerals are chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S), covellite (CuS) and bornite (Cu_5FeS_4).

Chalcopyrite ($CuFeS_2$) is the most difficult to oxidize sulphide copper mineral. Under the influence of *T. ferrooxidans*, the rate of chalcopyrite oxidation increased 6—12-fold as reported by Duncan, Torma et al. [49—51]. The process of its chemical oxidation, as reported by Yakhontova et al. [48], depends on the pH value and can be described by a number of equations:

at pH 1 to 3:

$$CuFeS_2 + 3^1/_4O_2 + 1^1/_2H_2O \rightarrow [CuHSO_4]^+ + Fe^{2+} + HSO_4^- + H^+ + 3\bar{e}; \quad (12)$$

at pH 3 to 7:

$$CuFeS_2 + 4O_2 \rightarrow Cu^{2+} + SO_4^{2-} + [FeSO_4]^+ + \bar{e}; \quad (13)$$

at pH 7 to 9:

$$CuFeS_2 + 3.9O_2 + 0.2H_2O \rightarrow Cu^{2+} + SO_4^{2-} + [FeSO_4]^+ + 0.4H^+ + 1.4\,\bar{e}. \quad (14)$$

During bacterial oxidation of $CuFeS_2$, Fe^{3+} is formed as an end product. Ferric iron, in its turn, interacts with chalcopyrite [52] according to the formula:

$$CuFeS_2 + Fe^{3+} \rightarrow Cu^{2+} + Fe^{2+} + 2S^0. \quad (15)$$

Secondary sulphide copper minerals, such as chalcocite (Cu_2S), bornite (Cu_5FeS_4) and covellite (CuS), are more readily oxidized under the impact of bacteria and chemical factors. The chemistry of their oxidation, according to Yakhontova and Grudev [44], may be expressed as follows:

at pH<4:

$$Cu_2S + 1^3/_4O_2 + 6^1/_2H_2O \rightarrow [CuHSO_4]^+ + Cu(H_2O)_6^{2+} + 3\bar{e}; \quad (16)$$

at pH>4:

$$Cu_2S + 2O_2 + 12H_2O \rightarrow 2Cu(H_2O)_6^{2+} + SO_4^{2-} + 2\bar{e}; \quad (17)$$

$$Cu_5FeS_4 \xrightarrow{O_2+H_2O} Cu(H_2O)_6^{2+} + 4[CuHSO_4]^+ + Fe(H_2O)_6^{3+} + 9\bar{e}. \quad (18)$$

The aqua-complex $Cu(H_2O)_6^{2+}$ will be further transformed into $[CuHSO_4]^+$ due to the presence of H^+ ions, which seems to be the main responsible for increased pH of the medium. Therefore, oxidation of Cu_2S and Cu_5FeS_4 is proton-consuming and results in an increase in pH. Both bacterial and chemical oxidations of Cu_2S will stop when pH reaches 4.6—4.7. Thus, unlike pyrite and chalcopyrite oxidation, bacterio-chemical oxidation of Cu_2S and Cu_5FeS_4 consumes sulphuric acid [3, 45, 53]. As shown by Beck [53], *T. ferrooxidans* catalyzes the oxidation of Cu_2S to $CuSO_4$ and CuS:

$$2Cu_2S + O_2 + 2H_2SO_4 \rightarrow 2CuS + 2CuSO_4 + 2H_2O. \quad (19)$$

The rate of this reaction in Beck's experiments was increased by the bacteria about 40-fold over that of the sterile control.

Another distinctive feature of secondary sulphide minerals of copper is the fact that they are relatively easy to oxidize by trivalent iron:

$$Cu_2S + Fe^{3+} \rightarrow Fe^{2+} + CuS + Cu^{2+}. \quad (20)$$

According to Razzell and Trussell [54], in the presence of bacteria and Fe^{2+}, which is oxidized to Fe^{3+}, the amount of copper solubilized is about 6 times greater, and about 3 times greater in the presence of bacteria alone, than that in the control without iron and bacteria. In Groudev's [55] experiments, the highest rate of copper extraction from covellite (CuS) in the presence of *T. ferrooxidans* reached 170 $mg \cdot l^{-1} \cdot h^{-1}$. By adding ferrous iron to the leach solution, copper extraction was further accelerated by 15 to 20%.

Thermophilic microorganisms were reported to participate in the oxidation of sulphide minerals of copper. For example, at 60° C and pH 2.5, they were observed to extract up to 50% of Cu from a copper ore with particle size in the range of 1.0 to 0.3 mm and Cu content of 0.32% [56]. Under similar leach conditions, the rate of copper extraction from a chalcopyrite concentrate (with particle size varying from 0.105 to 0.07 mm and containing about 27.6% Cu) was about 10—16 $mg \cdot l^{-1} \cdot day^{-1}$, as contrasted to 1.0—1.8 $mg \cdot l^{-1} \cdot day^{-1}$ without bacteria. Investigations by Chakrabarty and Murr [57] revealed that at 50° C in the presence of thermophilic bacteria only the same amount of copper could be extracted from a low-grade ore as was obtained at 30° C in the presence of *T. ferrooxidans*.

4. Oxidation of Sulphide Minerals of Other Metals and Metalloids

Zinc

Sulphide minerals of zinc can be oxidized by *T. ferrooxidans*, *T. thiooxidans* and *T. thioparus* [27, 58, 59]. Wurtzite is the most difficult to oxidize sulphide mineral of zinc. Marmatite was oxidi-

zed by *T. ferrooxidans* and *T. thiooxidans* faster than sphalerite either in the presence or absence of Fe^{3+}. Iron-free synthetic sulphide of zinc was oxidized faster by *T. thiooxidans* than by *T. ferrooxidans* or *T. thioparus*. The oxidation process may be expressed by the formula:

$$ZnS + 2O_2 \rightarrow ZnSO_4. \quad (21)$$

The rate of oxidation of zinc sulphides in the presence of bacteria in the experiments by Trussell and Duncan increased 4 to 5-fold as compared to control tests without bacteria [59]. The rate of zinc extraction from polymetallic ores in the presence of *T. ferrooxidans* was two to three-fold faster by comparison to experiments without bacteria [60]. The intensity of bacterial leaching of Zn from sulphide minerals was also increased in the presence of pyrite and Fe^{3+}. According to Kulebakin [61], the bacterial oxidation of kleiofan and marmatite was 19.3—20 times faster than the chemical oxidation by trivalent iron.

Nickel

Nickel is leached from sulphide minerals (pentlandite and millerite) and from ores in the presence of *T. ferrooxidans* about 2—17 times faster as compared to the chemical process [11, 62—66]. Oxidation of pentlandite follows the scheme:

$$(Ni, Fe)_9S_8 + 17^5/_8O_2 + 3^1/_4H_2SO_4 \rightarrow$$
$$\rightarrow 4^1/_2NiSO_4 + 4^1/_4Fe^{3+} + 6^3/_4SO_4^{2-} + 3^1/_4H_2O. \quad (22)$$

According to Torma [64], nickel is intensively leached from ores and concentrates in the presence of bacteria. The rate of Ni extraction from concentrates may reach ca. 200 $g \cdot l^{-1} \cdot h^{-1}$. In 66 hours, 73—97% of Ni were extracted from a low-grade ore containing 0.2% nickel. Over 90% of Ni were extracted in 8 days at the temperature of 50° C in the presence of thermophilic bacteria isolated from the Icelandic thermal springs [67]. The same authors found that in the presence of *T. ferrooxidans* at 30° C the same amount of nickel was extracted only in 14 days.

Antimony

T. ferrooxidans was reported to oxidize antimonite (Sb_2S_3) at pH 1.75 and 35° C according to the scheme [6]:

$$Sb_2S_3 + 6O_2 \rightarrow Sb_2(SO_4)_3. \quad (23)$$

Antimonite is also oxidized by *B. thioparus var. antimoniticus* at pH 8.0 and by *T. thiooxidans* at low pH values [26, 28]. Antimony sulphate is partially hydrolyzed forming an insoluble oxide of trivalent antimony according to the scheme:

$$Sb_2(SO_4)_3 + 2H_2O \rightleftarrows (SbO)_2SO_4 + 2H_2SO_4. \quad (24)$$

The trivalent antimony sulphate is partially oxidized to pentavalent antimony according to the scheme:

$$Sb_2(SO_4)_3 + O_2 + 2H_2SO_4 \rightarrow Sb_2(SO_4)_5 + 2H_2O. \quad (25)$$

Presumably, the reaction occurs under the influence of *T. ferrooxidans* but there is no direct proof for this statement. Sulphate of Sb^{5+} hydrolyzes with the formation of an insoluble oxide-sulphate:

$$Sb_2(SO_4)_5 + 4H_2O \rightleftarrows (SbO_2)_2SO_4 + 4H_2SO_4. \quad (26)$$

It has also been shown that Sb_2S_3 is oxidized by thionic bacteria to Sb_2O_3 [28]. Further oxidation, up to pentoxide, can be achieved with *Stibiobacter senarmontii* [68]. Due to the very low solubility of the hydrolysis products of antimony (V) oxide-sulphate, the amount of antimony dissolved in the medium is, as a rule, negligible.

Lead

T. ferrooxidans was reported to oxidize galenite, lead sulphide (PbS), and Tomizuka [69] proposed the following scheme of this process:

$$PbS + H_2SO_4 + {}^1/_2O_2 \rightarrow PbSO_4 + H_2O + S^0; \quad (27)$$

$$PbS + H_2SO_4 \rightarrow PbSO_4 + H_2S; \quad (28)$$

$$S + 1{}^1/_2O_2 + H_2O \rightarrow H_2SO_4. \quad (29)$$

T. ferrooxidans is a more effective oxidizer of galenite than Fe^{3+}. However, in the presence of bacteria and ferric iron the PbS oxidation is intensified because Fe^{3+} contributes in the process according to the next equation:

$$PbS + Fe_2(SO_4)_3 \rightarrow PbSO_4 + 2FeSO_4 + S^0. \quad (30)$$

Fe^{2+} produced in reaction (30) will be oxidized to Fe^{3+} by *T. ferrooxidans* as indicated in equation (3). Nevertheless, solubilization of lead virtually does not occur because the lead sulphate $PbSO_4$ is insoluble in the aqueous acid media. This fact can be used for selective separation of a number of elements from lead.

Tin

Data on the oxidation of tin sulphides by *T. ferrooxidans* are scarce. According to Duncan [70], 12% of Sn were extracted from stannite in the presence of bacteria as compared to 4% in the sterile control. Besides, it was stated that Sn^{2+} could be oxidized by *T. ferrooxidans* [16]. The optimum pH for this reaction was reported to be 2.2 at 37 to 40° C.

Molybdenum

T. ferrooxidans is known to oxidize molybdenite (MoS_2) according to the scheme:

$$2MoS_2+9O_2+6H_2O\rightarrow 2H_2MoO_4+4H_2SO_4. \quad (31)$$

In the presence of bacteria the process of oxidation of MoS_2 occurs faster than in the sterile control sample. However, molybdenum was found to be toxic to bacteria which perish at concentrations as low as 9 to 12 $mg\cdot l^{-1}$ [71, 72].

The thermophilic *Sulfolobus*-like bacteria are more promising for the leaching of molybdenum as indicated by Brierley [37]. A distinctive feature of these bacteria is that they are resistant to 2 $g\cdot l^{-1}$ of Mo and grow at its 750 $mg\cdot l^{-1}$ content in the medium. The rate of Mo extraction from MoS_2 at 60° C reached 26.5 $mg\cdot l^{-1}\cdot day^{-1}$, and exceeded the intensity of molybdenum leaching in the sterile controls about 130-fold [35, 37, 73]. In 30 days, 3.3% of Mo were extracted from a molybdenum concentrate (98.5% Mo) with particle size from 0.012 to 0.062 mm at 60° C and pH 2.5. When adding 0.02% yeast extract or yeast extract and 1% $FeSO_4$, 8.3% and 13.3% of Mo were extracted respectively. Without bacteria only 0.1% of Mo were extracted [7]. Molybdenite (MoS_2) may be oxidized by trivalent iron but the process results in the formation of insoluble products of oxidation.

Arsenic

More complete studies are available about the role of bacteria in the oxidation of arsenopyrite (FeAsS) [10, 74—77]. Like pyrite, arsenopyrite is a semiconductor with either electron (*n*-type) or hole (*p*-type) conductivity. In the electron-type arsenopyrite the conductivity is due to the abundance of sulphur present in the compound, while the conductivity in the hole-type arsenopyrite is due to the abundance of arsenic. According to Yakhontova and Nesterovich [45], arsenopyrite of the *p*-type, within the range of pH 2 to 6, is oxidized as expressed by the following equation:

$$4FeAsS+12^3/_4O_2+6^1/_2H_2O\rightarrow 3Fe^{3+}+Fe^{2+}+$$
$$+2H_3AsO_4+2H_2AsO_4^-+H_2SO_4+3SO_4^{2-}+H^++4\bar{e}. \quad (32)$$

Arsenopyrite of the *n*-type at the same pH values is oxidized according to the following scheme:

$$2FeAsS+4^1/_2O_2+5H_2O\rightarrow 2Fe^{2+}+SO_4^{2-}+$$
$$+HAsO_2+H_3AsO_4+HSO_4^-+5H^++6\bar{e}. \quad (33)$$

Arsenopyrite also interacts with trivalent iron, probably, accor-

ding to the scheme:

$$FeAsS + Fe^{3+} \rightarrow 2Fe^{2+} + As^{3+} + S^0; \quad (34)$$

$$As^{3+} + 3H_2O \rightarrow H_3AsO_3 + 3H^+. \quad (35)$$

Arsenic acid, Fe^{2+} and S^0 are unstable in the medium, and are oxidized according to the scheme [78]:

$$H_3AsO_3 + H_2O \rightarrow H_2AsO_4 + 3H^+ + 2\bar{e}, \quad (36)$$

$$Fe^{2+} \rightarrow Fe^{3+} + \bar{e}; \quad S^0 \xrightarrow{4\,H_2O} SO_4^{2-} + 8H^+ + 6\bar{e}. \quad (37)$$

In the presence of Fe^{3+}, iron arsenite will be formed:

$$Fe^{3+} + H_2AsO_4^- \rightarrow FeAsO_4 + 2H^+. \quad (38)$$

Kinetics studies of bacterial oxidation of arsenopyrite in tin- and gold-bearing concentrates with the initial arsenic content up to 15% revealed that extractions as high as 80 to 90% of arsenic can be obtained using direct-flow Pachuca tanks in 70 to 80 hours [78].

Bismuth

Bismuthite is not oxidized by *T. ferrooxidans* [79]. In this process, the bacterial involvement is indirect and can be represented by the following scheme:

$$Bi_2S_3 + 6Fe^{3+} + 6O_2 \rightarrow Bi_2(SO_4)_3 + 6Fe^{2+}. \quad (39)$$

It was reported that solutions with Fe^{3+} and H_2SO_4 extracted about 80% of bismuth from copper ores containing about 0.4% of Bi [80]. In a second stage of treatment with sulphuric acid of a copper ore containing bismuthite, the bismuth content of the solution reached concentrations as high as 5 $g \cdot l^{-1}$. Furthermore, it was found that bismuthite can be partially oxidized by *T. thioparus* at pH 8.0 [26, 28].

Vanadium

The mechanism of bacterial leaching of vanadium is similar to that of bismuth. In this process, trivalent vanadium is oxidized to its pentavalent state by Fe^{3+}, and the latter is regenerated by *T. ferrooxidans*.

5. Leaching of Rare and Scattered Elements

Rare and scattered elements are present in the crystal lattice of many sulphide or silicate minerals. Minerals of these elements as such are seldom encountered. Rare elements are released as a result of oxidation of sulphide minerals or destruction of silicate minerals under favourable leach conditions [3].

Therefore, the efficiency of leaching of rare and scattered elements is determined by the rate of oxidation or destruction of respective minerals.

Gallium and Cadmium

As reported by Lyalikova and Kulikova [81], *T. ferrooxidans* accelerated the leaching of gallium and cadmium from sphalerite (the main carrier of these elements) respectively by 2 and 5—8 times relative to the sterile controls. In this process, indium can also be solubilized. In the presence of *T. ferrooxidans* up to 90—92% of Cd were extracted from a copper-zinc-bearing concentrate in leach suspensions containing 20% pulp density at pH 2.0—2.5 and 35° C in 72 hours in continuous tank process [82, 83].

Brissette et al. [84] used *T. thiooxidans* leaching cadmium from pure CdS in the presence of elemental sulphur. Under optimum conditions they realized cadmium extractions as high as 72% in six weeks. It was found that the presence of sulphur was beneficial to the extraction. The extraction of cadmium leached by sulphuric acid in the sterile control was only 13%. The above authors believed that in the presence of elemental sulphur, *T. thiooxidans* oxidized cadmium sulphide to sulphate.

Torma reported [85] that *T. ferrooxidans* can oxidize gallium sulphide (Ga_2S_3). He used in his experiments a chalcopyrite concentrate containing gallium sulphide and a pure synthetic gallium sulphide. 40.2 $g \cdot l^{-1}$ of Cu and 2.2 $g \cdot l^{-1}$ of Ga were extracted in 120 hours from the pulp of 25% solids at pH 1.8 and 35° C. The extraction of gallium and copper in the sterile controls amounted to 8 to 15%. The specific rate of O_2 uptake ($\mu l\ O_2 \cdot h^{-1} \cdot mg^{-1}$ of protein) during the oxidation of Ga_2S_3 in the presence or absence of bacteria was 17.8 ± 0.16 and 5.1 ± 0.25, respectively. This divergence demonstrates the influence of bacteria on the oxidation of Ga_2S_3, which is oxidized by *T. ferrooxidans* and by the trivalent iron according to the next equations:

$$Ga_2S_3 + 6O_2 \rightarrow Ga_2(SO_4)_3; \quad (40)$$

$$Ga_2S_3 + 3Fe_2(SO_4)_3 \rightarrow Ga_2(SO_4)_3 + 6FeSO_4 + 3S^0. \quad (41)$$

Germanium and Cobalt

Germanium isomorphically substitutes copper, zinc and lead in their respective sulphides, and can be leached within a wide pH range. According to Lyalikova and Kulikova [81], during the oxidation of galenite by *T. thioparus var. antimoniticus*, there is about six times as much germanium in solution, than this is in the sterile controls. Respectively, germanium concentration was 100 and 17 $\mu g \cdot l^{-1}$ in the inoculated and sterile controls. Cobalt is always associated in varying amounts with pyrite, pyrrhotite,

arsenopyrite, sphalerite and other minerals, and will be leached during the oxidation. In bacterial oxidation of cobaltite [10], the rate of extraction of Co increased about 75 times as compared to the control. In experiments by Torma [64, 86], 68—76% of Co were leached from a nickel concentrate. The detection of Co, along with other elements, in acid mine drainage waters is the evidence of its leaching under natural conditions [87].

Rhenium

Rhenium is present in deposits of molybdenite, pyrite, chalcopyrite and galenite. Although no data are available on bioleaching of this element, it is known that in the natural oxidation of sulphides, rhenium is largely dissolved. Its content in the mine waters of corresponding deposits varies from 5 to 500 $mg \cdot l^{-1}$.

Selenium and Tellurium

Selenium and tellurium are present in pyrite, chalcopyrite, sphalerite, galenite and elemental sulphur. Selenium is also found in the elemental form as well as in the form of selenides. Tellurium is also found as a constituent of minerals, tellurides. The leaching of these elements is evidenced by their transport with waters and by a decrease in elements concentration in oxide ores. The role of bacteria in the leaching of selenium has been so far poorly studied. Lipman and Waksman [88] pointed out as early as 1922 that some autotrophic bacteria could obtain energy through oxidation of selenium into selenic acid. Torma and Habashi [12] reported that *T. ferrooxidans* could use the energy available from oxidation of copper selenide. As a result of this process, copper is dissolved in the leach medium, and selenium is oxidized to its elemental form:

$$CuSe + 2H^+ + {}^1/_2O_2 \rightarrow Cu^{2+} + Se^0 + H_2O. \quad (42)$$

Titanium

The role of lithotrophic bacteria in the leaching of titanium has been studied inadequately. In acid media below pH 2.0, titanium is dissolved from ores. This is indicated by its transport from the oxidative zone in copper-pyrite deposits. Presumably, *T. ferrooxidans* takes part in the process.

Uranium

The role of microorganisms in the leaching of uranium has been well studied. The data are summarized in a number of reviews and books [89, 90]. The essence of uranium leaching by

T. ferrooxidans is the oxidation of pyrite or Fe^{2+} with the formation of H_2SO_4 and Fe^{3+}. Ferric iron in the acid medium oxidizes U^{4+} to U^{6+}, which is soluble in sulphuric acid solutions. The leaching of uranium follows the scheme:

$$UO_2+Fe_2(SO_4)_3 \rightarrow UO_2SO_4+2FeSO_4; \quad (43)$$

$$UO_3+H_2SO_4 \rightarrow UO_2SO_4+H_2O. \quad (44)$$

Consequently, the presence of pyrite as a supplier of Fe^{3+} plays an important role in the bacterio-chemical leaching of uranium. When 3% of FeS_2 and 1.5% of Fe^{2+} were added to a uranium ore, uranium extraction exceeded 90% in 18 weeks. In experiments without addition of FeS_2 and Fe^{2+}, there was practically no extraction of uranium achieved despite the presence of thionic bacteria in the leach suspension [91]. *T. ferrooxidans* is known to oxidize also U^{4+} [18]. Tomizuka et al. [92] conducted continuous leaching of uranium in media containing $FeSO_4$ and 10% of ore at pH 2.0 and 30° C. The highest extraction of uranium was reached at the dilution rate about 0.060 $hour^{-1}$, when the highest rates of *T. ferrooxidans* growth and formation of Fe^{3+} ions were observed. However, to obtain 80% uranium extraction, the authors suggested performing the continuous leaching process at a dilution rate of 0.011 $hour^{-1}$. This method of leaching uranium as well as other metals from ores and concentrates seems to be the most progressive one.

Chapter III

INTENSIFICATION OF THE LEACHING OF METALS

The rate of extraction of metals from sulphide-bearing minerals depends largely on physico-chemical factors (medium acidity, redox potential, temperature, etc.), technological conditions (pulp density, mixing, gas transfer phenomena, etc.), and peculiarities of bacteria themselves.

1. Influence of Physico-Chemical Properties of Sulphide Minerals and of the Medium

Type of Conductivity of Sulphide Minerals

The theory of physico-chemical principles of oxidation of sulphide minerals has been summarized by Yakhontova and Grudev [44]. One of the major factors determining the intensity of oxidation processes is the redox potential (Eh) of the «electrolyte» (medium) and electrode potential (EP) of minerals as well as the type of their conductivity and structure of crystals. For example,

minerals of *n*- and *p*-type conductivity are oxidized with different efficiencies. According to Yakhontova and Nesterovich [45], pyrite and arsenopyrite of the *n*-type conductivity possess a lower energy of electron discharge, and are characterized by a high initial oxidation rate which rapidly slows down as reaction progresses. Sulphides of the *p*-type conductivity are initially oxidized at a lower rate but the process is more stable.

As shown by Silverman and Ehrlich [93], the intensity of oxidation of sulphide minerals is determined by the structure of crystals. Later Berry, Murr et at. [56, 94—96] reported that the distribution of bacteria on sulphide minerals depends on the structure of crystals and presence of dislocations. Obviously, the structure, conductivity and electrochemical properties of crystals are interrelated, and will determine the kinetics of their oxidation by bacteria. The relation between the Eh of the medium, EP and conductivity of a sulphide mineral can be seen from an example of pyrite oxidation in Figs. 2 and 3 [44, 97—99]. Experiments were carried out on pyrite with the electron (FeS_2^-) and hole

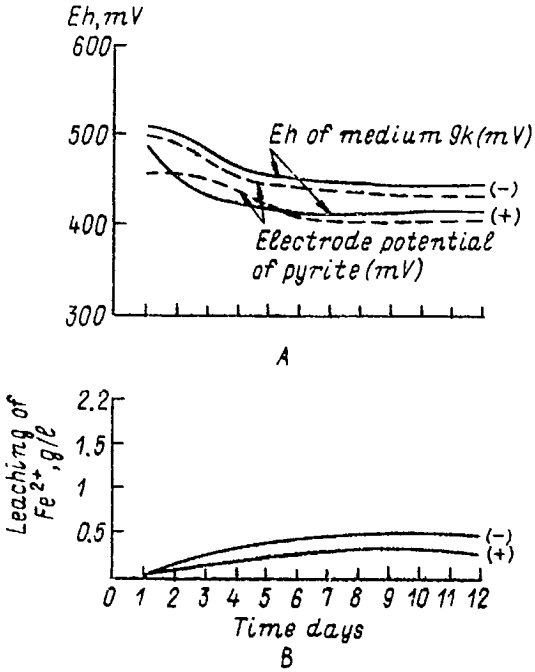

Fig. 2

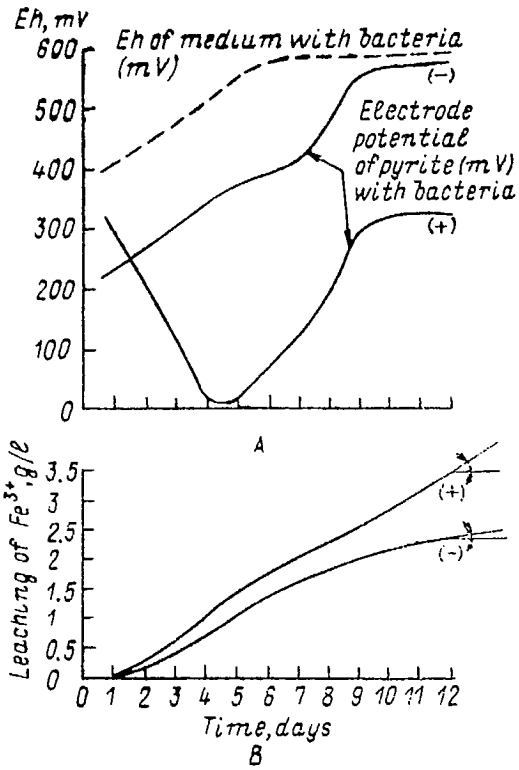

Fig. 3

Fig. 2. Changes in EP of pyrite with positive (+) and negative (—) conductance; in Eh of the 9 K medium without bacteria (pH 2.5) (A); and in Fe^{2+} leaching (B) [97]

Fig. 3. Changes in EP of pyrite with positive (+) and negative (—) conductance; in Eh of the 9 K medium with *T. ferrooxidans* (pH 2.5) (A); and in Fe^{3+} leaching (B) [97]

(FeS_2^+) type conductivity. It is clear from Fig. 2 that without bacteria there is no oxidation of pyrite regardless of its conductivity. This is due to the fact that the electrode potential of both types of pyrite is close to the Eh of the medium. The absence of any difference between EP and Eh indicates that both the medium and the mineral are energetically at equilibrium. Therefore, the latter cannot be oxidized under these conditions. In the presence of *T. ferrooxidans*, pyrite is oxidized intensively and behaviour of sulphides with different types of conductivity is different (Fig. 3). The difference between Eh and EP for $FeS_2(+)$ was about 600 mV and about 200 mV for $FeS_2(-)$. The initial difference between Eh and EP was rather large, but gradually disappeared and the leaching of Fe practically stopped. Thus, these findings suggest that the adsorption of bacteria on the mineral surface sharply decreases the EP and ensures a high oxidative capacity of the system.

Table 1 contains experimental Eh and EP values during the

Table 1

Optimum Eh and EP values for the bacterial oxidation of sulphide minerals [7, 45, 82, 83, 92, 100]

Minerals	Eh of the medium, V	EP of minerals, V
$CuFeS_2$	0.5—0.7	0.4—0.5
Cu_2S, Cu_5FeS_4	0.6—0.5	0.3—0.4
FeAsS	0.77—0.8	0.62—0.64
+		
$CuFeS_2$		
in concentrate		0.76—0.77
ZnS	0.6—0.7	0.23—0.43
		(pH 2.3—1.5)
+		
$CuFeS_2$		0.68—0.60
in concentrate		(pH 2.3—1.5)
U^{4+}/U^{6+}	0.75	0.41

oxidation of some sulphide minerals. According to Yakhontova and Nesterovich [45], the leaching of chalcopyrite stopped when the EP and Eh curves reached the stable level (0.5 and 0.7 V). For Cu_2S and Cu_5FeS_4, the leaching of copper stopped after the following changes: EP from 0.3 to 0.4 and Eh from 0.6 to 0.5 V.

Electrochemical Interaction of Sulphide Minerals

Sulphide minerals in the pulp or in ores interact electrochemically, i. e. there occur galvanic currents between them. In this case, the electrode potentials of some minerals may substantially differ from the stationary potentials measured individually.

The intensity of oxidation of a metal sulphide in the mixture

of sulphides depends both on the difference between the electrode potentials of the sulphides and on the difference between the sulphide EP and the medium Eh. The sulphide mineral that has the lowest EP, i. e. the sulphide-anode, is more readily oxidized chemically. To illustrate, the following examples may be cited. When extracting metals from a concentrate containing arsenopyrite and chalcopyrite, it was established that microorganisms most actively oxidize arsenopyrite, i. e., the sulphide-anode possessing the lowest EP (Table 1, Fig. 4). The difference between EPs of FeAsS and $CuFeS_2$ amounts to 0.14—0.15 V. Thus, in the examined case, chalcopyrite favoured the microbiological oxidation of arsenopyrite. Consequently, the microbiological oxidation of sulphide minerals has the same trend as the electrochemical dissolution, but is accelerated to a large extent due to the presence of bacteria [100, 101].

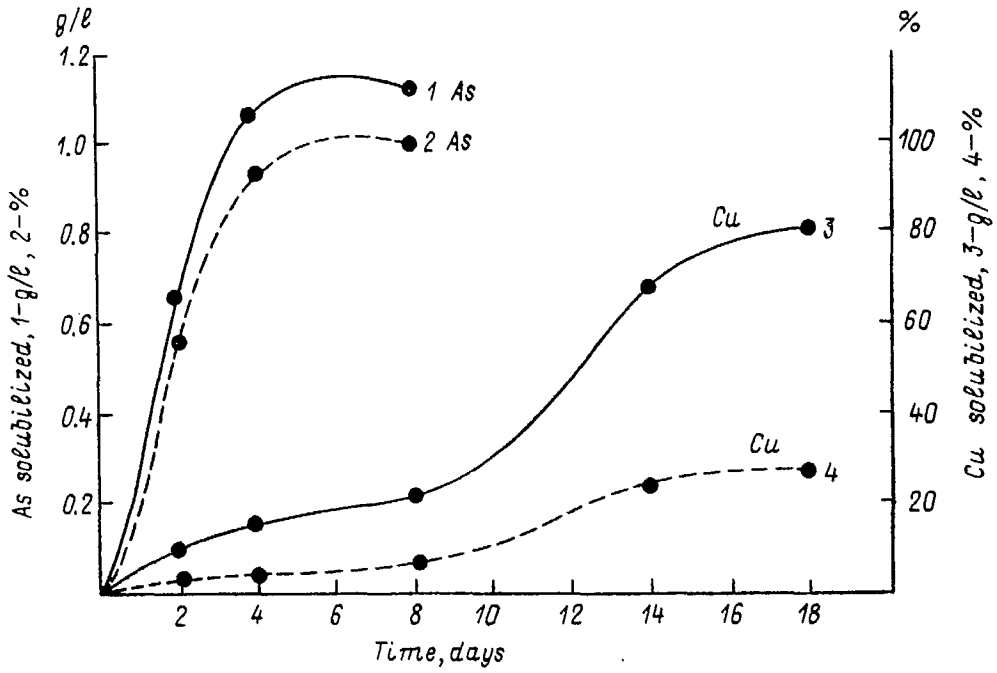

Fig. 4. Oxidation of arsenopyrite in the presence of chalcopyrite in a tin-containing concentrate [100]

Data by Yakhontova and Grudev [44] suggest that in the presence of several sulphide minerals the ore which will be oxidized preferentially by the bacteria depends on the type of its conductivity. For example, it was observed that arsenopyrite of the *p*-type acted as the cathode and favoured the destruction of chalcopyrite. The galvanic couple FeAsS of the *n*-type — FeS_2 favours the oxidation of arsenopyrite. However, the use of samples of the mixed composition (at the weight ratio of 1 : 1) proved, according to Yakhontova and Nesterovich [45], that arsenopyrite-pyrite couples in the bacterial solution had a minor difference of EP

($\sim$0.05 V) and only slightly deviated from the Eh of the solution (0.60—0.65 V). The «work» of these galvanic couples was negligible.

According to various authors [in ref. 56], when pyrite (EP $\sim$0.6 V) contacts chalcopyrite (EP $\sim$0.5 V) the latter corrodes more readily. Oxygen is reduced on the surface of pyrite (cathode):

$$O_2+4H^++4\bar{e}\rightarrow 2H_2O. \quad (45)$$

Chalcopyrite acts here as the anode:

$$CuFeS_2\rightarrow Cu^{2+}+2S^0+4\bar{e}. \quad (46)$$

Hence, the galvanic reaction may be expressed by the equation:

$$CuFeS_2+O_2+H^+\rightarrow Cu^{2+}+Fe^{2+}+2S^0+2H_2O. \quad (47)$$

When the two minerals were treated separately the results were reversed, i. e., pyrite was oxidized faster than chalcopyrite both in the presence and absence of bacteria.

During the oxidation of ZnS and $CuFeS_2$ concentrates the electrode potential of sphalerite varied from 430 mV at pH 1.5 to 230 mV at pH 2.3, while the potential of chalcopyrite amounted to 600 mV at pH 1.5 and 680 mV at pH 2.3. Thus, the electrode potential of $CuFeS_2$ is by 170—450 mV higher than that of ZnS, the lower pH favouring oxidation of chalcopyrite while the higher pH oxidation of sphalerite (Table 1, Fig. 5) [82]. Therefore, sphalerite is less resistant in the described system, and is oxidized more readily. Here 92% of Zn and only 20—25% of Cu passed into solution.

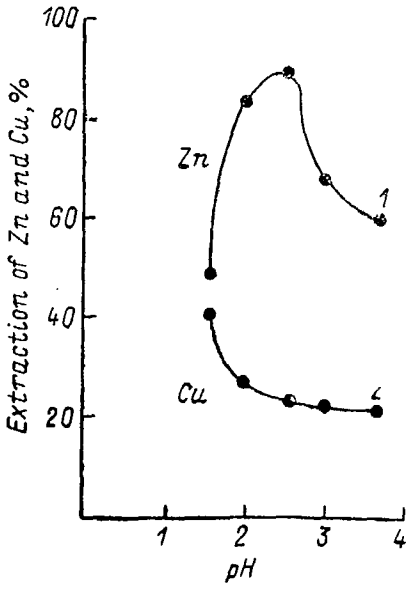

Fig. 5. Extraction of zinc (1) and copper (2) as a function of the pH of the medium in bacterial leaching of a copper-zinc concentrate [5]

The oxidation-reduction potential of U^{4+}/U^{6+} is equal to 0.41 V, and the highest rate of chemical oxidation of U^{4+} is realized at Eh of the medium about 0.75 V in the presence of Fe^{3+}.

Sulphide-cathode cannot be considered as absolutely inert in this system, either. It is partially oxidized, apparently due to the formation of microgalvanic couples and development of bacterial processes isolated on the mineral where a decrease in EP is observed. But the process seems to be marginal.

Existing data suggest that bacterial leaching of metals, at least in the tank process, may be intensified by controlling the oxidation-reduction conditions of the pulp. Furthermore, leaching intensity can be increased through appropriate selection of the concentrate for leaching with respect to its composition in order to achieve a desired interaction between minerals.

Medium Acidity

The maximum rate of oxidation of sulphide minerals, sulphur and Fe^{2+} correlates with the optimum growth of bacteria and ranges within pH 2.0—3.0 (the optimum pH 2.3—2.5). However, depending on other factors of the medium, the optimum pH varies. The following optimum pH values have been reported in the literature for the oxidation of sulphide minerals: $CuFeS_2$ — 2.2—2.5 (the optimum 2.3) [102], Cu_2S — 1.7—2.3; CuS — 2.3 [3, 6, 7, 102, 103]; ZnS — 2.0—3.0 (the optimum 2.5) [82, 83, 104], FeAsS — 2.0—3.0 (the optimum 2.5) [75, 76]. Nickel sulphides are readily oxidized at pH 2.5. Kamalov reported on cultures of *T. ferrooxidans* oxidizing Fe^{2+} and sulphide minerals at pH 1.5. The oxidation of galenite occurs most actively at pH 3.0—3.5 [69].

In dump and underground leaching of metals from ores the pH is controlled by adding sulphuric acid to solutions. In tank leaching of metals from concentrates, pH as a function of time sharply decreases (unless controlled), which results in suppressed life activity of bacteria [82]. In order to avoid this adverse effect, lime or calcite should be added to the leach suspension.

Thus the basic thermodynamic regularities typical of the oxidation of sulphide minerals are not altered by the biochemical activities of microorganisms. Bacteria merely influence the kinetics of these processes.

Role of Ferric and Ferrous Iron in the Bacterial Oxidation of Sulphide Minerals

Fe^{2+} and Fe^{3+} are the constant components of leach media for the biogenic extraction of metals from ores and concentrates. They exert a decisive influence on the oxidation-reduction capacities of the system. These ions are engaged in complex interre-

lations both between themselves and with sulphide minerals. As shown by Kelly and Jones [105], the rate of oxidation of Fe^{2+} by *T. ferrooxidans* depends on the Fe^{2+}/Fe^{3+} ratio in the medium. At different concentrations in the system, Fe^{3+} either accelerates the oxidation of Fe^{2+} or acts as an inhibitor.

Furthermore, the degree to which the rate of oxidation of Fe^{2+} is inhibited by the trivalent iron, depends on the age of culture, its adaptation to Fe^{3+} and the concentration of the latter in the medium. In experiments by Landesman et al. [106], 2.5—10 μmoles of Fe^{3+} (in 28 ml) decreased the duration of the lag-phase and increased the rate of breathing by 12%. However, addition of over 50 μmoles of Fe^{3+} caused the inhibition of breathing of *T. ferrooxidans* cells. In experiments by Kelly and Jones [105], the concentration of Fe^{3+} up to 50 mM (2.8 $g \cdot l^{-1}$) stimulated the consumption of oxygen by *T. ferrooxidans*. Yet, the Fe^{3+} ion depressed the oxidation of Fe^{2+} in the concentration of 100 mM (over 5.6 $g \cdot l^{-1}$).

Limited data are available on the role of Fe^{3+} and Fe^{2+} in bacterial oxidation of sulphide minerals. For instance, in the presence of *T. ferrooxidans* but without Fe^{3+}, 5.2 $g \cdot l^{-1}$ of arsenic were extracted from arsenopyrite [75, 76], while in the presence of bacteria and Fe^{3+} end concentrations as high as 1.0—3.3 $g \cdot l^{-1}$ of As were realized. In the presence of 10^{-4}—10^{-2} M of Fe^{3+}, the rate of bacterial oxidation of NiS, CoS, Cu_2S and CuS increased more than two-fold; higher concentrations of Fe^{3+}, however, yielded no effect [6]. The optimum concentration of Fe^{3+} for leaching copper from a copper-molybdenum concentrate was 1 $g \cdot l^{-1}$ [107]. Fe^{3+} is known to be a weak oxidizer of chalcopyrite. Nevertheless, from a finely ground sulphide concentrate ($\sim$0.5 μm) in the presence of Fe^{3+} alone 90% of copper were extracted in three hours [52]. The optimum concentrations of Fe^{3+} for the oxidation of Cu_2S and CuS are 0.004—0.01 M and 0.004—0.02 M respectively [103]. At Fe^{3+} concentrations above 1 $g \cdot l^{-1}$, the rate of leaching of copper did not increase. However, increased Fe^{3+} concentrations in solutions, up to 8 $g \cdot l^{-1}$, enhanced the extraction of Zn from a copper-zinc concentrate by 27% [108]. Duncan and Drummond [109] showed that pyrite was oxidized only by *T. ferrooxidans* and added Fe^{3+} in the presence of bacteria did not accelerate its oxidation. Addition of Fe^{2+} to the medium may either intensify or depress the bacterial oxidation of sulphide minerals. For instance, the rate of oxidation of $CuFeS_2$ was depressed 1.5-fold in the presence of 2 $g \cdot l^{-1}$ of Fe^{2+}, and the rate of FeAsS oxidation decreased upon addition of 1.0 $g \cdot l^{-1}$ and 2.5 $g \cdot l^{-1}$ of Fe^{2+} by 1.5 and 3 times respectively [3, 76]. The oxidation of PbS by *T. ferrooxidans* occurred most intensively upon addition of 0.0005—0.0025 moles of $FeSO_4$ per 100 ml of the medium. Both higher and lower $FeSO_4$ content decreased the rate of bacterial oxidation of PbS.

The mechanism of the inhibitory effect of Fe^{2+} and Fe^{3+} on bacterial oxidation of sulphide minerals is not yet completely clear. However, it was suggested that the competitive oxidation of sulphides by Fe^{3+} and *T. ferrooxidans*, as well as the prevention of bacterial attack on sulphides were due to the precipitation on the mineral surface of iron hydroxides [110]. As shown by Karavaiko, one of the reasons for the depression of bacterial activity of sulphide oxidation by iron ions is the formation of an unfavourable oxidation-reduction potential of the medium and EP of sulphide minerals.

The existing data demonstrate that a number of sulphide minerals may be oxidized by Fe^{3+}, e. g., cubanite, energite, chalcocite, covellite, tetrahedrite, sphalerite as well as tetravalent uranium and trivalent vanadium. In these cases, sulphuric acid solutions containing Fe^{3+} can be considered as the main leaching agent. Oxidation of other sulphide minerals may be accelerated in the presence of Fe^{3+}. Therefore, one of the conditions to be fulfilled in order to intensify the bacterio-chemical oxidation of sulphide minerals is to establish optimal concentrations of Fe^{3+} and Fe^{2+} in the leach solutions.

Effect of Temperature

The optimum temperature of oxidizing Fe^{2+}, S^0 and sulphide minerals by mesophilic bacteria ranges within 28—35° C. When the temperature deviates from the optimum, the rate of growth and oxidation decreases. Experiments by Karavaiko et al. [3] showed that the rate of ferrous iron oxidation in mine waters by mesophilic microflora decreased about 2—4-fold and the rate of CO_2 fixation 4—5-fold when the temperature decreased by 10° C within 12—16° C to 22—26° C. When other factors, such as pH, deviated from the optimum value, the intensity of CO_2 fixation and oxidation of Fe^{2+} by autochthonous microflora decreased 9- and 7-fold respectively at a drop of temperature by 10° C.

Obviously, such a sharp decrease in the rate of *T. ferrooxidans* growth and of Fe^{2+} oxidation is due not only to the temperature (Q_{10} usually equals 2) but also to other unfavourable factors. According to the impact of temperature on *T. ferrooxidans* in the oxidation of Fe^{2+}, Kovalenko and Karavaiko [111] noted two temperature ranges, 26—15° C and 15—5° C. As the temperature drops to 15° C, regardless of the initial Fe^{2+} concentration, the specific rate of bacterial growth and the rate of oxidation of Fe^{2+} decrease 1.5—2.5 and 1.1—2.3-fold, respectively. However, as the temperature drops from 15 to 5.5° C, the specific rate depends on the initial Fe^{2+} concentration, and will be depressed 4.2—16.3-fold in respect of bacterial growth and only 2.4—5.2-fold in respect of Fe^{2+} oxidation. At 15° C, the range of optimum Fe^{2+} concentrations is wider than this is at 26° C and 5.5° C, which are 1.2 to

12.5 $g \cdot l^{-1}$, 1.6 to 8—9 $g \cdot l^{-1}$ and 3—4 to 12 $g \cdot l^{-1}$, respectively. At 5.5° C, one can observe a significant growth of the saturation constant (R_s) (2.9 $g \cdot l^{-1}$ of Fe^{2+}) as compared to the temperatures 15 and 26° C (0.71 and 0.42 $g \cdot l^{-1}$, respectively). The low Fe^{2+} content of 1.6 $g \cdot l^{-1}$ at 5.5° C does not provide enough energy for the growth of *T. ferrooxidans* which needs a large amount of energy at low temperatures in order to support the life of bacteria. The effects of temperature and of pH of the medium on bacterial growth and Fe^{2+} oxidation are also interrelated [112, 113]. At 28° C, the range of pH values that do not affect bacteria, is wider and varying from 2.5 to 2.8 than that at 12° C varying from 2.3 to 2.4. Thus, the effect of temperature on the activity of *T. ferrooxidans* depends on a number of factors (pH, substrate concentration, ion concentrations, and so on).

It is also evident that the growth of bacteria at lower temperatures is depressed to a larger extent than the oxidation of Fe^{2+}. Hence to accelerate the oxidation processes at lower temperatures one should, first and foremost, increase the number of bacterial cells at least to 10^7—10^8 per 1 cm^3 [114].

A number of facultative thermophilic and thermophilic bacteria actively oxidize sulphide minerals within temperature range of 40 to 90° C [7, 32, 33]. In natural conditions, the facultative thermophilic bacteria, e. g. *S. thermosulfidooxidans*, are found at 20—25° C in quantities of 10^7 cell $\cdot cm^{-3}$ and take an active part in the oxidation of sulphides in ores at 20—50° C (the optimum 45—50° C).

Effect of Light

The effect of light on *T. ferrooxidans* was investigated by Le Roux and Marshall [31], and Groudeva et al. [115]. They proved that both visible light and UV light depressed the activity of thionic bacteria regardless of the substrate used. In experiments with visible light using wave lengths 430—560, 480—610 and 600 nm it was found that the degree of inhibition of bacterial activity depended on the wave length. In the visible range, the most inhibitory is the blue light, while the red light does not affect bacteria. Irradiation within the range of 300—400 nm during 1 to 8 minutes decreased the oxidation activity of bacteria by 4 to 100%. More bactericidal for *T. ferrooxidans* was found to be UV light with wave length of 200—300 nm. Short-wave light during 30 and 45 seconds depressed bacterial oxidation of Fe^{2+} by 14 and 100% respectively. Light resistance of *T. ferrooxidans* increases with the increase in the cell concentration of the medium. Trivalent iron also possesses protective properties in respect of bacterial activity.

Effect of Oxygen and Carbon Dioxide

Intensive oxidation of Fe^{2+} and of sulphide minerals may take place only with the optimum supply of oxygen and carbon dioxide. The O_2 consumption depends on the type of the substrate used and its concentration. Tuovinen and Kelly [116] calculated O_2 and CO_2 consumption by *T. ferrooxidans* in the medium containing Fe^{2+}, and demonstrated that O_2 and CO_2 consumption normally was about 183 and 81 times greater than the maximum amounts of oxygen and carbon dioxide soluble in the medium. The rate of copper leaching from a low-grade ore was proportional to the amount of oxygen consumed [7].

The growth limitation of *T. ferrooxidans* by CO_2 in direct-flow situation was shown by Egamberdieva et al. [117]. McDonald and Clark [118] proved that the specific rate of bacterial growth was independent of CO_2 concentration within 0.01—10%. However, Beck and Schaffia [119], and Kodama and Mori [120] found that increased CO_2 content of the air used for aeration resulted in an increase in the biomass of thionic bacteria and their activity.

It is known that the amount of CO_2 required will be varied with the modifications of the environment. For instance, the oxidation of a sphalerite concentrate demonstrated the existence of an interrelation between the pulp density, specific surface of particles and CO_2 consumption of *T. ferrooxidans*. According to Torma et al. [50], at the CO_2 content equal to 0.03% (normal air condition) the rate of zinc extraction increased linearly with the increase in the pulp density only up to 12%. At higher pulp densities, the low CO_2 concentration (in normal air) limited the oxidation of sulphides. At the CO_2 content amounting to 0.23—1.03%, the rate of Zn extraction from the concentrate increased linearly until the pulp density reached 24%. The above factors are closely connected with the specific surface of particles. At the CO_2 content amounting to 0.03% and 1%, the extraction of zinc from the concentrate occurred until the specific surface of particles reached 1 $m^2 \cdot g^{-1}$ and 3 $m^2 \cdot g^{-1}$, respectively. In these conditions, the rate of zinc extraction was about 0.4 and 1 $g \cdot l^{-1} \cdot h^{-1}$, respectively [50]. The supply of CO_2 into the pulp may be combined with the pH control through adding chalk according to the formula:

$$CaCO_3 + H_2SO_4 \rightarrow CaSO_4 + H_2CO_3. \quad (48)$$

It is evident that the intensity of bacterial oxidation of both Fe^{2+} and sulphide minerals depends on the rate of supply and dissolution of O_2 and CO_2 under existing technological conditions.

The highest rate of Zn extraction (96.5 $mg \cdot l^{-1} \cdot h^{-1}$) and Cu extraction (82.4 $mg \cdot l^{-1} \cdot h^{-1}$) from a lead concentrate was ob-

tained at the specific surface of particles exceeding 1.65 $m^2 \cdot g^{-1}$ [121]. Studying Zn leaching from a flotation concentrate, Torma et al. [104] proved that one of the many factors limiting the oxidation process is the total surface of mineral particles per unit volume of leach solution. It was reported that with the increase in the specific surface area of solid particles, the rate of Zn extraction was increased.

2. Influence of Technological Conditions

Size of Particles and Pulp Density

The rate of bacterial oxidation of sulphide minerals was found to increase with the decrease in the size of particles [50, 54, 102, 103, 121—124]. The highest rate of oxidation of sulphides in the pulp is observed when the diameter of particles is from 2.2 to 40 μm. The maximum extraction of Zn (98.1%) and Cu (96.5%) from a lead sulphide concentrate was obtained at particle size less than 5 μm in diameter and the pulp density of 12% and higher [121]. Studying zinc leaching from concentrate, Torma proved that the rate of its extraction under the above conditions exponentially increased with the decrease in the size of particles according to the equation:

$$V = V_m \cdot e^{(cd)},$$

where V_m — maximum rate of leaching of Zn, c — proportionality constant, and d — average diameter of particles.

The size of particles determines their surface area which also affects the rate of oxidation of sulphides. For instance, at the 16% pulp density, pH 2.3, temperature 35° C and 1% CO_2 content, the rate of Zn extraction increased linearly with the increase in the specific surface up to 3 $m^2 \cdot g^{-1}$.

Finally, the rate of leaching of metals from concentrates depends on the pulp density. For example, the extraction of Zn from a zinc concentrate was most intensive until the pulp density reached 16% [104]. The final Zn concentration in the solution was 72 $g \cdot l^{-1}$.

Experiments by Pol'kin et al. [108] proved that the rate of Zn extraction from a copper-zinc concentrate increased proportionally with the pulp density up to 25% concentration. The highest copper concentration (14 $g \cdot l^{-1}$) was obtained at the pulp density of a chalcopyrite concentrate equal to 26.7% [125].

In dump and underground leaching it is impossible to use a finely ground ore as particle size is closely connected with water permeability and aeration of the ore in dumps or in the ore body. In this case, to estimate the effect of particle size on bacterial oxidation of sulphide minerals, Bruynesteyn and Duncan [126] have introduced the notion of «active leaching volume»

which is a product of the ore's particles surface and the depth of penetration of bacteria and of the solvent. The rate of extraction of metals in this case is a hyperbolic function of the ore particles.

Solubility of the Final Products of Oxidation of Sulphide Minerals

Torma and Sakaguchi [127] have indicated that the highest rate of oxidation of a sulphide mineral by *T. ferrooxidans* is obtained when the product of oxidation possesses the highest solubility. The relationship between the rate of oxidation of a metal (dM^{2+}/dt) and the solubility of the product of a sulphide mineral (K_{sp}) may be expressed by a formula:

$$V = \frac{dM^{2+}}{dt} = AK_{sp} = A\,[M^{2+}]\,[S^{2-}],$$

where A — proportionality factor.

The direct relationship between the rate of oxidation of a sulphide and solubility of the product indicates that bacteria should remain in a close contact with the surface of substrate where the dissociation depends on the solubility of its oxidation product:

$$MS \rightleftarrows M^{2+} + S^{2-}. \qquad (49)$$

The sulphide portion is quickly oxidized by bacteria to the sulphate:

$$S^{2-} + 2O_2 \rightarrow SO_4^{2-}. \qquad (50)$$

When the dissociation equilibrium (in equation 49) is shifted to the right, theoretically this process may last until the complete dissolution of the substrate (MS) takes place. However, upon accumulation of the oxidation products, particularly metals, in the solution, bacterial activity is inhibited. Hence, the process may be completed at a low concentration of the initial substrate, or else the oxidation products should be removed from the system. The following succession of dissolution rates of sulphides has been established:

$$NiS > CoS > ZnS > CdS > CuS > Cu_2S.$$

Effect of Chemical Elements

General principles of metals toxicity for *T. ferrooxidans* have been formulated by Norris and Kelly [128]. Essentially, toxicity of metals depends on the physiological state of the microorganism, chemical form of metals and degree of their interaction in the environment.

Karavaiko et al. [129] reported that the activity of *T. ferrooxidans* in mine waters would be determined by the complexity of

metal ions and chemical composition of solutions which may depress or intensify the activity of bacteria. Research data on the influence of a number of chemical elements on *T. ferrooxidans* are rather of a theoretical interest and show the resistance of a given strain only under specific experimental conditions. Qualitative data on the resistance of *T. ferrooxidans* to heavy metals can be found in a number of publications. Various strains of *T. ferrooxidans* are resistant to 0.37 M (10 $g \cdot l^{-1}$) of Al, 0.15 M (10 $g \cdot l^{-1}$) of Zn, 0.17 M (10 $g \cdot l^{-1}$) of Co, 0.17 M (10 $g \cdot l^{-1}$) of Ni, 0.18 M (4 $g \cdot l^{-1}$) of Mn and 0.16 M (10 $g \cdot l^{-1}$) of Cu. The maximum inhibition by Au ions was at 10^{-7} M. Silver and anions of selenium, tellurium and arsenic depressed bacterial activity in a concentration range varying from 0.2 to 0.9 mM (from 50 to 100 $mg \cdot l^{-1}$) and molybdenum in a concentration over 0.03 mM (5 $mg \cdot l^{-1}$) [7, 78, 116, 130, 131]. However, thermophilic bacteria close to *Sulfolobus* develop at the content of Mo in the medium up to 2 $g \cdot l^{-1}$ [35, 73].

Minimal concentrations of As^{3+} and As^{5+} ions depressing the oxidation of Fe^{2+} by *T. ferrooxidans* were found to be 1.0 and 2.0 $g \cdot l^{-1}$, respectively [78].

Anions of Se, Te, As and Mo are more toxic for *T. ferrooxidans* than most cations of metals. However, the most toxic metals for these bacteria are Cd, Ag, Hg and U.

Mercury ions inhibit *T. ferrooxidans* in a concentration of $5 \cdot 10^{-4}$ mM [Le Roux, in ref. 7] and uranium ions in a concentration of 10^{-3}—$5 \cdot 10^{-4}$ M. The activity of *T. ferrooxidans* in the medium containing Fe^{2+} is depressed upon addition of 0.1—10.0 $mg \cdot l^{-1}$ of Ag^{+} [132, 133]. However, quantitative values of metal toxicity are affected by physico-chemical factors of the medium and bacterial culture condition. The following examples were cited by Norris and Kelly [24]: $AgNO_3$ in a concentration of 10^{-9}—10^{-8} M depressed the growth of *T. ferrooxidans* which was evidenced by an increase in the lag-phase. After addition of $AgNO_3$ in a concentration of 10^{-7} M there was no growth of bacteria. However, after addition of $AgNO_3$ in the same concentration (10^{-7} M) to the growing culture, its growth was at first somewhat depressed but resumed after 100 hours in the presence of $5 \cdot 10^{-2}$ M of K^{+}. Toxicity of $AgNO_3$ added in a concentration of $5 \cdot 10^{-8}$ M was also reduced upon pH increase from 1.7 to 2.0 which was indicated by the lag-phase dropping from 15 to 1 hour. When *T. ferrooxidans* was grown on sulphur and sulphides its resistance to Ag^{+} considerably increased. After addition of 10^{-5} M of $AgNO_3$ to the culture growing on pyrite, the extraction of iron from FeS_2 was not depressed. This is due to a close affinity of Ag^{+} to sulphur leading to a partial binding of the silver ion in the form of sulphide Ag_2S. Toxicity of other heavy metals (Zn, Ni, Co) was lower than that of Ag when bacteria were cultured on sulphur, and increased when thiosulphate was the substrate [38]. There

exist other mechanisms of interaction between Ag^+ and sulphide minerals which is indicated by enhanced activity of *T. ferrooxidans*. Bacterial activity was enhanced upon addition of Ag^+ ($AgNO_3$) during the oxidation of sulphide minerals of copper and zinc, according to McElroy and Duncan [134], McElroy and Bruynesteyn [135]. Austrian scientists suggested intensifying the leaching of copper from ore by adding 0.2% sodium lignosulphonate and 0.1% $AgNO_3$ or Ag_2SO_4. This resulted in copper extractions up to 93% (Patent No. 319616). Recently, Snell and Morgan [136] reported that silver catalyzed the oxidation of copper sulphide minerals. This indicates that silver interacts with sulphide minerals favouring both their bacterial and chemical oxidation.

As pointed out above, uranium is toxic to *T. ferrooxidans* in concentrations above 10^{-3}—$5 \cdot 10^{-4}$ M, depressing the fixation of CO_2 to a greater extent than the oxidation of Fe^{2+}. Addition of 100—200 mM of Zn^{2+} and Ni^{2+}, Mg^{2+} or Mn^{2+} to the medium partially reduced the toxicity of 2 mM of UO_2. Nevertheless, the cation concentrations of 2—20 mM did not reduce the toxicity of uranyl sulphate.

Inhibition of the growth of *T. ferrooxidans* in the presence of 0.7 $g \cdot l^{-1}$ of uranyl sulphate was partially reduced in the presence of 200 mM of potassium, sodium, lithium or NH_4^+ in the form of sulphates [130]. Zinc in the system containing copper reduced its toxicity for *T. ferrooxidans* [137]. Toxicity of Tl was also reduced in the medium with a high K^+ concentration. Thallium and rubidium in a concentration of 10^{-4} M inhibited the growth of *T. ferrooxidans* in a phosphate-free medium and were non-toxic in a concentration of 10^{-3} M in the normal medium [130]. Chlorides of sodium or potassium, according to the same authors, inhibited bacterial development in the absence of sulphates and their inhibitory concentration was dependent on the content of phosphorus in the medium.

Kovalenko and Karavaiko [111] demonstrated that the inhibitory effect of copper ions on the rate of Fe^{2+} oxidation by *T. ferrooxidans* depended on the stage of bacterial culture development, substrate concentration and temperature. The level of inhibition of Fe^{2+} oxidation in the presence of 4.4 $g \cdot l^{-1}$ of Cu^{2+} was lowered with the increase in substrate concentration regardless of the temperature variations within 6—28° C. The constants of Cu^{2+} inhibitor are as follows: at 28° C: from 0.5 to 0.6 $g \cdot l^{-1}$; at 12° C: from 2.5 to 2.8 $g \cdot l^{-1}$; at 6° C: from 4.0 to 4.4 $g \cdot l^{-1}$ of Cu^{2+}. Therefore, toxicity of the inhibitor decreases with the decrease in the temperature.

Toxicity of metals may be reduced in the presence of metal-complexing agents. For example, ethylenediaminetetraacetic acid in a concentration of 20 mM reduced the inhibition of Fe^{2+} oxidation in the presence of 1.5—2.0 mM of UO_2^{2+} [131]. Addition of cysteine (10^{-4} M) protected *T. ferrooxidans* against the inhi-

bitory action of $AgNO_3$ (10^{-5} M) on the Fe^{2+}-containing medium [128]. Yeast extract seems to have a similar effect on the media for thermophilic bacteria. The high resistance of thermophilic bacteria to metals is attributed to the presence of yeast extract [128].

According to Lazaroff [138], some anions, such as TeO_4^{2-}, WO_4^{2-}, AsO_4^{3-}, and PO_4^{3-}, increased the rate of Fe^{2+} oxidation by *T. ferrooxidans* in the presence of SO_4^{2-} or SeO_4^{2-} and produced no effect when the latter were absent. Under these conditions, low concentrations of Cl^-, $B_4O_7^{2-}$ and ClO_3^- produced no significant effect while Br^-, NO_3^- and MoO_4^{2-} inhibited the oxidation of Fe^{2+}.

The mechanism of cation and anion effect on *T. ferrooxidans* has so far been studied inadequately. The inhibitory effect of metals is attributed to their accumulation in the cell. They block the enzymes and disrupt cation transport through the membranes [128]. Kelly and Jones [105], and Kovalenko and Karavaiko [111] proved the competitive mechanism of inhibition of bacterial oxidation of Fe^{2+} by Fe^{3+} and Cu^{2+} ions, respectively. Apparently, this mechanism of inhibition of bacterial oxidation of Fe^{2+} and hence, of metal sulphides, is dominant in the presence of other metals as well.

When estimating the technological aspects of the problem, it is obvious that the influence of chemical elements on bacteria in dump, underground and tank leaching should be determined taking into consideration all physical factors as well, which are created during the technological process.

Sources of Nutrients

Nitrogen and phosphorus are present in mine waters mostly in minor quantities which often causes the depression of bacterial oxidation. Apatite-containing ores provide an exception. *T. ferrooxidans* uses practically only ammonium salts as a source of nitrogen. When added to the leach solutions, they, as well as phosphorus, accelerate the growth of *T. ferrooxidans* and the rate of oxidation of Fe^{2+} and of sulphide minerals. For instance, adding $(NH_4)_3PO_4$ to the solutions up to final concentrations of 17 $mg \cdot l^{-1}$ of NH_4^+ and 50 $mg \cdot l^{-1}$ of PO_4^{3-} increased the number of cells 10-fold according to Tuovinen et al. [139]. Optimum concentrations of KH_2PO_4 and $(NH_4)_2SO_4$ in mine waters of the Degtyarsky deposit according to Karavaiko et al. [3] were 400 and 300 $mg \cdot l^{-1}$, respectively, and in some cases, as high as 2 $g \cdot l^{-1}$ of $(NH_4)_2SO_4$ [140]. Optimum concentrations of nitrogen and phosphorus in metal leaching should be established for each particular case. According to MacIntosh [141], *T. ferrooxidans* is able to fix N_2. However, Tuovinen et al. [139] proved that the acid medium absorbs ammonium from the atmosphere enabling

T. ferrooxidans to grow in a nitrogen-free medium. Thus, the ability of these bacteria to fix atmospheric nitrogen has not been proved. Chakrabarty [142] also noted the importance of introducing the RP_4 plasmids of *Klebsiella* which contain the nitrogen-fixing genes into *T. ferrooxidans* cells. As pointed out above, the oxidation rate of sulphide minerals of copper and zinc on a nitrogen-free medium increased in the presence of N_2-fixing *Beijerinckia lacticogenes* [143].

Sulphates are used by thionic bacteria for biosynthesis and certain fermentative functions. Schnaitman et al. [144] proved that addition of SO_4^{2-} in a concentration from 3.6 to 50 mM increased the oxidation of Fe^{2+} by *T. ferrooxidans* two-fold. Sulphates are also important as complexing agents in the consumption of some elements and in the oxidation of ferrous iron. Hence, large amounts of SO_4^{2-} are required for an intensive oxidation of Fe^{2+} by *T. ferrooxidans* [138, 145].

Magnesium and other elements are important for bacterial development as microelements. They are present in sufficient quantities in mine waters and leaching solutions.

Influence of Organic Solvents

Microbiological leaching of metals from low-grade ores is a cyclic process. When leaching copper from ores, copper may be recovered from the solution by solvent extraction and electrowinning. However, the organic solvents used may inhibit the growth of *T. ferrooxidans*. As reported by Torma and Itzkovitch [146], reagents may be arranged in the following sequence according to the degree of inhibitory effect: Lix 70<Lix 73<Lix 71<Lix 64N<Lix 65N<TBP~isodecanol~nonylphenol<Lix 63 <<< <<< D_2EHPA~Kelex 100<Kelex 120^<< alamin 336~alamin 308 ~alamin 310<alamin 304~adogen 381~aliquat 336<adogen 364. To offset the toxic effect of extractants, solutions should be treated with activated carbon.

3. Influence of Microbiological Factors

Use of Active Bacterial Cultures

In close relation to the practical use of microorganisms for the leaching of metals from ores is the problem of obtaining highly active bacterial strains, which are resistant to extreme conditions. Resistance of «wild» strains of *T. ferrooxidans* to metals varies depending on their habitats and adaptation under natural conditions [47].

From the zones of sulphide mineral oxidation, various authors have isolated strains of *T. ferrooxidans* which are resistant to high metal concentrations in solutions: Cu — 0.5—10 $g \cdot l^{-1}$; Zn — 9—

30 $g \cdot l^{-1}$; Al — up to 6 $g \cdot l^{-1}$; Ni — up to 20 $g \cdot l^{-1}$; Co — up to 5 $g \cdot l^{-1}$; Cl — up to 0.25 $g \cdot l^{-1}$; Ag — 0.05—0.1 $g \cdot l^{-1}$; and As — up to 1 $g \cdot l^{-1}$.

Kuznetsova and Kuligina [147] reported that the introduction of exogenous bacteria in uranium leach suspension was less effective than the addition of nutrients in activating the autochthonous microflora. Further investigations revealed that in the processes of dump and underground leaching of metals the most urgent problem was how to optimize the activity of autochthonous microflora adapted to the specific conditions of leaching of minerals. Another urgent problem is how to increase the number of bacterial cells in solutions and ores up to 10^7—10^8 $cell \cdot cm^{-3}$ ($cell \cdot g^{-1}$), especially at low temperatures [3, 111—114].

Experimental isolation of highly active cultures of bacteria including the thermophilic ones promises well for tank leaching of minerals. In this process, stable and adequate conditions can be created for bacterial growth. For this purpose both adaptation and classical selection methods may be applied. Methods of gene engineering seem to be promising as well. It is known that only those cultures of *T. ferrooxidans* are kinetically stable which have been obtained in the presence of high concentrations of heavy metals and sulphide concentrates [100]. These bacteria also possess a higher ability to adsorb the oxidized substrates [83]. According to the same authors, the leaching of metals from concentrates in a dense pulp occured readily at the content of 10^9—10^{11} cells per 1 ml.

Bacterial cultures were derived under conditions of a gradually increasing concentration of a number of elements. For example, information is available about *T. ferrooxidans* strains resistant to the following concentrations of various elements and compounds: Cu — 50 $g \cdot l^{-1}$; Zn — 120 $g \cdot l^{-1}$; Co — 30 $g \cdot l^{-1}$; As — 6—10 $g \cdot l^{-1}$; Cl — 10 $g \cdot l^{-1}$; Fe — 160 $g \cdot l^{-1}$; Mo — 200 $mg \cdot l^{-1}$; Al — 20 $g \cdot l^{-1}$; Ni — 72 $g \cdot l^{-1}$; Ag — 1.0—10 $mg \cdot l^{-1}$; F — 100 $mg \cdot l^{-1}$; Cd — 120 $mg \cdot l^{-1}$ and U_3O_8 — 12 $g \cdot l^{-1}$ [6, 50, 100, 102, 128, 132, 148, 149—151].

Dave et al. [137] provided data on the oxidation of Fe^{2+} by *T. ferrooxidans* in the presence of 160 $g \cdot l^{-1}$ of $CuSO_4$ and 640 $g \cdot l^{-1}$ of $ZnSO_4$. The mechanism of *T. ferrooxidans* resistance to metals has not yet been studied adequately. There exist probably two mechanisms, i. e., physiological adaptation and development of mutants resistant to the extreme conditions. According to Tuovinen and Kelly [131], the frequency of mutants resistant to 1.0 and 1.5 mM UO_2^{2+} is about unit per $1.3 \cdot 10^6$ or $9.0 \cdot 10^8$ of cells, respectively. The frequency of mutants was increased by adding 15—150 mM of Ni, Zn and Mn.

It has of late been reported that plasmids are important for the resistance of bacteria to metals, and suggested that metal-resistant

T. ferrooxidans strains can be obtained by introducing appropriate plasmids into them [142].

Finally, another important technological problem to be solved is the culturing of a large biomass of active bacteria to be added to the leach solutions. This will have a beneficial effect in dump, underground and tank leaching of minerals. A possible method is the separation of bacteria from pregnant solutions. This will produce a biomass of bacteria adapted to the leach conditions. The culturing of *T. ferrooxidans* according to Kovrov et al. [42, 43] also seems to deserve attention. This method is based on the bacterial oxidation of Fe^{2+} in the conditions of continuous electrochemical reduction of Fe^{3+}.

Role of Mixed Bacterial Cultures in the Leaching of Minerals

In natural or tank leaching of ores associations of microorganisms may be beneficial. This can be illustrated by the leaching of Cu and Ni from a flotation copper-zinc concentrate on a nitrogen-free medium by mixed culture of *T. ferrooxidans* and *Beijerinckia lacticogenes* [143, 152]. In the presence of mixed culture 50% Cu and 100% Ni were extracted in 150 days, while in the presence of *T. ferrooxidans* alone only 18% Cu and 58% Ni were solubilized during the same period.

Typical of the thermophilic lithotrophic bacteria is that they readily oxidize sulphide minerals only in the presence of yeast extract or some other organic substances, e. g. glucose. It may be assumed that thermophilic bacteria can obtain organic substances that are necessary for their growth from other lithotrophic and organotrophic bacteria. Brierley [153] proved that *T. ferrooxidans* can provide organic compounds for the facultative thermophilic bacteria growing on the medium with Fe^{2+}. Data obtained by Golovacheva [154] also indicate that there is a synergism between *S. thermosulfidooxidans* and *L. ferrooxidans* grown on a copper-zinc sulphide ore. In this case, intensive oxidation of sulphide minerals occurs in the absence of yeast extract. Another example of the combined effect of bacteria is the oxidation of sulphide minerals by mixed cultures of *L. ferrooxidans* and *T. organoparus* or *L. ferrooxidans* and *T. thiooxidans* [23, 24]. Pure cultures of these bacteria do not oxidize sulphide minerals. A mixed culture oxidizes sulphide minerals at the same rate as *T. ferrooxidans*.

Chapter IV

TECHNOLOGICAL, ECONOMIC AND ECOLOGICAL ASPECTS OF BACTERIO-CHEMICAL LEACHING OF METALS

1. Technological Aspects

Dump and Underground Leaching

The leaching of copper from ores has been known for a long time. For example, as far back as 1497 copper was leached and recovered by the cementation method in Northern Hungary [155]. A complete leaching cycle is known to have been in operation there in 1566. Around 1750 some 200 tons of cement copper were produced annually in this way by employing the irrigation technique. In Germany, leaching of copper from dumps was practised also in the sixteenth century [156]. At the Rio Tinto mine in Spain, dump leaching of copper ores was started in 1725, while the accumulation of copper from mine waters dates back to 1670 [59]. In Kedabek (the Azerbaijan SSR), dump leaching was in use at the close of the last century. In 1939, practically simultaneously, underground leaching of copper was begun in the Novo-Levinsky and Belorechensky mines in the Urals [3].

At present, dump leaching of copper is used in the USSR, USA, Mexico, Australia, Peru, India, Yugoslavia, Bulgaria, and elsewhere [3, 6, 7, 157—159]. Roughly 5% of the world output and 11.5% of the U. S. copper production are obtained by leaching of low-grade ores [Wadsworth 1975; in ref. 160]. In a number of countries large quantities of uranium are being extracted by this method [7, 90, 161]. Leaching of uranium from low-grade ores in dumps was started in Canada in 1960.

The first research reports on bacterial leaching of metals from sulphide ores were published in 1922 by Rudolfs and Helbronner [162] who used unidentified bacteria. They put forward the notion that microorganisms might be used for leaching metals from low-grade sulphide ores. Somewhat later, in 1947, Colmer and Hinkle [8] isolated *T. ferrooxidans*, a microorganism with unprecedented physiological properties, which was capable to oxidize sulphur, ferrous iron and sulphide-containing minerals at low pH values.

The first patent for the use of *T. ferrooxidans* in the leaching of metals from ores was issued in the USA in 1958 [163]. In the USSR, the physiology and geochemical activity of microbes in ore deposites were first studied by S. I. Kuznetsov at the Institute of Microbiology of the ASSU, and the first work on bacterial leaching of metals dates back to the late fifties and was

done by V. I. Ivanov and B. A. Stepanov at the URALMEKHANOBR Institute. At present, a number of other genera and species of bacteria and their associations are known to take part in oxidation of sulphide-containing minerals and in leaching of minerals. In the USSR, first field tests of bacterio-chemical underground leaching of copper were carried out in 1964 [3].

The entire research cycle on underground and dump bacterio-chemical leaching of metals involves normally four consecutive steps: laboratory exploratory experiments, scaled-up laboratory experiments, tests on a semi-industrial plant, and investigations

Tabie 2

Research steps involved in developing bacterio-chemical dump and underground leaching of metals from ores

Research step	Experimental conditions	Research objectives
I	Weight of ore samples from 5 to 15 kg. Rigid-vinyl or acrylic plastic percolators: D-100 to 150 mm H-400 to 700 mm	Analysis of the phase and chemical composition of the ore and of its mineralogy and petrography; measurements of the specific water and acid retention of the ore, of its porosity, density and bulk density, etc.. Trying different spraying densities and different time intervals between spraying.
II	Same conditions.	By designing a full factorial experiment, find optimal parameter values for leaching of the metal from the ore in question.
III	Total weight of ore samples up to several tons. Ore size up to 400 mm Metal columns: D-0.7 to 1.2 m H-6 to 7 m	Improvements on the optimal leaching conditions found in the preceding step. Economic evaluation of the method.
IV	Ore dumps or ore bodies in deposits	Testing various irrigation techniques and ways of recovering the metal from solutions. Measuring the permeability to solutions of the soil or specially prepared dump sites. Testing different ways of increasing the rate of leaching in field conditions. Studying hydrodynamic characteristics of the material undergoing leaching. Solving the environment protection problems involved and commercial assesment of the entire system.

on a pilot-industrial facility. One example is the research on leaching of copper from ores of the Kounrad deposit which was carried in four steps listed in Table 2 [in ref. 164].

A schematic diagram of dump leaching of copper, showing equipment requirements of the facility, is presented in Fig. 6 [164]. Such technical arrangement of bacterio-chemical dump and underground leaching of metals is, in fact, similar to that involving the sulphuric acid process and is used in many countries [3, 7, 165].

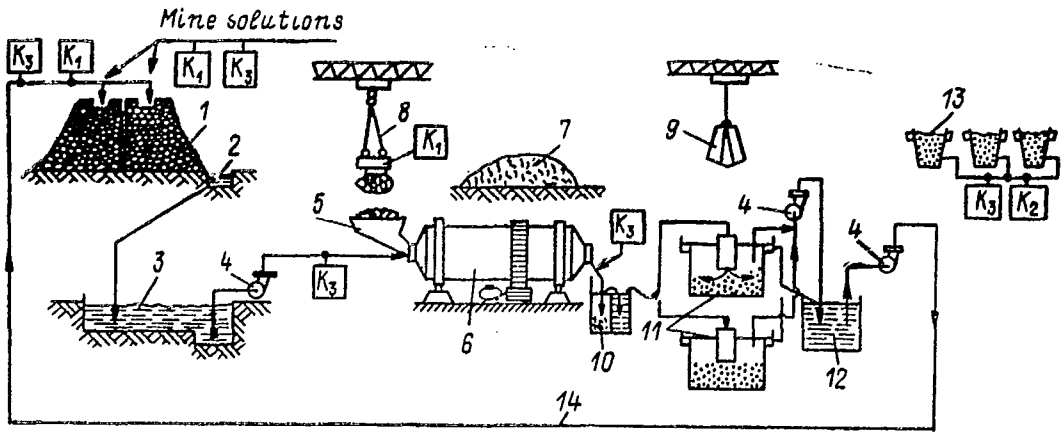

Fig. 6. A schematic diagram of a plant for leaching and cementation of copper: 1 — an ore dump leached; 2 — flumes for solutions; 3 — a settling pond for inflow solutions; 4 — pumps; 5 — an inlet for loading iron scrap; 6 — a cementation drum; 7 — a scrap pile; 8 — a travelling bridge magnetic crane with a telpher; 9 — a grab bucket for discharging cement copper; 10 — a trap for small-sized iron scrap; 11 — a settling basin for cement copper; 12 — a sump for recycle solutions; 13 — containers with output cement copper; 14 — pipe-lines

The flowsheet provides for a cyclic leaching process. Sulphuric acid solutions of ferrous and ferric iron containing *T. ferrooxidans* and other bacteria are normally used as leaching liquor (see Chapter I). Sulphide minerals contained in the ore are oxidized by bacteria and ferric iron according to the reactions considered in Chapter II.

Next, the solution coming from the ore dump is fed to a cementation unit where copper is precipitated as a result of the following reaction with the iron scrap:

$$CuSO_4 + Fe \rightarrow Cu + FeSO_4.$$

The cement copper is then transported to a copper smeltery.

Prior to further irrigating the ore, the solution containing Fe^{2+} is to be partially or entirely regenerated in a special pond. This involves the oxidation of ferrous iron by microorganisms and a partial removal of solutions from the cycle with their replacement by fresh water. If necessary, sulphuric acid could be added, as well as nitrogen and phosphorus salts. Thereafter the cycle is repeated.

The purpose of regenerating the leach liquor is to increase the content of bacteria and of the oxidizing agent, Fe^{3+}, as well as to reduce to an appropriate level the content of other elements that may accumulate in the solution during the process. In the leaching of Ni, it is recovered from the solution by electrolysis and the sulphuric acid formed in the process is used as the recycle solution.

Also under study is metal recovery from solutions using various sorption processes.

The expediency of leaching of copper and other metals from sulphide ores depends on the type of the ore, on technical factors, (such as preparation of dumps or ore body, supply of oxygen to the ore, maintaining optimal pH values, hydrodynamic characteristics of objects affected, etc.), and also on the number and the activity level of bacteria [1, 3, 4, 126, 166]. The main factors affecting biological oxidation of sulphide minerals in field conditions are summarized below [4].

1. *Physical characteristics of the rock body containing sulphide minerals*
   Geometry of the rock body
   Particle-size distribution
   Permeability to water and gases
   Thermal conductivity and heat capacity
   Location as related to influent water, drainage and the water table
2. *Characteristics of sulphidic material*
   (a) Relating to sulphidic component of the ore
      Presence or absence of iron pyrite
      Diversity and types of other metallic sulphides
      Particle size of individual sulphides
      Distribution of individual sulphides
   (b) Relating to the gangue
      Permeability to water and gases
      Capacity for acid neutralization
      Stability to acid and heat degradation and nature of degradation products
      Sorptive capacity for water, H^+, metal ions, and other chemical compounds (including organic matter)
      Ion-exchange properties
      Redox characteristics
3. *Composition of influent water*
   Solutes present (oxygen, carbon dioxide, carbon compounds, nitrogen compounds, other nutrients for bacteria)
   Redox status (largely governed by oxygen level and the ferrous/ferric ion ratio)
   Microbial populations (diversity and magnitude)
   Presence of natural toxic materials (e. g. mercury and molybdenum compounds)

4. *Climatic influences*

Rainfall and run-off patterns influencing the availability of water, water-logging, and the erosion of the rock body
Ambient temperature variations (seasonal and daily)

Studies on *T. ferrooxidans* distribution in dumps and in ore deposites have shown that the number of cells, within the oxidation region, varies from 10^5 to 10^8 per 1 g of ore or 1 ml of the solution depending on the season, technical arrangement of the leaching process, and on the temperature [3, 47, 87, 167—169]. The activity level of bacteria depends on the temperature, pH value, and on chemical composition of leach solutions [165].

During the leaching of copper from industrial dumps of sulphide ores their temperature has been observed to increase to 40—60° C [32, 166, 169—175]. The same process in columns takes it up to as high as 50 to 60° C [172]. Laboratory studies have shown that regeneration of Fe^{3+} and oxidation of sulphide minerals by bacteria are not impeded by high temperatures [30, 32, 35, 57, 67, 73]. It is evident that thermophilic bacteria can play an important role in industrial leaching of ores.

The distribution of thermophilic bacteria oxidizing Fe^{2+} and reduced sulphur compounds (S^0, sulphides) has not as yet been adequately studied. As mentioned above, *S. thermosulfidooxidans* [33] and some thermophilic bacteria close to thionic bacteria [30, 169] were isolated from leach dumps. As reported by Golovacheva and Karavaiko [33], the number of cells of *S. thermosulfidooxidans* in ores and leach solutions amounts to cially from high-grade sulphide ores and at lower temperatures, (20 to 25° C).

The results of ecological and laboratoty studies can indicate a general solution to the technological problems involved in increasing the efficiency of the whole cenosis of microorganisms in dump and underground leaching of metals. It should be emphasized, however, that an active process of metal leaching, especially from high-grade sulphide ores and at lower temperatures, would require concentration of bacteria of the order of 10^9 cells per 1 ml of solution. This can be achieved by incorporating into the system particular operation stages such as solution regeneration or growing biomass either in electrochemical cultivators or by means of the Bacterial Film Oxidation method [42, 43, 173, 174].

Tank Leaching

Developing an industrial process of tank bacterio-chemical leaching of metals from concentrates normally involves several research steps listed in Table 3.

A prototype of tank leaching is the extraction of metals from concentrates in fermenters or Pachuca tanks [17, 78, 135, 151,

Table 3

Research steps involved in developing a tank bacterio-chemical leaching system [175]

Research step	Experimental conditions	Research objectives
I Exploratory laboratory studies	Sample weight up to 1 kg; batch cultivation	Understanding the participation of microorganisms in oxidation of sulphide minerals in concentrates. Identifying underlying conditions, e. g., activity level of bacteria, their tolerance to the concentrate and metals, their adaptation, the pH range, the pulp density, etc..
II Scaled-up laboratory tests	Samples of a few kilograms in weight; continuous culture	Finding optimal conditions (parameter values) for the bacterial leaching process with recycling of regenerated solutions. Obtaining a working culture adapted to the entirety of extreme environment conditions of the leaching system. Economic evaluation of the process
III Pilot industrial tests	Production output exceeding 60 kg·day^{-1}; solid to liquid phase ratio = 1 : 4—5	Obtaining and refining data for feasibility evaluation and for designing a pilot commercial system. Stages: 1. Obtaining an active biomass; 2. Putting the plant in the projected operation mode, 3. Leaching operation with complete recycling of biomass and solutions

175, 176]. In the USSR, a direct-flow scheme is used with solid and liquid phases passing simultaneously through a successive series of Pachuca tanks (Fig. 7) [151]. Table 4 lists the parts of a pilot system of this type made from acid resistant materials. Bacterial oxidation of sulphide minerals takes place in the 120-liter Puchuca tanks (items 1—3 in Fig. 7). Next the liquid and solid phases are separated and valuable components are extracted from the solution. The solid sediment (cake) is dumped or is to undergo further processing and the solution is regenerated and recycled to the first Pachuca tank. Thus, the treatment of concentrate in Pachuca tanks is also a closed-circuit process. The active culture of *T. ferrooxidans* adapted to the concentrate in question is grown in the zero Pachuca tank.

A distinctive feature of the tank bacterio-chemical metal leaching from sulphide concentrates is that high-density pulps provide for high rates of oxidation of sulphide minerals and for selective element recovery. The highest rate of oxidation

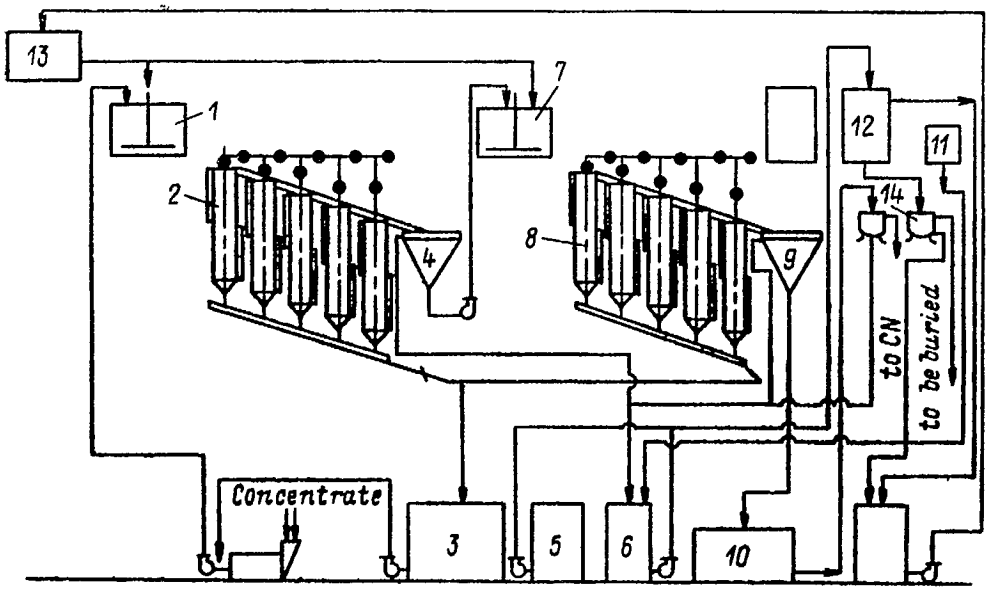

Fig. 7. A schematic diagram of metal leaching in Pachuca tanks [151]:
1 — contact tank No. 1; 2, 8 — Pachuca tanks; 3 — a tank for bacteria-containing solutions; 4, 9 — dehydrating cones; 5, 6 — vats for dehydrating cones underflow following the first and the second leaching stages; 7 — contact tank No. 2; 10 — a collector vat for output metal; 11 — a lime milk feeder; 12 — a settling vat; 13 — a collector vat for recycle solutions; 14 — a Nutch filter

Table 4

A parts list of a pilot leaching system [175]

Units	Capacity, liter	Number of units	Material
Ball mill 400×400 mm	—	1	—
Contact tank	900	1	X18H10T
Pachuca tank for leaching	200	5	—»—
Dehydrating cone	300	1	—»—
Contact tank	900	1	—»—
Pachuca tank for leaching	200	4	—»—
Dehydrating cone	300	1	—»—
Leach liquor vat	500	1	X18H10T
Liquid separator OST-3	—	1	—
Pachuca tank for regeneration of bacterial solutions	200	1	X18H10T
Vat for liquor treatment	500	1	—»—
Stand-by vat	500	1	—»—
Settler vat	500	2	—»—
Nutch filter	60	1	ceramics
Nutch filter	40	1	—»—
Vat for recycle liquor	500	1	X18H10T
Vat for recycle liquor	900	1	—»—
Agitator	30	1	St. 3
Vat for Pachuca tank discharge	900	1	X18H10T
Compressor RMK-2	—	2	—
Receiver	—	1	—

of sulphide minerals is obtained at 15 to 20% pulp density, pH 2.3, at temperatures within 30 to 35° C, with strong aeration in the presence of CO_2 (0.2 to 1.0%), fine grinding of minerals and with biomass content from 2.0 to 10 g (wet wt) per 1 ml of pulp. In tank bacterial leaching, the leach solution accumulates metals at concentrations high enough to be extracted by the electrolysis (see Table 5).

Table 5

Kinetics of bacterial extraction of metals

Concentrates	Pulp density, %	CO_2, %	Maximum rate of leaching		Tank capacity, l	Metal content of solution, $g \cdot l^{-1}$
			$mg \cdot l^{-1} \cdot h^{-1}$	metal		
Chalcopyrite (27.3% 23.8 %) [124, 176]	20 to 40 final density	0.1 0.1	725 500—700	Cu	50 50	50 20—50
Zinc [50, 104, 124]	40 16	0.1 1.0	1300 517 635 631	Zn Zn	10 0.25 30 12	98 70—120
Nickel [86]	25 15	0.2 0.2	175—222 155	Ni	8 8	72
Tin-copper-arsenic [78]	20	aeration	95.7 % in 96 hours	As	3	up to 10—15
Gold-bearing [78]	20	—»—	80—90 % in 70—80 hours	As	3	up to 10—15
Pyrite-bearing uranium ore [178]	30—40	—»—	up to 91.1—95 % in 6—9 days	U	0.5—2	—

Apart from those listed in Table 5, the following concentrates may be suitable for bacterial leaching: copper-nickel [11, 64, 86], lead [17, 121], and copper-bismuth [177].

Several flow charts, tested recently in scaled-up experiments, can be effectively implemented for tank extraction of metals from concentrates [78, 101, 121]. For example, tests run on a pilot industrial plant have shown that arsenic content as a toxic ingredient in tin-containing concentrates, which can occasionally reach 15%, can be reduced to 0.17—0.20% in 96 hours (Fig. 8). During a two-stage treatment of gold-arsenite concentrates (10% arsenic) with an intermediate removal of arsenic from the solution and with the solid to liquid phase ratio averaging 1 : 5, about 80 to 90% of FeAsS and 40% of FeS_2 is oxidized in 78 hours. With low arsenic content of the concentrate, the process in completed in a single stage and as much as 93 to 95% of arsenic is extracted in 72 hours. Gold recovery from the residue by the cyanidation method is as high as 92.5% (Fig. 9).

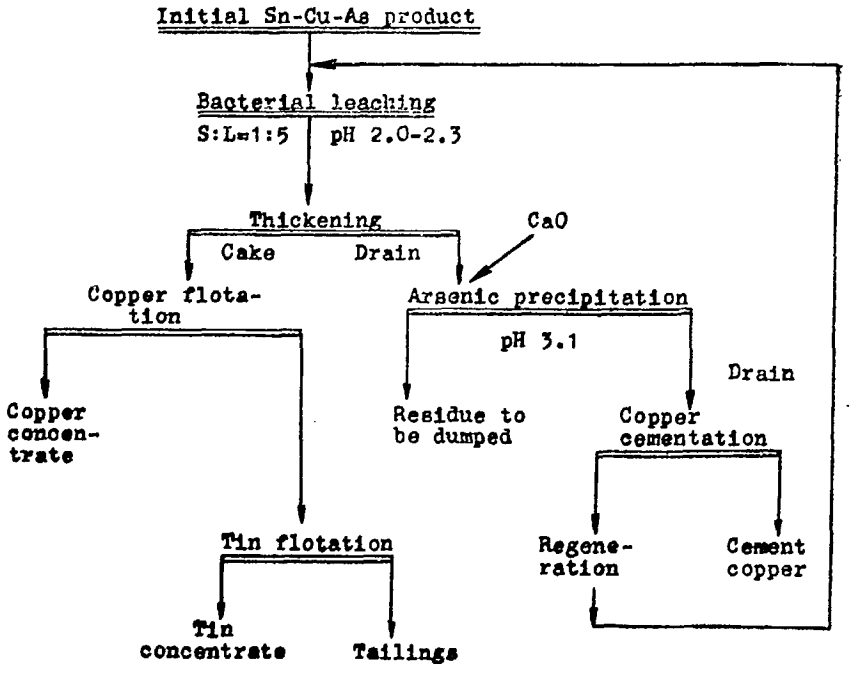

Fig. 8. A flowsheet for bacterial leaching of difficult-to-dress tin-copper-arsenic materials [78]

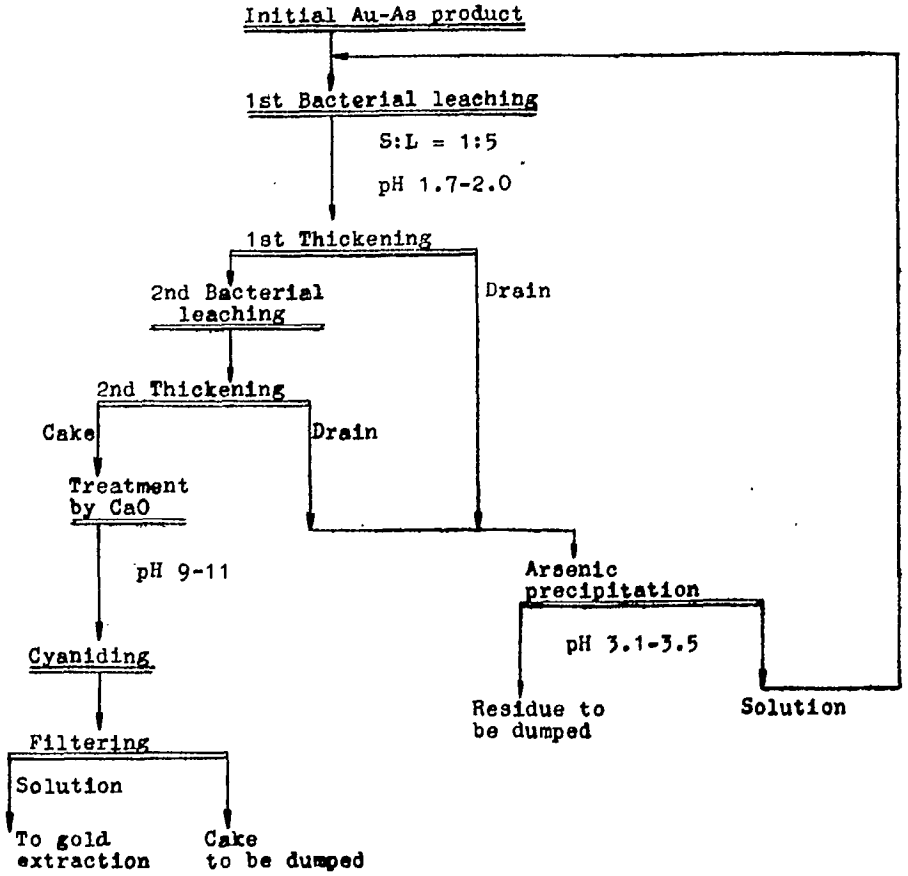

Fig. 9. A flowsheet for bacterial leaching of a gold-arsenic concentrate [78]

To recover copper and zinc from metacolloid composite concentrates, a combined approach has been used. The treatment includes selective bacterial leaching of zinc, cadmium and partially copper, recovery of metals from solutions, and cake flotation after the bacterial leaching, producing high-grade copper concentrate (Fig. 10). The total extraction was 92% of copper, 90% of zinc and 89% of cadmium [82, 83]. According to Torma and Subramanian [121], and Torma [17], a virtually full extraction of copper, zinc and cadmium from lead concentrates can be realized in the presence of *T. ferrooxidans*. Lead remains in the solid phase in the form of $PbSO_4$ and partially in the form of

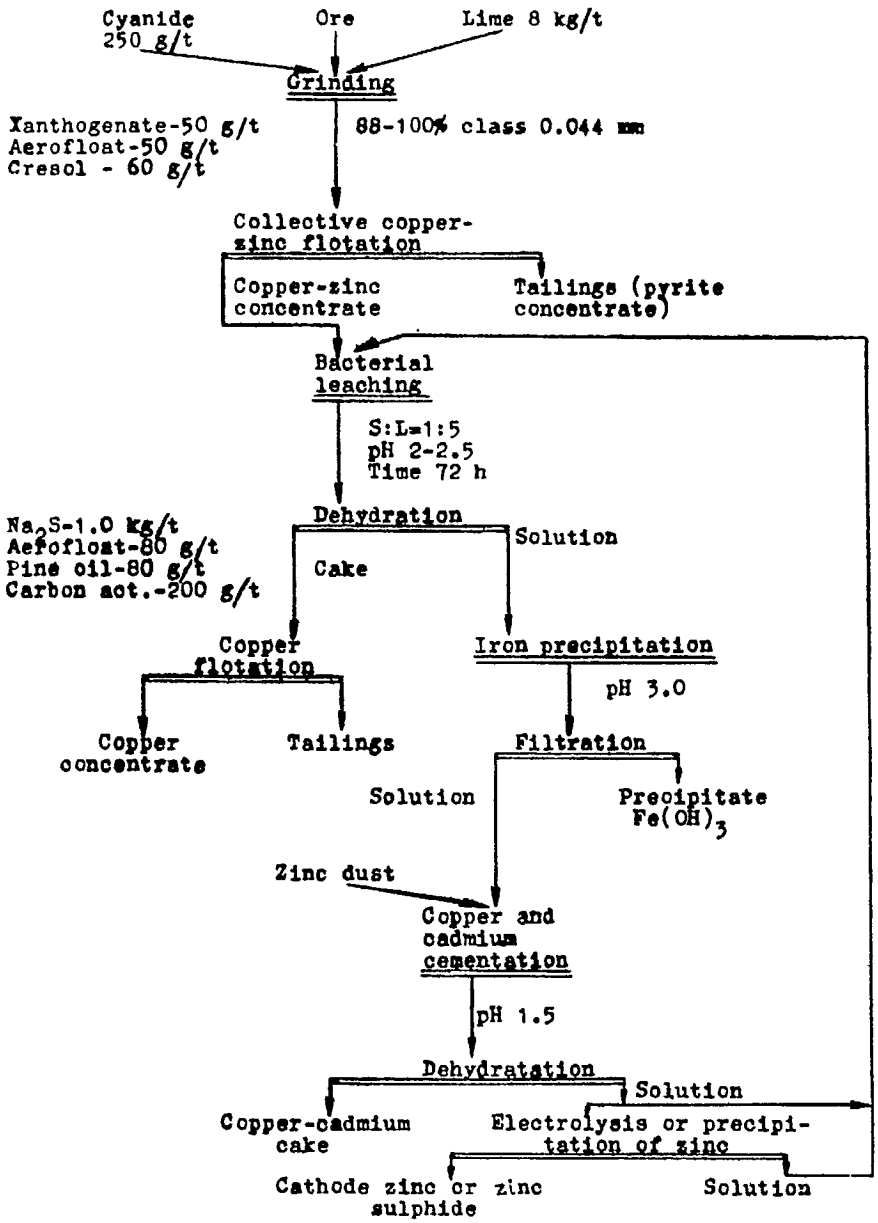

Fig. 10. A flow chart for combined processing of metacolloid copper-zinc ores [83]

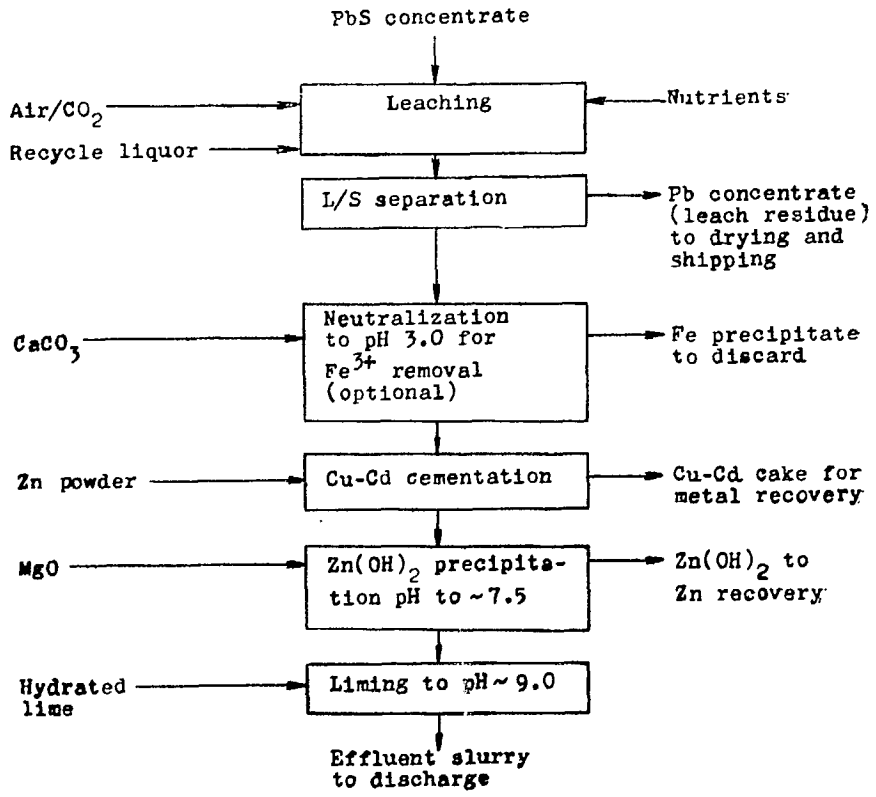

Fig. 11. A suggested flowsheet for leaching and extraction of copper, cadmium and zinc from a PbS concentrate [121]

non-oxidized PbS (Fig. 11). Both iron hydrate and lead sulphate can deposit on sulphide surfaces inhibiting bacterial activity [17]. As reported by Ilyaletdinov et al. [179], treatment of a copper-zinc concentrate with Fe^{3+}-containing bacterial solutions (20 to 25 $g \cdot l^{-1}$) at temperatures within 60 to 65° C resulted in 96 to 98% extraction of lead and 70% extraction of zinc in 3 to 4 hours. Thus, lead can also be extracted hydrometallurgically from concentrates.

Regeneration of Fe^{3+} can be carried out by using thermophilic bacteria *S. thermosulfidooxidans* which oxidize Fe^{2+} at 50° C.

Bismuthite, Bi_2S_3, is known not to be oxidized by *T. ferrooxidans*. For this reason, only a selective extraction of copper occurs during the treatment of copper-bismuth concentrates with a culture of *T. ferrooxidans*. According to Batyrbekova et al. [177], up to 68% of copper is extracted in a 3 to 4 stage treatment of concentrate (in 9 to 10 hours). The residual copper and bismuth can be recovered from the cake by addition of HCl and $CaCl_2$. The total copper extraction amounts to 92 to 95%. *T. ferrooxidans* can also be used to leach nickel and cobalt from ores and concentrates.

Studies conducted on various facilities have shown a high efficiency of *T. ferrooxidans* in uranium leaching. During lea-

ching of uranium from pyrite-containing ores in Pachuca tanks at pH 1.5 to 1.6 for 6 days, 91.1% of uranium was extracted [178]. Under semi-continuous cultivation conditions, 100% of uranium was extracted at 20% pulp density in 5 days [180, 181]. In experiments reported by Derry et al. [182], extraction of uranium from an ore containing 0.12% U_3O_8 was as high as 95%. Their technique makes use of *T. ferrooxidans* to regenerate Fe^{3+}. The process is run at 30° C.

Studies are under way to develop techniques for recovery of non-ferrous metals from various industrial wastes, copper carbonate shales, oil-containing shales, bitumenous sands [180, 183, 184] as well as for coal desulphurization. According to Ebner [185], up to 70% of copper and 95% of zinc can be recovered from industrial waste materials.

In experiments by Dugan and Apel [186], about 97% of py-

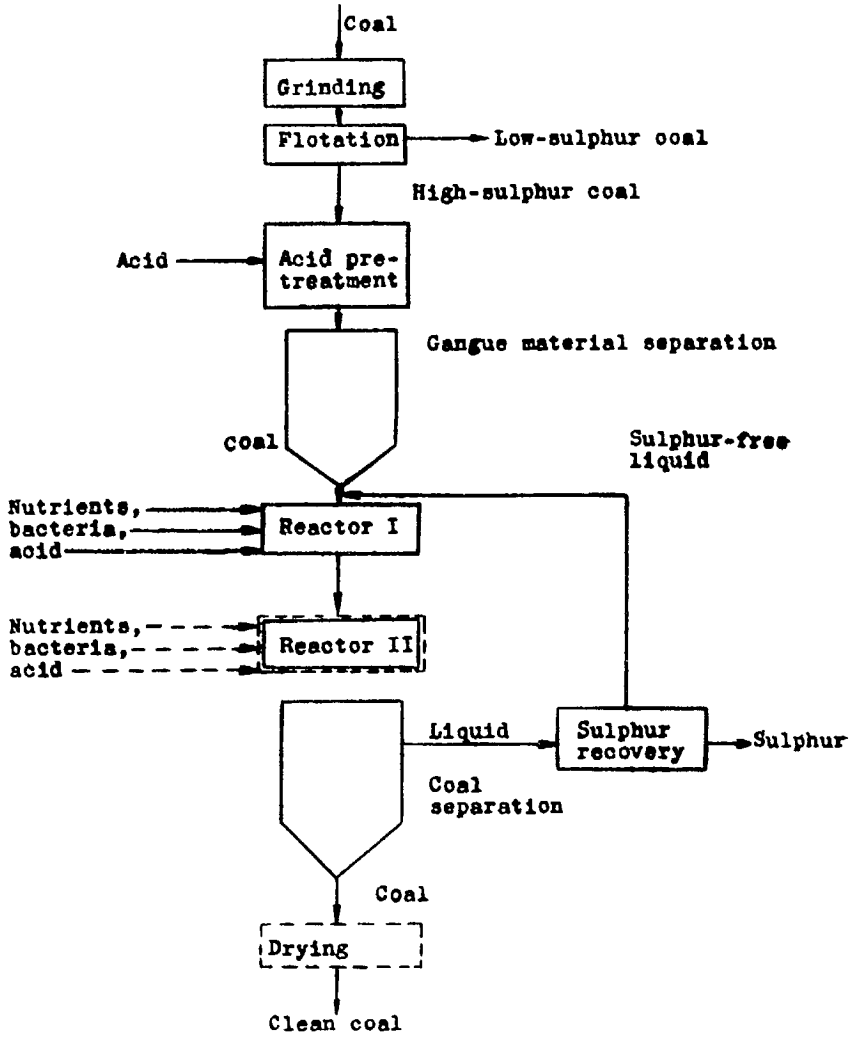

Fig. 12. A possible flowsheet for bacterial desulphurization of coals. Broken lines indicate optional steps [187]

ritic sulphur was removed from coal in 5 days by using *T. ferrooxidans*.

A schematic diagram of bacterial desulphurization of coals using *T. ferrooxidans* is shown in Fig 12. [187]. A two-stage treatment procedure is employed for coal containing not only pyrite but also organic sulphur compounds. At the first stage, FeS_2 is oxidized by autotrophic bacteria; at the second one, a mixed population of heterotrophic bacteria is used to oxidize organic sulphur compounds.

As seen from the above, such metals as copper, uranium, lead, bismuth, zinc, arsenic, nickel and cobalt can be extracted from composite concentrates by means of bacterio-chemical tank leaching.

Bacterial Biomass Production Methods

As pointed out above, the kinetics of bioleaching of metals from ores and concentrates depends on the bacterial biomass density in the pulp or in the ore body. A number of methods of biomass production have been proposed to date.

One such technique is based on cultivation of *T. ferrooxidans* on a Fe^{2+}-containing medium with continuous electrochemical reduction of Fe^{3+}. A schematic diagram of a system for continuous cultivation of *T. ferrooxidans* is shown in Fig. 13 [188]. The system comprises a reactor, being, in fact, an electrochemical cell of the pressure filter type where bacteria are grown, and also a number of equipment units designed to maintain optimum cultivation conditions (Fig. 14). All parts of the reactor casing are acrylic plastic while the cathode and anode are made of platinum and platinum-plated titanium respectively (items 3 and 4 in Fig. 14). The cathode and anode spaces are separated by a cation-exchange membrane of the MK-40 type.

Denisov et al. [188] report the daily biomass output of such a system to be 100 g dry weight per 1 m^2 of cathode area. From this, given an output level, the appropriate dimensions of the reactor can be found. The temperature is maintained by a water jacket and aeration is provided by a membrane type compressor (see Fig. 13). In the reactor, the rates of electrochemical reduction of Fe^{3+} to Fe^{2+} and of bacterial oxidation of Fe^{2+} to Fe^{3+} are balanced by regulating automatically the cathode voltage.

In this method, the major cost of biomass production is that of electric power. In laboratory conditions, 150 kWh of electric power was needed to produce 1 kg of dry cell biomass [189]. A theoretical estimate shows that the required electric power can be reduced by a factor of 2 to 3. This method of biomass production has not, however, been tested in industrial conditions.

In our experiments performed on a pilot tank leaching system it was shown that biomass of *T. ferrooxidans* can be obtained

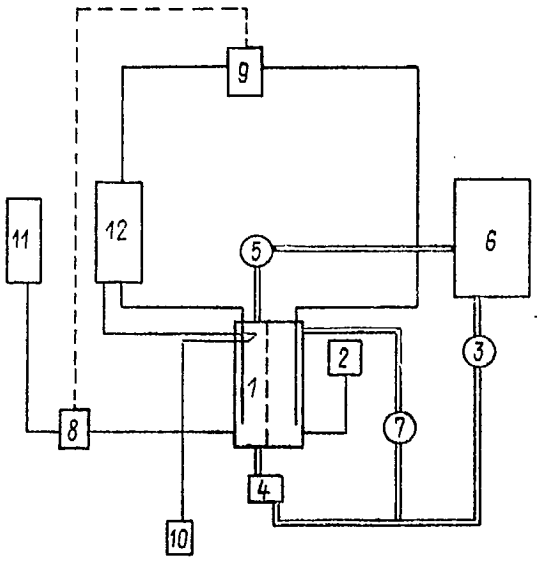

Fig. 13

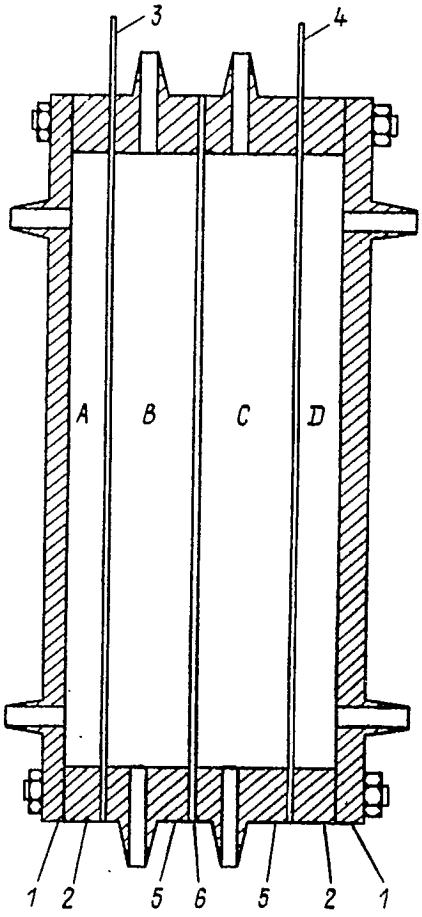

Fig. 14

Fig. 13. A schematic diagram of a system for continuous cultivation of *T. ferrooxidans* with electrochemical reduction of ferric iron [188]:

1 — a reactor; 2 — a storage tank for distilled water; 3 — compressor; 4 — a flow governor; 5 — a cooler; 6 — receiver; 7 — gas flowmeter; 8 — feed meter; 9 — a current integrator; 10 — a tank for output biomass; 11 — a storage tank for nutrient medium; 12 — an adjustable direct voltage source

Fig. 14. The schematics of the reactor from Fig. 13 [188]

A and D are a water jacket; B is the cathode space; C is the anode space; 1 — an outer wall of the water jacket; 2 — the water jacket frame; 3 — the cathode; 4 — the anode; 5 — frames of the cathode and anode spaces; 6 — an ion-exchange membrane

by separating bacterial cells from pregnant solutions. This technique seems to have a somewhat higher potential for industrial implementation than the one discussed above because it has the advantage of recycling into the process the biomass of active bacteria already adapted to leaching conditions. No cost estimate of this biomass production method has yet been carried out.

An innovating technique, termed Bacterial Film Oxidation (BACFOX Process), to increase bacterial content of solutions intended for dump and underground leaching has been recently developed [173, 174]. Basically, the method is as follows. A surface coated with *T. ferrooxidans* bacteria is to be submerged in

a solution containing Fe^{2+} through which fresh air is sparged. Oxidation of Fe^{2+} takes place while the inflow solution passes through the tank. A schematic diagram of a plant implementing this technique is presented in Fig. 15. Under such conditions,

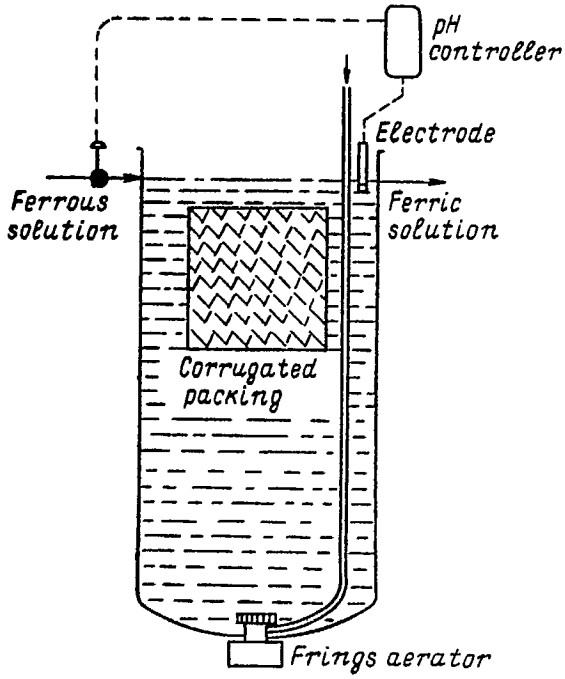

Fig. 15. Submerged corrugated pack unit — forced aeration [173]

bacteria have been shown to bind with precipitated jarosite with a capacity to form a bacterial-jarosite film on surfaces of different materials (e. g., glass, plastics). In experiments by Livesey-Goldblatt et al. [173], best results were obtained when a bacterial film coated a corrugated plastic surface. In this case, the specific rate of Fe^{2+} to Fe^{3+} oxidation was as high as 7.5 $g \cdot h^{-1}$ per 1 m^2 of bacterial film area.

Biosorption of Metals by Microorganisms

A major task in hydrometallurgy is the recovery of metals from solutions and treatment of wastewater from different industries. Today, only a few metals are being recovered from solutions, particularly those with relatively high content. However, it is in metal extraction from diluted solutions that the major problems are encountered. A new approach to dealing with these problems has recently evolved. There are many microorganisms with capacity either to sorb metal ions or to precipitate them. Table 6 lists several mechanisms of metal ion precipitation by means of bacterial activity. These include:

1. Biosorption;

2. Precipitation of metals in the form of sulphides;
3. Reduction of Cr^{6+} and of some other elements.

Precipitation of metals in the form of sulphides has been known for a long time. The method consists in using sulphate reducing bacteria to produce H_2S that has a capacity to precipitate practically the total metal contained in a solution. In one study, 98.5% of copper was recovered in this way, its initial content being 8.6 $g \cdot l^{-1}$ [209]. This technique has also been successfully tested on industrial scale, in mine conditions in the USSR [206].

The process of Cr^{6+} to Cr^{3+} reduction in solutions has also found industrial applications in the USSR [208]. The method is based on the ability of *Bacterium dechromaticans* to reduce Cr^{6+} to Cr^{3+} in anaerobic conditions; Cr^{3+} then precipitates. The process occurs at pH values between 8 and 9, and municipal sewage water is used as organic nutrient.

A novel approach to the problem is to employ biosorption of metals from solutions.

The results of experimental studies indicate that by using microorganisms up to 100% of lead, mercury, zinc, copper, nickel, cobalt, manganese, chromium, uranium, etc. can be reclaimed from diluted solutions (Table 6).

Table 6

Metal sorption and precipitation by microorganisms [190—212]

Microorganisms	Metal precipitation process
Fungi (biomass), yeasts, bacteria, algae	1. Biosorption of radioactive (U, Ra) and other elements: Al, Mo, Ag, Cu, Cd, Cr, Mn, Co, Ni, Zn, Hg, Pb, Au, Pt, Pd
Chitin and chitosan	Sorption of Ce, Zr, Hf, Ru from water circulating in a cooling system of a nuclear reactor
Sulphate reducing bacteria	2. Precipitation of metals from solutions: $C_{org.} + SO_4^{2-} \xrightarrow{\text{bacteria}} S^{2-} + CO_2$ $S^{2-} + Me \rightarrow \downarrow MeS$
Chromium reducing bacteria	3. Metal reduction: $Cr^{6+} \rightarrow \downarrow Cr^{3+}$

Using fungi makes it possible to recover 96 to 98% of gold and silver, up to 84% of platinum and 92% of palladium from dilute residue solutions used in the process of gold and silver refining [205].

A mixed bacterial culture under anaerobic conditions enabled

the extraction of as much as 81% of uranium and 93% of selenium from effluent solutions [211].

Algae have also proved to be very helpful in biosorption of metals from solutions [211].

Bacterial polysaccharides can also be effectively used in removing from solutions radioactive elements, copper and cadmium [212].

Sorption of metals from solutions leads to their accumulation in the biomass. It can be seen from Table 7 that the content of various metals in the biomass can become quite considerable.

Table 7

Metal accumulation in microorganisms

Solutions	Microorganisms	Metal content of cells	Reference
Solutions containing radio-active elements	Denitrifying	Uranium: 140 mg per 1 g of biomass dry weight	214
	Rhizopus arrhizus	Uranium and thorium: more than 180 mg per 1 g of biomass dry weight	215, 216
	Saccharomyces cerevisiae, Pseudomonas aeruginosa	Uranium: within 10 to 15% of cell dry weight	194, 213
	M-biosorbent (*Penic. chrysogenum*)	Uranium: 80 to 120 g per 1 kg of biosorbent dry weight at uranium density of 1 $g \cdot l^{-1}$. Upon treatment uranium density below 0.05 $mg \cdot l^{-1}$	217
Silver-containing solutions	Consortia of bacteria: *P. maltophila, Staphylococcus aureus* and unidentified cultures	Silver: up to 30% of the biomass dry weight	218

The mechanism of sorption of metals from solutions by microorganisms is for the most part well understood. Basically, it is due to the involvement of the cell wall. In fungi metal sorption, a very important role is known to be played by chitin and chitosan. For this reason, they should be used as such for metal extraction. One limitation to this is that an ever increasing catch of crustacea threatens to disrupt an ecological balance of the world ocean. A different approach to obtaining chitin and chitosan is their production from fungi.

There are a number of ways of biosorbents application.

Firstly, they can be utilized in biological reactors (Fig. 16), which are essentially biological filters using, e. g., coal as biosorbent carrier [214].

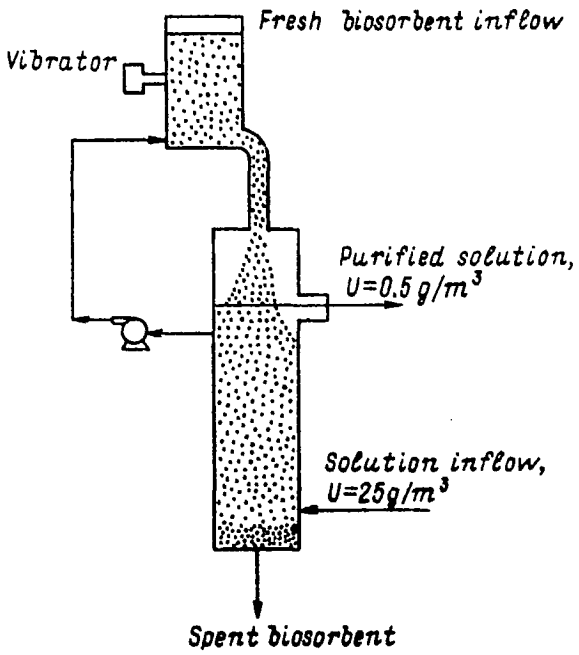

Fig. 16. A counter-flow contactor for continuous extraction of uranium using denitrifying bacteria carried by coal particles (biosorbent is in contact with the solution for about 8 min.) [214]

Another example relates to the use of the M-biosorbent produced in Czechoslovakia [217]. Its composition includes mycelium itself, *Penic. chrysogenum*, and also various reaction products of carbamide and formaldehyde polycondensate, used to fix the biosorbent to its carrier, and of mycelium constituents. The M-biosorbent is produced in the form of ground material with grain size between 0.3 and 0.8 mm. It can be loaded in plants that are designed for and normally run on ion-exchange resins.

Finally, in natural conditions, biosorption of metals by bacteria, algae, etc. occurs in ponds. As reported by Brierley and Brierley [211], pond sediments contained: uranium, from 430 to 1900 ppm; selenium, from 6 to 80 ppm; molybdenum, from 7 to 320 ppm. Thus, it becomes clear that not only plankton but also the sediments should be removed from ponds either to reclaim the metals or simply to bury them in the case the water treatment is our sole objective.

One of the essential merits of metal biosorbents is that they can be widely used in natural environments.

A well operating production of the biomass or, for example, of bacteria-synthesized polysaccharides can become a new source of selective ion-exchange materials. For instance, mixed algae cultures had a cation-exchange capacity greater than 640 μ equivalents per 1 g of dry weight [219].

The capacity of fungi cells exceeded by 2.5 times the capacity of common anionic exchange resins (IRA-400) used by uranium

production companies for selective separation of uranium from other ions in solution [215, 216].

The capacity of the M-biosorbent was as high as approximately 5 g uranium per 1 kg of its dry weight (the maximum capacity being in the range 80 to 120 g per 1 kg dry weight at uranium concentration in solution of 1 $g \cdot l^{-1}$).

A few other advantages of biosorbents can be pointed out. These include their availability, ease of their production, etc.

2. Economic Aspects

Commercial viability of dump, underground and tank leaching is closely related to the expenditure pattern. There are reasons to believe that this pattern will remain basically unchanged from place to place although the expenses themselves may to some extent vary.

Below we consider several examples of the metal leaching economics.

Dump and Underground Leaching

As a first example, we consider an estimate of the cost of dump leaching in Miami, USA, under the following conditions [220]:

— acid soluble copper	—0.5—1.0%
— stripping ratio	—1:1
— ultimate copper recovery	—60%
— copper to be recovered by cementation using scrap iron at $ per ton	—50—52.

The capital cost of dump leaching can be relatively low. The major expenses are usually those covering ore mining, consumption of sulphuric acid, and the cost of copper cementation process. The leaching business will be commercially more attractive if the costs of mining of low-grade ores and of their dumping are already included in the overall cost of mining operations. The expenses involved in production of roughly 30 tons of copper per day at a Miami mine are as follows (in $):

Supervision and salaried	140.00
Labor — 10 shifts per day at $40.00	400.00
Power — 4000 kW4 at $0.015	60.00
Iron for cementation — 30×1.5 at $52 per ton	2,340.00
Water	100.00
Miscellaneous supplies	50.00
Total	3,090.00

The production cost of 1 kg of copper was 10.3 cents. Adding here freight to the smelter and smelter charges (15.0 cents·kg^{-1}) gives the net production cost of 1 kg copper by dump leaching at 25.2 cents, i. e. $252 per ton.

The expenses involved in producing 1 ton of copper by dump leaching with mining and smelter charges included are as follows (in $):

Mining	110—260
Acid	90—320
Smelting	140
Scrap iron for cementation	76
Labor, power, water and miscellaneous supplies	26
Total	450—850

The expenses involved in copper production in India are listed in Table 8 [221].

Annual production of 2,000 tons of copper is projected, with 96% recovery.

Table 8

Biohydrometallurgical copper powders: indicative cost profile

Operation / Major items	Cost	
	Rs/ton Cu	$/ton Cu
Mining cost (overall)	100.00*	10
Heap leaching and cementation:		
Supervision and labour	200.00	
Power	150.00	
Water	40.00	
Maintenance, etc.	110.00	
Miscellaneous supplies	100.00	
Sulphuric acid**	—	
Scrap iron	2500.00	
Estimated cement copper costs	3200.00	300
Hydrorefining costs (overall)	4000.00	
	7200.00	
Capitalized costs: Depreciation, Interest, Insurance	5000.00	
Total powder production costs	12 200.00	1200

* Actuals will depend on geochemistry, topography, etc.

** External input not normally required for pyrites deposits.

As can be seen from Table 8, for this type of ores no sulphuric acid is needed, which is usually the case with pyrite-containing sulphidic ores, while in the former example it constituted one of the major cost items. According to the authors, such a net cost of dump copper production is commercially acceptable. This cost, however, tends to vary, specifically, it increases as the metal content of the ore falls down. For example, the net cost of 1 ton of copper produced in the initial years of operation of the dump leaching facility at the Nikolaev deposit amounted to 232.9 roubles, in 1972, and ten years later it exceeded 400 roubles [164].

The net cost of underground leaching of copper was $75—85 per ton in Bore [158] and approximately $80 per ton at the Bingham mine [222].

It was shown [3, 90, 161] that bacterial dump and underground leaching of uranium from ores can also be commercially profitable.

Tank Leaching

The economic aspects of tank leaching of metals are under consideration in the following series of papers [17, 85, 124, 135, 158, 165, 179, 221, 223].

The chalcopyrite concentrate. Capital and operating costs estimates for a flow-through plant to treat 200 tons/day of chalcopyrite concentrate, assuming 350 operating days a year and 96% copper recovery [135], are summarized in Table 9.

Table 9

Summary of capital cost estimates

Facility	Cost ($)	$/Annual net ton Cu
Leach plant	9.4×10^6	560
Electrowinning plant	4.2×10^6	250
Total	13.6×10^6	810

Direct operating costs involved in copper leaching are summarized in Table 10 [135].

Let us assume that the total investment (fixed+working capital) for a 200 tpd plant is to be repaid by annuity at 10% interest over a 15 year period. On this basis, financing cost per pound of copper produced is 5.95 cents. Adding the estimated direct operating cost (7.31 cents, see Table 10) produces a total treatment cost 13.26 cents per pound of copper ($292 per 1 ton).

According to McElroy and Bruynesteyn, in 1973 smelter charges per pound of copper in concentrate were in the range of 8.5 to 14.5 cents ($187 to $319 a ton), i. e. in rough correspondence

Table 10

Summary of estimated direct operating costs

Operation	Costs		% of Total	Cumulative % of Total
	$/net t Cu	cents of pound Cu		
Labour	41.83	2.09	28.6	28.6
Power (10 mills/kWh)	41.20	2.06	28.2	56.8
Maintenance (5% of fixed capital)	40.47	2.02	27.7	84.5
Limestone ($5.00/t)	15.00	0.75	10.2	94.7
Flocculants	4.17	0.21	2.8	97.5
Nutrients	2.47	0.12	1.7	99.3
Grinding balls (cast steel)	1.20	0.06	0.7	100
Total	146.34	7.31	100	

with costs of bacterial leaching under different obtaining conditions. McElroy and Bruynesteyn [135] stress the following virtues of bacterial leaching for processing of chalcopyrite concentrates:

— negligible atmospheric emissions;

— production of refined copper (i. e. potentially lower transport cost and increased market flexibility);

— feasibility for small scale operations (minimum ~40 tpd of concentrate);

— potentially increased returns for silver, gold and (possibly) molybdenite in concentrates;

— production of dilute sulphuric acid suitable for leaching of oxide ore and/or acid consuming waste dumps.

Estimates made earlier by Bruynesteyn and Duncan [124] for a plant to process 100 tons of chalcopyrite concentrate per day and produce annually 21,900,000 pounds of copper (i. e. 9932.6 tons) showed that the cost of solubilization of 1 pound of copper from concentrates could be as low as 2.89 cents. Taking into account electrowinning expenses (2.75 cents) establishes the total cost of copper production at 5.72 cents a pound ($126 a ton). By expanding the business to process 1000 tons of concentrate per day, the total cost of copper production can be brought down to 3.9 cents a pound ($86 a ton).

The lead concentrates. Another example is economic evaluation of bacterial processing of low-grade lead sulphide concentrates containing 42.9% lead, 29.6% sulphur, 16.7% iron, 7.7% zinc, 2.4% copper and 0.02% cadmium [17].

It is projected that 100 tons of the concentrate would be leached for five days at temperature 35° C, pH 2.3, and with pulp density 20%. Minimum extraction of zinc, copper and cadmium is projected to be 95%. Lead in the form of $PbSO_4$ is to remain in the solid phase. The capital cost of this process is estimated to be $7,300,000. This includes the costs of leach tanks, pumps, aera-

tion, regrinding equipment, thickeners, filters, dryer, electrolysis equipment, their installations, and the building.

The yearly metal production is shown in Table 11.

Table 11

Yearly metal production

Metal	Concentrate treated, kg	Metal content, %	Recovery, %	d/Y	Yearly metal production, kg
Zinc	100,000	7.7	95	365	2,700,000
Copper	100,000	2.4	95	365	830,000
Cadmium	100,000	0.02	95	365	7,000

The total market value of zinc, copper, and cadmium produced is estimated at $3,460,000. The yearly expenses are as follows (in $):

Direct Costs:	
Administration	
Labour	
Reactives (oxygen, limestone, hydrated lime, magnesia, H_2SO_4, K_2HPO_4, $(NH_4)_2SO_4$, and zinc powder)	
Electricity	800,000
Indirect Costs:	
Maintenance	
Electricity	
Water	
Depreciation at 10%	
Interest at 10%	
Insurance	1,600,000
Total	2,400,000

The gain ($/kg) of metal produced is given in Table 12.

Table 12

The gain of metal produced

	Costs of metal production, $/kg		
	Zn	Cu	Cd
Costs of mining concentration (estimated)	0.11	0.14	0.39
Costs of leaching, regrinding, cementation, electrowinning	0.53	1.13	5.71
Total expenses (A)	0.64	1.27	6.10
Selling price (B)	0.76	1.62	8.36
Gain (B-A)	0.12	0.35	2.26

These estimates do not take into account any expenses for lead which make up the major part of the costs.

The projected yearly profit of leaching copper, zinc and cadmium amounts to $630,000 (see Table 11). In other words, an investment of 7.3×10^6 produces the yearly return of revenue of about 6.3×10^5. This revenue could be further increased by selling the lead concentrate produced by the leaching process.

Gold-containing arsenopyrite concentrates. An economic analysis of leaching treatment of concentrates of this type has been reported by Livesey-Goldblatt et al. [223]. The analysis has been based on an experiment with a continuous culture grown in 20-liter vessels at temperature 30° C, pH of the medium in the range 1.7 to 1.9, and with the solid to liquid phase ratio of 1 : 8. The arsenic content of the concentrate amounted to 6.31% while the contents of gold and silver were 161.0 and 5.8 $g \cdot t^{-1}$ respectively. The economic estimates summarized

Table 13

Capital and operating costs in gold recovery

Gold extraction parameters	Treatment of 650 tons of concentrate	
	Bacterial leaching	Roasting
	(%)	(%)
Available gold	100.0	100.0
Loss of gold	8.2	8.2
Loss of gold in roasting	—	1.0
Loss of gold in cyanidation process	2.0	4.8
Gold recovery	89.8	86.0
Running expenses	(rand/t)	(rand/t)
Consumption of reagents	6.1	1.0
Power and water	4.3	1.6
Total	10.4	2.6
Capital costs	(thousand rands)	
Flotation	1410	1410
Roasting	—	2400
Bacterial leaching	1455	—
Cyanidation and smelting	2836	2786
Maintenance, etc.	2006	2229
Total	7707	8825

Note: In 1981, $=0.9667 R. S. A. rands.

in Table 13 relate to a plant treating 650 tons of concentrate per month. As can be seen from the Table, bacterial processing leads to a net cost of production that is lower than the one of conventional roasting technique and also to a higher percenage of gold recovery.

Leaching of other raw materials. According to estimates by Derry et al. [182], the cost of 1 kg of uranium (U_3O_8) produced by tank bacterial leaching would be about $2.75. For the conventional production method it is known to be as high as $4.85.

The microbiological process of coal desulphurization is also to be preferred on economic grounds to the chemical treatment. In desulphurization of coals containing 2% sulphur, the net cost of the biochemical process for a plant with a daily capacity of 8,000 tons is $14 a ton, while that of the chemical leaching is as high as $20 a ton. Expenditures for grinding excluded, the cost of coal desulphurization was $10.5 a ton in the presence of *T. ferrooxidans* and reduced to less than $9 when thermophilic bacteria were used [224].

A comparison examination of data on the dump, underground, and tank leaching economics reveals a common pattern of expenditures required for production of different metals. In fact, the following parameters can be identified as important from the economic viewpoint: the metal content of the ore or concentrate, percentage of metal recovery, the production scale, the leaching period, the cost of mining, the cost of copper cementation, the amount of capital to be invested in installing and operating the tank leaching facility.

As shown above, the rate of a metal leaching process is closely related to the biomass density.

In dump and underground leaching of metals, as pointed out above, wild strains of microorganisms are «at work». Their content in solutions and in ores is often too low to increase markedly the rates of oxidation processes, particularly so at low temperatures.

The economic analysis of biomass production has not yet been performed. Moreover, it is not clear which of the previously discussed processes of biomass production will prove to be the most profitable. Neither has been solved the problem of combined recovery of different metals from solutions in dump and underground leaching. Extraction of accompanying metals will undoubtly affect favourably the commercial viability of the entire production process.

3. Ecological Aspects

In discussing ecological impact of hydrometallurgy, the attention is to be primarily focussed on technical characteristics of

this method of metal production. Since all the flowsheets provide for closed-circuit operation, no major biospheric emissions of solutions should occur. Atmospheric emissions of noxious gases are also excluded.

In underground leaching, no large areas of land are to be used as sites for mining plants. This preserves natural landscape.

Nevertheless, each type of biological leaching process does give rise to a certain amount of particular environmentally contaminating waste materials. In hydrometallurgical industries, these are primarily solutions containing heavy metals. Typically, effluent solutions, no longer in the process, are neutralized and discharged into special ponds for settling and natural purification, upon which they are drained into rivers. Although this treatment procedure would normally provide for the removal from effluents of a major fraction of metals contained, the ultimate objective is still the removal of their entire metal content.

As pointed out above, microbiological sorption and precipitation of metals can apparently be very instrumental in solving this problem. Of equal importance is the problem of decontamination of solid waste materials from hydrometallurgical industries. According to Bruynesteyn and Hackl [225], the greatest potential danger to the environment is presented by dumps of leached ones and by rock spoil heaps.

Finally, there are a few problems in utilization and/or burying of certain waste materials, e. g. arsenic compounds (iron and calcium arsenates), produced in the tank leaching process. Not all of these problems have as yet been satisfactorily solved. Fortunately, the microorganisms important for biogeotechnology of metal production are not pathogenic and as such are no danger to the environment.

CONCLUSION

Application of microbiological and other hydrometallurgical methods for the extraction of metal values from low-grade ores introduces considerable changes into the existing practice of processing raw materials.

First and foremost, vast reserves of refractory and lost ores as well as wastes of enriching factories and composite sulphide concentrates will become eligible for processing. The bacterial leaching technology may present a solution for the utilization of refractory deposits of rich ores and large deposits in remote regions. This new method of metal extraction seems economically feasible. It ensures a higher standard of production technology, and provides for an integrated and more comprehensive utilization of mineral raw materials as compared to the classical methods of metal extraction. It also eliminates to a large extent the necessity for a large number of people working underground and the discharge of noxious gases into the atmosphere.

REFERENCES

1. Kalabin, A. I., 1969. Mineral Mining by Underground Leaching Technique. Moscow. Atomizdat (in Russian). (Kalabin, A. I., 1981. Mineral Mining by Underground Leaching and Other Geochemical Methods. Moscow. Atomizdat) (in Russian).
2. Corrans, I. J., Harris, B., and Ralph, B. J., 1972. Bacterial Leaching: an Introduction to Its Application and Theory and a Study on Its Mechanism of Operation. J. South African Institute of Miner. and Metallurg., 72, 8, 221.
3. Karavaiko, G. I., Kuznetsov, S. I., and Golomzik, A. I., 1972. Role of Microorganisms in the Leaching of Metals from Ores. Moscow, Nauka (in Russian). (Bacterial Leaching of Metals from Ores. Technicopy Ltd., 1977).
4. Ralph, B. J., 1979. In: Biogeochem. Cycling of Mineral-forming Elements. Eds. P. A. Trudinger, D. J. Swaine. Elsevier Sci. Publ. Company. Amsterdam—Oxford—New York.
5. Pol'kin, S. I., Adamov, E. V., and Panin, V. V., 1982. Technology for the Bacterial Leaching of Non-ferrous and Rare Metals. Moscow, Nedra (in Russian).
6. Torma, A. E., and Bosecker, K., 1982. Bacterial Leaching. *Progr. Ind. Microbiol.*, 16, 77.
7. Brierley, C. L., 1978. Bacterial Leaching. In: *Critical Rev. in Microbiol.*, 6, 3, 207.
8. Colmer, A. R., and Hinkle, M. E., 1947. *Science*, 106, 253.
9. Colmer, A. R., Temple, K. L., and Hinkle, M. E., 1949. *J. Bacteriol.*, 59, 317.
10. Lyalikova, N. N., 1966. Trudy Moskovskogo Obschestva Ispytatelei Prirody, 24, 211.
11. Torma, A. E., 1971. *Rev. Can. Biol.*, 30, 209.
12. Torma, A. E., and Habashi, F., 1972. *Can. J. Microbiol.*, 18, 1780.
13. Imai, K., Sakaguchi, H., Sugio, T., and Tano, T., 1973. *J. Ferment. Technol.*, 51, 12, 865.
14. Silver, M., and Torma, A. E., 1974. *Can. J. Microbiol.*, 20, 141.
15. Torma, A. E., and Gabra, G. G., 1977. *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 43, 1, 1.
16. Lewis, A. J., and Miller, J. D. A., 1977. *Can. J. Microbiol.*, 23, 2, 319.
17. Torma, A. E., 1978. In: Metallurg. Appl. Bacter. Leaching and Related Microbiol. Phenomena. Eds. L. E. Murr, A. E. Torma, J. A. Brierley. Acad. Press. New York, 375.
18. Soljanto, P, and Tuovinen, O. H., 1980. In: Biogeochemistry of Ancient and Modern Environments. Eds. P. A. Trudinger, M. R. Walter, B. J. Ralph. Austr. Acad. of Sci. and Springer Verlag, 469.
19. Lundgren, D. G., 1980. *Ann. Rev. Microbiol.*, 34, 263.
20. Markosyan, G. E., 1972. *Biologicheskii zhurnal Armenii*, 35, 2, 26.
21. Markosyan, G. E., 1973. *DAN SSSR*, 2P, 5, 1205.
22. Guay, R., and Silver, M., 1975. *Can. J. Microbiol.*, 21, 3, 281.
23. Markosyan, G. E., 1976. In: Ecology and Geochemical Activity of Microorganisms. Puschino, 160 (in Russian).
24. Norris, P. R., and Kelly, D. P., 1978. *FEMS Microbiol. Letters*, 4, 1, 143.
25. Waksman, S. A., and Joffe, I. S., 1922. *J. Bacteriol.*, 7, 2, 239.
26. Sokolova, G. A., and Karavaiko, G. I., 1964. Physiology and Geochemical Activity of Thionic Bacteria. Moscow, Nauka (in Russian).

27. Khalid, A. M., and Ralph, B. J., 1977. In: Round Table Conf. Bacter. Leaching. Braunschweig-Stöckheim. Ed. W. Schwartz, 165.
28. Lyalikova, N. N., 1967. *DAN SSSR, Mikrobiol.,* 176, No. 6, 1432.
29. Abdrashitova, S. A., Mynbaeva, B. N., and Ilyaletdinov, A. N., 1981. *Mikrobiologiya,* 50, 1, 41.
30. Brierley, J. A., and Le Roux, N. W., 1977. In: Round Table Conf. Bacterial Leaching. Braunschweig-Stöckheim, 51.
31. Le Roux, N. W., and Marshall, V., 1977. In: Round Table Conf. Bacter. Leaching. Braunschweig-Stöckheim, 21.
32. Brierley, J. A., 1978. *Appl. and Environm. Microbiol.,* 36, 3, 523.
33. Golovacheva, R. S., and Karavaiko, G. I., 1978. *Mikrobiologiya,* 47, 5, 815.
34. Brock, T. D., Brock, M. L., Belly, R. T., and Weiss, R. L., 1972. *Arch. Mikrobiol.,* 84, 54.
35. Brierley, C. L., and Murr, L. E., 1973. *Science,* 179, 4072, 488.
36. De Rosa, M., Cambacorta, A., and Bu'Lock, J. D., 1975. *J. Gen. Microbiol.,* 86, 1, 156.
37. Brierley, C. L., and Brierley, J. A., 1973. *Can. J. Microbiol.,* 19, 183.
38. Tuovinen, O. H., Niemela, S. I., and Gyllenberg, H. G., 1971. *Antonie van Leeuwenhoek,* 37, 4, 489.
39. Unz, R. F., and Lundgren, D. G., 1961. *Soil Sci.,* 92, 302.
40. McGoran, G. I. M., Duncan, D. W., and Walden, C. C., 1969. *Can. J. Microbiol.,* 15, 1, 135.
41. Tuovinen, O. H., 1972. Atomic Energy Review, 10, 2, 251.
42. Kovrov, B. G., Denisov, G. V., Sekacheva, L. G., and Belyi, A. V., 1978. In: Chemosynthesis in Continuous Culture. Ed. I. A. Terskov, Moscow, Nauka (S), 34 (in Russian).
43. Kovrov, B. G., Denisov, G. V., and Sekacheva, L. G., 1978. *Mikrobiologiya,* 47, 3, 400.
44. Yakhontova, L. K., and Grudev, A. P., 1978. Zone of Hypergenesis in Ore Deposits. MGU (in Russian).
45. Yakhontova, L. K., and Nesterovich, L. G., 1978. *Geokhimiya,* 8, 1267.
46. Arkesteyn, G. M., 1980. Contribution of Microorganisms to the Oxidation of Pyrite. Wageningen.
47. Kuznetsov, S. I., Ivanov, M. V., and Lyalikova, N. N., 1962. Introduction to the Geological Microbiology. Izd. AN SSSR, Moscow (in Russian).
48. Yakhontova, L. K., Grudev, A. P., and Nesterovich, L. G., 1978. *Vestnik MGU, Geologiya,* 3, 66.
49. Duncan, D. W., and Trussell, P. C., 1964. *Can. Metallurg. Quarterly,* 3, 1, 43.
50. Torma, A. E., Walden, C. C., Duncan, D. W., and Branion, R. M. R., 1972. *Biotechnol. Bioeng.,* 14, 777.
51. Torma, A. E., 1976. In: Proc. of the Third Intern. Biodegr. Symp. Eds. J. M. Sharply, A. M. Kaplan. Appl. Sci. Publ. Ltd. London, 937.
52. Beckstead, L. W., Munoz, P. B, Sepulveda, L, Herbst, J. A., Miller, J. D., Olsen, F. A., and Wadsworth, M. E., 1976. In: Extractive Metallurgy of Copper. V. 2. Eds. J. C. Vanpoulos, G. C. Agarwal. Amer. Inst. Mining Metallurg. and Petroleum Engin., New York, 611.
53. Beck, J. V., 1977. In: Round Table Conf. Bacterial Leaching. Braunschweig-Stöckheim, 119.
54. Razzel, W. E., and Trussell, P. C., 1963. *Appl. Microbiol.,* 11, 2, 105.
55. Groudev, S. N., 1980. Doklady Bolgarskoi AN. Comptes rendus de l'Académie bulgare des Sci., 33, 6, 829.
56. Berry, V. K., Murr, L. E., and Hiskey, J. B., 1978. *Hydrometallurgy,* 3, 309.
57. Chakrabarty, N., and Murr, L. E., 1979. *Biotechnol. Bioeng.,* 21, 1685.
58. Ivanov, V. I., Nagiriyan, F. I., and Stepanov, B. A., 1961. *Mikrobiologiya,* 30, 4, 688.
59. Trussell, P. C., Duncan, D. W., and Walden, C. C., 1964. *Can. Mining J.,* 85, 3, 46.
60. Gol'braikht, A. J., and Ilyaletdinov, A. N., 1970. Izv. Akad. Nauk Kaz. SSR, 1, 40.

61. Kulebakin, V. G., 1978. Izvestiya Sibirskogo Otdeleniya AN SSSR, Ser. biol., Novosibirsk, No. 51, 26.
62. Duncan, D. W., Walden, C. C., and Trussell, P. C., 1967. Transactions of SME, June 238, 1.
63. Moshnyakova, S. A., Karavaiko, G. I., and Schetinina, E. V., 1971. *Mikrobiologiya,* 40, 6, 1100.
64. Torma, A. E., 1972. Biohydrometallurgy of Cobalt and Nickel. A-72-7. Am. Inst. Min. and Petr. Eng., New York, 1—22.
65. Bosecker, K., 1977. In: Round Table Conf. Bacterial Leaching. Braunschweig-Stöckheim, 139.
66. Golovko, E. A., Rozental', A. K., Sedel'nikov, V. A., and Sukhodrev, V. M., 1978. Chemical and Bacterial Leaching of Copper-Nickel Ores. Moscow, Nauka (in Russian).
67. Le Roux, N. W., and Wakerly, D. S., 1979. In: Fourth Intern. Symp. on Environm. Biogeochem. and Conf. on Biogeochem. in Relation to Mining Industry and Environm. Pollution (Leaching Conf.) (Program and Abstracts). Austr. Acad. Sci., 99.
68. Lyalikova, N. N., 1974. *Mikrobiologiya,* 43, 6, 941.
69. Tomizuka, N., 1976. Microbiological Oxidation of a Lead Sulphide. Report of the Fermentation Research Institute, 48, 51.
70. Duncan, D. W., 1967. *Austral. Mining,* 15, Nov.
71. Bhappy, R. B., Reynolds, D. H., and Roman, R. J., 1965. *J. Metals,* N.Y., 17, 1199.
72. Trussell, P. C., 1965. The Microbiological Leaching of Metal Sulfide.
73. Brierley, C. L., 1972. Target Explor. Report E-2, Apr.
74. Ehrlich, H. L., 1964. *Econ. Geol.,* 59, 7, 1306.
75. Pol'kin, S. I., Karavaiko, G. I., Tauzhnianskaya, Z. A., and Panin, V. V., 1969. *Bulleten' Tsvetnaya metallurgiya,* No. 6, 35.
76. Pol'kin, S. I., Karavaiko, G. I., Tauzhnianskaya, Z. A., and Panin, V. V., 1970. In: Proceedings of the International Congress on Enrichment of Ores. Prague.
77. Pinches, A., 1971. *Mining and Miner. Eng.,* 7, 9.
78. Pol'kin, S. I., Panin, V. V., Adamov, E. V., Karavaiko, G. I., and Cherniak, A. S., 1975. Theory and Practice of Utilizing Microorganisms in Processing Difficult-to-Dress Ores and Concentrates. XI Intern. Miner. Proc. Congr., Cagliari, Paper 33.
79. Lyalikova, N. N., 1968. In: Application of Bacterial Leaching of Non-ferrous Metals from Submarginal Ores. Institut Tsvetmetinformatsii (in Russian).
80. Mizoguchi, T., Izaki, K., Takahashi, H., and Okabe, T., 1970. *Koguo Kogaku Zasshi,* 73, 8, 1811.
81. Lyalikova, N. N., and Kulikova, M. F., 1965. *DAN SSSR,* 164, No. 3, 674.
82. Pol'kin, S. I., Adamov, E. V., Panin, V. V., and Kazintseva, S. I., 1979. *Izvestiya Vysshikh Uchebnykh Zavedenii. Tsvetnaya Metallurgiya,* 3, 5.
83. Pol'kin, S. I., Adamov, E. V., Panin, V. V., Karavaiko, G. I., Andreev, P. I., and Kazintseva, S. I., 1979. Rudy i metale nieżelazne. Numer specjalny. XIII Międzynarodowy kongres, Przeróbki kopalin, Warszawa, 15.
84. Brissette, C., Champaque, J., and Jufras, L. R., 1971. *Can. Mining and Metallurg. Bull.,* 64, 714, 85.
85. Torma, A. E., 1978. *Can. J. Microbiol.,* 24, 7, 888.
86. Torma, A. E., 1972. Patent (Canada) 1 382 357.
87. Karavaiko, G. I., and Moshnyakova, S. A., 1971. *Izvestiya AN SSSR, Ser. biol.,* 3, 314.
88. Lipman, C. B., and Waksman, S. A., 1923. *Science,* 57, 58.
89. Zajic, J. E., 1969. Microbial Biogeochemistry. Acad. Press. New York, 345.
90. Kuznetsova, E. G., 1970. *Uspekhi mikrobiologii,* 6, 153.
91. Miller, R. P., Napier, E., Wells, R. A. I., Audsley, A., and Daborn, G. R., 1963. *Bull. Inst. Mining and Metallurgy,* 674, 217.
92. Tomizuka, N., Jagisawa, M., Someya, J., and Takahara, Y., 1976. *Agr. Biol. Chem.,* 40, 5, 1019.

93. Silverman, M. P., and Ehrlich H. L., 1964. *Advances Appl. Microbiol.*, 6, 153.
94. Berry, V. K., and Murr, L. E., 1975. *Metall. Trans.*, B 6, 488.
95. Murr, L. E., and Berry, V. K., 1976. *Hydrometallurgy*, 2, 11.
96. Berry, V. K., and Murr, L. E., 1977. In: 35th Ann. Proc. Electron Microscopy Soc. Amer., Boston, Ed. G. W. Bailey, 156.
97. Yakhontova, L. K., Sergeev, V. M., Karavaiko, G. I., and Sukhantseva, V. S., 1976. In: Ecology and Geochemical Activity of Microorganisms. Puschino, 99 (in Russian).
98. Yakhontova, L. K., and Karavaiko, G. I., 1977. *Vestnik MGU, ser. Geologiya*, 1, 49.
99. Yakhontova, L. K., Nesterovich, L. G., and Grudev, A. P., 1980. *Vestnik MGU, ser. Geologiya*, 1, 53.
100. Pol'kin, S. I., Panin, V. V., Karavaiko, G. I., and Adamov, E. V., 1976. In: Ecology and Geochemical Activity of Microorganisms. Puschino, 115 (in Russian).
101. Karavaiko, G. I., Pol'kin, S. I., Panin, V. V., and Adamov, E. V., 1976. In: Hydrometallurgy. Leaching in Autoclaves, Sorption, Extraction. Moscow, Nauka, 37 (in Russian).
102. Sakaguchi, H., Silver, M., and Torma, A. E., 1976. *Biotechnol. Bioeng.*, 18, 1091.
103. Sakaguchi, H., Torma, A. E., and Silver, M., 1976. *Appl. Environm. Microbiol.*, 31, 7.
104. Torma, A. E., Walden, C. C., and Branion, R. M. R., 1970. *Biotechnol. Bioeng.*, 12, 501.
105. Kelly, D. P., and Jones, C. A., 1978. In: Metallurg. Appl. Bacter. Leaching and Related Microbiol. Phenomena. Eds. L. E. Murr, A. E. Torma, J. A. Brierley. Acad. Press, New York, 19.
106. Landesman, J., Duncan, D. W., and Walden, C. C., 1966. *Can. J. Microbiol.*, 12, 1, 25.
107. Mubarakova, K. Yu., 1976. In: Ecology and Geochemical Activity of Microorganisms. Puschino, 131 (in Russian).
108. Pol'kin, S. I., Adamov, E. V., Kazintseva, S. I., and Grishin, S. I., 1977. *Izvestiya Vysshikh Uchebnykh Zavedenii. Tsvetnaya Metallurgiya*, 1, 3.
109. Duncan, D. W., and Drummond, A. D., 1973. *Can. J. Earth Sci.*, 10, 467.
110. Le Roux, N. W., North, A. A., and Wilson, J. C., 1973. Bacterial Oxidation of Pyrite. 10th Intern. Mineral Process. Congr., Paper 45. London.
111. Kovalenko, T. V., and Karavaiko, G. I., 1981. *Mikrobiologiya*, 50, 2, 326.
112. Moshnyakova, S. A., and Karavaiko, G. I., 1979. *Mikrobiologiya*, 48, 1, 49.
113. Petrova, T. A., Galaktionova, N. A., Karavaiko, G. I., Krylov, Yu. M., and Moshnyakova, S. A., 1979. *Mikrobiologiya*, 48, 2, 235.
114. Mikhailova T. L., and Pestovskikh, N. V., 1980. *Prikladnaya biokhimiya i mikrobiologiya*, 16, 4, 621.
115. Groudeva, V. G., Markov, K. G., and Gentchev, G. J., 1978. Doklady Bolgar. AN. Comptes rendus de l'Académie bulgare des Sci., 31, 6, 735.
116. Tuovinen, O. H., and Kelly, D. P., 1972. *Z. Allg. Mikrobiol.*, 12, 311.
117. Egamberdieva, K. Yu., Pis'man, T. I., Elkina, T. V., Furiaeva A. V., and Pechurkin, N. S., 1976. *Prikladnaya biokhimiya i mikrobiologiya*, No. 6, 871.
118. McDonald, D. G., and Clark, R. H., 1970. *Can. J. Chem. Eng.*, 48, 669.
119. Beck, J. V., and Schaffia, F. M., 1964. *J. Bacteriol.*, 88, 4, 850.
120. Kodama, A., and Mori, T., 1968. *Plant Cell Physiol.*, 9, 709.
121. Torma, A. E., and Subramanian, K. N., 1974. *Int. J. Miner. Process*, 1, 125.
122. Bryner, L., and Anderson, R., 1957. *Industr. and Eng. Chem.*, 49, 1721.
123. Duncan, D. W., Walden, C. C., and Trussell, P. C., 1966. *Transactions*, 69, 329.
124. Bruynesteyn, A., and Duncan, D. W., 1971. *Can. Met. Quart.*, 10, 1, 57.
125. Torma, A. E., Ashman, P. R., Olsen, T. M., and Bosecker, R., 1979. *Metall. Intern. Zeitschr. Tecknik und Wirtschaft*, H 5, 33, 479.

126. Bruynesteyn, A., and Duncan, D. W., 1977. In: Round Table Conf. Bacter. Leaching. Braunschweig-Stöckheim, 129.
127. Torma, A. E., and Sakaguchi, H., 1978. *J. Ferment. Technol.*, 56, 3, 173.
128. Norris, P. R., and Kelly, D. P., 1978. In: Metallurg. Appl. Bacter. Leaching and Related Microbiol. Phenomena. Eds. L. E. Murr, A. E. Torma, J. A. Brierley. New York, 83.
129. Karavaiko, G. I., Pivovarova, T. A., Schetinina, E. V., and Lazebnyi, A. F., 1974. *Mikrobiologiya,* 43, 6, 1098.
130. Tuovinen, O. H., and Kelly, D. P., 1974. *Arch. Mikrobiol.,* 98, 2, 167.
131. Tuovinen, O. H., and Kelly, D. P., 1974. *Arch. Mikrobiol.,* 95, 2, 153.
132. Hoffman, L. E., and Hendrix, J. L., 1976. *Biotechnol. Bioeng.,* 18, 1161.
133. Yudina, I. N., 1976. In: Trudy TsNIGRI, Moscow.
134. McElroy, R. O., and Duncan, D. W., 1974. Copper Extraction by Rapid Bacteriological Process. U. S. Patent 3856913.
135. McElroy, R. O., and Bruynesteyn, A., 1978. In: Metallurg. Appl. Bacter. Leaching and Related Microbiol. Phenomena. Eds. L. E. Murr, A. E. Torma, J. A. Brierley. Acad. Press. New York, 441.
136. Snell, G. J., and Morgan, S. C., 1977. *Engineer and Mining J.,* 178, 10, 100.
137. Dave, S. R., Natarajan, K. A., and Bhat, J. V., 1979. *Inst. Mining Metallurg. Sect. C,* 88, 234.
138. Lazaroff, N., 1977. *J. Gen. Microbiol.,* 101, 1, 85.
139. Tuovinen, O. H., Niemela, S. I., and Gyllenberg, H. G., 1971. *Biotechnol. Bioeng.,* 13, 517.
140. Golomzik, A. I., Mikhailova, T. A., and Abakumov, V. V., 1976. In: Ecology and Geochemical Activity of Microorganisms. Puschino, 104 (in Russian).
141. McIntosh, M. E., 1976. *Proc. Soc. Gen. Microbiol.,* 4, 23.
142. Chakrabarty, A. M., 1978. In: Metallurg. Appl. Bacter. Leaching and Related Microbiol. Phenomena. Eds. L. E. Murr, A. E. Torma, J. A. Brierley. Acad. Press. New York, 137.
143. Tsuchia, H. M., 1977. In: Round Table Conf. Bacter. Leaching. Braunschweig-Stöckheim, 101.
144. Shnaitman, C. A., Korzynski, M. S., and Lundgren, D. G., 1969. *J. Bacteriol.,* 99, 552.
145. Lazaroff, N., 1963. *J. Bacteriol.,* 85, 78.
146. Torma, A. E., and Itzkovitch, I. J., 1976. *Appl. Env. Microbiol.,* 32, 102.
147. Kuznetsova, E. G., and Kuligina, I. P., 1972. *Atomnaya energiya,* 32, 2.
148. Duncan, D. W., and Bruynesteyn, A., 1971. *Can Mining Metallurg. Bull.,* 74, 110.
149. Bruynesteyn, A., and Duncan, D. W., 1974. In: Solution Mining Symposium. Eds. F. F. Alan, W. A. McKinney, A. D. Pernichele. The Inst. Mining, Metallurg. and Petrol. Eng., New York, 324.
150. Golodyayev, G. P., and Shevchenko, L. S., 1977. In: Exploration of Mineral Resources of the Far East Region. Vladivostok, 168 (in Russian).
151. Yudina, I. N., Kostyukova, O. N., Gorenkov, N. L., Panin, V. V., and Kim, D. Kh., 1979. *Tsvetnye metally,* 8, 105.
152. Tsuchia, H. M., Trivedi, H. C., and Schuler, M. L., 1974. *Biotechnol. Bioeng. J.,* 16, 7, 991.
153. Brierley, J. A., 1979. In: Fourth Intern. Symp. on Environm. Biogeochem. and Conf. on Biogeochem. in Relation to Mining Industry and Environm. Pollution (Leaching Conf.) (Program and Abstracts). Austr. Acad. of Sci., 78.
154. Karavaiko, G. I., Kovalenko, T. V., and Golovacheva, R. S., 1980. Microbiological Aspects of Leaching Copper from Ores. In: Use of Microorganisms in Hydrometallurgy. Pécs, Hungary.
155. Podanyi, T., 1980. In: Use of Microorganisms in Hydrometallurgy (Thesis). Intern. Conf. Pécs, Hungary, 34.
156. Power, R. L., 1964. Copper Dump Leaching at Asarec's Silver Bell Unit Arizone. Unit Processes in Hydrometallurgy. New York—London, 806.

157. Fletcher, A. W., 1970. Metal Winning from Low-Grade Ore by Bacterial Leaching. Trans. Inst. of Min. and Metallurg., 79, C—247.
158. Hovanec, G. M., 1970. Review on Basic Economic Aspects of Acid Leaching of Copper-Bearing Ores. Symposium pracouniku banskeho prumyslu Hornicka Pribram ve vede a Technice.
159. Trudinger, P. A., 1971. Microbes, Metals and Minerals. Min. Sci. Eng., 3, 13.
160. Kingma, J. G., and Silver, M., 1980. *Appl. and Environm. Microbiol.*, 39, 2, 635.
161. Marrit, R. C., and Pings, W. B., 1969. Processing of Uranium Ores. Part II. Colo. Sch. Mines Miner. Ind. Bull., 12, 6, 20.
162. Rudolfs, W., and Helbronner, A., 1922. *Soil Sci.*, 14, 1—6, 459.
163. Zimmerley, S. R., Wilson D., and Prater, J., 1958. Patent USA, No. 28229964.
164. Shalamov, V. I., Khalezov, B. D., Novomlintsev, A. M., Nezhivykh, V. A., Fedorov, N. Ya., and Efimova, V. I. 1974. *Bull. Tsvetnaya metallurgiya,* No. 23, 17.
165. Adamov, E. V., and Panin, V. V., 1974. In: Minerals Dressing, VINITI, 8, 5 (in Russian).
166. Karavaiko, G. I., Abakumov, V. V., Krasheninnikova, S. A., Mikhailova, T. A., Piskunov, V. P., and Khalezov, B. D., 1981. *Prikladnaya biokhimiya i mikrobiologiya*, 27, 1, 73.
167. Beck, J. V., 1967. *Biotechnol. Bioeng.*, 9, 487.
168. Gol'braikht, A. I., Skripchenko, L. N., Malygina, L. V., and Mitrofanov, A. A., 1978. In: Activity of Microorganisms in Natural Substrates of Kazakhstan. Alma-Ata, 38 (in Russian).
169. Groudev, S. N., Gentchev, F. N., and Gaidarjiev, S. S., 1978. In: Metallurg. Appl. Bacter. Leaching and Related Microbiol. Phenomena, Eds. L. E. Murr, A. E. Torma, J. A. Brierley. Acad. Press. New York, 253.
170. Khalezov, B. D., Nezhivykh, V. A., Novomlintsev, A. M., Pavlichenko, G. A., and Shalamov, V. I., 1977. In: Trudy Ural'skogo nauchno-issledovatel'skogo i proektnogo instituta mednoi promyshlennosti, 20, 129.
171. Daniel, J. A., Harries, J. R., and Ritchie, A. I. M., 1979. In: Intern. Symp. on Environm. Biogeochem. and Conf. on Biogeochem. in Relation to Mining Industry and Environm. Pollution (Leaching Conf.) (Program and Abstracts). Austr. Acad. of Sci., 87.
172. Murr, L. E., and Brierley, J. A., 1978. In: Metallurg. Appl. Bacter. Leaching and Related Microbiol. Phenomena. Eds. L. E. Murr, A. E. Torma, J. A. Brierley. Acad. Press, New York, 492.
173. Livesey—Goldblatt, E., Tunley, T. H., Nagy, I. F., 1977. In: Round Table Conf. Bacter. Leaching. Braunschweig-Stöckheim. Ed. W. Schwartz, 175.
174. Groudev, S. N., 1981. *Dokl. Bolgar. Akad. Nauk,* 84, 10, 1437.
175. Aslanukov, R. Ya., Yudina, I. N., Gorenkov, N. L., Dudareva, T. E., and Filimonova, L. A., 1982. Practice of work on semi-industrial installation of tank bacterial leaching. USSR/UNEP/PRB/CMEA Project «Microbiological Leaching of Metals from Ores» (Lecture). Moscow—Sofia—Tula.
176. Pol'kin, S. I., Panin, V. V., Yudina, I. N., Adamov, E. V., Grishin, S. I., and Aslanukov, R. Ya., 1982. Technological schemes of processing of concentrates with application of the method of bacterial leaching. USSR/ /UNEP/PRB/CMEA Project «Microbiological Leaching of Metals from Ores» (Lecture). Moscow—Sofia—Tula.
177. Batyrbekova, S. A., Ilyaletdinov, A. N., Polyviannyi, I. R., and Kamalov, M. R., 1974. *Vestnik Akad. Nauk Kaz. SSR,* No. 12, 67.
178. McGreedy, H. H., Harrison, V. P., and Gow, W. A., 1969. *Can. Mining and Metallurg. Bull.*, 62, 682, 135.
179. Ilyaletdinov, A. N., Kamalov, M. R., and Bozhko, N. A., 1978. Izvestiya Akad. Nauk Kaz. SSR, ser. biol., No. 2, 2.
180. Zajic, J. E., and Ng, K. S., 1970. Developments in Industrial Microbiology.
181. Guay, R., Silver, M., and Torma, A. E., 1977. *Biotechnol. Bioeng.*, 19, 727.
182. Derry, R., Garret, K. H., Le Roux, N. W., and Smith, S. E., 1976. In:

Geology, Mining and Extractive Processing of Uranium. Ed. M. J. Jones. London, 56.
183. Findley, J., Appleman, M. D., and Yen, T. E., 1974. *Appl. Microbiol.*, 28, 460.
184. Meyer, W. C., and Yen, T. F., 1976. *Appl. Environm. Microbiol.*, 32, 4, 610.
185. Ebner, H. G., 1977. In: GBF Conf. Bacterial Leaching, Ed. W. Schwartz, Verlag Chemie, Weinheim, New York, 217.
186. Dugan, P. R., and Apel, W. A., 1978. In: Metallurg. Appl. Bacter. Leaching and Related Microbiol. Phenomena. Eds. L. E. Murr, A. E. Torma, J. A. Brierley. Acad. Press. New York, 223.
187. Kargi, F., 1982. *Enzyme Microb. Technol.*, 4, January 13, 13.
188. Denisov, G. V., Kovrov, B. G., and Sedel'nikov, S. M., 1982. Methods of growing *Thiobacillus ferrooxidans* in a cultivator with electrochemical reduction of Fe^{3+} to Fe^{2+}. USSR/UNEP/PRB/CMEA Project «Microbiological Leaching of Metals from Ores» (Lecture). Moscow—Sofia—Tula.
189. Kovrov, B. G., and Denisov, G. V., 1982. Technology of *Thiobacillus ferrooxidans* biomass production on the basis of Fe^{3+} electrochemical reduction. USSR/UNEP/PRB/CMEA Project «Microbiological Leaching of Metals from Ores» (Lecture). Moscow—Sofia—Tula.
190. Madgwick, J. C., and Ralph, B. J., 1977. In: Conf. Bacter. Leaching, GBF Monograph Series No. 4, Ed. W. Schwartz, Verlag Chemie, Weinheim, 93.
191. Beveridge, T. J., and Murray, R. G. E., 1980. *J. Bacteriol.*, 141, No. 2, 876.
192. Norris, P. R., and Kelly, D. P., 1979. *Dev. Ind. Microbiol.*, 30, 299.
193. Sakaguchi, T., Horikoshi, T., and Nakajima, A., 1978. *J. Ferment. Technol.*, 56, 561.
194. Strandberg, G. W., Shumate, S. E., and Parrot, J. R., 1981. *Appl. and Environm. Microbiol.*, 41, 1, 237.
195. Danch, A., Kasprzak, A., and Chmielovski, J., 1981. *Fizykochem. probl.*, 13, 245.
196. Muzzarelli, R. A. A., Tanfani, F., and Emanuelli, M. 1981. *Appl. Biochem.*, 3, 4, 322.
197. Chopra, I., 1971. *J. Gen. Microbiol.*, 63, 265.
198. McLean, O. J., Lucis, Shanh, Z. A., and Zansz, E. R., 1972. *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 31, 699.
199. Doyle, J. J., Marshall, R. J., and Pfander, W. H., 1971. *Appl. Microbiol.*, 29, 562.
200. Macaskie, L. E., and Dean, A. C. R., 1982. *Environm. Technol. Letters*, 3, 49.
201. Norris, P. R., and Kelly, D. P., 1977. *J. Gen. Microbiol.*, 99, 317.
202. Hassett, P. R., Jennet, J. C., and Smith, J. E., 1980. In: Contaminants and Sediments. Ed. R. A. Baker. Ann Arbor Sci. Publ. Inc. USA, 2, 409.
203. Olafson, R. W., Apel, K., and Sim, R. G., 1979. *Biochem., Biophys. Res. Common.*, 89, 36.
204. Mineyev, G. G., Tchernyak, A. S., and Semyonova, L. P., 1972. *Prikladnaya biokhimiya i mikrobiologiya*, 8, 3, 358.
205. Mineyev, G. G., Semyonova, L. P., Tchernyak, A. S., and Abakumov, D. N., 1972. *Nauch. Trudy Irgiredmeta*, 27, 122.
206. Ilyaletdinov, A. N., Enker, P. B., and Loginova, L. V., 1977. *Mikrobiologiya*, 46, 1, 113.
207. Brierley, J. A., and Brierley, C. L., 1979. In: Fourth Intern. Symp. on Environm. Biogeochem. and Conf. on Biogechem. in Relation to Mining Industry and Environm. Pollution (Leaching Conf.) (Program and Abstracts). Austr. Acad. of Sci., 100.
208. Romanenko, V. I., Kuznetsov, S. I., and Koren'kov, V. N., 1977. Improvements in/or relating to the purification of aqueous industrial effluents containing chromate and/or bichromate as impurity. Patent No. 1475369. London.
209. Solozhenkin, P. M., Lyubavina, L. L., and Levchenko, P. G., 1980. In: Proc. Intern. Conf. «Use of Microorganisms in Hydrometallurgy», Pécs, 135.
210. Jackson, T. A., 1978. *Environm. Geol.*, 2, 173.

211. Brierley, J. A., and Brierley, C. L., 1983. In: Biomineralization and Biological Metal Accumulation. Eds. P. Westbroek. E. W. de Jong, 499.
212. Norberg, A., 1981. In: 5th Intern. Symp. on Environm. Biochem. (Abstracts), Stockholm, Sweden, 89.
213. Shumate II, S. E., Strandberg, G. W., and Parrot, J. R., 1978. In: Biotechnol. Bioeng. Symp., No. 8, 13.
214. Shumate II, S. E., Strandberg, G. W., McWhirter, D. A., Parrot, J. R., Bogacki, G. M., and Locke, B. R. 1980. In: Biotechnol. Bioeng. Symp., No. 10, 27.
215. Tsezos, M., and Volesky, B., 1981. *Biotechnol. Bioeng.,* 23, 583.
216. Tsezos, M., and Volesky, B., 1982. *Biotechnol. Bioeng.,* 24, 385.
217. Biosorbent M. A new type of selective sorbents. Published by Department of scientific and technical information for Czechoslovakian Nuclear Power Committee. Zbraslav-on-Vltava,
218. Charley, R. C., and Bull, A. T., 1979. *Arch. Mikrobiol.,* 123, 239.
219. Gale, N. L., and Wixson, B. G., 1979. *Dev. Ind. Microbiol.,* 20, 259.
220. Shoemaker, R. S., and Darrah, R. M., 1968. *Mining Eng.,* 20, 12, 68.
221. Shahani, M. J., Altekar, V. A., 1983. Geomicrobiometallurgical powders. A status of the Art Review of an Emerging Composite Technology. Intern. Symp. on Biohydromet. Associazione Mineraria Sarda. Cagliari, Italy, 1—31.
222. Tauzhnianskaya, Z. A., 1973. Bacterial Leaching Technology Abroad. Tsvetmetinformatsiya, Moscow (in Russian).
223. Livesey-Goldblatt, E., Norman, P., Livesey-Goldblatt, D. R., 1983. In: Recent Progress in Biohydrometallurgy. Eds. G. Rossi and A. E. Torma, Assoc. Miner. Sarda—Iglesias, Italy, 627.
224. Murr, L. E., and Mehta, A. G., 1982. *Biotechnol. Bioeng.,* 24, No. 3, 743.
225. Bruynesteyn, A., and Hackl, R. P., 1982. Evaluation of acid production potential of mining waste materials. USSR/UNEP/PRB/CMEA Project «Microbiological Leaching of Metals from Ores» (Lecture). Moscow—Sofia—Tula.

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IN-PLACE LEACHING OF URANIUM AT DENISON MINES LTD.

ABSTRACT

In 1984, Denison Mines Limited initiated a major demonstration of bacterially-assisted, in-place underground leaching of uranium ore at their Elliot Lake mine. The project involved identification of the test stopes, establishment of an underground laboratory, preparation of the test stopes, including bulk heading, design and installation of pumping and control systems, optimization of microbial environment, start up and operations. The mine configuration and available mine water enhanced the feasibility of the project. Problems of low temperature, radon levels, competitive microorganisms, ore fragmentation and water management were addressed. This report presents the background, development and current status of the project with a preliminary assessment of the long-term potential.

1. INTRODUCTION

Denison Mines Limited has been producing uranium from ores at Elliot Lake, Ontario since the late 1950's. Over that period of time there have been a number of modifications to the milling operation from crushing through concentrate drying and packing (1). There have been several mill expansions, the latest taking

place in 1982 to reach the current mill capacity of 13,600 tonnes per day (2) of an ore containing approximately 1 kg per tonne.

Denison has used underground room and pillar techniques as their standard mining practice. The sedimentary quartz pebble conglomerate occurs in relatively flat lying beds. Long-hole drilling and blasting are used. Ore is removed using trackless machinery and hoisted to the surface for uranium recovery.

The Denison mill uses semi-autogenous grinding and pebble mills (Figure 1). Ore at 50% -200 mesh is leached in sulphuric acid with sodium chlorate as an oxidant in air agitated pachucas. Drum filters remove the leached residue. Moving bed ion exchange has been augmented by a fixed bed system in the recent expansion. Nitrate elution is followed by ammonia precipitation. Yellow cake is dried and packed in steel drums.

Over the years Denison has used mine water as mill process water. This mine water has been of particular interest because it contains about 100 ppm uranium. This relatively high uranium content arises from bacterial oxidation of pyrite (5-7% in Denison ore). This reaction generates acid and ferric ion in solution which leaches uranium from the conglomerate. This phenomenon was first identified and explained in the early 1960's (3). With volumes of up to 5000 litres per minute, this mine water added over 100 tonnes of uranium to mill production each year. On occasion, completely mined stopes were washed down with mine water to promote this phenomenon, but no concerted or carefully engineered approach was used.

In the early 1980's when the highly optimistic projections for increasing uranium demand were unfulfilled, uranium prices dropped precipitously. Denison sought process economies via a number of measures. One such measure was to consider increasing the concentration of uranium in mine water by enhanced in-place leaching.

The concept of well-engineered, in-place leaching had already been well demonstrated at the full scale operations of Agnew Lake Mines Limited (4). In 1976, that mine began development using bacterially-assisted leaching exclusively as the uranium recovery process. Leaching was performed on broken ore underground as well as in surface heaps of the "swell" created during blasting. This operation ran for 5 years ceasing production in 1983 in the face of declining ore grades (from 0.043 to 0.028% U), poor underground leaching recoveries and reduced uranium prices. Principal constraints to high uranium recoveries were the steep incline of the ore pockets and the large size of the broken ore. Nevertheless, the more carefully controlled size in the flat-lying surface heaps gave excellent results.

Active bacterial oxidation does take place underground at Denison as is demonstrated by the high uranium content of mine water. All of these factors led Denison to consider the design of a major project to increase uranium recovery by in-place underground leaching.

3. DEVELOPMENT PLANS

In-place leaching had been conducted as a small and interesting adjunct to mining operations but the recovery of uranium was not highly significant. The process worked when conditions were right and helped gain value from waste materials underground. However, there had never been any major strategy or design in the process.

In 1983, Denison recognized the need to further reduce costs. Uranium in mine water was virtually free since it involved no mining cost, no additional pumping costs and could be treated in the existing mill. Perhaps this could be expanded. However, to do so required more broken ore underground. Denison therefore embarked on a project to design, develop and operate a demonstration of in-place leaching of ore broken specifically for the purpose. This demonstration would be used to provide criteria for full scale design, costs for development and operation and identification of any major problems.

2. DENISON MINES

The quartz-pebble conglomerate at Denison lies in two major reefs. The lower reef which is about 10 metres in thickness has been mined for over 25 years. It has an grade of between 0-75 and 1.0 kg per tonne. The relatively flat lying bed of ore has enabled the use of conventional mining techniques. Pillars amounting to about 20% of the ore volume have been left for roof support. This mine has produced over 50 million tonnes of ore since its start-up.

Operations at Denison have required some selective mining. A chloritic ore zone is avoided. Chloritic ore has a very high acid consumption and causes grave filtration problems. In addition, the upper reef zone of the mine is below cutoff grade at about 0.4 kg U per tonne. This 8 metre thick zone parallels the lower reef and is separated from the lower zone by about 50 cm of barren rock. The upper reef contains an estimated 50 million tonnes of material. Chloritic ore represents perhaps 15,000 tonnes of uranium at more than 1 kg/tonne.

The Denison mine accumulates water underground at a rate of about 5,000 litres per minute. This water comes from mine machinery such as drills, wetting-down ore to prevent dusting, condensation from ventilation air in summer, leakage from the surface, and drainage from backfill operations. Water is pumped constantly to the surface with the daily amount approaching 7,500 tonnes in the summer.

The objectives of the project were specifically:

1. verify the optimal underground blasting procedures to fragment ore into sufficiently small size for in-place leaching;
2. test the best way of leaching the fragmented ore trickling, percolation or flooding;
3. develop the proper methods for protecting workers from the higher level of radon emanating from the large volume of fragmented ore;
4. perform bacteriological studies in universities to understand and optimize nutrient needs and temperature behaviour of uranium leaching bacteria.

3.1 Fragmentation

The Denison mine provided an ideal situation for in-place leaching. The lower reef had been removed as ore for surface treatment. Drilling could be carried out from the lower reef and the upper reef material could be blasted into the existing cavity. There was no need for any removal of material from the stopes.

Denison established four test stopes. These stopes were approximately 80 metres long with a slope of about 10° and 25 meters wide with some central pillars for roof support. Each represented about 35,000 tonnes of upper reef ore.

Three drilling patterns were established: 4 feet by 3 feet (1.22 m x 0.91 m), 4 feet by 2 feet and 3 feet by 3 feet. These had been established based on experience and a predictive computer model developed by the explosives manufacturer, Canadian Industries Limited (CIL). A rubble size of minus 13 inches (33 cm) was sought with the bulk of the material in finer (minus 5 cm) sizes. The three blasting patterns were to be tested in the large stopes to assess their effects. The size was to be analysed using a CIL technique called Blaspha, an analysis of photographs of broken ore. This would be correlated with bulk samples taken with a scoop-tram mining machine. This sample would also be run in an underground laboratory column test.

3.2 Leaching

Laboratory studies at the British Columbia Research Council and at Denison had indicated that the best leaching results were obtained in a trickle or percolation leaching system similar to surface heap leaching. In this system the mine water would be sprayed or trickled over the surface of the ore pile to keep the ore wet but not flooded. However, this approach presented a problem; the roof of the stope would require support so that men could work in the stope putting in the piping and sprays. Roof support with long steel rods is expensive.

As an alternative, Denison decided to evaluate flood leaching as well. Flood leaching required that the lower end of the stope be completely sealed with a waterproof bulkhead. Mine

water would then completely fill the stope containing the broken ore. Once filled, the stope would be allowed to drain. When empty, a short rest period in air would encourage bacterial growth on the wetted ore surfaces. The flooding cycle would then be repeated until the discharge solution was below economic levels.

3.3 Radon emanation

One concern about the in-place leaching was that the presence of large quantities of broken ore underground could raise the radon gas levels above the acceptable working levels. With a mining rate of about 10,000 tonnes per day the four stopes would represent the equivalent of 15 days of mining operations. Furthermore, if the demonstration was successful, Denison would require some basis for determining the contribution to radon levels as new stopes were added. This would provide ventilation design data.

Radon levels would be checked in each stope during the progress of development and operation. Careful comparison would be made between the trickle leach and flood leach stopes.

3.4 Bacteriological studies

Denison had relied exclusively on the indigenous strains of the bacterium: Thiobacillus ferrooxidans for leaching of residual underground wastes. In developing the demonstration project, it was decided to allocate a portion of the funds to university

research on developing an improved understanding of the bacteria and to optimize nutrition and temperature response of the microbes.

Two university groups were identified, Dalhousie University (Dr. R.G.L. McCready) in Halifax, Nova Scotia and Laurentian University (Dr. G. Ferroni) in Sudbury, Ontario. Dalhousie undertook to optimize the nutritional requirements for indigenous Thiobacillus ferrooxidans. Laurentian set out to isolate from the underground environment and elsewhere different strains of this same bacterium in order to examine the effect of temperature on growth rates, and to study bioleaching rates over the range of 6 to 25°C.

4. PERFORMANCE TO DATE

The project began in May 1984. The stope development work, laboratory tests, and university studies were to be completed in 12 months. However, the true test will be the overall recovery of uranium from these test stopes over a 12 to 24 month leaching period. Denison based its projections on 70% recovery in 12 months based on the so-called Ottawa curve (Figure 2) developed by Mines Branch (CANMET) in the 1960's (5). The following provides a brief summary of results to date.

4.1 Stope Development

Stope development took approximately 6 months per stope. These were scheduled 2 months apart. Three stopes drilled and blasted on 4' x 3', 3' x 3' and 4' x 2' were prepared for flood leaching. Two types of drill rig were chosen: air operated bar and arm drills, and a new electric hydraulic "uppers" jumbo. Drilling was in fact completed one month ahead of schedule.

Blasting of stopes began in October 1984. Small blasts of 2000 tonnes each were designed to allow full testing of the fragmentation parameters. However, this procedure was abandoned in the interest of safety. Blasts as large as 15,000 tonnes were carried out with excellent results using proper timing procedures.

Photographs were taken of all blasts and sent to CIL for analysis. Unfortunately loose fractured rock from the roof fell on top of the broken ore and dominated the photographs. The analyses were not as satisfactory as had been anticipated. Broken ore was scooped out of these stopes for screen analysis. Such an analysis is shown in Table I. However, with a stope 250 feet long, a single scoop from one end is not necessarily representative. It would appear that the actual size of the rubblized ore may never be adequately determined unless a leached-out stope is completely sampled after completion of the project.

Bulkheads were built to seal up the lower end of the flood leaching stopes. This lower end was purposely left narrow to

reduce the size of the bulkhead. Each stope had two bulkheads capable of withstanding static heads of up to 100 feet (30 meters). These are reinforced concrete bulkheads set into a channel cut into the rock at the end of the stope. They are about 40 cm thick with the necessary plumbing fittings for sampling, emptying the stope, and aeration. Aeration pipes had been laid on the floor of the stope prior to blasting.

The lower end of the trickle leach stope was fitted with a dam about 1.5 meters high to provide a small reservoir at the discharge end for surge capacity, sampling and safety. Rock bolts, of about 1.5 meters in length, were installed in the roof of the trickle leach stope to prevent rock falls. A network of hoses was laid on the surface of the broken ore and sprays were installed in a regular pattern.

4.2 Laboratory studies

Denison established an underground laboratory in which samples could be prepared, simple analyses could be performed, and as a site for column tests. Six columns about 60 cm diameter and three meters high were set up to test various leach parameters. Two columns were set up using ore from the 3' x 3' and 4' x 3' blast patterns as controls. Two other columns were set up with ore from these blasting patterns with optimized nutritional supplements in one and massive inoculation of Thiobacillus in the other. These tests were started in February 1985. Significant leaching rates were established as shown in

Table II. The optimization of nutrients, massive inoculation and closer blasting pattern all gave significant improvements.

This underground laboratory work was the on-site testing area for parmeters established based on university research. Indigenous mine drainage was being used as the leach liquor in the Denison mine. Chemical analyses of several mine water samples was made to determine the concentration of normally required nutrients for Thiobacillus ferrooxidans. These analyses are shown in Table III. The high concentration of nitrogen compounds is due to the use of ammonium nitrate based explosives in stope development.

Recent studies (6) have indicated that the growth medium (called 9K) used in laboratory culturing of Thiobacillus contain excessive concentrations of phosphate, magnesium and ammonia. Work on leaching of pyrite from coal (7) had shown that T. ferrooxidans would be active in media considerably more dilute than considered optimum under laboratory conditions. Bacteria were very active in the Denison mine under conditions as shown in Table III. Studies were undertaken to determine whether nutritional supplements would enhance bacterial growth and the rate of uranium solubilization.

Work on coal had indicated that 0.1 M phosphate was required for T. ferrooxidans growth. Therefore a series of media was tested as outlined in Table IV. For comparison the compositions of 9K (laboratory) medium is shown.

Growth and iron oxidation rates were compared and medium A was found to be optimum. Five Denison mine water isolates were then grown in this medium with iron oxidation rates as shown in Figure 3. It appeared that all grew well in this medium. It appeared that the mine water at Denison was lacking only in phosphate with average levels of 3.5 ppm (Table III) versus recommended levels of 10 ppm. Phosphate supplementation was therefore recommended to Denison and applied in one of the underground test columns.

Having optimized nutritional requirements, work proceeded to determine whether some of the isolates from Denison had better cold tolerance (psychrophilic response) than others. Figure 4 shows the effect of temperature on the most active of the isolates. This culture was taken from an area adjacent to the ventilation shaft and therefore most subject to temperature variation. Of the five isolates and various other cultures tested, only three grew at temperatures less than 15°C. The indigenous strains of Thiobacillus obviously have much greater temperature tolerance than others. Nevertheless temperature has a profound effect on iron oxidation rates which will undoubtedly have an impact during winter months when underground temperatures are in the 10-12°C range.

Work at Laurentian University confirmed the psychrophilic nature of Thiobacillus ferrooxidans. It was estabished that the mean generation time roughly doubled for each 6°C rise in temperature from 6-25°C as shown in Table V. It would appear

that temperatures below 12°C are generally unfavourable, but in the normal seasonal cycle the temperature range of 12-20°C should sustain active indigenous population of bacteria. This temperature effects data and can be utilized in scheduling the rest periods between flood leaching cycles.

The concentration of bacterial cells in laboratory leaching tests demonstrated the effect of increasing population (Table VI). These results have led to larger scale tests using inoculation with cultures of Thiobacillus ferrooxidans.

4.3 Leaching operations

It is too early to judge the results on the stope leaching. Leaching was begun in May and June on the four test stopes. Preliminary results after about 100 days are shown in Table VII for the four stopes. In addition, a number of interesting facets of the operation have been indicated.

4.3.1 Water

The substantial increase in the total volume of water underground has led to concern about the water balance. There is no accurate measure of the amount of water entering the mine. Recent mine developments and increasing use of backfill (in slurry form) seem to have increased the influx of water. Sump capacity is being taxed. Operation of the flooded stopes requires adequate sump capacity. During the summer of 1985,

stope flooding was delayed due to the demand for balancing the underground water influx with pump capacity.

Stope leakage caused difficulties. Leakage from a flooded stope into an adjacent parallel stope had to be checked by extensive grouting. One of the bulkheads was leaking around the perimeter and had to be repaired.

The quality of leach solution presented some problems. Backfill drainage at about pH 10 was neutralizing water in the sump. This drainage was diverted to another sump.

Water temperatures even in May and June were considerably lower than anticipated. Winter temperatures of 12°C persisted even into June so that start-up of the stopes was slow. Denison is considering using ion exchange barren from the surface mill to increase the temperature of the leach solution and so accelerate bacterial growth. However, there is some concern about potential contamination by other ions and organics.

Denison has plans to expand the in-place leaching operation. However pumping capacity is limited and new pumping capacity is expensive. Therefore underground concentration of the solutions from the current 300 ppm level to perhaps 1000-2000 ppm is being considered. Three options are being evaluated: ion exchange, reverse osmosis and biosorption. Ion exchange is preferred because Denison has considerable experience in this field. Reverse osmosis seems to be feasible using recently developed membranes. Biosorption does not seem to be sufficiently well developed to be a serious contender.

4.3.2 Aeration

The demand for oxygen for the biological oxidation of the pyrite is substantial. From experience in leaching waste underground, it would appear that this demand is easily met with ventilation air. However, in the flooded stopes air access is limited. Air lines have been installed under the ore but some have been crushed or broken. Denison therefore consulted one of the oxygen manufacturers to develop a comprehensive analysis of aeration demands.

In the underground laboratory, tests have been conducted in one column using forced aeration with oxygen enriched air. Aeration under pressure is being considered to increase the level of dissolved oxygen in the leach solutions.

4.3.3 Monitoring

Discharge solutions from the stopes are being monitored on a regular basis. Solutions are typically 300 ppm. However, it is very difficult to determine the true rate of leaching. The average analysis of the ore in the stope is based on geological and mine development drilling. Approximate grades are known but the ore is variable. The only measure of progress is the amount of uranium in solution. Neither the grade nor the volume of ore is accurately known.

Various strategies are in place to use better predictive techniques. Data from laboratory and column leach tests are being collected. Mineralogical analyses of leached ore particles

are providing insights into leaching rates and depth of penetration. Denison is now working with Queen's University in Kingston, Ontario on developing a computer model based on these data.

4.3.4 Biotechnology

During the course of the work at Denison, leaching on one of the older stopes seemed to stop. The presence of a yellow precipitate gave rise to the suspicion that it was jarosite. However, upon analysis little jarosite was detected. Samples sent to one of the authors (McCready) were soon determined to be fungi. These fungi were heterotrophs, i.e. required organic carbon for growth. Upon investigation this stope was found to have been an underground maintenance area and have high concentrations of petroleum products, the carbon source. Conditions in the stope remained compatible with the growth of Thiobacillus however no uranium was present in the discharge water. Tests revealed that the fungi were in fact adsorbing the uranium. This phenomenon is now being investigated more fully. Uranium loadings of up to 12% on a dry-weight basis have been achieved in the laboratory at pH 2.3 and with 15 gpL of iron. While Denison must avoid conditions under which these fungi will grow in the leaching stopes, it may well be a route to concentration of uranium.

5. CONCLUDING COMMENTS

This Denison in-place underground leaching demonstration has been set up at cost of about $1,500,000. The National Research Council of Canada provided financial support for half of these costs under its Program for Industry/Laboratory Projects (PILP). This program is designed to help industry acquire new technology from government and other public laboratories with the risks being shared by the Federal Government.

Denison Mines Limited senior management gave this project full and unqualified support. The potential for cost reduction in uranium production was so substantial that the project was given high priority.

Federal government and university researchers in turn gave full cooperation to the work. Excellent communication was essential.

The final results are as yet unknown but all parties are sufficiently encouraged to plan a new phase of mine development and research. Research plans include further investigations of improved monitoring systems, aeration parameters, solution treatment options, impact of varying mineralogy, use of IX barren solution, characterization of other microorganisms such as fungi and mathematical modelling.

REFERENCES

1. Larocque, E. and Pakkala, E. "Current leaching and product recovery practice at Denison Mines Limited"; CIM Bull. 72 804 (April 1979) 172-176.
2. Pakkala, E. "Denison Mines Limited: Mill Expansion"; CUPMC Proceedings, CANMET Division Report ERP/MSL 80-158 (TR).
3. Harrison, V.F., Gow, W.A. and Ivarson, K.C.L. "Leaching of uranium from Elliot Lake ore in the presence of bacteria"; Extraction Metallurgy Division Report EMA 66-1, Dept. of Mines and Technical Serveys, Ottawa (now CANMET, Energy, Mines and Resources Canada).
4. Heyman, L. "Metallurgical aspects of the operation at Agnew Lake Mines Limited"; CUPMC Proc., ERP/MSL 80-158 (TR), CANMET, Energy, Mines and Resources Canada; 1980.
5. McCreedy, H.H., Harrison, V.F. and Gow, W.A. "A proposed method, using bacteria, for the continuous leaching of a uranium ore"; CIM Bull. 63 682 (February 1969) 135-140.
6. McCready, R.G.L., Wadden, D. and Marchbank, A. "Nutrient requirements for the in-place leaching of uranium by Thiobacillus ferroxidans"; Submitted to Hydrometallurgy, October 31, 1985.
7. McCready, R.G.L. "Microbiological studies on high-sulphur coals"; Atlantic Research Lab. Tech. Report 50, NRC 23601, 1984.

FIGURE CAPTIONS

Fig. 1. Simplified Denison Flowsheet

Fig. 2 Uranium Extraction by Iron-Oxidizing Bacteria

Fig. 3. Growth of Denison Mine Isolates at 28°C on Low Phosphate Medium

Fig. 4. Growth of T. ferrooxidans E.L. #5 at Various Temperatures on Low PO Medium at 150 rpm, (Average of Triplicate Cultures) 10 ppm Mg & 10 ppm NH^+ at pH 2.3

FIGURE 1

SIMPLIFIED DENISON FLOWSHEET

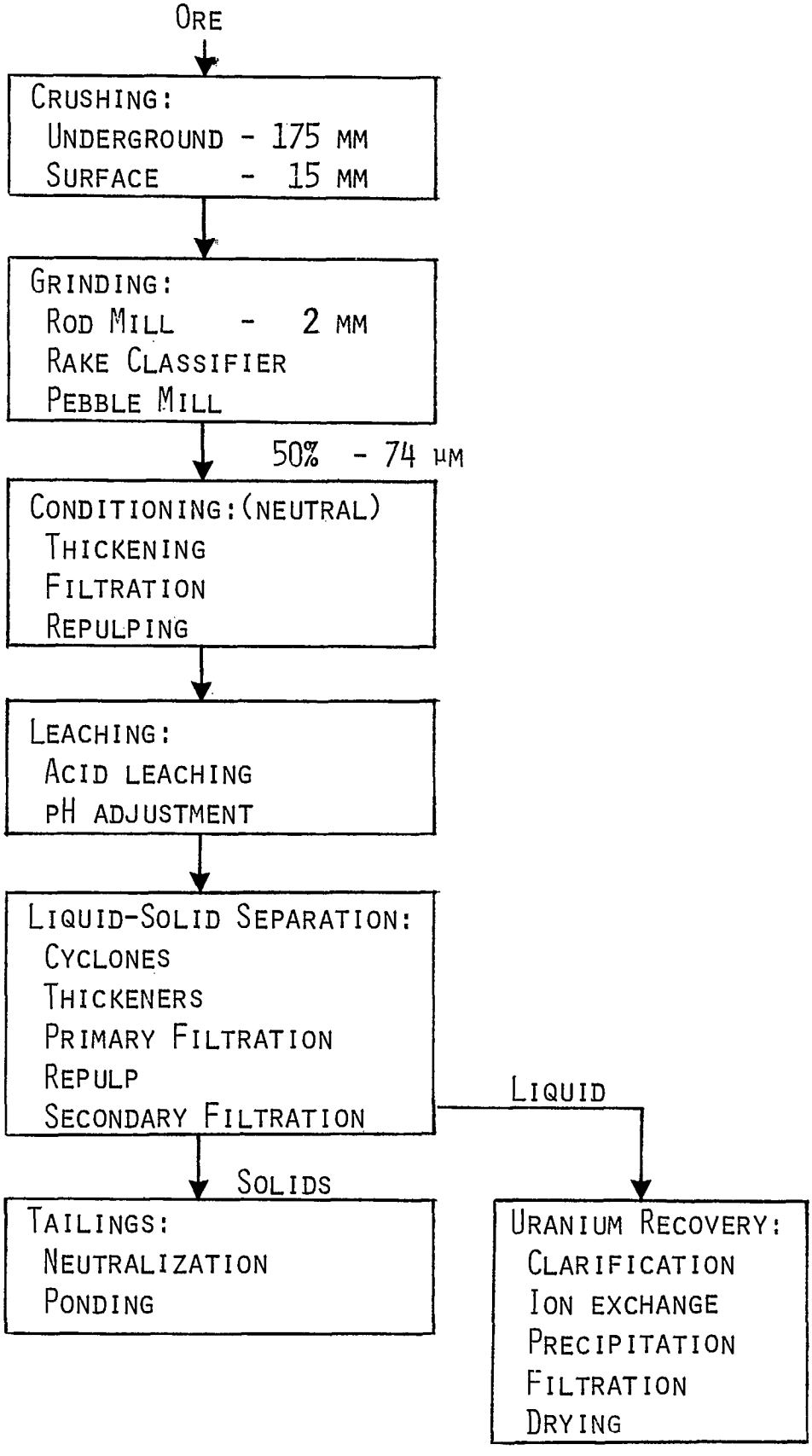
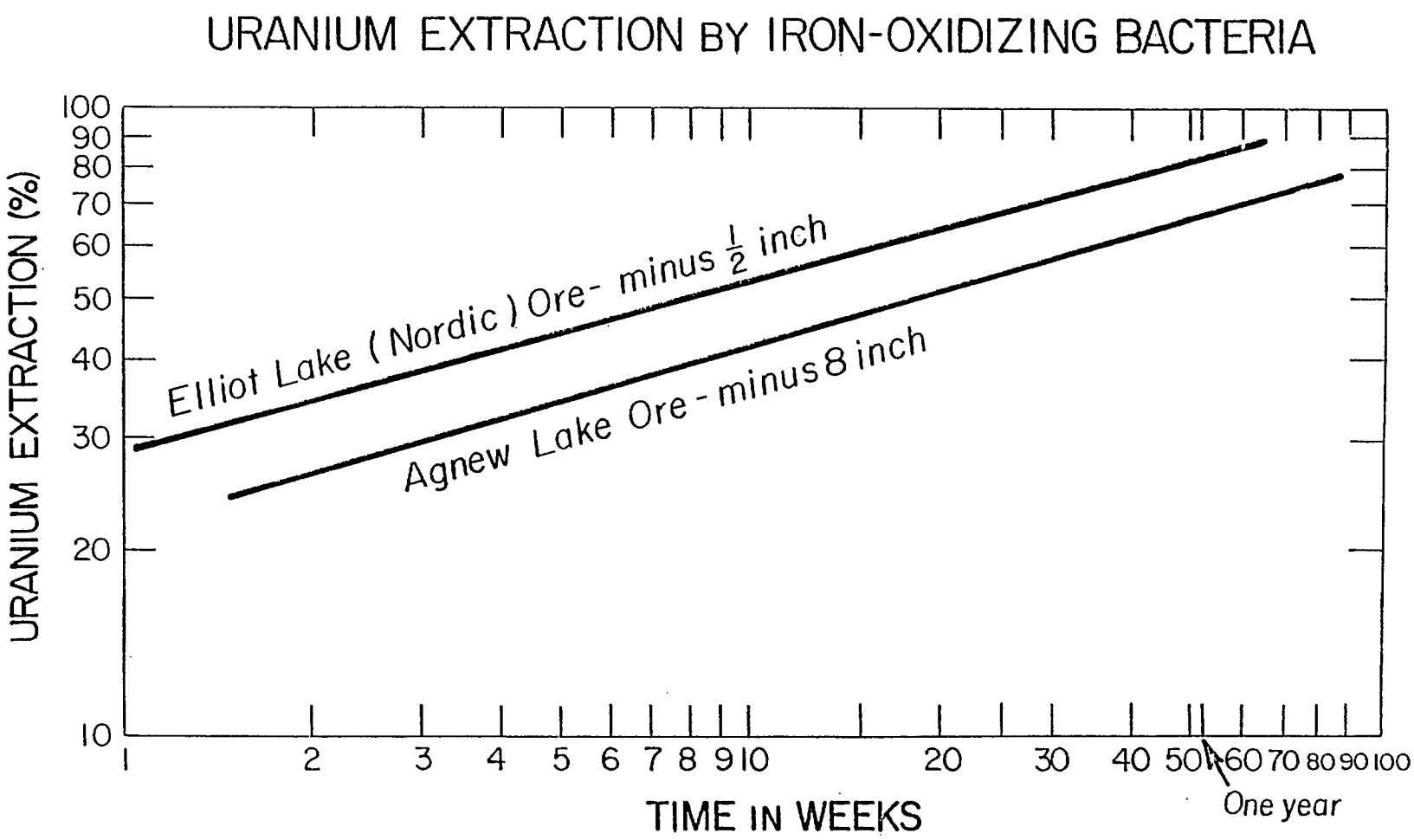


FIGURE 2


URANIUM EXTRACTION BY IRON-OXIDIZING BACTERIA
URANIUM EXTRACTION (%)
100
90
80
70
60
50
40
30
20
10
Elliot Lake (Nordic) Ore- minus 1/2 inch
Agnew Lake Ore - minus 8 inch
1
2
3
4
5
6
7
8
9
10
20
30
40
50
60
70
80
90
100
One year
TIME IN WEEKS

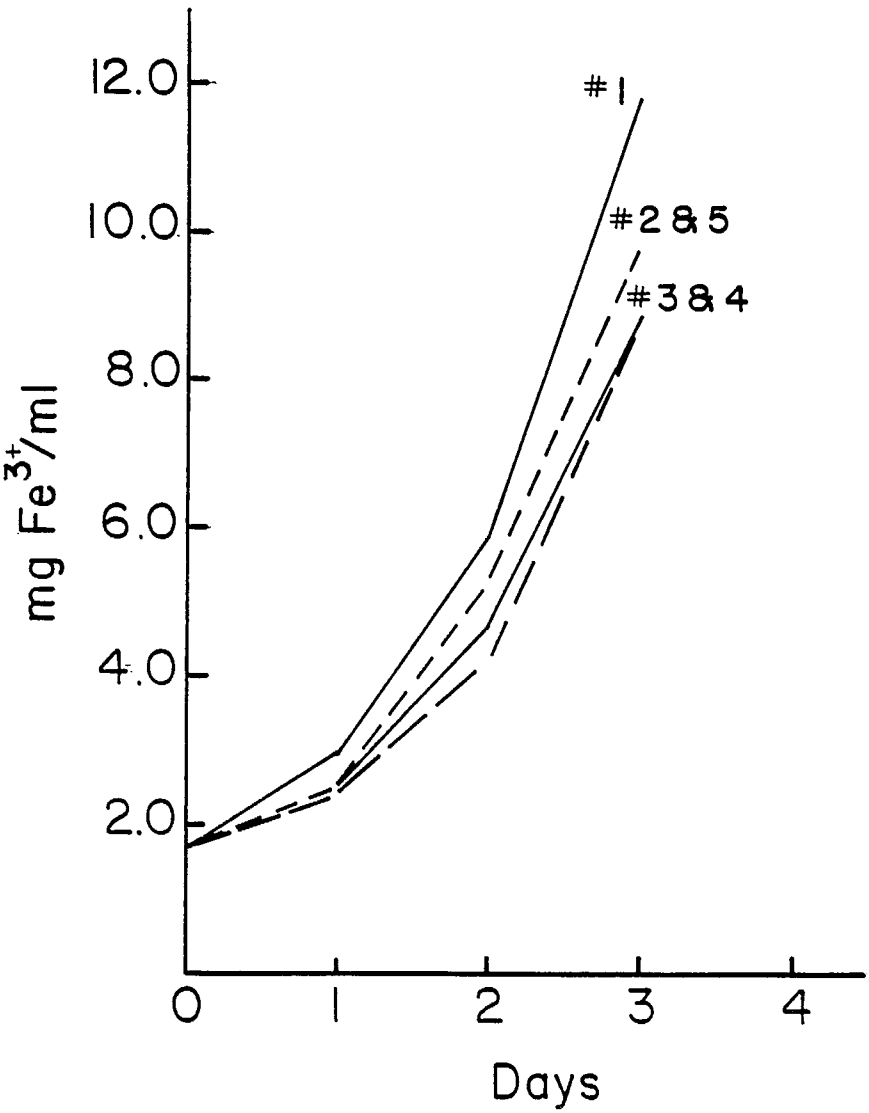


FIG. 3. GROWTH OF DENISON MINE ISOLATES AT 28° ON LOW PHOSPHATE MEDIUM (6)

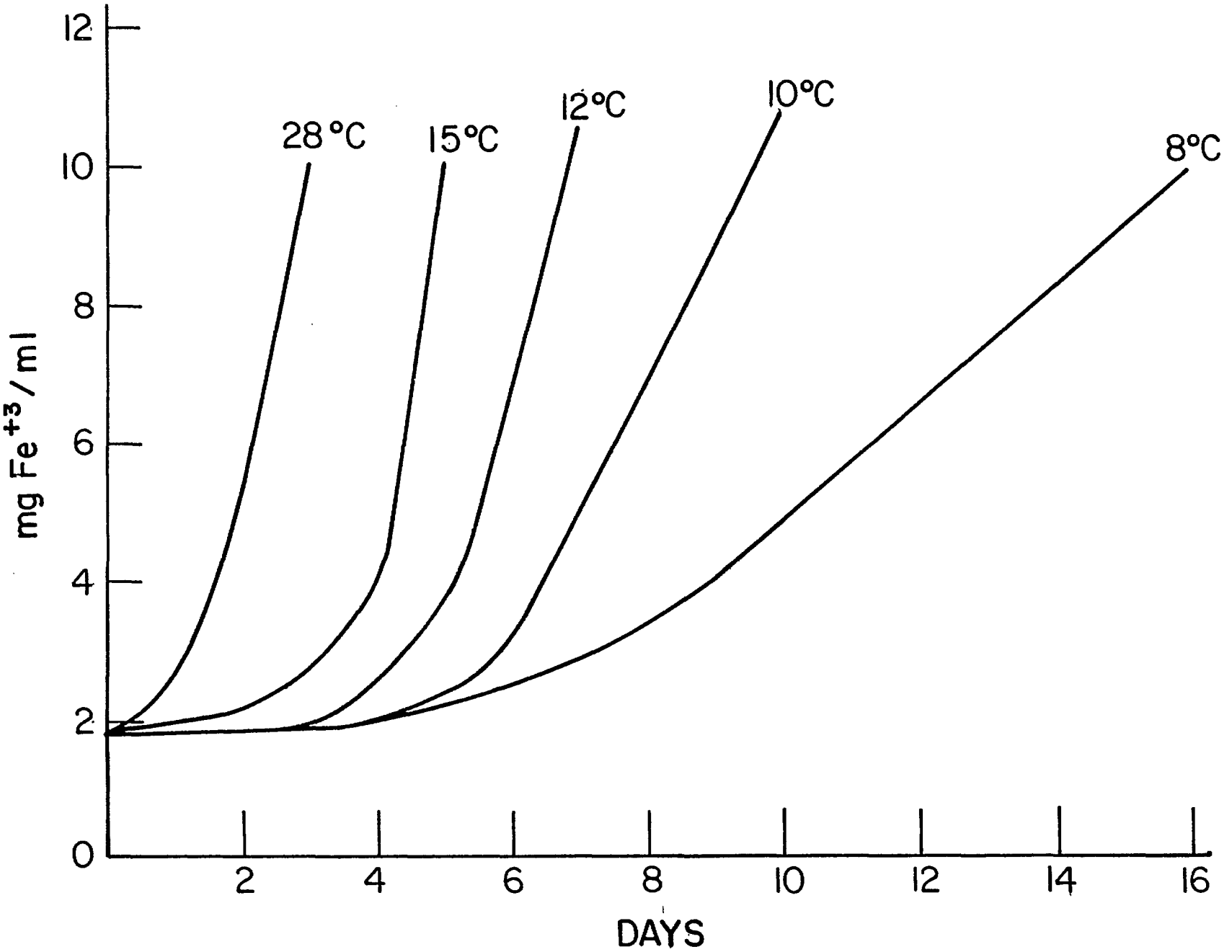


FIG. 4. GROWTH OF T. FERROOXIDANS E.L. #5 AT VARIOUS TEMPERATURES ON LOW PO_4 MEDIUM AT 150 RPM. (AVERAGE OF TRIPLICATE CULTURES) 10 PPM PO_4, 10 PPM MG & 10 PPM NH_4^+ AT PH 2.3

TABLE I

SCREEN AND CHEMICAL ANALYSIS

DENISON TEST STOPE

(0.91 M X 1.22 M BLASTING PATTERN)

SIZE FRACTION	WT (KG)	% WT	U3O8 (%)	U3O8 (G)	U3O8 DISTRIBUTION
+101.6 MM	489	34.50	.033	161.1	20.7
+ 50.8 MM	187	13.22	.039	72.9	9.4
+ 25.4 MM	186	13.16	.055	102.4	13.2
+ 12.7 MM	158	11.14	.066	104.0	13.4
+ 6.4 MM	117	8.28	.079	92.5	11.9
- 6.4 MM	279	19.70	.088	245.3	31.4
TOTAL	14L6	100	.055	778.2	100

TABLE II

LABORATORY COLUMN RESULTS

COLUMN	BLASTING PATTERN	TREATMENT	TIME ELAPSED DAYS	% EXTRACTION
1	0.91 M X 1.22 M	CONTROL	187	22.1
2	"	INOCULATED	187	37.7
3	0.91 M X 0.91 M	INOCULATED	209	51.0
4	"	CONTROL	209	38.7

Table III

Bacterial Nutrient Content of Mine Water Samples in ppm

Sample No.	PO_4^{3+}	Mg^{2+}	NH_4^{+}	NO_2^{-}	NO_3^{-}
30D + 32N discharge	3	15	50	7	170
46078 discharge	9	12	75	11	350
46076 discharge	4	10	50	7	190
Total Mine H_2O	1	15	30	3	170
32881 Sump Discharge	3	7	50	6	162
26305 Sump Discharge	1	8	20	2	95
Average values	3.5	11.2	45.8	6	189.5

Table IV

Composition of the Various Growth Media Tested (ppm)

Medium	(PO_4^{3+})	(NH_4^{+})	(Mg^{2+})	($FeSO_4$)
A	10	10	10	18,000
B	10	10	2	"
C	10	20	1	"
D	10	10	0.5	"
E	10	10	1	"
9 K Standard	235	820	95	24,000

TABLE V

MEAN GENERATION TIMES OF THE NATURAL ISOLATES FOR THE TEMPERATURE RANGE 35 TO 2°C

TEMPERATURE (°C)	NUMBER OF EXPERIMENTS	G (HOURS)	S.D.	S.E.	% E
35	3	19.4	1.41	0.8	4.1
30	4	11.5	2.05	1.0	9.1
25	5	11.7	2.58	0.7	6.0
18	66	22.7	1.59	0.6	2.7
12	35	43.7	6.41	2.4	5.5
6	20	103	29	13	12.6
2	5	247	46	21	8.5

TABLE VI

THE EFFECT OF INOCULUM SIZE ON THE AMOUNT OF U_3O_8 LEACHED AND ON THE RATE OF U_3O_8 LEACHING

INOCULUM SIZE (mL)	% LEACHED	RATE (mg U_3O_8 L^{-1} H^{-1})
0	45	0.9
0.1	48	0.8
1.0	51	1.4
10.0	62	1.9

EACH VALUE IS THE AVERAGE OF TWO REPLICATES.

TABLE VII

Preliminary Leach Results from Production Stopes

Block	Leach Method	Comment	Time Elapsed	% Extraction
46072/73	Flood	Inoculated	125	32.2
46074/75	Flood	Control	135	23.1

TABLE VIII

FUTURE RESEARCH NEEDS

- OPTIMIZE OXYGEN DEMAND PARAMETERS AND SUPPLY SYSTEMS
- IMPROVE WATER BALANCE UTILIZING SORPTION OR MEMBRANE TECHNOLOGIES
- RECYCLE WATER TO MAINTAIN HIGHER TEMPERATURES
- OPTIMIZE NUTRIENT SUPPLY TO LEACHING STOPES
- TEST LEACHING OF CHLORIC ORE BLOCK
- CONTROL FUNGAL GROWTH
- ANALYSE LEACHED STOPE UPON COMPLETION FOR TRUE PARTICLE SIZE AND RESIDUAL URANIUM

BIOADSORBANTS

Energy, Mines and Resources Canada | Énergie, Mines et Ressources Canada

CANMET

Canada Centre for Mineral and Energy Technology | Centre canadien de la technologie des minéraux et de l'énergie

REVIEW OF BIOADSORPTION RESEARCH TO RECOVER URANIUM FROM LEACH SOLUTIONS IN CANADA

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Project: 3.0.4.5.02
Biological Unit Operations

MINERAL SCIENCES LABORATORIES
DIVISION REPORT MSL 86-45 (OP&J) DRAFT

REVIEW OF BIOADSORPTION RESEARCH TO RECOVER URANIUM FROM LEACH SOLUTIONS IN CANADA

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ABSTRACT

Treatment of dilute uranium containing liquors by ion exchange techniques has been practiced for sometime.

Kerr-McGee, United Nuclear, the Homestake Sapin and Eldorado Nuclear Limited have, at various times, evaluated the treatment of mine water by ion exchange techniques to recover the uranium values.

Recently increased attention has been paid to evaluate the potential of biotechnology, by the use of various biomasses, as applied to the mining industry. In Canada, the Canadian Government has initiated various joint Government, Industry and Research organization programs to evaluate such options. The recovery of uranium by biomasses such as Rhizopus, Penicillium and Actinomycetes have been studied. The present paper will address the results of these studies with a comparison of ion exchange, reverse osmosis and

biosorption techniques to recover uranium from mine water produced at a Canadian mine.

Introduction:

In Canada and especially in the Elliot Lake area, there are extensive low grade deposits of uranium. Operators such as Denison Mines have found that underground leaching of the ore is economically attractive, however, the leaching produces vast quantities of low grade (200-500 mg U/L) process solutions which require concentration underground or pumping to the surface for uranium recovery.

Several alternative processes are being considered for uranium concentration underground including ion exchange, reverse osmosis and bioadsorption. Solvent extraction is not a viable alternative due to the low concentration of uranium in the leachate and because of the potential fire hazard due to the volatile nature of the reagents.

Results:

In Canada a variety of organisms have been tested for their ability to adsorb uranium from dilute leach liquors obtained from an underground mining operation. A typical chemical analysis of uranium leach liquor is presented in Table I.

Table 1. Effect of pH adjustment on the ion content of process leach liquors

Ions		As received	pH adjusted to			
		pH 2.0	3.0	3.5	4.0	5.0
Uranium	mg/L	234-224	233	184-256	221	43-127
Iron	mg/L	1056	n.d.	13	n.d.	19
Zinc	mg/L	12	n.d.	8.3	n.d.	9
Copper	mg/L	9	0	2.2	n.d.	1
Sulphate	mg/L	2543	2534	2543	2543	2543

n.d. - not determined

The organisms studied to date include, Rhizopus arrhizus, Saccharomyces cerevisiae, Streptomyces levoris, Chlorella vulgaris, a mixed culture derived from sewage, a Penicillium spp, and a Tritirachium spp.

All of the organisms tested for their bioadsorption capabilities were grown in air-lift fermentors in a continuous fed-batch mode which produced high growth yields with minimal problems.

Stirred tank adsorption tests were utilized to assess the uranium loading behaviour of the various candidate biomasses. Loading rates and equilibrium adsorption tests were carried out.

The bioadsorption capacities of the various biomasses were both pH and temperature dependent. The adsorption maximum for uranium was in the range of pH 3.5-5.0. An increase in temperature from 4°C to 35°C resulted in a 75% increase in the bioadsorption capacity of viable cultures and ≈55% increase in thermally inactivated cultures.

The presence of contaminating ions such as Fe^{+3}, SO_4^{-2}, Co^{+2}, Cu^{+2}, Ni^{+2} and Zn^{+2} greatly reduce the bioadsorption capacity. Fifteen mg of Fe^{+3}/L reduced the uranium adsorption capacity of Strept. levoris by 64%. Similarly, 15 mg/L of

cobalt, copper, nickel or zinc reduced the adsorption capacity for uranium by 31%, 25%, 24% and 26% respectively.

Ions such as Fe^{+3} which compete for the uranium binding sites on the biomass are precipitated from solution by adjusting the pH of the leach liquor from pH 2.0 to pH 3.5 to 5.0. Biomass loading should not be attempted at a pH >5.0 as basic uranyl sulfate may precipitate from solution.

Elution of uranium from the loaded biomass was investigated with potential eluants such as NaCl, Na_2SO_4, $MgCl_2$, Na_2CO_3, $NaHCO_3$, H_2SO_4, EDTA and $(NH_4)_2SO_4$. Although 1-2N H_2SO_4 effectively stripped uranium from the biomass, it also resulted in extensive biomass degradation. Of the eluants tested alkaline carbonate or bicarbonate proved to be the most effective and are preferred over nitrogenous compounds which are environmentally unacceptable.

The data obtained for uranium loading at pH 3.5 and 20°C for various organisms and the stripping efficiency of 0.1M $NaHCO_3$ or Na_2CO_3 are presented in Table 2.

Table 2. Comparison of the bioadsorption of uranium by various organisms

Organism	Loading of uranium mg U/g dry wt at pH 3.5 and 20°C	% efficiency of stripping with 0.1M $NaHCO_3$
Rhizopus arrhizus	42.3	>80
Strept. levoris	62.3	>90
Mixed Culture	49.9	>90
S. cerevisiae	50.8	80
Chlorella vulgaris	28.5	>90
Penicillium spp.	20.5	-
Tritirachium spp.	21.0	>95*

*0.1M Na_2CO_3

The biomass uranium loading capacities of 20-60 mg/g dry weight compare favorably to the uranium loadings observed with ion exchange resins contacted with dilute solutions. Generally, with dilute solutions, the maximum resin loading will be 1-3 g U/L of wet settled resin (w.s.r.).

Based upon the preliminary adsorption and elution results a conceptual flowsheet to recover an impure uranium product is presented in Figure 1.

A preliminary relative cost comparison between stirred-tank biomass contact and elution, underground Ion Exchange Loading and surface stripping and underground reverse osmosis procedures for recovery of uranium are shown in Table 3.

Table 3. Relative cost comparison of ion exchange, bioadsorption and reverse osmosis for uranium recovery

	Ion Exchange	Bioadsorption	Reverse Osmosis
Relative Capital Cost	1.0	2.6	2.1
Relative Operating Cost	1.0	7.0	1.0
% Reduction in Pumping	84%	86%	70%
Overall Relative Operating Costs	1.0	7.0	1.5

As the current economic comparison of the three processes is based on the use of biomass in a stirred tank reactor, a classifier and a centrifuge are required as part of the capital equipment and the energy costs for this equipment are incorporated into the operating costs also. Also, at present, the biomass is assumed to be efficient for 10 adsorbant cycles, therefore the cost of biomass replacement is an added operating cost.

The early assessment of the economics of a biomass adsorption recovery system for the recovery of uranium from dilute leach liquors has indicated the following improvements in the technology are required to make such a system economically competitive with the available ion exchange technology.

1. The useful life of the biomass needs to be increased by immobilization and pelletization in order to develop a system similar to an ion exchange column.

2. Organisms with a higher loading capacity, equivalent to an ion exchange resin, are required.

3. An economical stripping agent must be found, or a system in which the stripping agent ($NaHCO_3$) may be recycled must be developed.

If such improvements can be attained, the use of bioadsorbants should be economically competitive with current ion exchange technology.

References:

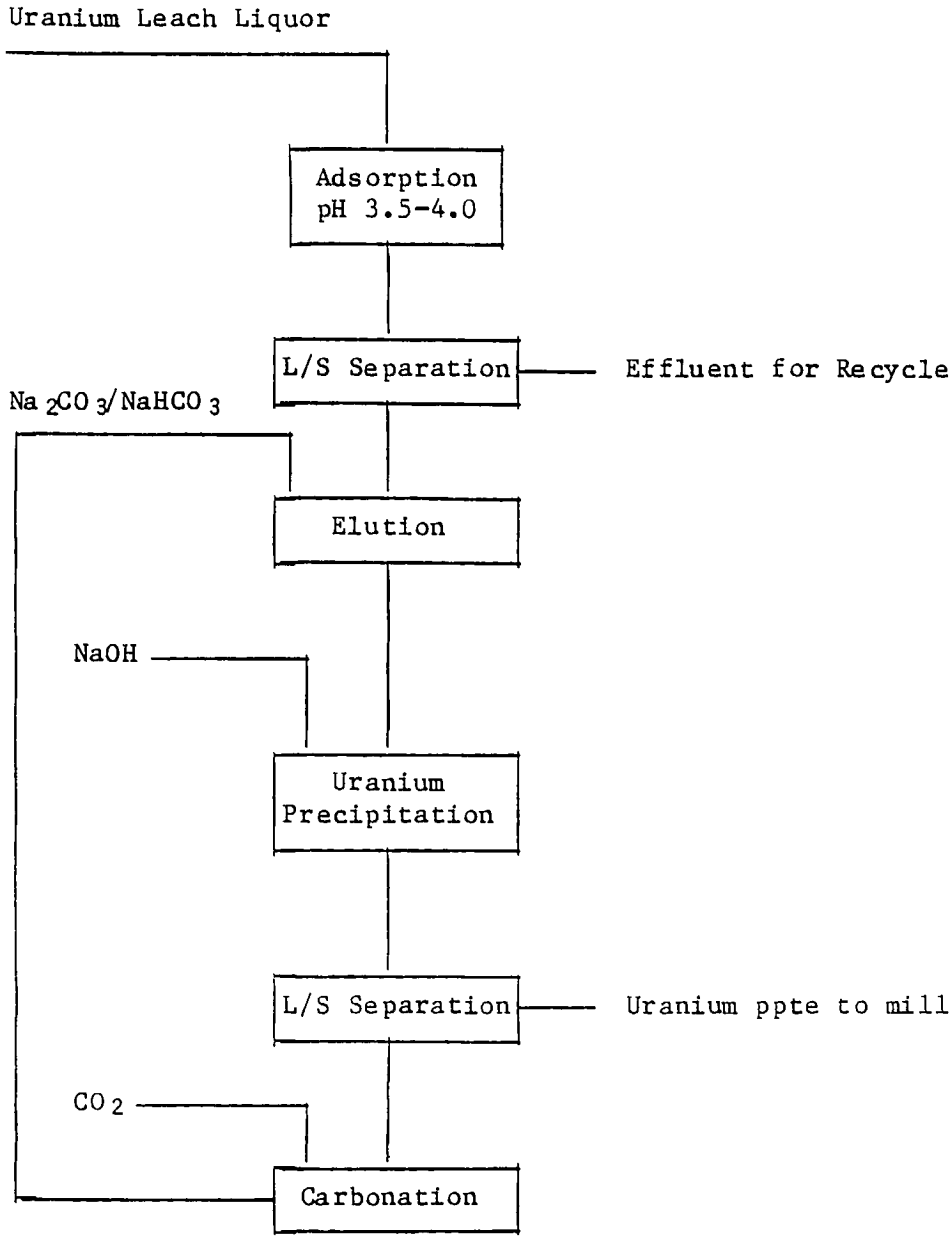
[1] Acres Davy McKee Engineering Inc. 1986. Bioadsorption Capacity of Denison Mines Biomass Isolates. DSS Contract Serial Number; OSQ85-00223.

[2] Melis Consulting Engineers Ltd. 1986. Technical and Economic Comparison of Uranium Recovery from Bioleach Solutions by the use of Biomass, Ion-Exchange and Reverse Osmosis. DSS Contract Serial Number; OSQ85-00159.

[3] Senes Consultants Ltd. 1985. Evaluation of Biomass for Uranium Recovery from Process Streams. DSS Contract Serial Number; OSQ84-00277.

Figure 1

Conceptual Flowsheet to Recover Uranium From Dilute Leach Liquor



SESSION III: PAPER 13

A REVIEW OF BIOADSORPTION TECHNIQUES TO RECOVER HEAVY METALS FROM MINERAL-PROCESSING STREAMS

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ABSTRACT

The processing of ores to recover primary metals generates "waste" streams containing low (ppm) concentrations of either the primary or secondary metals. Concentration techniques, which permit the recovery of valuable primary metals or the removal of trace concentrations of toxic secondary metals from mineral process streams or effluents, will benefit many mining companies.

Various microorganisms, including fungi, bacteria, and algae, have been found to concentrate heavy metals effectively from low-concentration aqueous solutions. In this paper, a number of bioadsorption methods for metal recovery are reviewed, with particular emphasis on the process design aspects of the techniques. Finally, the candidate processes are compared and recommendations are made for future test work in the use of biosorbents as metal extractants from mine process streams.

SESSION III: PRÉSENTATION 13

REVUE DES DIFFÉRENTES TECHNIQUES DE BIOADSORPTION UTILISÉES POUR RÉCUPÉRER LES MÉTAUX LOURDS DES EFFLUENTS PROVENANT DES INSTALLATIONS DE TRAITEMENT MINÉRAL

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RÉSUMÉ

Le traitement des minerais en vue de récupérer les métaux primaires et secondaires génère un écoulement d'eaux résiduelles contenant de faibles concentrations (ppm) soit de métaux primaires ou secondaires. Les techniques de concentration qui permettent de récupérer les métaux primaires précieux et d'éliminer les concentrations trace de métaux secondaires toxiques des eaux usées ou effluents provenant des installations de traitement de minerais seront utiles à plusieurs entreprises.

Des micro-organismes divers y compris les champignons, les cultures bactériennes et les algues sont utilisées efficacement pour la concentration des métaux lourds à partir de solutions aqueuses faiblement concentrées. Dans ce document, l'auteur présente un certain nombre de méthodes de biadsorption pour la récupération des métaux en accordant une attention particulière à l'élaboration des procédés de traitement. Enfin, il examine et compare des schémas de traitement hypothétiques et fait des recommandations concernant le travaux de recherche futurs portant sur l'utilisation de biosorbants en tant qu'agents d'extraction du métal se trouvant dans les eaux usées en provenance des installations de traitement.

A REVIEW OF BIOADSORPTION TECHNIQUES TO RECOVER HEAVY METALS FROM MINERAL-PROCESSING STREAMS

OVERVIEW

This paper presents a summary of a two-phase study sponsored by CANMET to assess bioadsorption technology in the mining industry. The first phase is a general overview of bioadsorption technology, and the second phase is a specific laboratory testing and evaluation program.

PHASE 1 - LITERATURE INVESTIGATION

Introduction

The processing of ores to recover primary metals generates "waste" streams containing low (ppm) concentrations of either the primary or secondary metals. The daily volumes of such wastes are usually high. The elements are commonly heavy metals such as copper, lead, zinc, cadmium, molybdenum, arsenic, mercury, uranium, radium, thorium, gold, or silver. At the trace concentrations involved, it is not economical to recover primary metals by conventional extraction procedures. However, these elements may represent an environmental hazard, so waste treatment (often expensive) may be required prior to discharge.

Concentration techniques, which permit the recovery of valuable primary metals or the removal of trace concentrations of toxic secondary metals from mineral process streams or effluents, will provide a significant benefit to many mining companies. Biological systems are potential candidates.

Various microorganisms and plants have been shown to concentrate heavy metals effectively from aqueous solutions containing low concentrations of the element in question. Much of the interest in this phenomenon has centred on the potential use of such species as biological monitors of heavy metal pollution. However, from these studies has come the realization that such biological agents can selectively concentrate heavy metals from aqueous solutions by factors as high as several thousandfold. Many species of bacteria, fungi, algae, and aqueous plants have been tested for their ability to concentrate heavy metals.

In this paper, focus will be placed on the process design aspects of the various bioadsorption processes for metal recovery described to date. The technical and economic factors of each system will be evaluated for their suitability to the Canadian mining environment.

This is important to note since many of the bioadsorption processes studied to date have involved relatively clean and well-defined solutions, rather than the variable streams prevalent in mining operations.

Key points in evaluating bioadsorption techniques for the recovery of heavy metals in mining processes include:

- availability of biosorbent;
- reproducibility of biosorbent, especially if it is not produced from a defined microbial culture;
- specificity of method to particular metal;
- reusability of biomass;
- capacity and efficiency of process;
- nutrient costs;
- adaptability of method to existing waste treatment equipment;
- sensitivity of process to operating parameters;
- sensitivity of method to other cations and anions in process streams;
- economic feasibility of more sophisticated bioadsorption processes;
- regeneration of biosorbent.

The assessment of bioadsorption processes requires some understanding of the mechanisms by which microorganisms accumulate and concentrate heavy metals from their immediate environment. A brief overview of the mechanisms of uptake of heavy metals by microbes is warranted.

The Concentration of Metals from Solution by Microorganisms

The ability of certain bacteria, fungi, and algae to accumulate and concentrate metals has long been recognized. Much of the research focuses on the potential use of these organisms as indicators of heavy metal pollution in the environment, where they have been shown to accumulate metal contents as high as 36-40% by dry cell weight. However, the mechanisms enabling these organisms to survive and grow in the presence of metal concentrations, which are toxic to other organisms, have also been studied. Significant variations are noted between the metal uptake ability of different genera, different species, and also different strains within a species.

The uptake of metals by microorganisms is a two-stage process. The first phase occurs very rapidly in most organisms (within 5-30 min of cell contact with the metal-containing solution) and involves the "passive" physiochemical adsorption/retention of metal cations and anions by the constituents of the cell wall. This "passive" process we define as bioadsorption. The second phase involves the "active" physiological absorption of metals via energy-dependent metabolic processes. This latter phase, identified as bioaccumulation, is dependent on the tolerance of the specific organism to relatively high concentrations of "toxic" elements in the intracellular cytoplasm and other subcellular components.

Evaluation of Bioadsorption Processes

The bioadsorption processes evaluated for use in recovering heavy metals from mineral-processing streams may be classified in terms of the nature of the biosorbent, as follows:

- activated sludge
- defined bacterial cultures

- fungi and yeasts
- algae
- microbial derivatives.

There is some overlap of information for these processes, but specific attributes of each type of adsorbent warrant a separate assessment.

At this point, it is necessary to distinguish between bioadsorption and bioaccumulation since this important aspect of process design has been poorly described by many researchers. The two processes have often been combined into the single heading of uptake because the predominant mechanism in effect is not known.

Bioaccumulation, which is the predominant uptake mechanism in many organisms, is less desirable in process design because it requires the cells to be actively metabolizing, and the poor release of heavy metal from the intracellular matrix makes it difficult to reuse the biosorbent.

Bioadsorption, on the other hand, involves the chelation of metals on the cell surface. The uptake is generally rapid, as well as it being easier to recover the metal(s) and reuse the biosorbent. These are important criteria for process design.

Two basic alternatives are available for bioadsorption processes. One approach involves a single-stage procedure, with actively growing, metal-tolerant organisms in the ore-processing stream, fortified with appropriate nutrients. The second approach involves producing the biomass in a separate reactor, and then bringing the isolated microbial biomass into contact with the process liquor in a controlled environment, i.e., a two-stage concept. The biomass may be used as produced; deactivated before use, then treated to improve physical characteristics (e.g., immobilization); or extracted to yield purified components with high complexing ability.

The use of actively growing culture (i.e., combined bioadsorption/bioaccumulation) has hitherto been more compatible with processes based on large storage systems or lagoons exposed to the natural elements. This approach has many limitations. Expensive storage facilities and lack of space at mine sites make it uneconomical. More compact systems that can be operated in controlled environments, with high adsorption of metals and short residence times by biosorbents, are preferable.

The attractive feature of bioadsorption is a certain specificity of the biosorbent for divalent and multivalent heavy metal cations. As noted earlier, the metal uptake may vary widely for different mutant strains within a species and between different genera. The nutrient status of the organism, the age of the cells, the temperature, pH, etc., are all important parameters affecting the performance of a biosorbent. An additional factor is whether enough biomass can be generated at an economical cost to meet the needs of a process.

Activated Sludge Systems

For the purpose of this study, activated sludge (AS) is defined as a biosorbent consisting of a naturally selected, undefined, mixed culture of microorganisms. In addition to bacteria, the biological constituents include algae, fungi, ciliated protozoa, rotifers, and nematodes.

At first glance, AS systems, which are applied primarily to the reduction of the organic content of wastes, do not seem to apply to the mining industry. However, their ability to remove heavy metals from municipal sewage has long been recognized, and AS systems are probably the best characterized of any of the bioadsorption processes. Most of the developments using pure cultures of microorganisms and isolated microbial polymers are, as discussed later, based on the behaviour of microorganisms in AS processes.

Data generated in municipal waste treatment plants have sparked much of the interest in the mechanisms of metal removal by AS systems. A number of studies using synthetic media have been conducted to determine the adsorption of a variety of metals by activated sludge. In general, the data indicate that this method is useful for the uptake of metals such as mercury, cadmium, lead, copper, cobalt, radium, and to a lesser degree zinc, nickel, uranium, and thorium.

Adsorption efficiencies of over 90% have been achieved for cadmium, copper, lead, and mercury. However, it is estimated that as much as 20% of the metal removal in these waste treatment systems is due to the filtration of metal particulates. Depending on the metal in question, actual metal recovery due to AS activity is about 10-80% (1).

Metal uptake by activated sludge was found to be affected significantly by pH (1) and age of the cells. In general, younger cultures were found to be more active adsorbents than older ones. Moderate temperature variations and deactivation of the sludge by heat sterilization did not significantly affect AS activity.

Bioadsorption by AS is a rapid process, generally complete within 10 min. The speed of the reaction indicates that the principal mechanism is "passive" bioadsorption.

As stated earlier, the testwork conducted to date has involved synthetic media. No processes using activated sludge to adsorb metals from ore-processing streams have been identified. However, this approach or its refinement using defined microbial species (discussed in the following section) warrants further investigation.

The following are some of the attractive features of this biosorbent:

- Bioadsorption, rather than bioaccumulation, is the predominant mechanism for the uptake of heavy metals such as mercury, lead, cadmium, copper, and cobalt by activated sludge.
- The bioadsorption is a rapid process.
- Deactivation of the sludge is not necessary.

- The natural selection process of activated sludge systems leads to a predominance of "hardy" strains of organisms.
- The organisms do not require a highly defined culture medium, e.g., sewage from the mining community would serve as one nutrient source.

However, before larger scale applications of AS to the removal of metals from ore process streams are considered, additional information is required, including:

- adaptation/acclimation of activated sludge culture;
- evaluation and optimization of the bioadsorption efficiency/kinetics with process liquor, e.g., effect of pH, temperature, contact time, age of biomass, nutrient requirements, effect of competing cations/anions, effect of chelating agents in the ore process liquor;
- evaluation of the efficiency of biomass recovery (filtration, sedimentation, particle size, etc.);
- evaluation of procedures to recover metals from biomass;
- adsorption capacity of regenerated biomass as well as physical characteristics.

The information in the literature indicates that bacteria, which have capsules or slime layers or which liberate polymers into the surrounding solution, are the more effective biosorbents. They tend to grow attached to the surface of reactors. If metal-laden sludge can be regenerated, the preferred process would be the two-stage one, incorporating a separate reactor to produce the biosorbent.

Defined Bacterial Cultures

This concept is a refinement of the activated sludge process. The major goal is to isolate bacterial species with enhanced capacities to adsorb heavy metals. The most successful sources for such organisms, to date, have been either activated sludge or metal-polluted soils, in which exposure to toxic metals has already induced a natural selection process favouring metal-tolerant strains.

With the exception of one organism, Zooglea ramigera 115 (2), test data have not been published on the behaviour of metal-accumulating organisms in liquors of similar composition to that of mine process streams. Most of the studies to date have used highly defined culture media that are not relevant to the mining situation. Many bacteria and fungi have been screened for their uptake of specific metals, e.g., Cu, Pb, and Cd (3,4,5,6). Bioaccumulation, however, appears to be the predominant mechanism of uptake.

Dugan and Pickrum (2) tested the ability of Z. ramigera 115 to remove cations and anions from several mine waters (pH 3.0). An estimated 25-33% of the cations and 25% of the sulphate was adsorbed by the biomass. As no data were presented for the adsorption of heavy metal cations, the results are inconclusive.

Data from investigations using synthetic heavy metal mixtures may be summarized as follows:

- Iron, cadmium, and mercury were well adsorbed by Z. ramigera 115 (2).
- Sphaerotilus natans was found to adsorb iron, cadmium, copper, and cobalt (19).
- Cadmium was accumulated by Citrobacter sp. (6).
- Lead was concentrated by Azotobacter sp. (7).
- Pseudomonas aeruginosa was found to adsorb uranium very rapidly (complete within one minute) from solution (8,9). However, the recovery of metal from biomass was not efficient.

Until more complete data are available on the rate of uptake of the respective elements, the adsorption isotherms, the rate of production of biomass relative to the total metal adsorption capacity, and the performance of these organisms in more realistic test conditions, it would not be justified to use such organisms directly in mill process streams. The approach of most research groups has been to identify candidate organisms for further laboratory investigations into their usefulness in future bioadsorption processes.

In terms of process design, defined bacterial cultures have the same limitations as outlined for activated sludge.

Fungi and Yeasts

Although both yeasts and fungi have been shown to adsorb metals from solution, from the standpoint of process design only fungi deserve serious consideration. Fungi have the important advantage over other biosorbents in that metals, particularly uranium, can be readily desorbed from the biomass by dilute alkali carbonates. This is a key factor in the design of process systems.

Investigations have shown that uranium, lead, cadmium, and a number of other heavy metals can be adsorbed effectively by spent fungal mycelium.

One of the most promising technologies for the bioadsorption of uranium and other radionuclides is the use of spent fungal mycelia from the fermentation industry (18). The potential exists for the use of deactivated fungal mycelium as an ion exchange material able to be reused many times. At this stage, however, the data on the interaction of heavy metals with mycelia of different fungal species have not been well enough documented to permit direct application to mine process streams.

The growth rate for fungi is generally lower than bacteria. Culture requirements and nutrients are also more fastidious. This poses the question of whether or not adequate amounts of biomass would be available. The physical handling characteristics will also be important but, if the biosorbent can be reused, the supply of biomass becomes less significant. At present, reuse would be limited to elements such as uranium and, possibly, radium.

Algae

Algae concentrate metals from the natural environment and show impressive accumulation factors. Algal cells have been found to adsorb cadmium, copper, and other cations.

Algae have been proposed as bioadsorbents to remove heavy metals from wastewater (10). The most successful applications have involved tailings ponds and meander systems (11), and this combination serves to overcome the major limitation of inadequate light intensity. Shallow water systems, large surface areas, adequate light intensity, and a suitable nutrient source will foster the growth of algal blooms (11). However, due to the large pond area required by the process and the limited yield of algal cultures, the feasibility of using growing algae for direct treatment of processing streams is poor.

Reuse of algal biomass is key to the application of algae to remove heavy metals from large daily volumes of mine-processing liquids. The use of algal derivatives (next section) may improve its value. At this time, however, an economical and practical process cannot be designed using this biosorbent.

Microbial Derivatives

This section will cover two types of products: immobilized cells and metal-chelating compounds (e.g., gelatinous polymers, polysaccharides) extracted from microbial cultures. The common goal of these separate approaches is to prepare a reusable chemical product, of natural origin, with a higher metal-complexing ability than crude biomass preparations.

Immobilized Cells

Bacterial and algal cells have been immobilized in gel supports such as toluene diisocyanate, glutaraldehyde, polyacrylamide, agar, cellulose, and alginate. Most investigations into heavy metal uptake by immobilized cells have been conducted using polyacrylamide because of its prior commercial use in other immobilized systems. However, Nakajima et al. (20) showed that toluene diisocyanate and glutaraldehyde systems gave the highest uranium adsorption.

Most investigations to date have concentrated on the adsorption of uranium by immobilized cells. However, lead has also been found to have limited adsorption characteristics (5). Data on desorption methods are limited.

A variant of the immobilization approach is the use of chemically stiffened fungal mycelium. This type of biosorbent has been patented for use in the recovery of uranium and radionuclides (12). However, there are indications that the adsorbent will also remove lead. No data on desorption techniques are available for this method.

At present, inadequate data are available to design a system that uses these advance bioadsorbents. With free cells/mycelia, major difficulties in a

large-scale system will be encountered in the production and physical recovery of the biomass. Costs of producing the biosorbent may be significant. However, immobilization of cells by entrapment or stiffening is a realistic approach to improving the physical characteristics for use in column reactors and the economics of reusability.

Process factors, which must be defined for this concept before it can be used in mining environments, include:

- the conditions/reagents needed to desorb heavy metals (other than uranium);
- the effect of desorbing agents on the physical characteristics of the adsorbent;
- the stability of the immobilized biosorbents in the presence of high ionic strength solutions and organic compounds/solvents liable to be encountered in mineral-processing streams;
- the effects of temperature and pH variations on the biosorbent;
- the effect of immobilization on the metal adsorption capacity and the kinetics of adsorption;
- the economics of the immobilization procedures.

Microbial Polymers

It has been demonstrated that microbial polymers (usually anionic or cellulosic-type polysaccharides) can efficiently bind heavy metals in synthetic solutions (13,14,2,15). Significant proportions of these complexes remain water-soluble.

A number of microbial polymers have been proposed to recover metals from mine drainage. Cell flocs of Z. ramigera 115 have been found to recover metals from acid mine water (2,16,17), and it is reported that the metals can be recovered from the gel by acid extraction. No studies were conducted on the extracted polymers of these compounds.

Other natural polyelectrolytes have also been proposed for heavy metal removal. However, all of these compounds are very specialized, as well as very limited in supply or expensive to produce.

The use of natural polyelectrolytes from organisms is an advanced process. As with immobilized derivatives, considerable information must still be obtained before these compounds can be used in actual process streams. No data have been published on the adsorption characteristics of these polymers in complex mine-processing liquors or in competition with other cations, e.g., Ca, Mg.

At present, the most practical way to use these polyelectrolytes is in natural association with the cells. However, there is considerable scope for future development. The isolation and culture of microbial strains that produce higher yields of polyelectrolytes, or improvements in technology to prepare polyelectrolytes for use in mining processes, are two areas for development. However, considerable research effort is necessary.

Conclusions

In the current literature, no bioadsorption process or concept has been identified for which adequate data are available to permit the direct design of a system to treat mine process streams. The limitations of available data include:

- lack of information on biosorbent activity in complex mixtures such as actual mine process streams;
- limited data on metal desorption characteristics and biomass regeneration;
- lack of research on the supply or generation of adequate amounts of biomass, and the nutrient requirements;
- no available data on process schematics and mass balance calculations.

These factors all significantly influence the likelihood that bioadsorption processes can be developed to the level of economically attractive routes to recover low concentrations of metals in mine process liquids.

Of the processes reviewed, algae appear to have limited potential in the Canadian mining environment. The economical generation of adequate amounts of algal biomass is an obvious practical limitation. The use of immobilized algal systems, however, may bypass this limitation.

Based on available information, the most promising target metal for further bioadsorption studies is uranium. This selection is influenced strongly by the efficient regeneration procedures noted for this element with several biosorbents (an important criterion for process design).

From this review, the various processes may be ranked in the following manner for their ability to recover uranium:

- fungal mycelium
- microbial derivatives (immobilized cells)
- activated sludge
- yeasts or defined microbial cultures
- algae.

Fungal mycelium have been found to have a high capacity for uranium in aqueous solutions. In addition, simple recovery of the metal from the biomass may be achieved by washing with dilute carbonate salts. Both these attributes are important in designing the bioadsorption process.

For the recovery of metals other than uranium, the process types are ranked in the following order of effectiveness:

- defined bacteria cultures
- activated sludge
- microbial derivatives
- fungi and algae.

The reversal of the ranking, compared with that for uranium, indicates that fungal mycelium may not be feasible for use as a general biosorbent.

The selection of the type of bioadsorption method to be used in mine process waters will probably be site-specific. Several methods have been identified to adsorb a number of heavy metals from solutions. However, the biosorbents can only be evaluated for performance based on the composition of the actual process liquids, so each case must be considered individually. No one method can be applied to all mining situations.

PHASE II - LABORATORY STUDY AND EVALUATION

Introduction

Five biomasses - Rhizopus arrhizus, Streptomyces levoris, a mixed culture sewage sludge, Saccharomyces cerevisiae, and Chlorella vulgaris - were evaluated for potential utilization in a uranium recovery process. These laboratory studies were conducted by the Institute of Biotechnology at the University of Waterloo. The major difference between these studies and many previous investigations was the use of a process-mining solution rather than a synthetic pure solution. This resulted in a major difference in bioadsorption rates and capacity.

Summary of Laboratory Findings

The principal findings of the laboratory study are highlighted below.

Biomass growth rates and growth yields

- The fungus Rhizopus arrhizus and the yeast Saccharomyces cerevisiae, which are very simple to cultivate in axenic culture, produce high growth yields. Rhizopus arrhizus is harvested with ease.
- Streptomyces levoris is also simple to cultivate, but requires a more complex growth medium.
- Chlorella vulgaris is an alga that possesses very low specific growth rate and low cell yield. As such, it is difficult to produce in large quantities and is subject to contamination. These aspects were troublesome in this study.
- Air-lift fermentation in a continuous fed-batch mode, which produces high growth yields with minimal problems, would be an excellent method for large-scale biomass production.

Uranium adsorption

- Rhizopus arrhizus, Streptomyces levoris, and the mixed culture proved to be effective biomasses for uranium adsorption. The data in this study suggest that Rhizopus oligosporous may be equivalent or superior to Rhizopus arrhizus.
- There may be many other organisms that would perform equally well as the organisms tested in this study.

- Immobilization of cultures, coupled with the retention of high bioadsorption capacity, is difficult to achieve and many failures can be expected. This area needs further work.
- Bioadsorption capacity is a strong function of pH. Optimum levels that maximize both adsorption capacity and uranium concentration in process solutions would be in the 3.5 to 4 pH range.
- Thermal inactivation and cell age have only a marginal effect on equilibrium bioadsorption capacity. However, thermal inactivation results in a doubling of the kinetic rate constant for microbes with mycelial growth habits and for sewage flocs. Unicellular microbes such as Chlorella do not appear to be affected.
- Presence of contaminants such as iron, sulphate, and heavy metals can greatly reduce bioadsorption capacity. For example, 15 mg/L of ferric iron added to synthetic solutions resulted in a 64% reduction in Streptomyces levoris bioadsorption capacity. Similarly, 15 mg/L of cobalt, copper, nickel, and zinc resulted in 31%, 25%, 24%, and 26% reductions in bioadsorption capacity, respectively.
- Sulphate levels in process liquor give rise to sulphato-complexes that are not adsorbed by any biomass.
- The adsorption temperature had a marked effect on both viable and thermally inactivated cultures. An increase in temperatures from 4°C to 35°C resulted in a 75% increase in bioadsorption capacity for viable cultures, and a 55% increase for inactivated cultures.
- Contact time is an important variable, especially at lower pH values.
- Process liquor (mine water) is not suited for direct uranium recovery by biomass. The pH level at 2.0 appears to degrade the cell structure, with the associated high contaminant levels (Fe^{3+}, for example) resulting in a marked reduction in bioadsorption capacity.
- Bioadsorption data from process liquors are not amenable to direct comparison with published data on synthetic solutions.

Uranium desorption

- $NaHCO_3$ is a simple and effective stripping agent for all biomasses tested.
- Repeated adsorption/desorption cycles do not greatly affect biomass loading or desorption capacity.
- Weak sulphuric acid was marginally effective as a stripping agent, while sodium EDTA and ammonium sulphate were ineffective.

Mechanisms

- Simple sorption isotherms, such as the Freundlich and Langmuir isotherm, describe the experimental data adequately.
- A kinetic model has been proposed to describe the time-dependent change of the uranium adsorption capacity. The model predictions compare favourably with experimental observations.

General comments

- Data on uranium bioadsorption must be interpreted cautiously. Factors such as temperature, biomass concentration, cell physiology, uranium content in test solutions, contaminant levels, pH, etc., can greatly distort results. Casual observations can lead to erroneous interpretations of the data.
- Although uranium bioadsorption appears to have a good potential for application with process solutions, this study has not delineated all pertinent factors for optimal design.

Potential Process Applications

General

Biomasses are not likely to replace conventional uranium concentration/purification processes, such as ion exchange or solvent extraction. These latter processes are highly efficient, well-developed, and very cost-effective in uranium mill applications. However, these processes are somewhat sophisticated and require rigourous operator attention.

Biomass applications are likely to be directed at low-grade solutions where minimal process control is possible. In addition, where biomass costs are low, one-time applications are certainly a possibility. Perhaps the most likely applications deal with the recovery of uranium from wastewater or bioleach solutions. The primary examples would be:

- acid mine waters
- acid tailings runoff
- underground leaching solutions.

A discussion of these potential applications follows.

Acid mine water

A typical acid mine water was described in the Phase II study as having a uranium content of 127 mg/L and a pH of 2.7. At these pH and uranium levels, most biomasses would have an effective net adsorption capacity of at least 20 mg U/g and, as such, 43 kg of biomass would be required per kg of uranium (U_3O_8) recovered. Biomass purchase cost ranges from nil for waste biomass, such as sewage sludge or brewery waste, to several dollars per kg.

Obviously, if the biomass could be obtained at no cost, one-time utilization and disposal of the biomass is possible if minimal alteration to the as-received biomass is required before use. However, if the biomass is purchased, it must be capable of repetitive use.

For one-time application, we foresee a material such as an inactivated, thickened sewage sludge applied to the inlet of underground sumps in the mine, and allowed to contact with mine-water solutions. Because of the long contact times, the biomass may reach substantially higher bioadsorption levels. Mine

water for use in underground leaching would be recycled from the sumps. As the sumps become full, the fluid-like biomass would be pumped to the surface with the mine water (at about 1% solids). Residual biomass would be mucked out with the sump sludges during routine sump clean-outs. Given a residual mine-water concentration of 100 mg U/L for every 100 000 L of solution with 1% biomass, one would get 11.7 kg of uranium (U_3O_8) in the water, and 23.8 kg of uranium in the biomass. On the surface, the biomass would be eluted with bicarbonate or a strong acid solution, discarded and/or recycled, and the soluble uranium sent to the mill for recovery.

The net effect would be:

- mine waters would be recycled underground
- effective uranium concentration in mine water pumped to the surface is tripled, i.e., three times more uranium goes to the surface in the same volume of mine water.

The major problem would be getting an adequate supply of biomass. For example, a typical municipal sewage plant would produce .5 kg of biomass per kg of BOD_5 applied. A community of 20 000 people could only produce 1000 kg of biomass per day. This would only support the production of 23 kg of U_3O_8 per day. In addition, the sludge would require thickening before pumping underground (10% solids would be reasonable). Using a centrifuge or vacuum filter, it should be possible to thicken and recycle the sludge. For a 10-cycle operation, this would increase the potential production to 230 kg/d or 84 000 kg/a. Total pumping capacity required at 1% solids to support this level of production would be 1 000 000 L/d. Obviously, if the adsorption capacity could be improved, much greater levels of uranium could be produced.

Acid tailings runoff

Acid runoff and seepages from uranium tailings areas contain elevated levels of uranium; however, these levels are in the range of <1-5 mg U/L with pH levels in the 2.2-3.5 range. These runoff and seepage streams require continuous long-term treatment at a substantial cost. With the low uranium values in solution, conventional recovery technologies might be applicable, but costs would be prohibitive. In these situations, biomass could prove to be effective for uranium recovery. The uranium recovered could offset the cost of treatment, with spent biomass being used as a surface soil conditioner for the tailings. This would provide:

- potential revenue from uranium
- a method for disposing of sewage sludge
- improved conditions for tailings area revegetation and rehabilitation.

For this application, sludges could be added upstream of the sedimentation pond, then allowed to settle and contact with the seepage. Once or twice per year, the sludge would be recovered, eluted for its uranium content, and the waste material disposed on the tailings surface.

Bioleaching solutions

One potential application is the recovery of uranium from heap-leaching or underground leaching operations. These operations produce a uranium content typically in the range of 100 to 300 mg/L. For a heap-leaching operation on the surface, ion exchange or solvent extraction technology would likely be the simplest operation, but underground recovery with biomass certainly has some potential. Again, the driving force would be to have an inexpensive adsorber that does not require major process controls. The bioleach process solutions tested in this study had a very high acid content, at pH of 2.0 and a uranium level of approximately 250 mg/L. At the low pH, the biomass was degraded. It will therefore be necessary to perform some pre-treatment to adjust the pH upwards prior to adsorption. This pre-treatment may also have some beneficial side effects, since under the current scheme the leaching solutions are too strong and can actually inhibit bacterial activity.

For this type of application, either a system similar to that discussed for the mine water would be employed, or possibly a contacting system using columns could also be considered.

Again, the benefits are:

- direct underground recycle of leach water
- higher production levels for the same quantity of mine water pumped.

Evaluation of Biomass Solids Contact/Separation Equipment

General

There are two basic types of contacting equipment: stirred reactors and columns. Both have potential application for uranium bioadsorption. Devices for the separation of solids include thickeners, filters, and centrifuges. Given the difficulty in dewatering many biomasses, the thickener and centrifuge systems are probably most applicable; however, filters can, and have, been used. The centrifuge is probably most applicable when wet recycle of the biomass is required.

In order to demonstrate potential requirements for contacting/separation for a uranium recovery system, two sample flowsheets have been developed. The basic design criteria for loading capacity, elution requirement, etc., have been derived from the laboratory testwork. The process solution is mine leach water at 250 mg/L of uranium and pH adjusted to the 2.5-3.5 range.

This section also includes an overall assessment of a range of other contact/separation devices and how amenable these devices are for each biomass tested.

Sample Biomass Recovery Flowsheets

The sample flowsheets were developed by A.H. Ross and Associates.

Contacting System

The size of the equipment necessary to remove soluble uranium from typical process mine water has been estimated for stirred tanks and gravity downflow column systems. The design parameters are considered reasonably conservative but require confirmation.

Stirred tank system

Design

The flowchart is shown in Figure 1. Mine water and biomass are contacted in a series of three stirred tanks for a total contact time of 30 min. Sufficient biomass is introduced to reduce the uranium content of the water by an average of 0.1 kg/m^3.

The water/biomass pulp gravitates from the final tank to a gravity clarifier. The water overflowing at the top of the vessel is recycled to a leaching stope. The solids settle to the bottom of the clarifier, then are raked to the centre to be pumped out as a 3% solids pulp. Typically, such clarifiers are provided with a deep centre well and with a thick bed of suspended solids that filter the upflowing solution. Progressing cavity pumps are used on biomass pulps to avoid physical degradation of the material. Flocculants are generally not used during clarifications, but they could prove beneficial in improving underflow densities.

The thickened biomass is directed to a solid bowl centrifuge. The extracted water is returned to the clarifier, and the biomass, now at 20% solids, is directed to the elution tanks. Uranium is eluted from the biomass with a 0.1 Molar (8.4 kg/m^3) solution of sodium bicarbonate. Contact time of fifteen minutes is provided to transfer uranium from the biomass into the solution.

A minor amount of bicarbonate is destroyed by the acid in the water introduced with the biomass. If proved advantageous, this amount could be reduced by repulping the solids with clean water and separating in a second centrifuge.

Pulp that overflows the second elution vessel is pumped to another centrifuge. It would be located above the contactors so that a screw conveyor could assist in returning the thickened biomass to the contactors. The centrate is directed to a mine sump and pumped to the surface.

The amount of uranium returning with the recycled biomass to the contactor could be reduced by simply repulping the biomass with water and centrifuging the pulp in a third machine. The consumption of sodium bicarbonate would thereby be reduced.

Comments

1. The system appears simple, the equipment of familiar type and size, and the operations straightforward.

2. Replacement of biomass with fresh material is readily accomplished.
3. The sodium bicarbonate consumption depends upon a sufficient per cent solids in the biomass discharge from centrifuges. The moisture in the material may vary with the age and condition of the biomass.

Equipment list

Contactor tanks	3	2.0 m diam x 2.4 m, agitated
Clarifier	1	8 m diam
Slurry pump	2	Moyno type, variable feed drive
Centrifuge	2	420 mm diam x 1675 mm, 37.5 kW
Elution tanks	3	1.0 m x 1.4 m, agitated
Bicarbonate mix tank	2	4.0 m diam x 4 m, agitated
Bicarbonate feed pump	1	centrifugal
Screw conveyor	2	225 mm diam x 3 m
Estimated sodium bicarbonate		11 kg/kg U 1040 kg/d
Estimated connected power		105 kW

Capital cost

A rough estimate of the capital cost would be $1 000 000.

Column system

Design

The flowsheet is shown in Figure 2. Mine water and biomass are contacted by passing the water, under gravity, through a bed of biomass. After the passage of a measured amount, the mine water is stopped and displaced from the bed with a flow of fresh water. The fresh water is followed by a sodium bicarbonate elution and a second water wash. The water washes reduce contact between the acidic mine water and bicarbonate solution, avoiding disruption of the bed that would be caused by the release of carbon dioxide.

The rate of water percolation through the bed, the low, and a large bed area is required to pass the design flow. Mine water would be sprinkled into a shallow pool on the top of the bed. Pressure on the bed is avoided in order not to compact the bed and thereby restrict the flow.

Treated mine water is collected with a bed support system at the bottom and recirculated to leaching areas. Outlet eluant is directed to mine sumps for pumping to the surface.

A spare column to permit normal operation during replacement of a bed is provided.

Comments

The column equipment is simple, with the system being cheaper to install and operate than the stirred tank system. Operation should require little attention, except for periodic replacement of the beds and routine makeup of the sodium bicarbonate eluant. The chances of a successful technical operation, however, appear less certain. Potential problems with high turbidity, resulting in column plugging, are also possible.

Incomplete separation of mine water and eluant, and the resulting CO_2 generation, may disrupt the bed and lead to significant channelling of solution flows. This would reduce adsorption/elution effectiveness and provide uranium recovery lower than expected.

The throughput is critically dependent on percolation into, and through, the bed. Stability of the flowthrough rate, as a function of prior treatment of the biomass and operating conditions, requires confirmation.

Equipment list

Operating cycle operation	Time h	Volume m^3	Rate m^3/h
Adsorption	19.2	240	12.5
Wash	1.44	18	12.5
Elution	1.92	24	12.5
Wash	1.44	18	12.5
TOTAL	24.00	300	12.5

Wash water consumption	1.5 m^3/kg
Sodium bicarbonate consumption	8.4 kg/kg U 806 kg/d
Biomass bed, 5 @	12 m^3, 5.2 m diam x 0.57 m
Eluant makeup tanks, 2	agitated
Eluant feed pump	centrifugal

Capital cost

A rough estimate of the capital cost for this type of application is $700 000.

Major Economic Hurdles

The primary economic hurdles are the biomass cost, capital cost of equipment, and the operating cost for desorbing agents and labour. A short discussion of each is provided.

Biomass cost

Although the two primary candidates, Rhizopus arrhizus and the mixed culture, are available either as waste by-products or they can be produced from waste materials, factors such as transportation, treatment, dewatering, etc., will still contribute greatly to the cost. A major factor will also be the scale of production. If the demand is small, the cost will be very high. Conversely, if demand is great, the economy of scale will play a major role in reducing costs. Realistically, a cost of $3 to $5/kg would be appropriate to permit culturing, inactivation, and transportation. For immobilization, an additional $1/kg would be appropriate.

From the laboratory study, it was concluded that 10 adsorption/desorption cycles was the best that could be expected for reusing the biomass. For this use level and a net effective 20 mg/g of biomass adsorption capacity, a total of 4.3 kg of biomass would be required per kg of U_3O_8. At $4/kg for biomass and $50/kg for U_3O_8, the biomass represents 34% of the cost of the uranium product or approximately $17/kg of U_3O_8.

Capital cost and equipment

A preliminary estimate for the capital cost of equipment for underground recovery of uranium has been undertaken, using the stirred reactor-type adsorption flowsheet. Approximate costs for this facility would be $1 000 000. Assuming a three-year amortization at 12%, borrowing and capital payback costs represent approximately $9.65/kg of U_3O_8.

Elution agent

Sodium bicarbonate is the most effective agent, with approximately 9 kg of bicarbonate required per kg of U_3O_8. Allowing 40¢/kg for bicarbonate, this represents approximately $3.60/kg of U_3O_8.

Labour

The labour requirement would likely be one full-time operator/labourer, two shifts/day. At $50 000 labour cost per person, this would represent approximately $2.43/kg of U_3O_8.

Miscellaneous

Ongoing maintenance and power costs would be added to the above costs, but are not expected to add substantially to production expenses.

From the preceding discussion, it can be seen that biomass and capital costs are the most significant factors. The potential cost could be in the range of $30-$35/kg of U_3O_8 recovered from a level solution.

Major Technical Problems

The major technical problems affecting the full-scale application of biomass for uranium recovery would be:

- large-scale production, at a reasonable cost, of an immobilized culture that has good bioadsorption characteristics in combination with mechanical strength;
- maintenance of the bioadsorption characteristics and mechanical properties in cyclic adsorption/desorption operations;
- small-scale production of biomass (with limited demand, costs for biomass could be very prohibitive);
- cost-competitive applications as compared to conventional technologies;
- for underground applications, concerns over handling of chemicals and CO_2 generation from bicarbonate;
- contamination and degradation of the biomass by facultative and anaerobic bacteria, thus causing the development of septic conditions and odours;
- instrumentation of the uranium recovery system for optimum performance.

CONCLUSIONS FROM PHASE I AND II STUDIES

- Bioadsorption technology has potential application for removal of metals from waste streams. In addition, potential also exists for the development of economical metal recovery processes.
- The current applications for metal recovery studies in these projects were not economical.

REFERENCES

1. Nelson, P.O.; Chung, A.K.; and Hudson, M.C. "Factors affecting the fate of heavy metals in the activated sludge process"; J Water Poll Cont Fed 53:1323-1333; 1981.

2. Dugan, P.R., and Pickrum, H.M. "Removal of mineral ions from water by microbially produced polymers"; Proc 27th Ind Waste Conf; Purdue University; 1019-1038.

3. Horitsu, H.; Nishida, H.; Kato, H.; and Tomoyeda, M. "Isolation of potassium chromate-tolerant bacterium and chromate uptake by the bacterium"; Agric Biol Chem 42:2037-2043; 1978.

4. Horitsu, H.; Kato, H.; and Tomoyeda, M. "Uptake of cadmium by a cadmium chloride-tolerant bacterium, Pseudomonas aeruginosa"; J Ferment Technol 57:273-279; 1979.

5. Aickin, R.M., and Dean, A.C.R. "Lead accumulation by Pseudomonas fluorescens and by a Citrobacter sp."; Microbios Lett 9:55-66; 1979.

6. MacAskie, L.E., and Dean, A.C.R. "Cadmium accumulation by microorganisms"; Environ Technol Lett 3:49-56; 1982.

7. Tornabene, T.G., and Edwards, H.W. "Microbial uptake of lead"; Science 176:1334-1335; 1972.

8. Strandberg, G.W.; Shumate, II, S.E.; and Parrott, Jr., J.R. "Microbial cells as biosorbents for heavy metals: accumulation of uranium by S. cerevisiae and P. aeruginosa"; Appl Environ Microbiol 41:237-245; 1981.

9. Strandberg, G.w.; Shumate, II, S.E.; Parrott, Jr., J.R.; and McWhirter, D.A. "Microbial uptake of uranium, cesium and radium"; Symp on Microbial Process for Metals Accumulation and S/N Removal from Fuels"; ACS Meeting; San Francisco, California; August 24-29, 1980.

10. Filip, D.S.; Peters, T.; and Adams, V.D.; and Middlebrooks, E.J. "Residual heavy metal removal by an algae-intermittent sand filtration system"; Water Res 13:305-313; 1979.

11. Gale, N.L., and Wixson, B.G. "Removal of heavy metals from industrial effluents by algae"; Dev Ind Microbiol 20:259-273; 1979.

12. Nemec, P.; Prochazko, H.; Stamberg, K.; Katzer, J.; Stamberg, J.; Jilek, R.; and Hulak, P. "Treatment of fungal myalia for retention of metals"; U.S. Patent 4,021,368; 1977.

13. Corpe, W.A. "Metal-binding properties of surface materials from marine bacteria"; Dev Ind Microbiol 16:249-255; 1975.

14. Brown, M.J., and Lester, J.N. "Metal removal in activated sludge. The role of bacterial extracellular polymers"; Water Res 13:817-837; 1979.

15. Strong, J.R.P.; Madgwick, J.C.; and Ralph, B.J. "Metal binding polysaccharide from the alga, Klebshordium fluitans"; Biotechnol Lett 4:239-242; 1982.

16. Norberg, A. Abstracts 28th Cong IUPAC; B123; 1981.

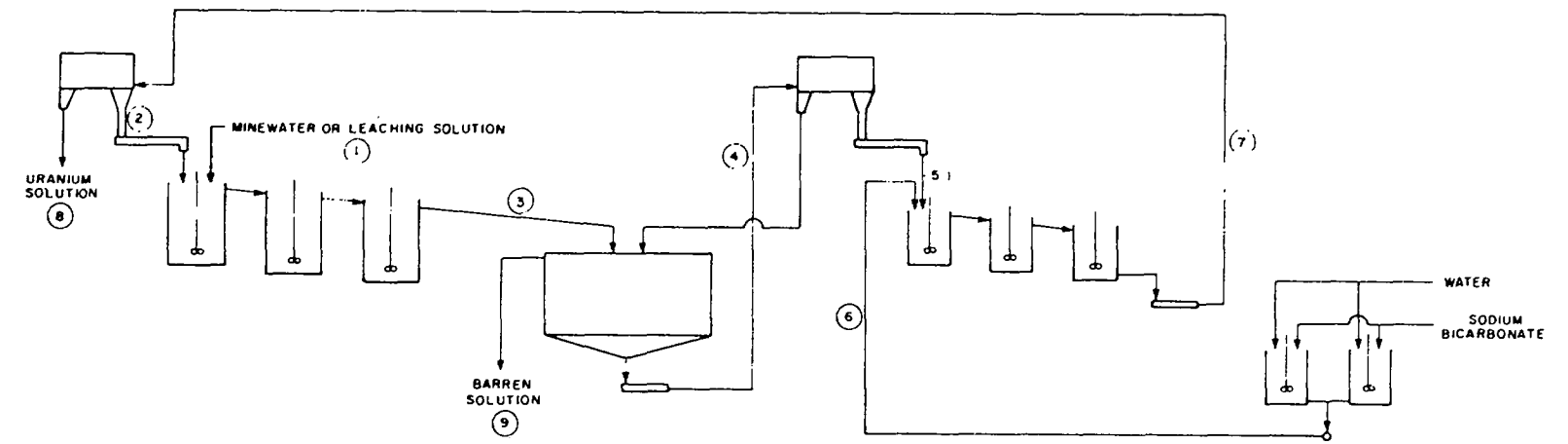
17. Brierley, J.A., and Brierley, C.L. "Biological accumulation of some heavy metals - biotechnological applications"; Biomineralization and Biological Metal Accumulation; Edited by P. Westbroek and E.W. deJong; D. Riedel Pub. Co; pp. 449-509; 1983.

18. Volesky, B., and Tsezos, M. "Separation of uranium by biosorption"; U.S. Patent 4,320,093; 1982.

19. Hatch, R.T., and Menawat,A. "Biological removal and recovery of trace heavy metals"; Biotechnol and Bioeng Symp 8:191-203; 1978.

20. Nakajima, A.; Horikoshi, T.; Sakaguchi, T. "Distribution and chemical state of heavy metal ions absorbed by Chlorella cells"; Agric Biol Chem 45:903-908; 1981.

FIGURES



	1	2	3	4	5	6	7	8	9
SOLUTION FLOW (m^3/h)	40	1 0	41	8 08	1 0	4·0	5 0	4 0	40
BIOMASS FLOW (kg/h)	-	250	250	250	250	-	250	-	-
URANIUM CONCENTRATION (kg/m^3)	0 250	·	0 15	-	-	-	1 0	1 0	0 15
URANIUM CONCENTRATION NET (kg/kg)	-	-	0 020	0 020	0 020	-	-	-	-

NOTE

SEE SECTION 4.2 2. FOR EQUIPMENT SIZING

Fig. 1 - Preliminary flowsheet for uranium recovery from minewater using biomass and stirred reactors

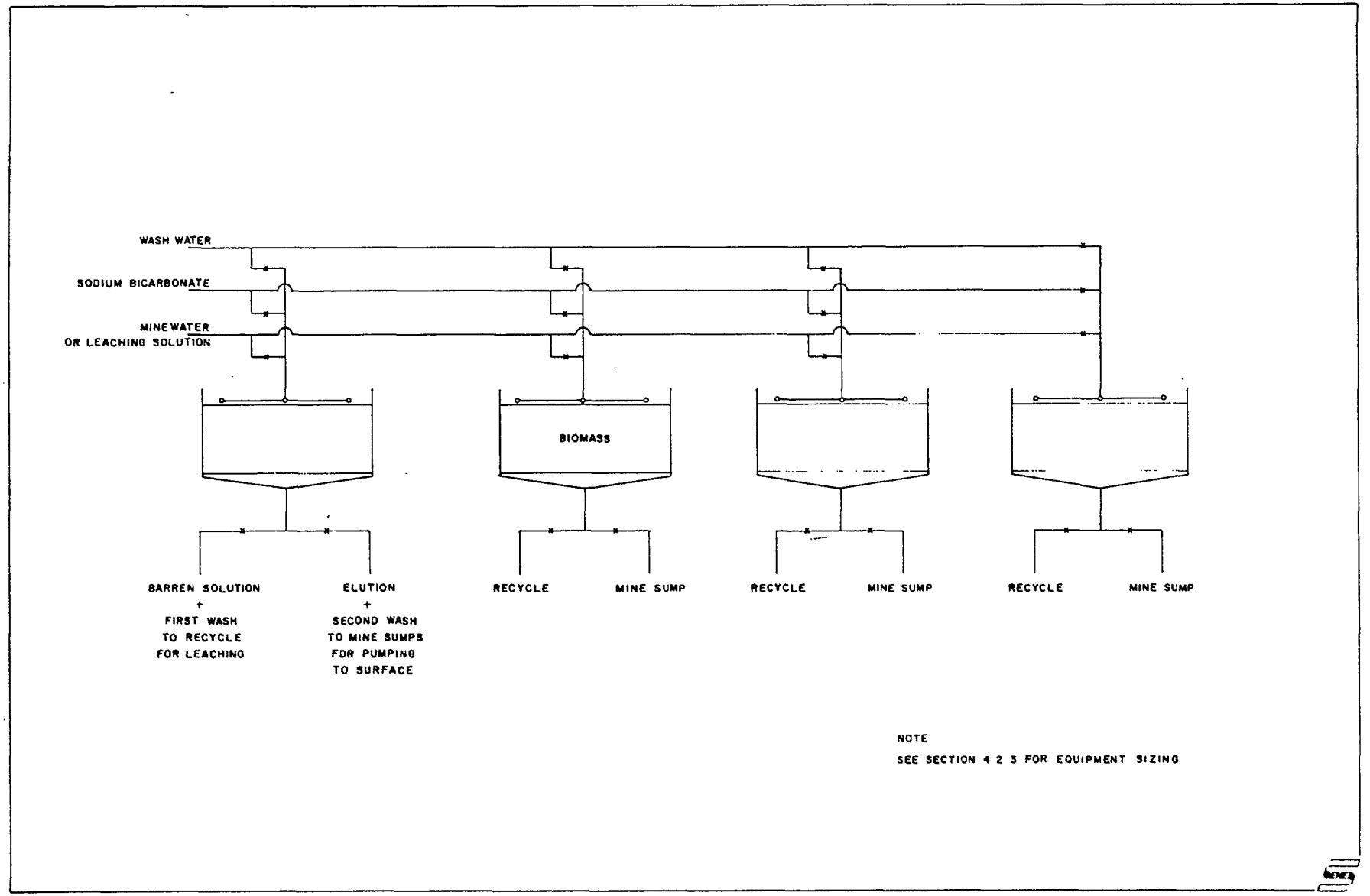


Fig. 2 - Preliminary flowsheet for uranium recovery from minewater using biomass and columns

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13. Anaerobic degradation of chlorinated aromatic hydrocarbons

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SUMMARY

Anaerobic microbial communities have recently been shown to degrade a variety of halogenated hydrocarbons, including several chlorinated aromatic compounds. This reductive dechlorination removes chlorine and replaces it with a hydrogen. Dechlorination of non-aryl Cl atoms, studied in the previous decade, occurs more readily, is catalyzed by a variety of common anaerobes, is rather non-specific and can be catalyzed by dead cells and reduced iron porphyrins. Dechlorination of aryl Cl atoms has been recognized in the past three years; it shows specificity between enriched community and substrate, is catalyzed only by living cells, and is carried out by unique or yet unknown anaerobic bacteria. We have demonstrated this reaction in anaerobic communities from sludges and sediments for halogenated benzoates and from sludges for chlorinated phenols, 2,4,5-T, and hexachlorobenzene. For *m* Cl-benzoates, and *o*, *m*, *p* Cl-phenols, anaerobic consortia have been successfully enriched using these substrates as the sole C and energy source. These consortia produce CH_4 and CO_2 as products and show specificity for the enrichment substrate or closely related analogue. The dechlorination does not occur in sterilized, inhibited or oxygenated samples. Other examples of aryl Cl dehalogenation are also presented. These include data on PCBs, and the pesticides techlofthalam, diuron, benthiocarb and chloronitrofen.

About 25 years ago it was first recognized that some of the chemicals that had been used or disposed of in the environment were not disappearing and were becoming a hazard to humans and other animals. This "environmental" concern stimulated the development of a body of knowledge on biodegradation and persistence of a variety of chemicals. Most of this research was focused on aerobic degradation, since the dogma was that aerobic microorganisms have a much more versatile metabolism. This was illustrated by the diverse substrate range of the pseudomonads and of many saprophytic fungi. Furthermore, oxygenases, which use activated oxygen as a powerful catalyst, were the best known mechanism for the cleavage of bonds not common to intermediary metabolism. Anaerobes not only lack these virtues but were difficult to culture and were slow-growing. Thus it is not surprising that the anaerobes were generally ignored as organisms that might make useful contributions to pollutant biodegradation. With the recent improvements in anaerobic methodology and the growing appreciation of the unexplored diversity of this physiological group, it was then reason-

able to ask the question: do anaerobes possess the capacity to carry out novel biodegradation reactions – especially reactions that are difficult or unknown under aerobic conditions?

Besides new, potentially useful reactions there are other reasons why anaerobic biodegradation may be advantageous. If the pollutant is bound to soil or sediment, the most cost-effective treatment is to degrade it in place. If this can be stimulated by making the site anaerobic, e.g. by flooding, the cost would be minimal. If a waste processing unit is to be constructed, anaerobic systems are often less expensive because the cost for pumping oxygen into the system is avoided, less hydraulic volume is required (i.e., a more concentrated biomass can be used), thus reducing capital construction costs, and there is usually the potential for recovering methane as a useful product. Operation of anaerobic systems may also be easier and more reliable, since less sludge accumulates, and the system is often more stable to influent and environmental perturbations. Thus, even if an aerobic community capable of biodegradation of the chemical of interest exists, there may be reasons why an anaerobic biodegradation unit is still preferred.

In our earlier work [15,33], we screened more than 100 different chemicals for biodegradation in anaerobic sludge or eutrophic lake sediments. From this survey we identified several classes of chemicals that were biodegraded and of particular interest. These included chlorinated aromatic compounds discussed further below, cresols [7,33], phthalates [32] and polyethylene glycols [10]. The anaerobic degradation of chlorinated chemicals was of particular interest, since this class has been the most ubiquitous and problematic of the chemical classes that have polluted the environment. Furthermore, the key reaction that we observed – replacement of the aromatic chlorine(s) with hydrogen – was a new and particularly promising biotransformation. In many cases, if only one chlorine atom were to be removed the compound would be more biodegradable and less toxic. Aerobic metabolism of highly chlorinated aromatic chemicals is often restricted, since two adjacent ring positions must be free for hydroxylation; the Cl is then removed after ring opening. Thus, the anaerobic dechlorination which occurs prior to ring opening provides a means to overcome the block preventing aerobic degradation.

Chlorine on non-aromatic carbon atoms

The phenomenon of reductive dehalogenation has been known for some time, but the previous evidence was for removal of chlorine from non-aromatic carbon atoms. This includes two groups of chemicals: the more complex structures often referred to as chlorinated hydrocarbons and the small molecular weight chlorinated solvents. Chemicals in the first class that are susceptible to reductive dechlorination are listed in Table 1 along with the habitats in which that activity has been demonstrated. The activity does not appear to be habitat-specific, since it is usually found in other anaerobic habitats if such a study is performed.

The reductive dechlorination of non-aryl Cl in those chlorinated hydrocarbons was first reported for DDT in 1967 [12], but the next 15 years of research on anaerobic dechlorination led to further examples of removal of non-aryl Cl and not of Cl from the aromatic ring (Table 1). The single exception is the dechlorination of pentachlorophenol

Table 1

Anaerobic microbial habitats that have shown reductive dechlorination of Cl from non-aromatic C-Cl bonds (summarized from reviews of Essac and Matsumura [11] and Sethunathan [31])

Chemical	Active anaerobic habitats[a]
DDT	Soil, rumen fluid, sewage sludge, sediments, microbial cultures
Lindane	Soil, sediments, microbial cultures
Toxaphene	Rumen fluid, sediment
Heptachlor	Soil, microbial cultures
Mirex	Sewage sludge
Endrin	Soil
Methoxychlor	Soil

[a] Soil used was made anaerobic by flooding, adding an anaerobic atmosphere and/or adding readily degradable organic matter. Some of the soils were paddy rice soils.

(discussed below). Thus, it seems that the aryl Cl is more difficult to remove than the non-aryl Cl.

The best studied examples of the non-aryl dechlorination are of DDT and lindane (for a review, see Ref. 11). DDT is readily converted to TDE (DDD or 1,1-dichloro-2,2-bis(chlorophenyl)-ethane) in most if not all anaerobic habitats and by many anaerobically grown microbial cultures. However, dead cells and reduced iron porphyrins are also known to carry out the same conversion. Lindane (BCH or γ-hexachlorocyclohexane) is readily converted to tetrachlorocyclohexane in anaerobic soils and by anaerobic microbial cultures. The most active microorganisms, *Clostridium* and *Citrobacter* species, showed nearly completed dechlorination presumably yielding benzene [18]. These authors noted that only bacteria with iron-sulfur protein-dependent H_2 evolution were active and that only substrates which were electron donors for ferredoxin-dependent H_2 evolution supported the dechlorination. This dechlorination may also be non-specific and perhaps directly catalyzed by low-potential electron donors. Thus, the reason why the non-aryl Cl atoms are more easily dehalogenated may be that they are more easily attacked by the low-potential electron carriers found in many microbial cells.

The chlorinated solvents, especially the chlorinated methanes, ethanes, and ethylenes, are important groundwater pollutants for which reductive dechlorination has also recently been shown [4,5,29,40]. Compounds which were reductively dechlorinated in methanogenic communities from sewage sludge and in anaerobic groundwaters include carbon tetrachloride, chloroform, tetrachloroethane, tetrachloroethylene, trichloroethane and trichloroethylene. Reduced iron porphyrins were also shown to dechlorinate some of these compounds [20].

The remainder of this chapter focuses on the work since 1982, when the reductive dechlorination of the aryl Cl became more widely known.

Chlorinated benzoates

Chlorinated benzoates are the most widely used model compounds to study aerobic chloroaromatic metabolism, but this class also is of some practical significance. Chlorobenzoates do occur at hazardous waste sites, are a product of certain pollutants, e.g. PCBs, and do have some congeners that are herbicides: Amiben (3-amino-2,5-dichlorobenzoic acid), Banvel (dicamba or 2-methoxy-3,6-dichlorobenzoic acid), and TBA (2,3,6-trichlorobenzoic acid).

Two independent observations lead to our interpretation that reductive dehalogenation of an aromatic compound by an anaerobic community must have occurred. Horowitz was using eutrophic lake sediments to study the fate of selected chemicals and observed that 4-amino-3,5-dichlorobenzoate was converted to 4-amino-3-chlorobenzoate [16]. At the same time Shelton was studying compounds that were converted to gas by anaerobic sludge and noted that in two of the nine sludges fed 3-chlorobenzoate 85% of the substrate carbon was converted to $CH_4 + CO_2$ [33]. This enrichment was later shown to produce benzoate as an intermediate [35] and, therefore, provided the evidence that this community also replaced the aryl Cl with H. Oxygen strongly inhibited the dechlorination in both communities.

The range of halogenated benzoate substrates dehalogenated by the sediment and sludge communities is summarized in Table 2. Without exception all chlorines in the *meta* position were removed and none were removed from other positions. For the bromo and iodo substituents the specificity is not apparent, as these halogens were removed from the *ortho* and *para* positions as well as the *meta* position. Thus, considerable specificity is shown by these chlorobenzoate dehalogenating communities for the *meta* chlorine. The number of chlorine substitutions does not seem to be important to the dechlorination since mono-, di-, and trichlorinated substrates all yielded to dechlorination.

The dechlorination of chlorobenzoates in sediments was characterized by a particularly lengthy lag period prior to the onset of rapid dechlorination. This period ranged from 3 weeks to more than 6 months depending on the chemical [16,22]. Since this lag period was more easily studied in sediment, this environment was used to characterize certain

Table 2

Specificity of fresh water sediments, sludge enrichment and dehalogenating strain (DCB-1) for position of halogen for dehalogenation (data summarized from Suflita et al. [35] and Linkfield [22])

Substrate, DCB-1	Position of halogen removal		
	sediments	sludge enrichment	pure culture
Monohalogen			
2- or 4-iodobenzoate	2-, 4-	2-, 4-	nt
3-iodobenzoate	3	3	3
2- or 4-bromobenzoate	2-, 4-	2-, 4-	nt
3-bromobenzoate	3	3	3
2- or 4-chlorobenzoate	–	–	–
3-chlorobenzoate	3	3	3
3- or 4-fluorobenzoate	–	–	–
2-fluorobenzoate	2	?	nt
4-amino-3-chlorobenzoate	–	3	3
Dihalogen			
3,5-dichlorobenzoate	3,5	3,5	3,5
3,4-dichlorobenzoate	3	3	3
2,5-dichlorobenzoate	5	5	5
2,4-dichlorobenzoate	–	–	–
2,6-dichlorobenzoate	–	–	–
5-bromo-2-chlorobenzoate	nt	nt	5
4-amino-3,5-dichlorobenzoate	5	3,5	5
Trihalogen			
2,3,6-trichlorobenzoate	3	3	3

nt = not tested; – = no activity.

aspects of the dehalogenation. The lag period was followed by the rapid onset of dechlorination which occurred both for substrates that were completely metabolized to CH_4 + CO_2 and for those that showed only a single dechlorination. In the latter case no substrate carbon was available for growth, and thus the lag period cannot be due to a population growth response. Once the acclimation occurred, subsequent dechlorination commenced without lag following the addition of new substrate.

The fact that dechlorination did not occur during this lengthy lag period is also strong evidence that the dechlorination is not a generalized chemical reaction perhaps caused by low-potential electron donors, such as occurred for the non-aryl dechlorinations described earlier. These donors should be just as prevalent if not more so early in the incubation (lag period) as well as later. There is also evidence that this acclimation was chemical-specific. Sediments acclimated to benzoate, 3-iodobenzoate or 4-amino-3,5-dichlorobenzoate resulted in an acclimation which degraded that or related substrates but not substrates from the other two groups [16]. Obviously, there is a specific response elicited by the particular chlorobenzoate addition that eventually selects for some biochemical capacity to carry out the dehalogenation. In our studies, sediments sterilized by autoclaving, gamma irradiation or formaldehyde did not show dechlorination [16].

The chlorobenzoate dechlorinating activity observed in sludge was studied primarily after enrichment [36]. This enrichment was obtained by repeated additions of 3-chlorobenzoate as the only

carbon source and by periodic transfer until a stable community existed free from the original sludge matrix. This enriched consortium showed dehalogenation rates that ranged from 0.31 μmol · h^{-1} · mg protein^{-1} for 3-chlorobenzoate to 0.04 μmol · h^{-1} · mg protein^{-1} for 4-amino-3,5-dichlorobenzoate [35]. The inhibition of dechlorination of the monochloro substrate by dichlorobenzoate was also observed [36]. The dichloro substrate, probably acting as a substrate analogue, caused a nearly stoichiometric accumulation of the monochloro product. When the dichloro substrate was depleted the inhibition was relieved, and the monochloro product was also degraded. The kinetic pattern of the accumulation and disappearance of these substrates and products showed the phenomenon to be one of competitive inhibition. Examples of the first substrate in the pathway being the competitive inhibitor of a second reaction in the pathway are rare, since this mechanism would prevent growth on such substrates. However, with mixtures of chemicals existing in waste sites such a phenomenon could be likely. If present it would result in the inhibition of degradation even if the biochemical pathway existed in the indigenous community.

The sludge community that carried out the dechlorination has been characterized [34]. Eight different organisms were isolated and a ninth, *Methanothrix*, can be easily recognized by its morphology. These organisms are illustrated in Fig. 1 and are placed according to their likely position in the path of carbon flow. Four of these organisms are thought not to play a central role in carbon flow but are probably present as scavengers. These are two anaerobic butyrate oxidizers and the two sulfidogens. The remaining five organisms are thought to have major roles in the carbon and hydrogen flow. At least four are necessary for the conversion of chlorobenzoate to CH_4 + CO_2: (1) the dechlorinating strain, DCB-1, that produces benzoate; (2) the benzoate-oxidizing strain, BZ-2 (probably *Syntrophus buswelli*), that produces H_2 plus acetate; (3) two H_2-consuming methanogens, strains of *Methanospirillum* and *Methanobacterium*, hydrogen consumers being necessary to provide thermodynamically favorable conditions for anaerobic benzoate oxidation to proceed; and (4) the acetoclastic methanogen, *Methanothrix*, which cleaves acetate into CO_2 and CH_4.

For the subject of this paper the particularly interesting member of this community is the dechlorinating bacterium. This isolate has no known close relatives. It is a rather large, non-motile, gram-negative rod with a unique collar surrounding virtually every cell [34]. The substrate range is extremely limited, with pyruvate being the only sub-

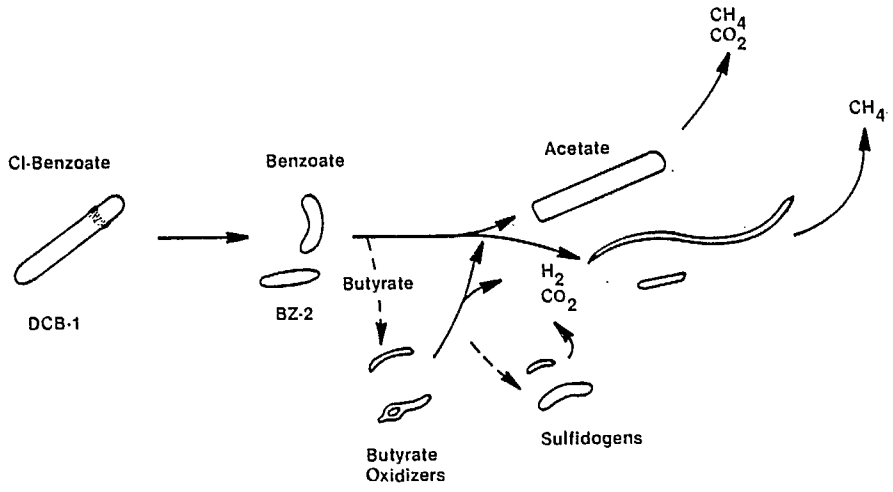


Fig. 1. Diagram of the bacteria present in the 3-chlorobenzoate degrading consortium. They are arranged according to their projected position in the food chain.

strate so far shown to support reasonable growth [22,34]. DCB-1 can probably also live as a scavenger, since it was isolated on rumen fluid and does respond with improved growth when rumen fluid or trypticase supplements are added to the medium. A particularly important contribution to the understanding of DCB-1 was made by Linkfield [22] when he found that this organism showed improved growth when thiosulfate or sulfite were provided as electron acceptors. The culture cannot, however, be maintained with sulfate as the electron acceptor. Thus, DCB-1 is a type of sulfidogen, but apparently not closely related to the presently known members of this class.

The unusual morphology, restrictive physiology, and unique dechlorinating abilities make this new organism particularly intriguing. At present neither the mechanism nor the basis of the dechlorinating property are known. The slow growth rate of DCB-1 (4–5 day generation time), the low cell yield, and a relatively low rate of dechlorination in pyruvate culture (0.1 pmol · bacterium^{-1} · day^{-1}) do not make this an easy process to study.

Chlorinated phenols

Pentachlorophenol (PCP) is the most widely studied chlorophenol congener, presumably due to its widespread use as a pesticide. Approx. 80 million pounds of PCP were manufactured in 1977 [8], making it the second most heavily used pesticide in the United States [13]. The single largest use of PCP is as a wood preservative. It has also been used as a herbicide, primarily in rice and sugar cane [27].

The use of PCP as a herbicide on rice paddies led to studies on the anaerobic degradation of PCP in flooded soils. Ide et al. [17] suggested reductive dechlorination as a degradative pathway for PCP. The following degradation products were detected in paddy soil: 2,3,4,5-, 2,3,5,6-, and 2,3,4,6-tetrachlorophenol, 2,4,5-, and 2,3,5-trichlorophenol, 3,4-, and 3,5-dichlorophenol, and 3-chlorophenol. In general it appeared that the chloro groups in the *ortho* and *para* positions of PCP were dechlorinated more easily than those in the *meta* position. PCP degradation did not occur in sterile reduced soil.

Several trichlorophenols (4 or 5), all three tetrachlorophenols, and pentachloroanisole (OH → OCH_3) were reported as products of PCP degradation in a similar study of ten flooded soils [21]. Pentachloroanisole and 2,3,4,5-tetrachlorophenol were the major products. 3,4,5-Trichlorophenol was not observed. The half-life of PCP (100 ppm) averaged 30 days under flooded conditions. PCP degradation was slower under "upland" (aerobic) conditions. Other studies have reported more rapid PCP degradation under aerobic conditions [9,23].

Reductive dechlorination of PCP on a "nitrogen-aerated" silty clay loam soil has also been observed by Murthy et al. [27]. Pentachloroanisole was observed in both aerobic and anaerobic soils, but was significantly greater in aerobic soil. The dechlorinated products observed in anaerobic soil were: 2,3,5,6- and 2,3,4,5-tetrachlorophenols and 2,3,6-trichlorophenol. Approx. 74% of the added ^{14}C was extracted with hexane at the end of the 24 day incubation. Of this, 85.3% was PCP and 5.3% was pentachloroanisole. The three dechlorinated products accounted for a total of 7% of the ^{14}C activity extracted.

The occurrence of a wide variety of dechlorinated products in the anaerobic soils described above suggests a non-specific process in some cases, perhaps mediated by biologically produced electron carriers as has been described for DDT and other compounds [25,42,43]. More specific, enzymatically mediated processes may also be involved. In general, reductive dechlorination reactions appeared to occur more easily in the higher chlorinated phenols.

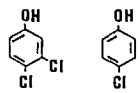
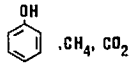
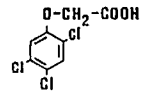
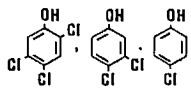
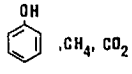
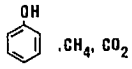
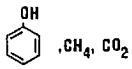
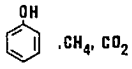
Chlorophenols (including PCP), chlorocatechols and chloroquaiacols were degraded in an anaerobic fluidized-bed reactor during the treatment of paper and pulp mill effluents [14,30]. Chlorophenol removal was more rapid in the anaerobic reactor than in either an aerated lagoon or an activated sludge plant.

The fate of PCP and other chlorophenols in anaerobic sewage sludges has also been studied, and these results are summarized in Table 3. In fresh (unacclimated) sludge from primary anaerobic digestors (Jackson, Michigan), the *ortho* positions of PCP (chlorine in the 2 and 6 positions) were rapidly dechlorinated giving 3,4,5-trichlorophenol,

which was subsequently converted to 3,5-dichlorophenol [24]. Monochlorophenol isomers were not observed. 2,4,6-Trichlorophenol followed this same pattern of *ortho* dechlorination yielding 4-chlorophenol as the final degradation product. The degradation of PCP by two other anaerobic sludges was considerably slower; whereas the Jackson sludge completely degraded 50 μM PCP within 14 days, only partial PCP degradation was observed after 70 days in the other two sludges [24]. Extensive PCP biodegradation in anaerobic sludge was also observed by Guthrie et al. [13]. PCP inhibited methanogenesis at a concentration of 200 $\mu g \cdot l^{-1}$ in fresh sludge and at 600 $\mu g \cdot l^{-1}$ in acclimated sludge.

Table 3

Fate of chlorophenols in anaerobic sewage sludge from Jackson, Michigan[a]

Compound	Source of activity	Product(s) observed
	fresh sludge	
	fresh sludge	
	fresh sludge	
	fresh sludge	
	sludge enriched on 3-chlorophenol[b]	
	sludge enriched on 3-chlorophenol[b]	
	sludge enriched on 2-chlorophenol[c]	
	sludge enriched on 2-chlorophenol[c]	

[a] Refs. 6, 7 and 24.

[b] This sludge also degraded 4-chlorophenol and 3,5-dichlorophenol.

[c] This sludge degrades 2- and 4-chlorophenol at equal rates. The same products were observed in sludge enriched on 4-chlorophenol.

Reductive dechlorination reactions have also been observed for the mono- and dichlorophenols in fresh and acclimated anaerobic digestor sludge. The initial monochlorophenol dechlorination was observed for 2-chlorophenol [7]. Complete conversion of 30 ppm 2-chlorophenol to phenol required approx. 3 weeks in fresh 10% anaerobic sludge. All three monochlorophenols inhibited methane production. In fresh whole sludge, the relative rates of disappearance were: 2-chlorophenol $\gg$ 3-chlorophenol > 4-chlorophenol [6]. For dichlorophenols in unacclimated sludge, reductive dechlorination of the Cl group *ortho* to phenolic OH was observed for each dichlorophenol isomer with such a Cl substituent. 3,4-Dichlorophenol and 3,5-dichlorophenol were persistent during the 6 week incubation [6].

The same sludge acclimated to either 2-, 3-, or 4-chlorophenol gave patterns of degradation distinctly different from those of fresh sludge [6]. For example, sludge acclimated to the degradation of 2-chlorophenol degraded 2- and 4-chlorophenol at equal rates whereas 3-chlorophenol was not degraded. Sludge acclimated to 3-chlorophenol also degraded 4-chlorophenol but not 2-chlorophenol. The rates of degradation were enhanced in the acclimated sludges and previously persistent compounds, viz. 3,4- and 3,5-dichlorophenol, were degraded quite rapidly. At the same time, the rapid *ortho* dechlorination of the dichlorophenols was less prevalent.

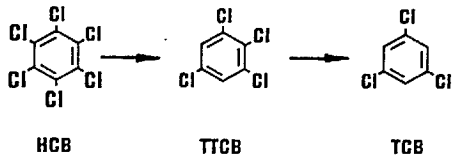
These studies have shown that the outstanding feature of chlorophenol degradation in fresh sludge was the reductive dechlorination of Cl *ortho* to phenolic OH. This dechlorination could result from enzymatic activity of a specific organism(s) or from a more non-specific process mediated by biologically produced electron carriers. At least two distinct chlorophenol-degrading populations were observed in sludges acclimated to 2- or 3-chlorophenol. These sludges were simultaneously acclimated to the degradation of other chlorophenols. The specificity of the cross-acclimation patterns, and the en-

hanced rates of degradation, are suggestive of the existence of a specific biological mechanism, which is presumably enzymatic.

Chlorinated benzenes and PCBs

The potential for reductive dechlorination of chlorinated benzenes and PCBs is of great interest due to their widespread occurrence in the environment. There is evidence that hexachlorobenzene (HCB), trichlorobenzene and a variety of PCB congeners are subject to reductive dechlorination reactions. In general, these reactions appear to occur at a much slower rate than those of the chlorobenzoates and chlorophenols described earlier. Reductive dechlorination may still be a significant detoxification mechanism in contaminated anaerobic environments such as sediments.

Studies on the reductive dechlorination of HCB have been conducted using anaerobic sludge from a source (Jackson, Michigan) that had previously shown the ability to dehalogenate chlorinated phenols. The HCB was dechlorinated as evidenced by the accumulation of 1,3,5-trichlorobenzene (TCB). Approx. 30% of the added HCB (50 $\mu g \cdot ml^{-1}$) was removed over a period of 12–14 weeks. At its maximum accumulation (14 weeks) 1,3,5-TCB accounted for approx. 40% of the HCB disappearance. Penta- and tetrachlorobenzenes were not detected. The same sludge which had been autoclaved showed no decrease in the HCB concentration. In the active samples, further dechlorination to give dichlorobenzene, chlorobenzenes, and benzene is possible; however, these compounds are easily lost by volatilization. In sterilized sludge inoculated (20% inoculum) with sludge previously exposed to HCB for 20 weeks, similar results were obtained except that both 1,2,3,5-tetrachlorobenzene (TTCB) and 1,3,5-TCB were observed. Sterilized sludge alone did not show the formation of either TTCB or TCB.



In conclusion, these results showed a significant biodegradation of HCB via reductive dechlorination under strict anaerobic conditions.

Bailey [3] has pointed out that dehalogenation of HCB in anaerobic sediments can be inferred from data by Oliver and Nicol [28] on the distribution of chlorobenzenes in the Great Lakes. Analysis of different HCB congeners in sediment cores revealed that in the older layers the ratio of dichlorobenzene (DCB) and TCBs to HCB and pentachlorobenzene (PCB) increased dramatically. For example, the ratio of 1,4-DCB to HCB increased as follows: 0.41 (0–1 cm; 1976–1980), 0.26 (1–2 cm: 1971–1976), 0.40 (2–3 cm; 1965–1971), 1.44 (3–4 cm; 1958–1965), 1.16 (4–5 cm; 1950–1958), 1.81 (5–6 cm; 1941–1950) and 20 (6–7 cm; 1932–1941). The pattern suggested a slow dehalogenation of HCB in the anaerobic sediment.

A study of PCB congener distribution in upper Hudson River sediments suggested that PCBs were being reductively dechlorinated by several populations of anaerobic bacteria [7a]. Sediments containing relatively high levels of PCBs (> 50 ppm) all showed losses of up to one-third of the chlorine originally present. This resulted in distinct changes in congener distribution, with the less chlorinated PCB congeners becoming relatively more prevalent. Several different patterns of dechlorination were observed at different sites. The high levels of individual mono-, di-, and trichlorobiphenyls in these samples could not be explained by simple physical partitioning of the original discharges. Apparently the reductive dechlorination of PCBs occurred in a stepwise fashion until lower chlorinated congeners were produced which were more difficult to reduce due to their increasing reduction potentials. This result was consistent with those described earlier for the chlorophenols. The PCB dechlorinations were suggested to be biological in nature, based on the observation of congener selectivity, which was similar to that observed for the dechlorination of chlorobenzoates [35].

The intestinal contents of rats have been reported to convert TCB to mono- and dichlorobenzene [38]. In a subsequent study, 12 bacterial strains were isolated and tested for their ability to convert

1,2,4-TCB to 1,2-DCB [39]. Although 1,2-DCB was detected in each of these 12 strains, the percent conversion of TCB was very low (approx. $<1\%$). The dechlorinating activity was highest in *Staphylococcus epidermidis* (strain 1) and proceeded only under an atmosphere of H_2. The dechlorinating activity of strain A was apparently stimulated by NADPH; however, the stimulatory effect was observed for dry, intact, and broken cells.

Other compounds

Reductive dechlorination has been observed for several pesticides in anaerobic sediments, soils and sludges. One of the earliest was the anaerobic dechlorination of techlofthalam (*N*-(2,3-dichlorophenyl)-3,4,5,6-tetrachlorophthalmic acid) in paddy soil [19]. Two or more monochlorinated products were observed after 2 weeks incubation.

Enrichment cultures obtained from pond sediment completely degraded the herbicide diuron ((3-(3,4-dichlorophenyl-1,1)dimethylurea C)) in 17–25 days. The mode of degradation was removal of the 4-Cl substituent giving 3-(3-chlorophenyl)-1,1-dimethylurea in stoichiometric amounts [1,2]. The monochloro derivative was not degraded.

The herbicide benthiocarb (thiobencarb, *S*-4-chlorobenzyl *N,N*-diethylthiocarbamate) used widely in rice fields has been observed to undergo reductive dechlorination in certain flooded soils. The dechlorinated product, *S*-benzyl *N,N*-diethylthiocarbamate, appeared to cause dwarfing of rice plants in some paddy fields [26]. Of 17 soils examined, the dechlorination occurred in two soils. Organic amendments to these soils were required for dechlorination. It was suggested that the dechlorinating activity in soil required free solution phosphate and was carried out by facultative anaerobic microorganisms.

In a related study, the reductive dechlorination of CNP (chloronitrofen, 4-nitrophenyl 2,4,6-trichlorophenyl ether) was compared with benthiocarb [41]. The primary dechlorinated product from CNP was 4-aminophenyl 2,6-dichlorophenyl ether which resulted from removal of the *para* Cl. Subsequent removal of Cl in the 2 and 6 positions occurred to lesser extents. The dechlorination of CNP in soil was slower than that of benthiocarb.

Anaerobic degradation of the pesticides 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) in sewage sludge has been observed [24]. The ether bonds of 2,4-D and 2,4,5-T were rapidly cleaved giving 2,4-dichlorophenol and 2,4.5-trichlorophenol. This reaction was followed by rapid removal of Cl groups in the *ortho* positions of 2,4-dichlorophenol and 2,4,5-trichlorophenol. 4-Chlorophenol released from 2,4-dichlorophenol was degraded slowly, and 3,4-dichlorophenol released from 2,4,5-trichlorophenol was partially dechlorinated to 4-chlorophenol. Suflita et al. [37] observed 2,5-dichlorophenoxyacetic acid as the sole product from the reductive dechlorination of 2,4.5-T. For that study, the active organism had been selected by prior growth on 3-chlorobenzoate.

Conclusions

Several general conclusions regarding the reductive dechlorination of aromatic structures can be drawn from the work summarized here.

(1) A wide variety of chlorinated aromatic compounds are subject to reductive dechlorination reactions. These include structures with (e.g., chlorobenzoates, chlorophenols) and without (e.g., chlorobenzenes, PCBs) polar functional groups.
(2) Reductive dechlorination reactions occur in a variety of anaerobic habitats including soil, anaerobic digestor sludge, and sediment.
(3) Microbial communities enriched on particular chlorinated substrates have activities that are generally specific for substituent and position.
(4) Chlorine removal is more facile with higher chlorinated structures.
(5) Reductive dechlorination of aryl Cl generally appears to be a specific, perhaps enzymatic process. This is in contrast to the reductive dechlorination of non-aryl Cl which occurs more readily, is more non-specific and can be catalyzed by low-potential electron carriers.

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REFERENCES

1 Attaway, H.H., N.D. Camper and M.J.B. Paynter. 1982. Anaerobic microbial degradation of diruon by pond sediment. Pesticide Biochem. Physiol. 17: 96–101.

2 Attaway, H.H., M.J.B. Paynter and N.D. Camper. 1982. Degradation of selected phenylurea herbicides by anaerobic pond sediment. J. Environ. Sci. Health B17: 683–699.

3 Bailey, R.E. 1983. Comment on "Chlorobenzenes in sediments, water and selected fish from Lakes Superior, Huron, Erie, and Ontario". Environ. Sci. Technol. 17: 504.

4 Bouwer, E.J. and P.L. McCarty. 1983. Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl. Environ. Microbiol. 45: 1286–1294.

5 Bouwer, E.J., B.E. Rittman and P.L. McCarty. 1981. Anaerobic degradation of halogenated 1- and 2-carbon organic compounds. Environ. Sci. Technol. 15: 596–599.

6 Boyd, S.A. and D.R. Shelton. 1984. Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. Appl. Environ. Microbiol. 47: 272–277.

7 Boyd, S.A., D.R. Shelton, D. Berry and J.M. Tiedje. 1983. Anaerobic biodegradation of phenolic compounds in digested sludge. Appl. Environ. Microbiol. 46: 50–54.

7a Brown, J.F., R.E. Wagner, D.L. Bedford, M.J. Brennan, J.C. Carnahan, R.J. May and T.J. Tofflemire. 1984. PCB Transformations in Upper Hudson Sediments. Northeastern Environ. Sci. Issues 34: 167–179.

8 Cirelli, D.P. 1978. Patterns of pentachlorophenol usage in the United States of America – an overview. In: Pentachlorophenol – Chemistry, Pharmacology, and Environmental Toxicology (Rao, K.P., ed.), pp. 13–18, Plenum Press, New York.

9 De Laune, R.D., R.P. Gambrell and K.S. Reddy. 1983. Fate of pentachlorophenol in estuarine sediment. Environ. Pollut. Ser. B 6: 297–308.

10 Dwyer, D.F. and J.M. Tiedje. 1983. Degradation of ethylene glycol and polyethylene glycols by methanogenic consortia. Appl. Environ. Microbiol. 46: 185–190.

11 Essac, E.G. and F. Matsumura. 1980. Metabolism of insecticides by reductive systems. Pharmacol. Ther. 9: 1–26.

12 Guenzi, W.D. and W.E. Beard. 1967. Anaerobic biodegradation of DDT to DDD in soil. Science 156: 116–119.

13 Guthrie, M.A., E.J. Kirsch, R.F. Wukasch and C.P.L. Grady. 1984. Pentachlorophenol biodegradation-II. Anaerobic. Water Res. 18: 451–461.

14 Hakulinen, R. and M. Salkinoja-Salonen. 1982. Treatment of pulp and paper industry wastewaters in an anaerobic fluidized bed reactor. Process Biochem. 17(2): 18–22.

15 Horowitz, A., D.R. Shelton, C.P. Cornell and J.M. Tiedje. 1982. Anaerobic degradation of aromatic compounds in sediments and digested sludge. Dev. Indust. Microbiol. 23: 435–444.

16 Horowitz, A., J.M. Suflita and J.M. Tiedje. 1983. Reductive dehalogenations of halobenzoates by anaerobic lake sediment microorganisms. Appl. Environ. Microbiol. 45: 1459–1465.

17 Ide, A., Y. Niki, F. Sakamoto, I. Wantanabe and H. Wantanabe. 1972. Decomposition of pentachlorophenol in paddy soil. Agric. Biol. Chem. 36: 1937–1944.

18 Jagnow, G., K. Haider and P.-Chr. Ellwardt. 1977. Anaerobic dechlorination and degradation of hexachlorocyclohexane isomers by anaerobic and facultative anaerobic bacteria. Arch. Microbiol. 115: 285–292.

19 Kirkpatrick, D., S.R. Biggs, B. Conway, C.M. Finn, D.R. Hawkins, T. Honda, M. Ishida and G.P. Powell. 1981. Metabolism of *N*-(2,3-dichlorophenyl)-3,4,5,6-tetrachlorophthalmic acid (Techlofthalam) in paddy soil and rice. J. Agric. Food Chem. 29: 1149–1153.

20 Klecka, G.M. and S.J. Gonsior. 1984. Reductive dechlorination of chlorinated methanes and ethanes by reduced iron (II) porphyrins. Chemosphere 13: 391–402.

21 Kuwatsuka, S. and M. Igarashi. 1975. Degradation of PCP in soils. II. The relationship between the degradation of PCP and the properties of soils, and the identification of the degradation products of PCP. Soil Sci. Plant Nutr. 21: 405–414.

22 Linkfield, T.G. 1985. Anaerobic reductive dehalogenation: The lag period preceeding haloaromatic dehalogenation, enrichment of sediment activity, and the partial characterization of a dehalogenating organism, strain DCB-1. Ph.D. Thesis, Michigan State University.

23 Liu, D., K. Thomson and W.M.J. Strachan. 1981. Biodegradation of pentachlorophenol in a simulated aquatic environment. Bull. Environ. Contam. Toxicol. 26: 85–90.

24 Mikesell, M.D. and S.A. Boyd. 1985. Reductive dechlorination of the pesticides 2,4-D, 2,4,5-T, and pentachlorophenol in anaerobes sludges. J. Environ. Qual. 14: 337–340.

25 Miskus, R.P., D.P. Blair and J.E. Casida. 1965. Conversion of DDT to DDD by bovine rumen fluid, lake water, and reduced porphyrins. J. Agric. Food Chem. 13: 481–483.

26 Moon, Y.H. and S. Kuwatsuka. 1984. Properties and conditions of soils causing the dechlorination of the herbicide benthiocarb (thiobencarb) in flooded soils. J. Pestic. Sci. 9: 745–754.

27 Murthy, N.B.K., D.D. Kaufman and G.F. Fries. 1979. Degradation of pentachlorophenol (PCP) in aerobic and anaerobic soil. J. Environ. Health Sci. B14: 1–14.

28 Oliver, B.G. and K.D. Nicol. 1982. Chlorobenzenes in sedi-

ments, water, and selected fish from Lakes Superior, Huron, Erie and Ontario. Environ. Sci. Technol. 16: 532–536.

29 Parsons, F., P.R. Wood and J. DeMarco. 1984. Transformations of tetrachloroethylene and trichloroethylene in microcosms and groundwater. J. Am. Water Works Assoc. 76: 56–59.

30 Salkinoja-Salonen, M., R. Valo, J. Apajalahti, R. Hakulinen, L. Silakoski and T. Jaakkola. 1984. Biodegradation of chlorophenolic compounds in wastes from wood processing industry. In: Current Perspective in Microbial Ecology (Klug, M.J. and C.A. Reddy, eds.), pp. 668–676, American Society for Microbiology, Washington, DC.

31 Sethunathan, N., V.R. Rao, T.K. Adhya and K. Raghu. 1983. Microbiology of rice soils. CRC Critical Reviews in Microbiology 10: 125–170.

32 Shelton, D.R., S.A. Boyd and J.M. Tiedje. 1984. Anaerobic degradation of phthalic acid esters in sludge. Environ. Sci. Technol. 18: 93–97.

33 Shelton, D.R. and J.M. Tiedje. 1984. General method for determining anaerobic biodegradation potential. Appl. Environ. Microbiol. 47: 850–857.

34 Shelton, D.R. and J.M. Tiedje. 1984. Isolation and partial characterization of bacteria in an anaerobic consortium that mineralizes 3-chlorobenzoic acid. Appl. Environ. Microbiol. 48: 840–848.

35 Suflita, J.M., A. Horowitz, D.R. Shelton and J.M. Tiedje. 1982. Dehalogenation: A novel pathway for the anaerobic biodegradation of haloaromatic compounds. Science 218: 1115–1117.

36 Suflita, J.M., J.A. Robinson and J.M. Tiedje. 1983. Kinetics of microbial dehalogenation of haloaromatic substrates in methanogenic environments. Appl. Environ. Microbiol. 45: 1466–1473.

37 Suflita, J.M., J. Stout and J.M. Tiedje. 1984. Dechlorination of (2,4,5-trichlorophenoxy)acetic acid by anaerobic microorganism. J. Agric. Food Chem. 32: 218–221.

38 Tsuchiya, T. and T. Yamaha. 1983. Reductive dechlorination of 1,2,4-trichlorobenzene on incubation with intestinal contents of rats. Agric. Biol. Chem. 47: 1163–1165.

39 Tsuchiya, T. and T. Yamaha. 1984. Reductive dechlorination of 1,2,4-trichlorobenzene by *Staphylococcus epidermidis* isolated from intestinal contents of rats. Agric. Biol. Chem. 48: 1545–1550.

40 Vogel, T.M. and P.L. McCarty. 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride and carbon dioxide under methanogenic conditions. Appl. Environ. Microbiol. 49: 1080–1083.

41 Yamada, T. and T. Suzuki. 1983. Occurrence of reductive dechlorination products in the paddy field soil treated with CNP (Chloronitrofen). J. Pestic. Sci. 8: 437–443.

42 Yoneyama, K. and F. Matsumura. 1981. Reductive metabolism of heptachloroparathione, 4,4'-dichlorobenzophenone, and carbophenothion by rat liver systems. Pestic. Biochem. Physiol. 15: 213–221.

43 Zoro, J.A., J.M. Hunter, G. Eglington and G.C. Ware. 1974. Degradation of *p,p'*-DDT in reducing environments. Nature (Lond.) 247: 235–236.

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THE ROLE OF BACTERIA IN THE FORMATION OF ORGANIC DEPOSITS ON THE SPIRAL CONCENTRATORS OF THE MOUNT WRIGHT PLANT, QUEBEC CARTIER MINING COMPANY

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Extractive Metallurgy Laboratory

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ABSTRACT

The formation of an organic deposit on the spiral concentrators of the Quebec Cartier Mining Company Mount Wright Plant, Fermont, Quebec, altered the geometry of the spirals, thereby decreasing the efficiency of the operation of the concentrators. The organic deposit and the process water at the plant were found to be contaminated with bacteria capable of slime formation. Analysis of samples obtained from various locations within the plant and from process water sources indicated that the source of contamination and the metabolites for the organisms arise from the percolation of sewage into Hesse Lake. Prevention of contamination of Hesse Lake with sewage, sterilization of the plant and chlorination of the process water until the metabolite levels in Hesse Lake drop to normal levels (less than 15 micrograms per litre) are recommended.

LE RÔLE DES BACTÉRIES DANS LA FORMATION LES DÉPÔTS ORGANIQUES SUR LES SURFACES DES CONCENTRATEURS EN SPIRALES DE L'USINE MOUNT WRIGHT DE LA COMPAGNIE MINIÈRE QUÉBEC CARTIER

par

M. Silver, V. Sanmugasunderam et R.G.L. McCready

RÉSUMÉ

La formation d'un dépôt organique sur les surfaces des concentrateurs en spirale de l'usine Mount Wright de la Compagnie Minière Québec Cartier (QCM), Fermont, Québec, a modifié la géométrie de ces surfaces et, par conséquence, a diminué l'efficacité de l'opération de ces concentrateurs. Des espèces de bactéries qui synthétisent des matériaux vaseux ont été trouvées dans le dépôt organique et dans l'eau du procédé. L'analyse des échantillons de l'eau du procédé provenant de différents lieux a démontré que la source de contamination et des métabolites essentiels pour les microorganismes provenait du dévesement l'induction des eaux-vannes dans le lac Hesse. On propose que la contamination du lac Hesse par les eaux-vannes cesse, que l'usine soit stérélisée, et que l'eau du procédé soit traitée avec du chlore jusqu'à ce que les concentrations des glucides reviennent à des niveaux normaux (moins que 15 micrograms par mL).

INTRODUCTION

The Quebec Cartier Mining Company, Mount Wright Plant (QCM), Fermont, Quebec, processes 130,000 to 150,000 tonnes per day of a fine grained 30% iron specular hematite ore. The ore is crushed and ground to less than (1.40 mm) 14 mesh, and pumped at 35% solids to a spiral concentrator unit (used for separating the crushed ore into concentrate, middlings and tailings) consisting of 8640 single spirals. Approximately 86% of the iron is recovered in the concentrate which contains approximately 66% iron in about 40% of the original mass of the ore. The tailings contain about 8% iron in approximately 60% of the original mass of the ore, and are discarded as waste. The middlings are returned to the grinding circuit and are reprocessed (1).

A problem that has arisen in the spiral concentrators decreases the efficiency of this operation. An organic deposit (crud) forms in the spirals, altering their geometry. This results in poor separation of the concentrate from the tailings and an increase in the proportion of the iron reporting to the middlings. As the middlings must be reground, the energy requirements of the grinding circuit are increased, thereby increasing processing costs. Furthermore, overgrinding can result in losses of fine iron minerals to tailings.

Spiral deposits have been a problem within the QCM circuit since 1977. Commercial laboratories have previously analyzed the organic deposits and have identified enteric coliform bacteria in a few of the samples. The problem of the

organic deposit formation was brought to the attention of a CANMET offical during a visit to the plant. One of the authors visited QCM and collected process water samples from various locations within the mill circuit. Analysis of these samples identified the source of contamination and the metabolites supporting the growth of the organisms.

In natural and industrial aquatic environments high concentrations of bacteria, surrounded by an extra-cellular polysaccharide component called the glycocalyx, are frequently found on surfaces. These glycocalyx-enclosed microcolonies form stable films which tend to enlarge and may contain one or a number of different species of bacteria. The bacterial population is attached on submerged surfaces, and withdraws nutrients very efficiently from the water flowing over the film. The glycocalyx also protects the bacterial cells from physical and chemical agents (3).

Figure 1 shows the diagrammatic representation of a bacterial cell. Capsules, slime and the cell wall and their relationship is shown clearly in the diagram. The slimes and capsules are composed of polysaccharides, lipopolysaccharides and other sugars. Because of their composition they act as strong adhesives under certain industrial situations as seen in the spiral deposit.

Samples of the organic deposits and water were obtained from various locations in the mill and the bacteria were

identified and the cellular concentration was determined to locate the source of contamination. These samples were also analyzed to determine the carbon and energy sources present which sustained the bacterial growth.

MATERIALS AND METHODS

Samples of organic deposit found on the surface of the spiral concentrators were obtained from QCM. Liquid samples were collected in sterile plastic bottles from eighteen locations at the plant. The sampling locations are listed in Table 1 and are indicated in Figures 2 and 3.

A suspension of the organic deposit was prepared using sterile water. A standard loopful of this suspension, streaked on to nutrient agar plates, was incubated for 72 hours. The individual colonies which developed on the plates were then subcultured. Gram-negative rod-shaped bacteria capable of growth on carbohydrates were identified using the Enterotube II and Oxi/Ferm diagnostic systems (Hoffman La Roche Limited, Mississauga, Ontario). Non-enteric bacteria (*Corynebacterium*, *Arthrobacter*, *Norcardia*, and *Streptomyces*) were identified from their morphology, acid-fast staining characteristics, and growth on sugars other than those contained in the above diagnostic systems. Slime generation was observed by growing the bacteria isolated from the organic deposit in a defined liquid medium (4) containing 0.1% glucose or sucrose (Table 2).

The bacteria were enumerated by the pour plate method (5), in which 1.0 mL aliquots of the liquid samples, or five

ten-fold serial dilutions thereof, were placed in sterile petri dishes with about 10 mL of melted agar medium (45oC). Levines EMB agar was used for the enumeration of enteric and faecal coliform bacteria, and nutrient agar was used for the enumeration of total bacteria. After 72 hours, the colony-forming units in each plate were counted with the aid of a Quebec Colony Counter. The average number of colonies was multiplied by the reciprocal of the dilution to give the bacterial concentrations per mL of original sample.

Carbohydrate concentrations were determined colorimetrically with anthrone (6), total organic carbon was determined by combustion, and total dissolved solids were determined gravimetrically.

RESULTS

The identification of microorganisms, isolated from the organic deposit and the water samples obtained from the plant, are shown in Table 3. Both enteric bacteria originating from the intestinal tract of warm-blooded animals and microorganisms commonly found in soil, dust and surface water were detected. Of the enteric bacteria, only the faecal coliform Klebsiella oxytoca produced copious amounts of capsular material. The soil bacteria were identified as Pseudomonas fluorescens, a Norcardia species, and two isolates of the genus Corynebacterium which also produced large amounts of capsular material, whereas all other microorganisms identified produced only minor quantities of

capsular material.

Table 4 shows the concentrations of faecal coliforms, enteric bacteria and total bacteria from the water samples of the mill. The concentrations of carbohydrate carbon, total organic carbon and total dissolved solids present in the liquid samples are presented in Table 5. The carbohydrate carbon is expressed as mg carbon per litre.

Bacteria in all samples were able to grow and produce capsular material when incubated in the presence of air at 20oC in a medium containing mineral salts and a sugar. Calgon M 502, the additive used by QCM for thickening, when used as the sole carbon-source did not support growth of these bacteria at concentrations between 0.001 and 10% (v/v).

DISCUSSIONS

Microorganisms which cause slime deposit formation are either of soil origin and belong to the Pseudomonas and Flavobacterium, or originate in the intestines of warm-blooded animals and belong to the genus Klebsiella (formerly known as Aerobacter) (2). All three genera are present in both the organic deposit from the spiral concentrators and in the process water. In addition, enteric bacteria originating from sewage were detected.

The presence of enteric bacteria , including faecal coliforms, in the organic deposit from the spiral concentrators

suggests sewage contamination of the mill process water as the probable cause for the formation of this deposit. The detection of large concentrations of these bacteria in the process water further reinforces this thesis.

The sources of make-up water determine the type and number of microorganisms introduced into the mill system(2). Surface water, free from sewage, generally contains small concentrations (usually less than 100 cells/mL) of microorganisms, mainly of soil origin. Water polluted by pasture run-off or sewage will contain bacteria of intestinal origin (enteric bacteria) which may be present in large concentrations (>1,000 cells/mL). When polluted water is used for industrial purposes, the bacteria enter the plant circuit, and grow and multiply by using the organic compounds present as their energy source.

Bacteria proliferate in plant locations such as holding tanks, sumps, or attached to the interior walls of pipes and exposed to a flowing stream of nutrient containing water (3). Biofouling of pipes is a very serious problem to many industries (7). When attached to a surface , bacteria can efficiently remove nutrients from solution, and multiply with the formation of a very concentrated cell mass (2). The process water of the Mount Wright mill contains 5 to 25 mg/L of total organic carbon. The carbohydrate carbon, which is available to organisms as a nutrient, varies from 0.6 to 5 mg/L. Carbohydrate content, measured as glucose, varies from 1.5 to 12.1 mg/L.

High concentrations of enteric bacteria and the presence of faecal coliforms at locations 1, 2 & 5 indicates that the Hesse Lake is contaminated with sewage. The origin of this pollution appears to be the sewage overflow from the settling ponds and filtration lagoons (locations 6 to 8) which percolates into Hesse Lake. The water sample obtained from the in-take sump in the pumphouse near Hesse Lake (sample 5) has a total organic carbon content of 5.9 mg/L of which 2.2 mg/L is in a form which can be readily utilized by the microorganisms as nutrient.

Table 4 shows that the samples obtained from Hesse Lake and the sump (in-take for the pumps), sample numbers 1, 2 & 5, have faecal coliform and enteric bacteria and the carbohydrate carbon, which is the nutrient for the bacteria, is more than 2 mg per litre. The maximum concentrations of sugars in Canadian lakes have been reported to be 10 micrograms of sucrose and 5 micrograms of glucose (8). Sample number 8, obtained from the filtration lagoon, which receives the overflow from the sewage sedimentation tank, had 4.8 mg/L of carbohydrate carbon and the concentrations of faecal coliform bacteria and enteric bacteria were 1,017/mL and 5,380/mL respectively. Sample numbers 13 to 16, from the surface of thickener # 1, and the head tanks of the rougher, cleaner and recleaner spirals, respectively, had over 4 mg/L of cabohydrate carbon. For practical purposes, the amount of nutrient available for the bacteria is at the same concentration as that found in the overflow from the sewage settling pond. The maximum concentration of enteric bacteria

were detected in samples 6 and 18, which were obtained from the settling pond and the mill discharge respectively. The concentration of total organic carbon was highest in the mill discharge, probably due to the introduction of soil organic matter from the open pit. Although, the organic carbon is high, the carbohydrate carbon was only about 1 mg/L, which may be due to its depletion by the organisms present. The grinding mill, being inside the plant building, is maintained at a temperature that will support active bacterial growth. Moreover, the water in the mill receives maximum aeration and the bacterial cells growing in this part of the circuit are not exposed to the same abrasion by fine particles that may occur later in the circuit. The high concentration of enteric bacteria in the mill discharge could be due to the fact that it is the most suitable location for the attachment and growth of enteric type organisms.

RECOMMENDATIONS

1. Ensure that the sewage does not contaminate Hesse Lake or any water that returns to the Hesse Lake.
2. Decontaminate the entire plant by removing all accumulated deposits and chemically sterilizing the entire circuit.
3. Continue the chlorination of the process water until the assimilable sugar concentration reaches normal levels (15 micrograms per litre). The analytical procedure, for the determination of the sugar concentrations in water samples, is detailed in the Appendix.

ACKNOWLEDGEMENTS

The authors express their sincere appreciation of the following persons for their work, advice and discussions throughout the course of this study: J. Salley and S. Rajan for their technical assistance; W. Cameron for his advice, coordination of sampling and encouragement; the staff of the Chemical Laboratory for the various chemical analyses and the staff of QCM for providing the samples and cooperation extended to CANMET.

REFERENCES

1. Staff, Quebec-Cartier Mining Company - Mount Wright; In: Milling Practice in Canada; Edited by D.E. Pickett; Montreal,Canadian Institute of Mining and Metallurgy (CIM Special Volume 16); 266-268; 1978.

2. Smith, A.L., and Muia, R.A. "Identify and Control Microbiological Organisms in Cooling Water Systems"; Power 17-19 and 91-95; July, 1973.

3. Costerton, J.W.G., Irwin, R.T. and Cheng, K.J. "The Bacterial Glycocalyx in Nature and Disease"; Ann Rev Microbiol 35:299-324; 1981.

4. Stanier, R.Y., Doudoroff, M. and Adelberg, E.A. "The Microbial World" 3rd Edition; Englewood Cliffs, Prentice-Hall; 1970.

5. Collins, C.H. and Lyne, P.M. "Microbial Methods" 3rd Edition; London, Butterworths; 1970.

6. Umbreit, W.W., Burris, R.H. and Stauffer, J.F. "Manometric and Biochemical Techniques" 5th Edition; Minneapolis, Burgess; 1972.

7. Costerton, J.W. and Geesey, G.G. "Microbial Contamination of Surfaces"; In Surface Contamination; Edited by K.L. Mittal; New York, Plenum; 211 - 221; 1979.

8. Kuznetov, S.I., Dubimina, G.A. and Lapetea, N.A. "Biology of Oligotrophic Bacteria"; Ann Rev Micrbiol 33:377-387; 1979.

TABLE 1

Sample Description

Sample #	Location (See Fig. 2 & 3)	Remarks
1	Hesse Lake	Surface sample
2	Hesse Lake	Two feet below the surface
3	Discharge from plant	Approx. 25 feet upstream from intake
4	Ground water	Supposedly from underground.
5	Sump	In-take location for the pumps
6	Settling pond # 1	Surface sample
7	Settling pond # 2	Sewage from primary filtration and settling pond # 2 find their way to this pond.
8	Filtration lagoon	Very strong odour
9	Filtration tank	Water from Mogridge prior to filtration and chlorination
10	Filtration tank	Same as 9 except that it contained algal scrapings
11	Tap	Potable Mogridge water after filtration and chlorination.
12	Process sump	Approx. 1 part from Hesse Lake 2 parts from thickeners and the overflow from Mogridge.
13	Thickener # 1	Surface sample
14	Head tank for rougher spirals	Location indicatedonschematic block diagram
15	Head tank for cleaner spirals	Location indicated on schematic block diagram
16	Head tank for re-cleaner spirals	Location indicated on schematic block diagram
17	Recycle feed	The water fractions from coarse cleaner and recleaner spirals collection.
18	Mill discharge	Slurry from the grinding mill

TABLE 2

Composition of Defined Liquid Medium(4)

Component	Concentration (g/L)
Carbon Substrate	Variable
K_2HPO_4	1.0
$MgSO_4.7H_2O$	0.2
$FeSO_4.7H_2O$	0.01
$CaCl_2$	0.01
NH_4Cl	1.0
$MnSO_4$	0.001
pH 6.5	

The medium minus sugar substrate is sterilized at 121°C for 15 minutes. Sterile glucose or sucrose solutions are added to yield a final concentration of 0.1%. Calgon M 502, when used as substrate, is added to the solution at concentrations of 0.001 to 10% before sterilization.

TABLE 3

Identification of Microorganisms from the Organic Deposit on the Spiral Concentrator Surfaces and in the Process Water of the Quebec Cartier Mining Company Mount Wright Mill

Group	Bacteria Isolated from Organic Deposit	Bacteria Isolated from Process Water
Faecal Coliform Bacteria	Escherichia coli	Escherichia coli
	Klebsiella oxytoca	Klebsiella oxytoca
		Klebsiella ozaenae
Other Enteric Bacteria	Citrobacter freundii	Citrobacter amalonaticus
	Shigella sp.	Enterobacter agglomerans
		Providencia stuartii
		Shigella sp.
		Yersina pseudotuberculosus
Oxidative-Fementative Bacteria	Flavobacterium sp.	Flavobacterium sp.
	Pseudomonas fluorescens	Pseudomonas aeruginoa
	Pseudomonas putida	Pseudomonas sp.
Gram-positive Pleomorphic Bacteria	Corynebacteirum sp.	Arthrobacter sp
	Norcardia sp.	Corynebacteirum sp.
		Norcardia sp.
Other Micro-organisms	Torulopsis sp.	

TABLE 4

Concentrations of Bacteria in the Water Samples
(organisms/mL)

Sample #	Coliform Bacteria	Enteric Bacteria	Total Bacteria
1	92	500	2,450
2	82	318	1,580
3	181	2,565	20,000
4	0	8	649
5	13	96	324
6	2,017	12,200	273,800
7	290	1,930	67,933
8	1,017	5,380	163,800
9	199	426	970
10	5	23	930
11	0	0	432
12	19	75	1,245
13	108	255	830
14	39	154	1,175
15	25	65	1,570
16	22	103	1,260
17	75	274	4,400
18	320	15,500	38,000

TABLE 5

Concentrations of Carbohydrate Carbon, Total Organic Carbon and Total Dissolved Solids in Milligrams per Litre

Sample #	Carbohydrate Carbon	Total Organic Carbon	Total Dissolved Solids
1	3.0	5.5	100
2	2.4	7.0	100
3	1.6	9.5	181
4	1.1	11.0	65
5	2.2	5.9	101
6	4.1	8.5	71
7	3.5	13.0	80
8	4.8	8.6	60
9	1.6	7.9	36
10	0.6	9.8	33
11	0.7	6.1	30
12	4.0	18.0	135
13	4.7	5.8	120
14	4.7	5.7	124
15	4.4	5.6	115
16	4.3	7.2	114
17	0.8	11.0	115
18	1.0	25.0	215

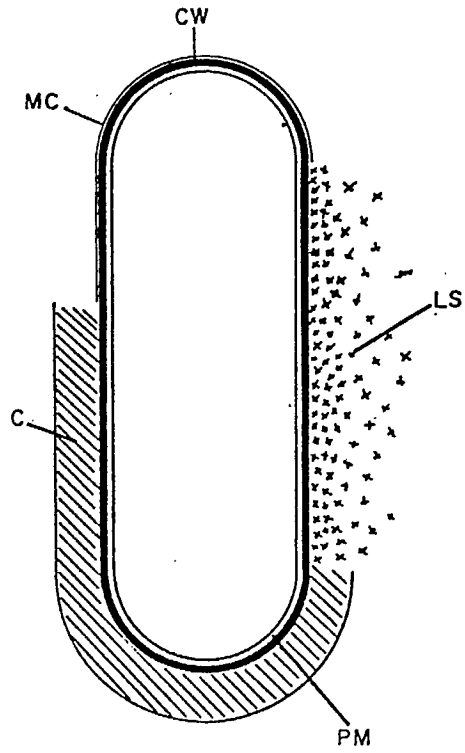

FIG. 1. A diagrammatic representation of the relationship between the capsules, slime, and microcapsular layers to the cell wall and protoplast membrane. CW = cell wall; PM = protoplasmic membrane; C = capsule; MC = microcapsule; LS = loose slime.

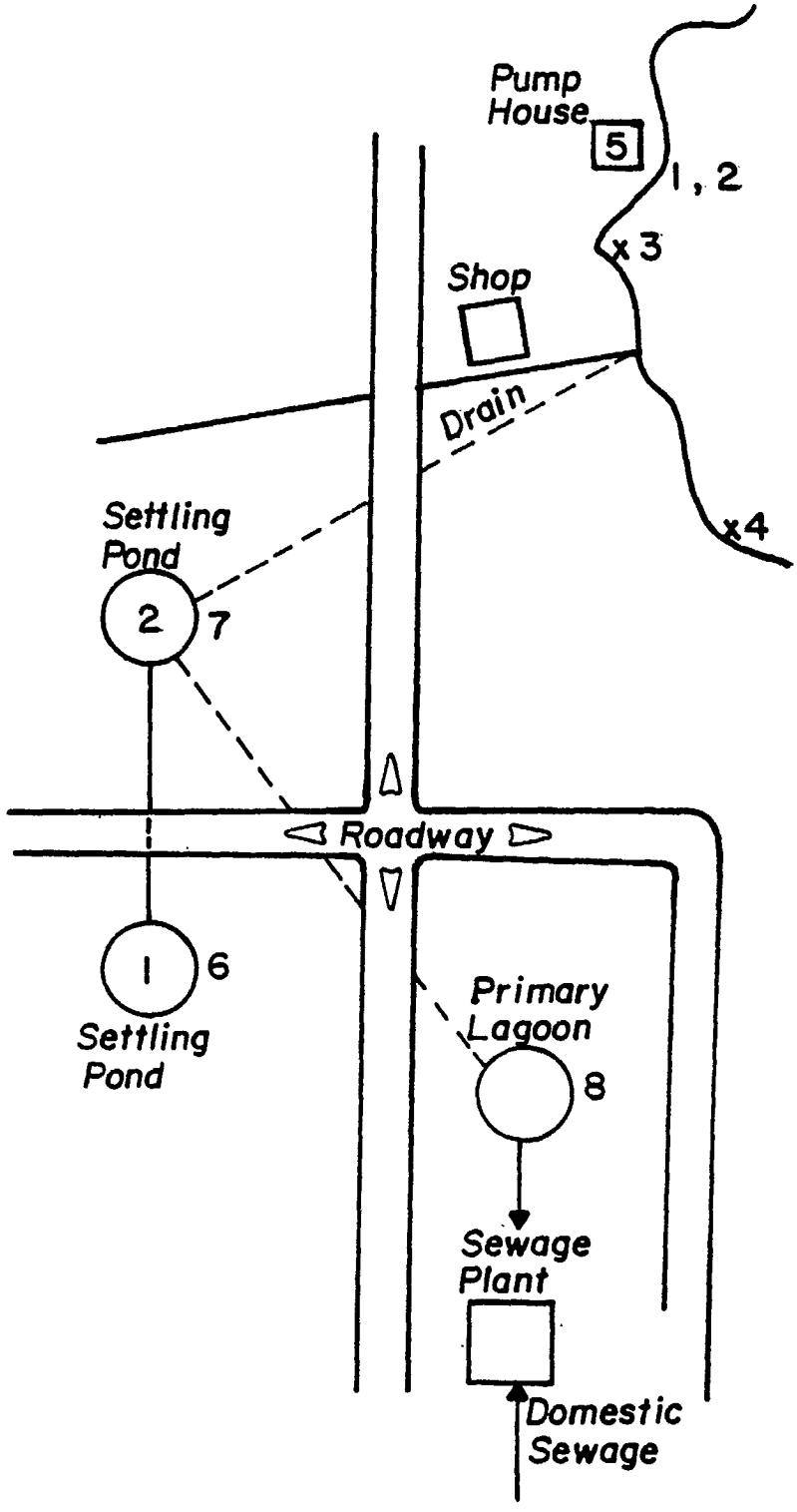

Figure 2. Sampling Locations 1 to 8

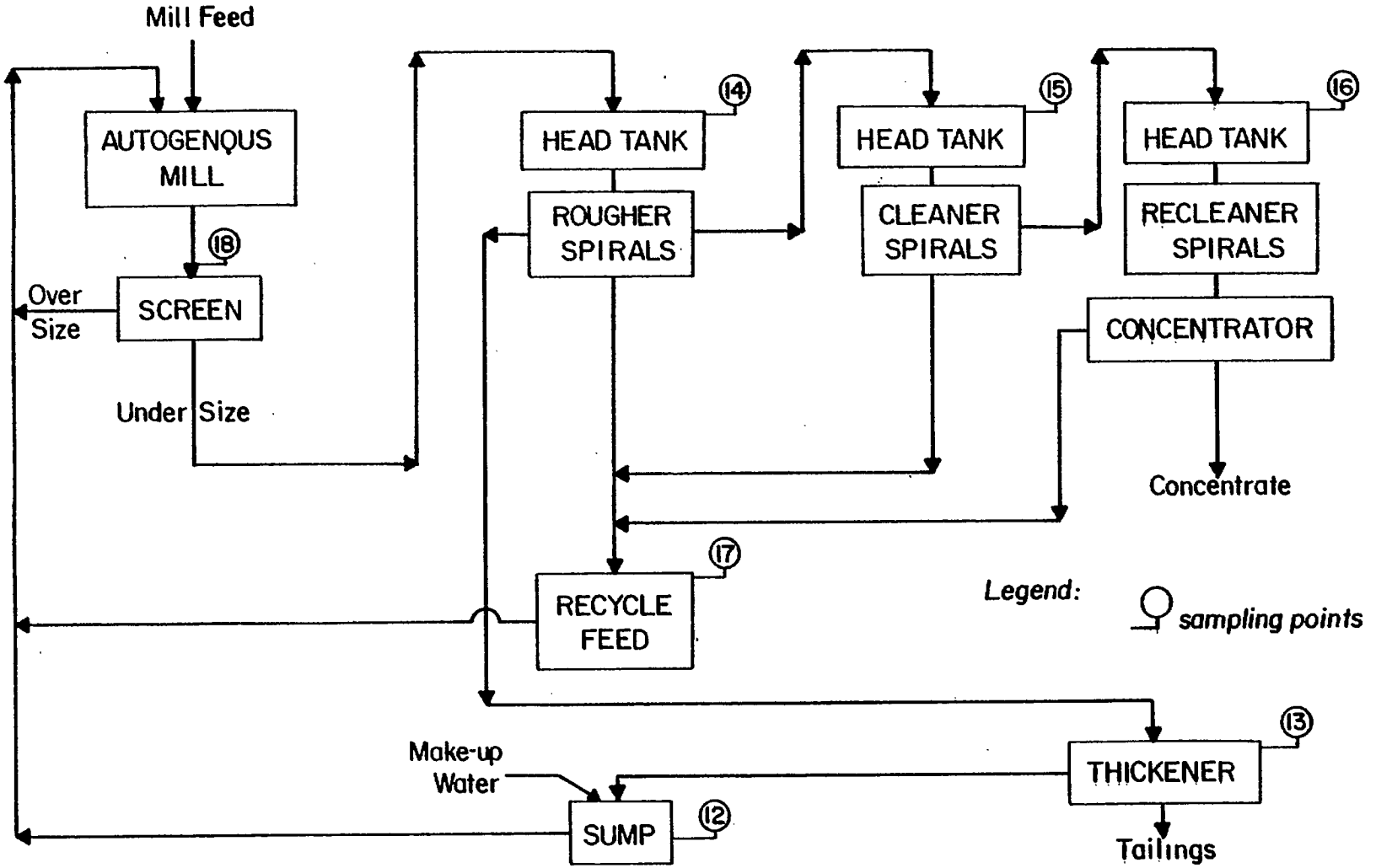

Figure 3 Schematic Block Diagram Showing Sampling Locations 12 to 18.

APPENDIX

METHOD FOR THE DETERMINATION OF TOTAL CARBOHYDRATE CONCENTRATION

Appendix - Method for the determination of total carbohydrate concentration

Reference: Umbreit, W.W., Burris, R.H. and Stauffer, J.F. "Manometric and Biochemical Techniques, 5th Edition. Minneapolis, Burgess (1972).

Anthrone (9-oxyanthracene) reacts with all carbohydrates (mono-, di-, oligo-, and polysaccharides) to give a characteristic blue colour, but the colour yield is not the same for all carbohydrates. As the standard used is glucose, the results can be described in terms of concentration of total carbohydrates as glucose, or can be multiplied by 0.4 and described as concentration of carbohydrate carbon.

Reagents: 1. The anthrone reagent consists of 2 g of anthrone (9-oxyanthracene) dissolved in 1 L of 95% reagent grade sulphuric acid (950 mL H_2SO_4 plus 50 mL distilled water). The reagent is unstable and must be prepared daily.

2. The glucose standard is prepared by adding 120 mg of reagent-grade glucose to 1 L of distilled water.

3. Distilled water.

Equipment and Supplies: 1. A spectrophotometer or colorimeter capable of use in determining optical density at 620 nm ($O.D._{620}$).

2. Acid washed test tubes of at least 15 mL capacity.

3. A matched set of spectrophotometer curvettes.

Procedure: To 3 mL of sample containing or diluted to contain 12 to 120 μg of carbohydrate (as glucose), add 6 mL of anthrone reagent and mix immediately. Heat for 3 min in a boiling water bath and cool to room temperature. Measure the optical density at 620 nm and compare to standards containing 12 to 120 μg of glucose.

Because the anthrone reagent is unstable and darkens with time, a fresh solution must be prepared daily and a set of standards must be included with each determination. Because of the corrosive nature of the reagent, which is prepared in 95% sulphuric acid, care must be exercised in handling of this reagent; spills must be avoided.

The colour developed by this method follows Beer's law, and the concentration can be calculated from the standard curve.

Calculation: mg/L carbohydrate as glucose = $\frac{AB}{C} \times \frac{1000}{D}$

where A is the total number of milligrams in the standard, B is the optical density of the sample, C is the optical density of the standards, and D is the number of millilitres (3) of the sample. To convert the number of mg of glucose per litre to the number of mg of carbohydrate carbon per litre, multiply by 0.4.

BACTERIAL CORROSION

Corrosion of Mild Steel in Cultures of Ferric Iron Reducing Bacterium Isolated from Crude Oil I. Polarization Characteristics*

C. O. OBUEKWE, D. W. S. WESTLAKE,* J. A. PLAMBECK,** and F. D. COOK****

Abstract

The polarization characteristics of mild steel were studied under microaerobic conditions in four media, including a synthetic medium and the produced water from an oil field. In all the media, anodic depolarization was always observed when a ferric iron reducing bacterium (*Pseudomonas* sp.) was present. In produced water, both anodic and cathodic depolarization of the mild steel could occur, although transiently. Addition of sodium lactate as a substrate to boost the total available energy in the produced water caused the anodic depolarization to be sustained while cathodic depolarization was not abolished. Anodic depolarization was accompanied by the bacterial reduction of ferric to ferrous compounds. In the absence of the ferric iron reducing bacterium, both the anode and the cathode were polarized. A mechanism for the bacteria induced anodic depolarization is suggested.

Introduction

The pipeline system serving the Pembina oil field of north central Alberta, a field which utilizes injection water to maintain production, frequently fails due to corrosion. The frequency of failure of this pipeline system is much higher than that reported in pipelines serving the Rainbow oil field in northwestern Alberta, which at the time of this study was still in primary production. The oils produced from these fields have similar physical/chemical characteristics but do differ in their bottom sediments and water (BS and W) and bacterial contents. Rainbow crude oil had higher BS and W levels than the Pembina crude oil, but had no bacterial contamination. In contrast, Pembina oil samples, which had lower BS and W values, supported a wide variety of bacterial populations.[1] If the corrosion failures reported in the two pipeline systems were due to direct electrochemical attack, greater corrosion would be expected in the Rainbow system because its BS and W value is higher. Since the observed corrosion in the two pipeline systems is greater in the Pembina pipeline system, other factors such as bacteria may be involved in the severe corrosion observed in the Pembina pipeline system.

The role of bacteria in the corrosion of ferrous metals is well documented.[2-7] The bacterial contaminants of Pembina oil include sulfate reducers and a variety of aerobic and facultative bacteria. The aerobes constitute a diverse group, within which occurs a group of nutritional by versatile and biochemically active bacteria which have been shown to be able to reduce SO_3^{2-}, $S_2O_3^{2-}$, and S^0 to S^{2-}. They also have the abillty to reduce ferric [Fe(III)] to ferrous [Fe(II)] compounds. The simultaneous production of Fe(II) and S^{2-} will undoubtedly add to the corrosivity of the environment. Booth, *et*

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al,[8] reported on the influence of Fe(II) on the corrosion of mild steel. High levels of Fe(II) in cultures of *Desulfovibrio* sp. prevented the formation of a protective FeS coating on the coupon, while the high amount of FeS formed caused cathodic depolarization. The corrosivity of soils has been associated with soluble iron and soils with Fe(II) content above about 333 μg/g of soil have proved very corrosive.[4] It was the constant occurrence of the ferric iron reducing bacteria in the Pembina crude oil and their potential corrosive activities that prompted investigation into their role in the corrosion of the Pembina crude oil pipeline system.

Materials and Methods

Bacterial Culture and Inoculum Preparations

The bacterium employed is a ferric iron [Fe(III)] reducing *Pseudomonas* sp., isolate #200.[1] The organism was isolated from crude oil samples from the Pembina oil field and was chosen for this investigation because of its corrosive activities.[9]

The bacterial inoculum for the studies was grown in a complex medium[10] containing nutrient broth. The organism was grown aerobically in 200 ml volumes in 500 ml Erlenmeyer flasks on a New Brunswick shaker (Model G-2; 295 rpm and one and one-half inch eccentricity) at 30° for 14 hours. The cells were recovered by centrifugation, washed three times in cold 0.1M phosphate buffer, pH 7.2, and suspended in the same buffer at the concentration of 1 g wet weight per 80 ml of the buffer, yielding a cell number of approximately 30×10^7 cells per ml.

Polarization Media

The bacterial growth and polarization experiments were undertaken in three different media (two complex media and a defined synthetic medium) and produced water from the Pembina oil field. The composition of the media are as follows, per liter: (1) modified Butlin's medium-K_2HPO_4, 0.5 g; NH_4Cl, 1.0 g; Na_2SO_4, 2.0 g; $MgSO_4.7H_2O$, 0.1 g; $FeSO_4.7H_2O$, 0.1 g; sodium lactate (60%), 1.5 ml; Yeast extract (Difco) 1.5 g; final pH 7.2; (2) B_{10} medium-K_2HPO_4, 0.8 g; KH_2PO_4, 0.2 g; $MgSO_4.7H_2O$, 0.2 g; Na_2SO_4, 0.4 g; $MnSO_4$, 0.001 g; $NaMoO_4$, 0.001 g; Yeast extract (Difco) 50 g; Peptone (Difco), 5.0 g; $FePO_4$, 4.7 g; final pH 7.2; (3) synthetic medium-K_2HPO_4, 0.5 g; Na_2SO_4, 2.0 g; NH_4Cl, 1.0 g; $CaCl_2.2H_2O$, 0.15 g; $MgSO_4.7H_2O$, 0.1 g; $FeSO_4.7H_2O$, 0.1 g; sodium lactate (60%), 3 ml; final pH 7.2.

Polarization Cell

The polarization cell consisted of a Pyrex glass corrosion chamber inserted into a larger auxiliary electrode chamber. The chambers were separated from each other by the 0.5 mm thick porous Pyrex seal of pore dimensions 0.32 to 10 μ (Figure 1), of a 33 mm diameter sealing tube. which housed the working (indicator) electrode (mild steel coupon), and the reference saturated calomel electrode. The working electrode was AISI 10-18 mild steel, 50 × 12 × 1.0 mm, chosen because it is closely related to the material that the pipeline was fabricated from. The test steel coupons were punched out on a die from sheared, cold rolled sheet metal. fine glass-blasted to give a bright finish, packaged in water proof sleeves and used as received. The coupons were never handled with bare fingers.

The auxiliary electrode (platinum flag of large area) was contained outside the corrosion chamber in a large surrounding chamber containing about 800 ml of medium. By this arrangement, the auxiliary electrode was physically separated from the working electrode but remained electrically connected by the porous seal through which ionic migration can occur. The large auxiliary electrode compartment, containing the bulk of the medium, which slowly diffused into the corrosion chamber, acted as a substrate reservoir for sustained bacterial growth and activity. A major advantage of this cell is that it is easy to construct and assemble for use. The whole unit, minus the reference and working electrodes, is autoclaved as one, thus reducing the chances of bacterial contamination—an asset in microbiological studies.

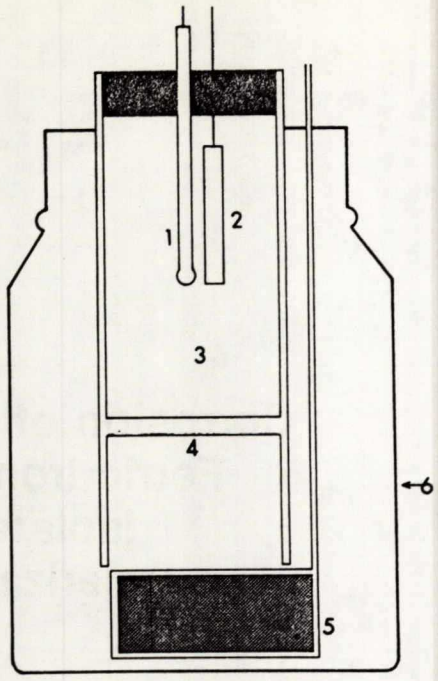

FIGURE 1 — Polarization cell. 1—Reference electrode (SCE); 2—working electrode (mild steel); 3—corrosion chamber; 4—porous seal; 5—auxiliary electrode (platinum); 6—auxiliary electrode chamber (medium reservoir).

Sterilization of Components

The SCE was sterilized by wrapping for 10 minutes in tissue paper soaked in 70% ethanol. The working electrodes (steel coupons) were sterilized by immersion in 70% ethanol, degreased by rinsing in 95% ethanol, and dried in a stream of warm air under U.V. All other components of the polarization cell were autoclaved, as a unit, at 121 C and 15 psi for 15 minutes.

Polarization Procedure

Before each polarization run, the medium was deaerated by passing a stream of deoxygenated nitrogen gas (N_2) for 15 minutes. Deoxygenation of N_2 was achieved by bubbling the gas through an acidic 0.82% vanadous chloride solution. No further deaeration of the cell was attempted so that the experiments could be conducted essentially under microaerobic conditions. The unimmersed portion of the working electrode was covered with Teflon tape. One milliliter of the washed cell suspension constituted the inoculum.

Immediately following inoculation (time 0), the value of the open circuit potential was recorded for two to five minutes during which the potential stabilized. The working electrode was then polarized potentiodynamically over a range of 0.4 V in the negative and positive directions (cathodically and anodically, respectively) with respect to the open circuit potential. The working electrode was polarized at the slow scan rate of 2 mV/second and the current output was read at intervals of 25 mV; the uninoculated cell being the control. Each polarization run was conducted on a fresh coupon sample.

The potentiostat used was a Princeton Applied Research (PAR), Model 173 Potentiostat/Galvanostat coupled to a PAR Model 175 Universal Programmer which provided the desired potential (as measured by Electrometer Probe Model 178) between the working and reference electrodes. All potentials

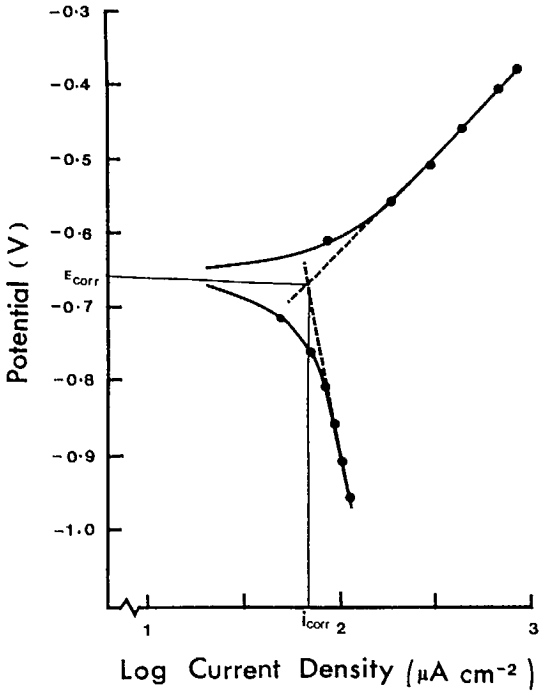

FIGURE 2 — Tafel plot constructed from data obtained during polarization of mild steel coupon in sterile B_{10} medium in the newly constructed electrochemical cell.

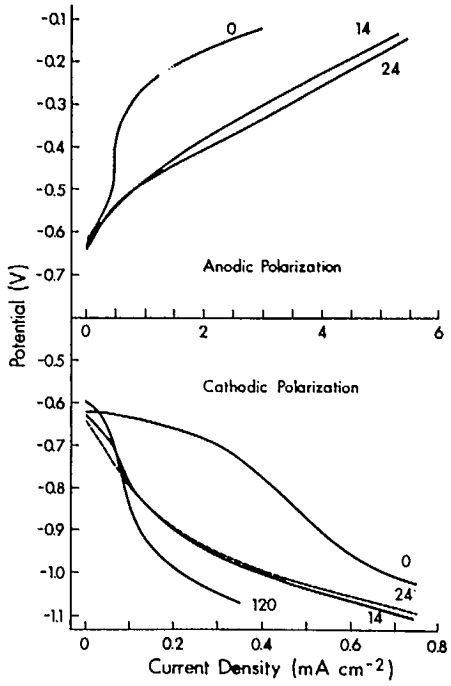

FIGURE 3 — Polarization curves for mild steel in B_{10} medium inoculated with isolate #200. Dashed lines are used for clarity only in distinguishing intersecting curves. (0, 14, 24, 120 denote incubation time (hours) at 25 ± 2 C).

were measured and the values reported are relative to the saturated calomel electrode (SCE) reference. The current was recorded on a Houston Instrument Omnigraphic Recorder Model 2000.

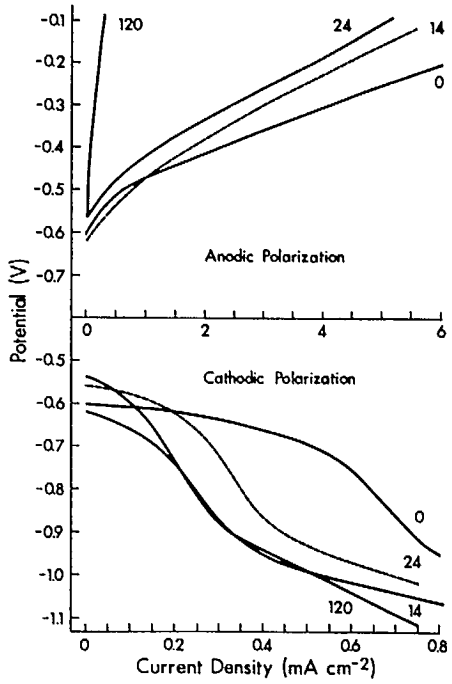

FIGURE 4 — Polarization curves for mild steel in uninoculated (control) B_{10} medium. Dashed lines are used for clarity only in distinguishing intersecting curves. (0, 14, 24, 120 denote incubation time (hours) at 25 ± 2 C).

Results

After the construction of the polarization cell, a trail run was undertaken to check the functionality of the cell. Data obtained during the polarization of mild steel in sterile B_{10} medium was used to construct Tafel plots (Figure 2) which showed well defined Tafel regions. Extrapolation of the anodic and cathodic branches to intersection gave a corrosion potential of −0.655 V, which was in good agreement with the measured open circuit potential of −0.66 V.

The polarization characteristics of a mild steel coupon in B_{10} culture of the bacterium (isolate #200) at room temperature are shown in Figure 3. The anodic polarization curves show that the metal became more active with incubation in cultures of the organism. The increase in corrodibility was greater after 14 hours of incubation but increased more slowly up to 24 hours. At the onset of the experiment, the inoculated medium appeared deep brown, but with incubation changed to a greenish color. This indicated the reduction of Fe(III) compounds present (*e.g.*, $FePO_4$) to the Fe(II) form. After 24 hours of incubation, a thick sediment was deposited at the bottom of the cell. In the absence of the organism (Figure 4), the coupon became more resistant to corrosion with exposure, which it did not in the inoculated medium. The increase in the current per unit potential change (dI/dE) was highest at the beginning of the experiment and decreased with time. Thus, inhibition of anodic corrosion occurred in B_{10} medium in the absence of the organism, while anodic depolarization was observed in the presence of the organism. The cathodic polarization curves are not affected by the organism (Figures 3 and 4).

The anodic and cathodic polarization curves of the steel specimen in inoculated Butlin's medium are shown in Figure 5. There was active anodic depolarization after 14 hours. Prolonged incubation, up to 6 days, yielded polarization curves which did not differ from the curve obtained within the first day of bacterial growth; however, the cathodic process was inhibited. Both the anodic and cathodic process were polarized in the absence of the organism (Figure 6).

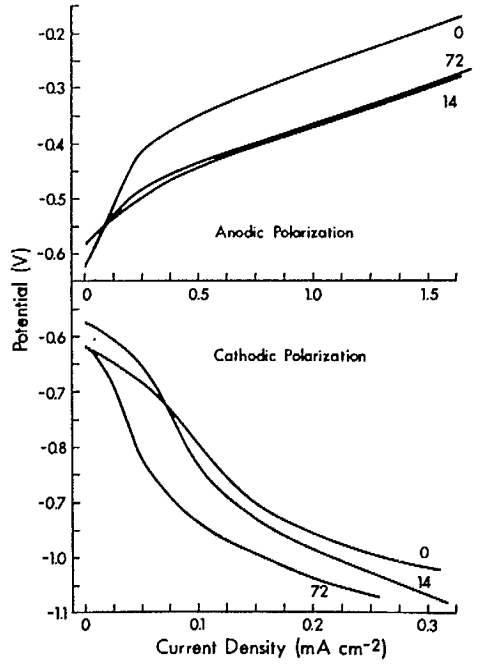

FIGURE 5 — Polarization curves for mild steel in Butlin's medium inoculated with isolate #200. (0, 14, 72 denote incubation time (hours) at 25 ± 2 C).

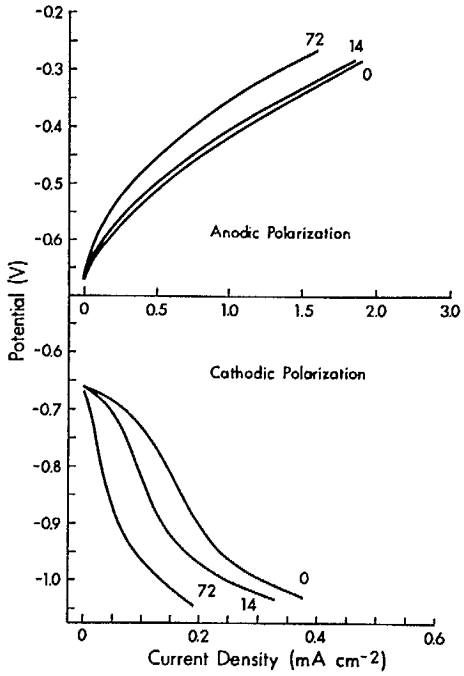

FIGURE 6 — Polarization curves of mild steel in uninoculated Butlin's medium (control). (0, 14, 72 denote incubation time (hours) at 25 ± 2 C).

In the synthetic (defined) medium, anodic depolarization was also recorded within 14 hours (Figure 7) when inoculated with the organism. The anodic and cathodic processes were polarized in sterile synthetic medium (control—Figure 8). In the three media studied so far, there was also anodic depolarization only in the presence of the organism.

The trend in the response of the mild steel coupons to anodic and cathodic polarization in produced water was more

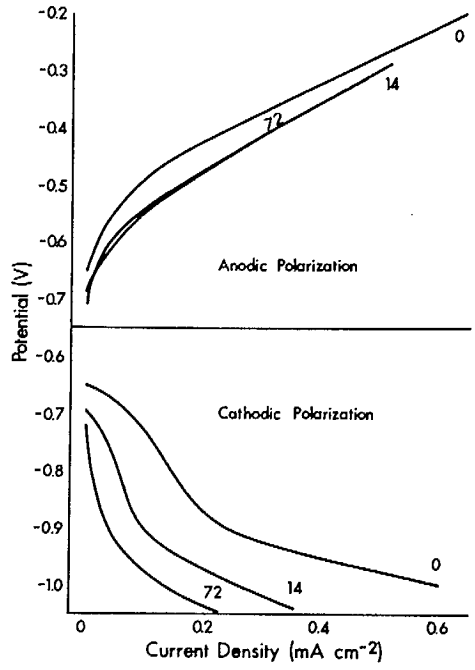

FIGURE 7 — Polarization curves for mild steel in synthetic medium (containing 1800 mg sodium lactate/1) inoculated with isolate #200. (0, 14, 72 denote incubation time (hours) at 25 ± 2 C).

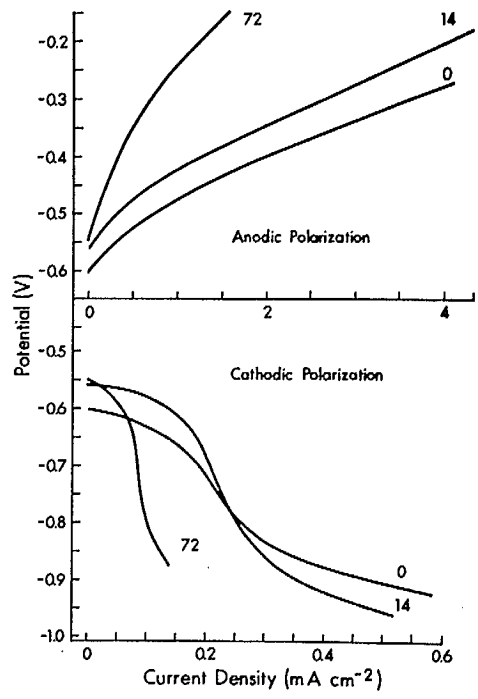

FIGURE 8 — Polarization curves for mild steel in uninoculated (control) synthetic medium (containing 1800 mg sodium lactate/1). (0, 12, 72 denote incubation time (hours) at 25 ± 2 C).

complex than observed in other media. The anodic polarization curves (Figure 9) showed that in fresh, untreated (unautoclaved, uninoculated) produced water, there was an initial anodic depolarization (14 hours) followed by stifling (polarization) of the anodic process. It appeared that in the untreated

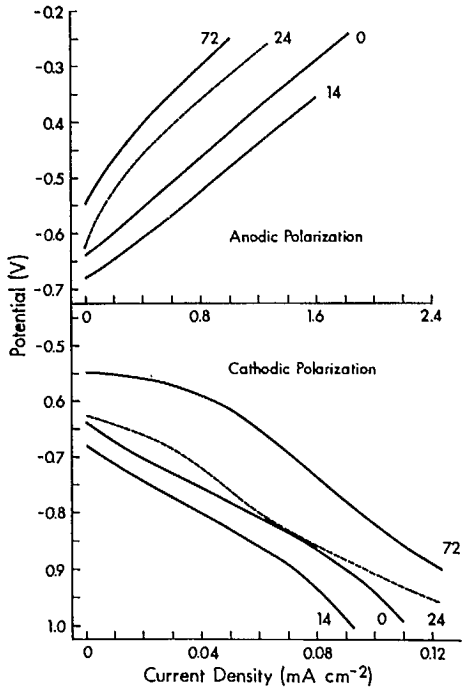

FIGURE 9 — Polarization curves for mild steel in unautoclaved, uninoculated (*i.e.*, with natural flora) produced water. (0, 14, 24, 72 denote incubation time (hours) at 25 ± 2 C).

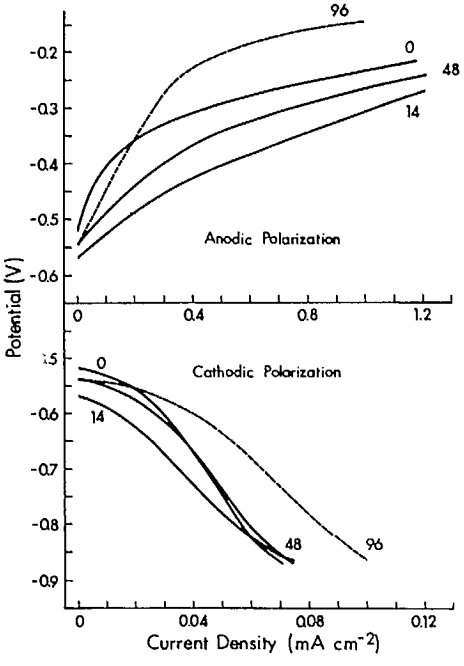

FIGURE 10 — Polarization curves for mild steel in autoclaved produced water inoculated with isolate # 200. (0, 14, 48, 96 denote incubation time (hour) at 25 ± 2 C).

produced water, the metal was initially corrodible but became less so with exposure. With the cathodic reaction, an opposite trend was observed; an initial polarization was succeeded by active depolarization.

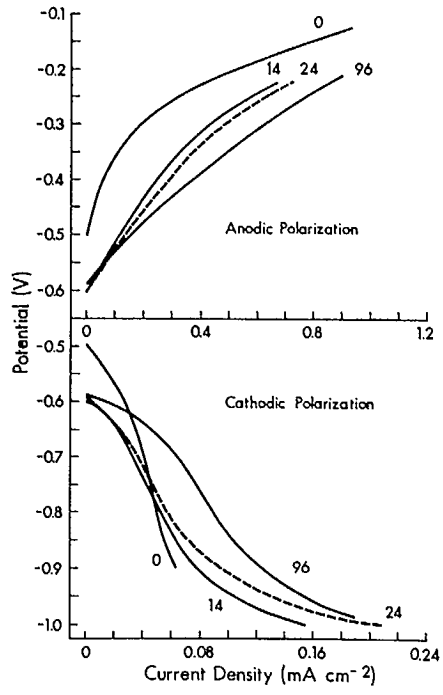

FIGURE 11 — Polarization of mild steel in produced water modified by the addition of 900 mg sodium lactate/1 and inoculated with isolate # 200. (0, 14, 24, 96 denote incubation time (hours) at 25 ± 2 C).

When the produced water was autoclaved (to kill indigenous organisms) and then inoculated with isolate # 200, the same trend in the polarization curves, an initial anodic depolarization followed by stifling of the reaction (Figure 10), was observed. With the cathodic process, there was an initial inhibition followed by depolarization. The similarity between the results obtained with the autoclaved produced water (later inoculated with isolate # 200) and untreated produced water indicated strongly that the electrode reactions were caused by the same type of organism. When the total available energy, utilizable by the bacteria, in the produced water was boosted by the addition of 900 mg of sodium lactate per liter, inhibition of the anodic process did not occur and anodic depolarization was sustained (Figure 11). The depolarization of the cathode still occurred.

Discussion

The polarization characteristics of mild steel in cultures of a ferric iron reducing bacterium have been studied. The organism was grown in three different media and produced water which was selected to cover a range of nutritional and environmental variables to which the organism may be exposed in nature. Medium B had a very high Fe(III) content to reflect an environment, *e.g.*, soil, rich in iron. Since much of the iron present in the soil is in the Fe(III) form, this form of iron was employed. Furthermore, it was hoped that the effects of the ability of the organism to reduce Fe(III) to Fe(II) would be most easily observed in this medium. Butlin's medium represented a general purpose medium (nonselective environment), while the synthetic medium was employed so that the composition would be sufficiently characterized and controlled. The decision to use produced water was a pragmatic one. Produced water was a component of the crude oil—water emulsion from which the organism was isolated, and constitutes the natural environment of the organism; which indicates what might actually occcur under field conditions. In all these

media, the presence of isolate # 200 caused anodic depolarization but the cathode was depolarized only in produced water.

The polarization characteristics of the mild steel in produced water samples were very interesting. Whether the produced water was autoclaved first and inoculated with isolate # 200 or left untreated, the polarization behavior of the steel specimen was the same. The observed depolarization of the anode coincided with the active growth period normally observed with the organism. Since the untreated produced water contains its natural flora (different organisms), including the iron reducing bacteria, the observed succession in the electrode processes could also be due to a succession in the natural population. As each group of bacteria grows and dies, making way for other types, a different metabolic demand is made on the environment of the coupon. Such changes in the metabolic process may explain the observed succession in the electrode reactions on the steel coupon. However, polarization of the steel specimens in autoclaved produced water (later inoculated with isolate # 200) gave rise to polarization curves, similar to those observed with untreated produced water. It appears, therefore, that succession in the natural population of the medium cannot adequately explain the succession in the electrode reactions observed. Rather, the results obtained indicated that the reactions appear to be catalyzed by the same organism. Increasing the total energy available in produced water abolished the inhibition of the anodic process, and anodic depolarization was sustained. The transience of the depolarization of mild steel in untreated produced water therefore must be due to the limited available energy for continued activities of the microorganism. At the exhaustion of the limited energy source in produced water, the organism presumably switched over to a different metabolic process which resulted in the cathodic depolarization. Metabolic processes, such as cathodic hydrogen utilization as found in *Desulfovibrio* sp.,[11] and other microorganisms[12-14] may result in cathodic depolarization. It is evident from the present studies that anodic depolarization is consistently expressed whenever isolate # 200 is actively growing. The operation of both the cathodic and anodic depolarization reactions in produced water may well account for the severe corrosion in the pipeline system (Pembina) carrying the crude oil from which the organism was isolated.

Isolate # 200 is essentially an aerobic organism. No further deaeration was conducted, after the initial deaeration, before the polarization runs. Thus, these experiments were carried out under microaerobic conditions. The corrosion of ferrous metal will initially produce Fe(II), some of which will be oxidized to Fe(III) forms by the small amount of O_2 dissolved in solution. Ferric compounds are generally insoluble and when deposited on the surface of the metal may prevent further corrosion by acting as a barrier between the metal and its environment; the inhibition of corrosion by the formation of some Fe(III) coating on a metal surface is well documented.[15-18] Lockte[19] reported that the formation of an insoluble ferric corrosion product in the presence of phosphate and that of dissolved air, inhibited the corrosion of iron by polarizing both the anode and cathode. This was supported by the work of Pryor and Cohen.[20] It is noteworthy, that in all the media used in this study (except produced water), there was no cathodic depolarization. Presumably, the small amount of dissolved O_2 present was used up in the oxidation of Fe(II) to Fe(III) or was prevented from reaching the cathode by the Fe(III) barrier formed, and consequently O_2 did not act as a cathodic depolarizer.

Isolate # 200 reduces Fe(III) to Fe(II). In the presence of this organism, the anode was consistently depolarized. Von Wolzogen Kuhr and van der Vlugt[3] and von Wolzogen Kuhr[2] proposed that sulfate reducing bacteria caused the corrosion of steel by the depolarization of the cathode. It is proposed, in the same light, that the iron reducing bacterium, isolate # 200, caused the corrosion of mild steel under microaerobic conditions by the depolarization of the anode. It is suggested that the ability to reduce and remove protective Fe(III) covering is the most important single factor in the depolarization of the anode.

Conclusion

The effect of a ferric iron reducing pseudomonad isolated from crude oil samples of the Pembina oil field upon the corrosion of mild steel has been investigated in produced water and three synthetic media. Sustained anodic depolarization is consistently observed when this bacterium is actively growing under microaerobic conditions. Its ability to reduce and thereby remove protective ferric coatings may be responsible for the high level of corrosion observed in the Pembina pipeline system.

Acknowledgments

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References

1. C. O. Obuekwe. Microbial Corrosion of Crude Oil Pipeline. Ph.D. Thesis, Dept. of Microbiology, University of Alberta, (1980).
2. C. A. H. von Wolzogen Kuhr. Unity of Anaerobic and Aerobic Iron Corrosion Process in the Soil. Corrosion, Vol. 17, p. 119-125 (1961).
3. C. A. H. von Wolzogen Kuhr and L. S. van der Vlugt. De grafiterring van dietijzer als electrobiochemisch process in anaerobe gronden. Water, Vol. 18, p. 147-165 (1934).
4. J. D. A. Miller and A. K. Tiller. Microbial Corrosion of Buried and Immersed Metal. p. 61-105 (1970). In Miller (Ed.) Microbial Aspects of Metallurgy. American Elsevier, New York.
5. D. M. Updergraff. Microbiological Corrosion of Iron and Steel. Corrosion, Vol. 11, p. 44-48 (1955).
6. D. E. Hughes. The Microbiology of Corrosion. In Shreir, L. L. (Ed.). Corrosion I. Newness-Butterworth, Vol. 2, p. 73-82 (1963).
7. A. N. Baumgartner. Microbial Corrosion—What Causes it and How It can be Controlled. Water Flooding. Soc. of Petroleum Eng. of Amer. Inst. Mech. Eng. Reprint Series No. 2a. p. 58-62 (1973).
8. G. H. Booth, J. A. Robb, and D. S. Wakerley. The Influence of Ferrous Ion on Anaerobic Corrosion of Mild Steel by Actively Growing Cultures of Sulfate-Reducing Bacteria. In 3rd Intern. Congress of Metallic Corrosion, Vol. 2, p. 542-544 (1967).
9. C. O. Obuekwe, F. D. Cook, and D. W. S. Westlake. Interactive Production of Sulfide in Crude Oil; a Possible Mechanism for a Severe Corrosion of Pipeline. Abstr. Annu. Meet. Can. Soc. Microbiol. Victoria, B. S., p. 13 (1979).
10. A. Torriani and F. Rothman. Mutants of *Escherichia coli* Constitutive for Alkaline Phosphatase. J. Bacteriol, Vol. 81, p. 835-836 (1961).
11. G. H. Booth and A. K. Tiller. Polarization Studies of Mild Steel in Cultures of Sulfate-Reducing Bacteria. Trans. Farad. Soc., Vol. 56, p. 1689-1696 (1960).
12. D. D. Mara and D. J. A. Williams. Corrosion of Mild Steel by Nitrate-Reducing Bacteria. Chem. and Ind., p. 566-567 May (1971).
13. D. A. Mara and D. J. A. Williams. Polarization of Pure Iron in the Presence of Hydrogenase-Positive Microbes—I. Nonphotosynthetic Bacteria. Corr., Sci., Vol. 11, p. 895-900 (1971 b).
14. D. D. Mara and D. J. A. Williams. Polarization Studies of Pure Fe in the Presence of Hydrogenase-Positive Microbes—II. Photosynthetic Bacteria and Microalgae. Corr. Sci., Vol. 12, p. 29-34 (1972).

15. K. H. Logan. Underground Corrosion. U.S. Department of Commerce. National Bureau of Standards Circular C-450. p. 312 (1945).
16. I. Olefjord. ESCA Studies of Passive Films Formed on Steel by Oxidizing Inhibitors. In Seventh Scandinavian Corrosion Congress. Trondheim, Norway. p. 434-439 (1975).
17. S. A. Ashton, R. A. King, and J. D. A. Miller. Protection Film Formation on Ferrous Metals in Semicontinuous Cultures of Nitrate-Reducing Bacteria. Brit. Corr., Vol. J.8, p. 132-136 (1973 a).
18. S. A. Ashton, J. D. A. Miller, and R. A. King. Corrosion of Ferrous Metals in Batch Cultures of Nitrate-Reducing Bacteria. Brit. Corr., Vol. J.8, p. 185-189 (1973 b).
19. H. L. Lockte. The Electrochemical Behavior of Iron in Corrosion Cells. III. The Current Carrying Capacity. Trans. Electrochem. Soc., Vol. 64, p. 181-189 (1933).
20. M. J. Pryor and M. Cohen. The Mechanism of Inhibition of the Corrosion of Iron by Solutions of Sodium Orthophosphate. J. Electrochem. Soc., Vol. 98, p. 263-272 (1951).

Corrosion of Mild Steel in Cultures of Ferric Iron Reducing Bacterium Isolated from Crude Oil II. Mechanism of Anodic Depolarization*

C. O. OBUEKWE, D. W. S. WESTLAKE,* J. A. PLAMBECK,** and F. D. COOK****

Abstract

A protective ferric covering was formed on mild steel coupons by reaction with the oxidative inhibitor, nitrite. In cultures of a ferric iron reducing bacterium, there was a loss of passivity of coupons and polarization studies revealed intense depolarization of the anode, while in the absence of the bacterium, the metal remained passive. The anodic depolarization was accompanied by conversion of ferric to ferrous compounds and was marked by the change in color of the medium from dark brown to a greenish hue. Electron micrographs revealed that in the absence of the organism, a dense, crystalline surface deposit covered the metal, but was extensively removed in the presence of the bacterium. The bacterium caused anodic depolarization of mild steel by removing or preventing the formation of a protective ferric covering.

Introduction

Polarization studies[1] showed that the ferric [Fe(III)] iron reducing *Pseudomonas* sp. (#200), isolated from produced liquids of the Pembina oil field, caused anodic depolarization of mild steel coupons. It was suggested that the observed depolarization was the result of the ability of isolate #200 to reduce Fe(III) to Fe(II) and prevent the formation of a protective surface coat. The Fe(III) was thought to have been formed on the metal surface by the oxidation of the primary corrosion product [Fe(II)]. This was an assumption, since no chemical analysis was conducted to determine the nature of the surface coat. The possibility that the surface coat formed was Fe(III) is supported by the observations of Pryor and Cohen[2] who reported the formation of insoluble Fe(III) coating in the presence of air when iron was immersed in sodium orthophosphate solution.

To test if the depolarization of the anodic process was due to the removal (dissolution) of a protective Fe(III) covering, it would be necessary to induce a protective Fe(III) covering on

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the metal surface and then study the effect of the organism (isolate #200) on the stability of the passivated metal. A protective Fe(III) covering can be conveniently formed on steel by treatment with nitrite or incorporation of nitrite into a culture medium. Nitrite is a strong oxidizing agent[3,4] and its oxidative property has been widely adopted in the inhibition (passivation) of ferrous metals.[5-8] The inhibitory effect of nitrite is due to the formation of the protective Fe(III) compound γ-Fe_2O_3 on the metal surface. Studies of biochemical activities of isolate #200[9] show that it is possible to generate nitrite *in situ* by the addition of nitrate to culture media because isolate #200 will reduce nitrate to nitrite in the absence of oxygen.

The object of this investigation is to determine the effect of *Pseudomonas* sp. (#200) on the corrodibility of mild steel already passivated by the formation of a protective Fe(III) film. Such information will help determine whether anodic depolarization is indeed caused by the dissolution of a protective ferric film or coat as postulated.

Materials and Methods

Bacterial Cultures and Polarization Studies

The growth of the organism and inoculum preparation were as previously described.[1] Polarization procedures[10] included deaeration of solutions by bubbling a stream of deoxygenated nitrogen gas through the medium prior to each polarization run and by blanketing the surface of the medium with deoxygenated nitrogen during the polarization experiment.

Nitrite Determination

Produced nitrite was determined by the spectrophotometric method of Montgomery and Dymock,[11] except that N-(1-naphthyl)-ethylenediamine hydrochloride was used and all readings were taken in a Spectronic 20 spectrophotometer.

Scanning Electron Microscopy

The mild steel specimens (coupons) on withdrawal from the culture medium were immediately rinsed in running distilled water to remove loose surface coverings. They were then dehydrated by sequential passage through a regime of increasing ethanol concentrations (30 to 100%). The dry specimens were coated with a 150 Å film of gold, in an Edmons sputter-coater and examined in a Cambridge Stereoscan Model S_4 electron microscope at an accelerating voltage of 20 kV.

Results

Isolate #200 can reduce nitrate to nitrite. The data in Table 1 show the time dependent formation of nitrite from nitrate by isolate #200. After prolonged incubation (*e.g.*, 5 hours), nitrous oxide, which is not a corrosion inhibitor, would be detected by gas chromatography. Thus, the concentration of nitrite would be reduced and the degree of corrosion protection decreased.

TABLE 1 — Induction of Nitrate Reductase in *Pseudomonas* Sp. (#200).

Incubation Time hours	NO_2^- – N[(1)] (mg/l) 10 μM NO_3^-	5 μM NO_3^-
0	0	0
1	1	—
3	—	10
5	45	47
7	72	49
9	99	40
11	110	35

[(1)]Formed by 1 ml washed cell suspension (3×10^8 cells) *Pseudomonas* isolate #200 using sodium lactate as energy source and a phosphate buffer pH 7.2.

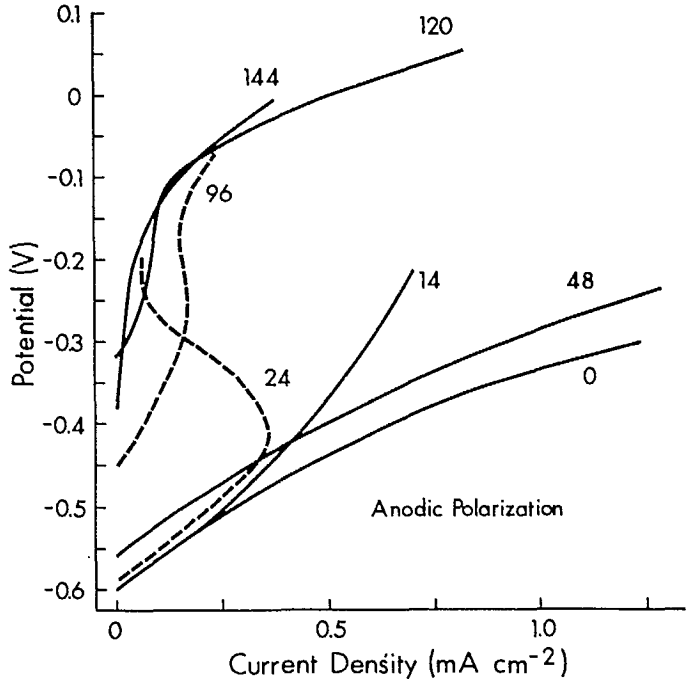

FIGURE 1 — Anodic polarization curves of mild steel in B_{10} medium containing KNO_3 (1 g/l) inoculated with isolate #200. (0, 14, 24, 48, 96, 120, and 144 denote the incubation time—hours at 25 ± 2 C).

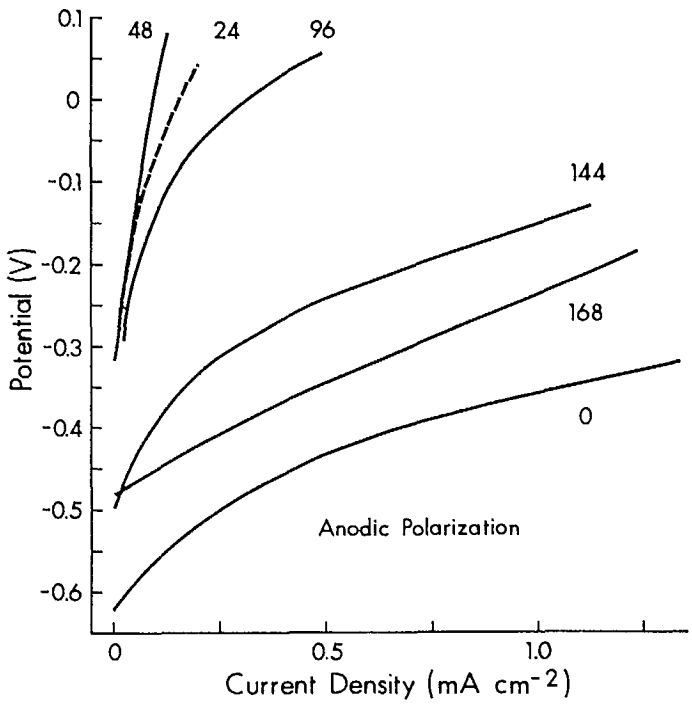

FIGURE 2 — Anodic polarization curves for mild steel in Butlin's medium containing KNO_3 (1 g/l) inoculated with isolate #200. (0, 24, 48, 96, 144, and 168 denotes incubation time—hours at 25 ± 2 C).

Anodic polarization curves for mild steel in a B_{10} medium containing 1 g/L KNO_3 and inoculated with isolate #200 is shown in Figure 1. The anodic current decreased continually within the first 24 hours of incubation. At 24 hours of incubation, the coupon became passivated with anodic current density of less than 0.1 mA cm^{-2}. With further incubation, however, the metal lost its passivity and the anodic current soared markedly after 24 hours. At this time, the medium changed from its characteristic deep brown color [due to Fe(III)] to a green color [due to Fe(II)]. Subsequently, there was a general decline in the anodic current. Comparison of the anodic polarization curves at 24 and 96 hours show a marked difference in the behavior of the steel specimen. At 24 hours, the critical passivating potential was −0.41 V but shifted to the more noble potential of −0.25 V after 96 hours. The critical anodic current density at 24 hours was 0.35 mA cm^{-2} which was double that (0.15 mA cm^{-2}) obtained at 96 hours of incubation. The shift in the critical passivating potential to more positive values would indicate that it was becoming more difficult to passivate the metal with time in the presence of isolate #200. By the end of the experiment, the open circuit potential of mild steel rose from −0.6 V to −0.32 V, an increase of 0.28 V.

When nitrate was added to inoculated Butlin's medium, the anodic polarization curves (Figure 2) revealed initial inhibition of the anodic process within the first 14 hours. Anodic inhibition was succeeded by very active anodic depolarization. The metal was not passivatable in Butlin's medium although it was in B_{10} medium. The cathodic reaction (not shown) was polarized in both media, in the presence or absence of the organism.

The changes in corrosion rate of the coupon in inoculated nitrate containing medium are shown in Figure 3. The corrosion rate was initially high (at time 0), but declined up to 24 hours later; thereafter it rose sharply, almost linearly, with incubation time. The trend in the changes in corrosion rate corresponded to the changes in the anodic current as shown in Figure 2. The similarity between the anodic current curves and the total corrosion rates indicated that the anodic process, rather than the cathodic process (not shown) controlled the overall corrosion of the coupon under the experimental conditions.

The open circuit potential changes observed in the nitrate containing medium differed markedly from those already described for B_{10} medium. The potential initially increased to more noble values (from −0.60 to −0.32 V) within 48 hours but subsequently declined to more active values (from −0.32 to −0.50 V). These changes (from active to noble and finally to active) in the potential also corresponded to the observed trend in the corrosion rate, as shown in Figure 3.

When nitrite rather than nitrate was incorporated in Butlin's medium inoculated with isolate #200, there was immediate passivation of the coupon (Figure 4). Extremely low anodic current was observed from the beginning until the transpassive zone (after +0.15 V). On further incubation, the passivity was lost and the metal became more corrodible. In the absence of the organism, the metal remained passive (Figure 5); in fact, the anodic current decreased with time and the potential remained more noble.

A similar active anodic depolarization was also observed in inoculated B_{10} medium containing nitrite (Figure 6). In the absence of the organism, the metal remained passive (Figure 7). It appears, therefore, that the presence of isolate #200 always caused the loss of the metal's passivity.

Scanning electron microscopic studies of unpolarized coupons were undertaken to parallel the electrochemical procedures. In uninoculated B_{10} medium, the metal was covered by a dense, crystalline surface coating (Figure 8). This coating did not dissolve when rinsed in tap water, and therefore could be considered insoluble but was extensively removed in cultures of isolate #200 (Figure 9) and did not occur at all on fresh coupons (control Figure 10). Therefore, isolate #200 either prevented the formation or removed such surface covering, exposing the metal to the environment. A closer view (Figure 11) shows that the crystalline surface covering is very closely packed and would constitute a protective barrier between the metal and the surrounding medium.

Discussion

Isolate #200 is classified as a member of the genus *Pseudomonas*.[10] Members of this genus are characterized as being strict aerobes with the exception of species, *e.g., P. denitrificans* which can use oxidized nitrogen compounds,

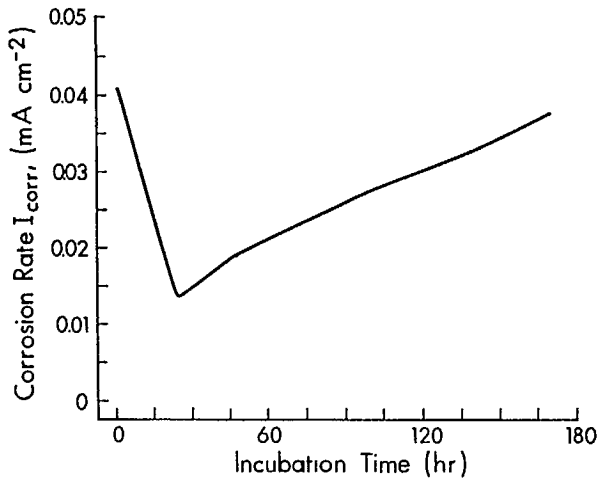

FIGURE 3 — Changes in corrosion rate of mild steel in Butlin's medium containing KNO_3 (1 g/l) inoculated with isolate #200. Corrosion current was estimated by the extrapolation of the Tafel slopes.

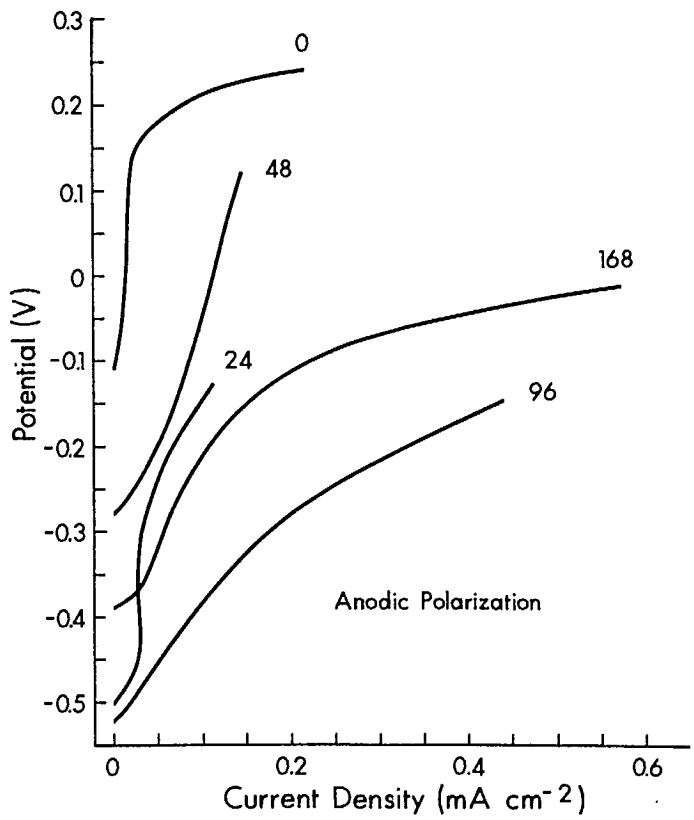

FIGURE 4a — Anodic polarization curves for mild steel in Butlin's medium containing 0.7 g/l sodium nitrite and inoculated with isolate #200. (0, 24, 48, 96, and 168 denote the incubation time—hours at 25 ± 2 C).

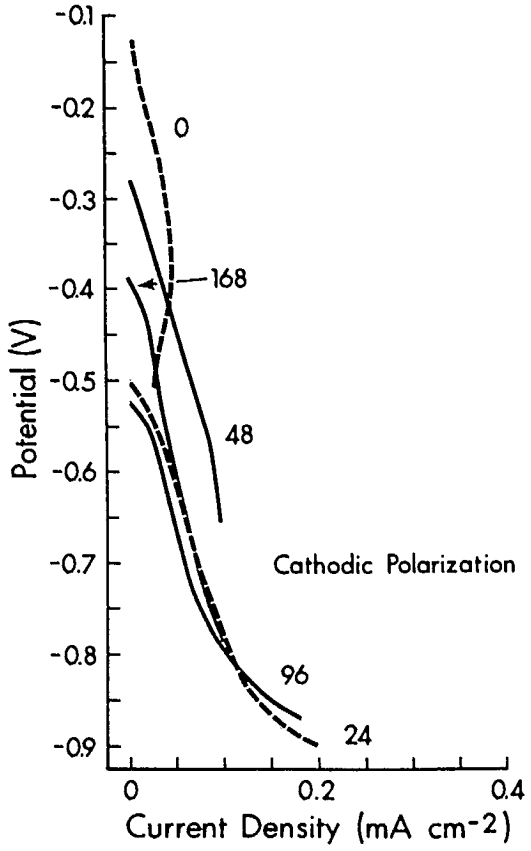

FIGURE 4b — Cathodic polarization curves for mild steel in Butlin's medium containing 0.7 g/l sodium nitrite inoculated with isolate #200. (0, 24, 48, 96, and 168 denote the incubation time—hours at 25 ± 2 C).

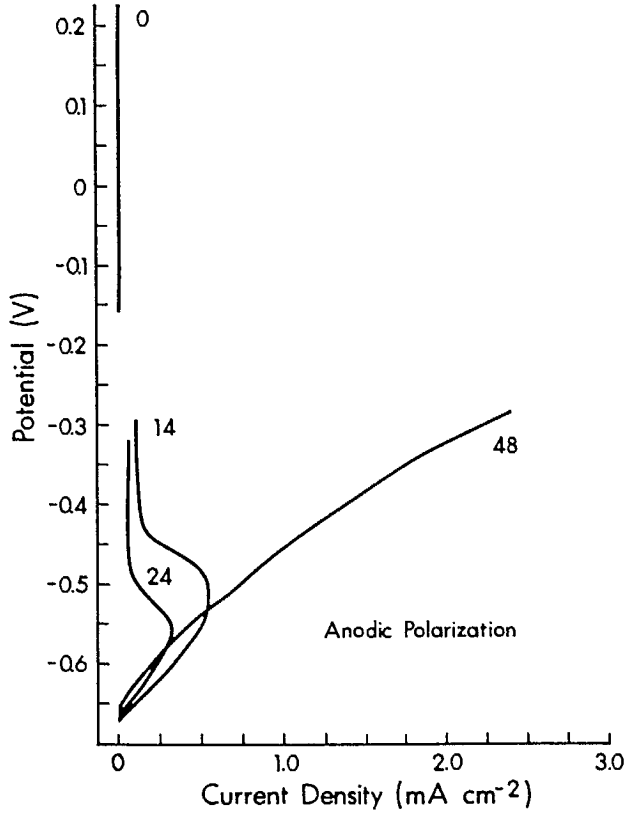

FIGURE 6 — Anodic polarization of mild steel in B_{10} medium containing 0.7 g/l sodium nitrite and inoculated in isolate #200. (0, 14, 24, and 48 denote incubation time—hours at 25 ± 2 C).

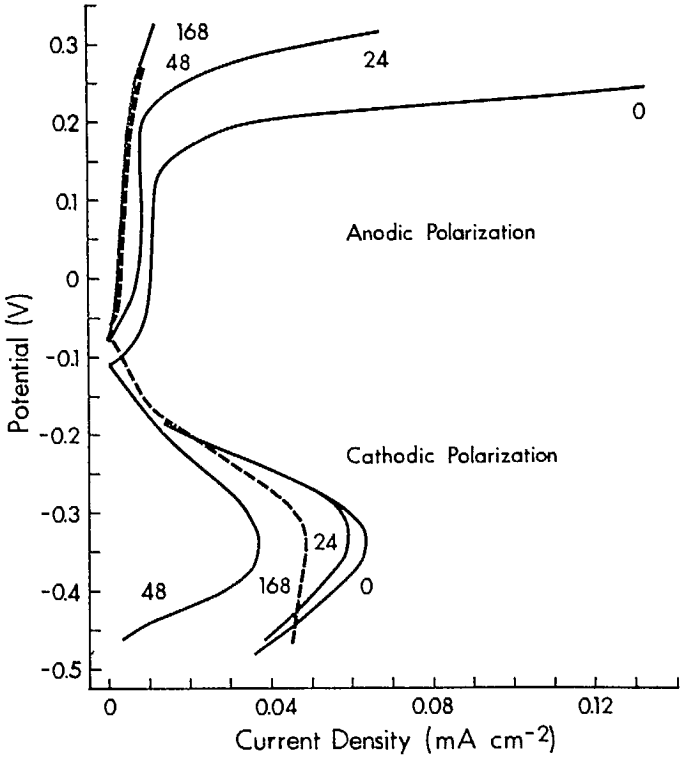

FIGURE 5 — Polarization curves of mild steeel in uninoculated (control) Butlin's medium containing 0.7 g/l sodium nitrite. (0, 24, 48, and 168 denote incubation time—hours at 25 ± 2 C).

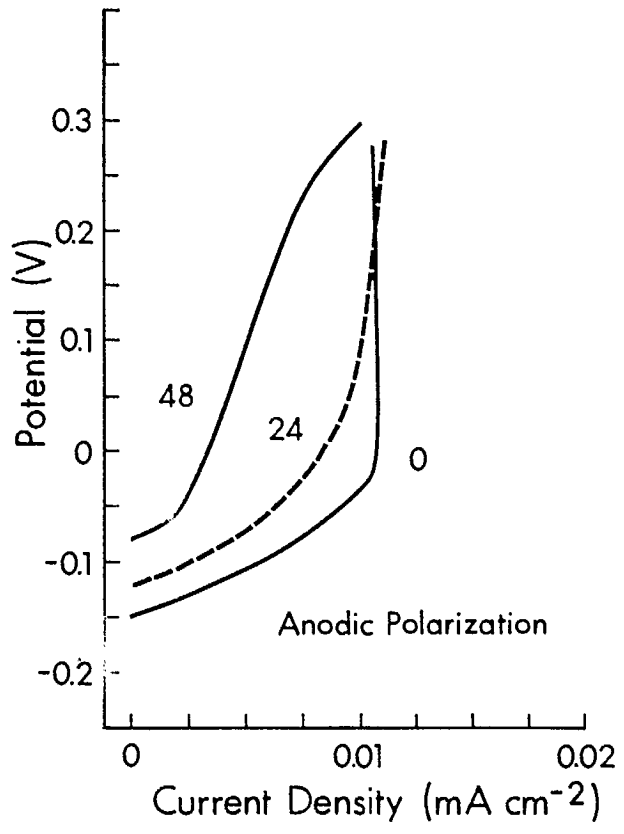

FIGURE 7 — Anodic polarization curves for mild steel in uninoculated (control) B_{10} medium containing 0.7 g/l sodium nitrite. (0, 24, and 48 denote incubation time—hours at 25 ± 2 C).

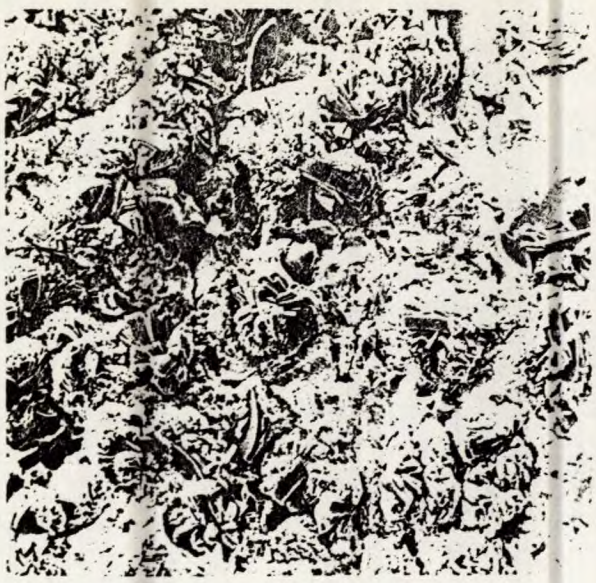

FIGURE 8 — Scanning electron micrograph (SEM) of mild steel coupon submerged in uninoculated (control) B_{10} medium for 48 hours. The coupon was first rinsed in distilled water and dehydrated in a regime of increasing ethanol concentration—30 to 100%. The micrograph shows formation of a protective densely packed crystalline surface covering in the absence of the organism (245X).

FIGURE 9 — SEM of mild steel coupon submerged in culture of isolate #200 (iron reducing bacterium) in B_{10} medium for 42 hours. The coupon was first rinsed in distilled water and dehydrated in increasing ethanol concentrations—30 to 100%. The micrograph shows extensive removal of the protective, dense, crystalline surface covering in the presence of the organism (245X).

FIGURE 10 — SEM of unsubmerged metal. Note the absence of any surface covering (1740X).

FIGURE 11 — Closer view of the protective densely packed, crystalline surface covering on submerged metal. Such covering will pose a protective barrier between the metal and its corrosive environment (1225X).

e.g., nitrate, as electron acceptors; that is, anaerobic respiration takes place. Isolate #200 also uses nitrate as an electron acceptor, as well as Fe(III) and various forms of oxidized sulfur compounds.[10] Because of the primary preferred electron acceptor of isolate #200 is oxygen, traces of oxygen (if present) in polarization experiments would be quickly removed and the cells would then use alternate electron acceptors (*i.e.*, anaerobic respiration takes place).

The effect of isolate #200 on mild steel passivated by the oxidizing inhibitor nitrate has been studied. In all media used, the effect of the organism was to destroy the passivity and cause increased corrosion. The incorporation of nitrate in the growth media has enabled us to observe the corrosion rate, initially in the presence of nitrate and later in the presence of nitrite formed from nitrate by this organism (Figure 3). The corrosion rate was initially high because the nitrate originally present is corrosive,[12] but the subsequent formation of nitrite resulted in the oxidation and passivation of the steel specimen, presumably due to the formation of the character-

istic γ-Fe_2O_3. Within 7 hours, isolate #200 ($\cong 3 \times 10^7$ cells) is capable of producing nitrite equivalent to about 1.8×10^{-4}M nitrate (as KNO_3). This amount of nitrite is much more than the amount (5×10^{-5}M) used by Olefjord[7] to passivate iron. It is evident that in the presence of nitrate, the organism is capable of generating enough nitrite to passivate the immersed coupons. Thus, the polarization of the anodic reaction evident between 24 and 48 hours (Figure 2) was due to nitrite formed from nitrate by the bacterium. At this time, the culture medium turned yellow, indicating the preponderance of Fe(III) compounds in the medium. The subsequent depolarization of the anode after 48 hours and the accompanying change in the color of the medium (from brown to green) signified that the protective Fe(III) film had been destroyed and the Fe(III) converted to a soluble Fe(II) form. Since the anodic depolarization occurred only in the presence of the organism, and this organism reduced Fe(III) to Fe(II), it can be inferred that the reduction of Fe(III) was the cause of the loss of the protective film and the consequent depolarization and increase in corrosion current. The changes in the corrosion rate (Figure 3) corresponded to the changes in the anodic current as shown in Figure 2, indicating the anodic reaction was the controlling factor in the overall corrosion process.

As is evident from Figures 1 and 2, the steel specimen was not passivatable in Butlin's medium although it was easily passivated before 48 hours in B_{10} medium. The difference in the passivatability of mild steel in these two media may be accounted for by the total oxidizing powers of Fe(III) and nitrite, and the oxidizing power of B_{10} medium must be greater than that of Butlin's medium which contained only the quantity of nitrite formed from nitrate by the bacterium. Strong oxidizing solutions passivate metals more easily than nonoxidizing solutions.[5] It is apparent, therefore, that isolate #200 can prevent or reduce the ease of passivation of the metal by decreasing the total oxidizing power both in terms of Fe(III) and in terms of nitrite.

Logan[13] reported that the corrosion of underground pipes was retarded by the oxidation of the corrosion products to form thick deposits. Such deposits either reduced the potential difference between the anodic and cathodic areas of posed an electrical resistance which reduced the corrosion current. The retardation of corrosion of iron in estuarine waters of the Thames was reported by Booth, *et al.*[14,15] Chemical analysis of the retarding layer showed that it was composed of Fe(III) compounds, α-$Fe_2O_3 \cdot H_2O$, γ-$Fe_2O_3 \cdot H_2O$ and Fe_2O_3. A coating of a mixture of Fe(III) and Fe(II) phosphates preserved buried ancient nails from corrosive soil.[16] In the absence of the organism, a dense, crystalline surface layer was formed (Figure 8). Coherent crystalline corrosion products inhibit corrosion.[2] On the other hand, such a continuous protective surface covering was lacking on coupons immersed in cultures of isolate #200, and the metal was exposed to its environment. The organism destroyed the protective ferric film formed on the passivated metal surface (Figure 9) and caused anodic depolarization. Further surface changes in mild steel coupons by bacteria are published elsewhere.[17]

Conclusion

It is concluded that anodic depolarization of mild steel by isolate #200 is due to its ability to reduce Fe(III) to Fe(II), thus converting the protective Fe(III) covering to soluble Fe(II) forms and exposing the metal to further corrosion.

Acknowledgment

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References

1. C. O. Obuekwe, D. W. S. Westlake, J. A. Plambeck, and F. D. Cook. Corrosion of Mild Steel in Cultures of Ferric Iron-Reducing Bacterium Isolated from Crude Oil. I. Polarization Characteristics. Accepted—Corrosion, Vol. 37, p. 461-467 (1981).
2. M. J. Pryor and M. Cohen. The Mechanism of the Inhibition of the Corrosion of Iron by Solutions of Sodium Orthophosphate. J. Electrochem. Soc., Vol. 98, p. 263-272 (1951).
3. J. T. Moraghan and R. J. Buresh. Chemical Reduction of Nitrite and Nitrous Oxide by Ferrous Iron. Soil Sci. Soc. Amer., J., Vol. 41, p. 47-50 (1977).
4. H. H. Uhlig. Passivity of Metals and Alloys. Corr. Sci., Vol. 19, p. 777-791 (1979).
5. M. G. Fontana and N. D. Greene. Corrosion Engineering, p. 391 McGraw-Hill (1967).
6. P. H. G. Draper. Structure and Mechanism of Formation of Oxide Films of Fe Single Crystals by $NaNO_2$. Corr. Sci., Vol. 7, p. 91-101 (1967).
7. I. Olefjord. ESCA Studies of Passive Films Formed on Steel by Oxidizing Inhibitors. In Seventh Scandinavian Corrosion Congress. Trondheim, Norway, p. 434-439 (1975).
8. J. B. Lumsden and Z. Szklarska-Smialowska. The Properties of Films Formed on Iron Exposed to Inhibitive Solutions. Corrosion, Vol. 34, p. 169-176 (1978).
9. C. O. Obuekwe, D. W. S. Westlake, and F. D. Cook. Effect of Nitrate on Reduction of Ferric Iron by a Bacterium Isolated from Crude Oil. Accepted-Can. J. Microbiology (1981).
10. C. O. Obuekwe. Microbial Corrosion of a Crude Oil Pipeline. Ph.D. Thesis, Dept. of Microbiology, University of Alberta (1980).
11. H. A. C. Montgomery and J. F. Dymock. The Determination of Nitrite in Water. Analyst, Vol. 86, p. 414-416 (1961).
12. A. Albu-Yaron and A. Semel. Nitrate Induced Corrosion of Tin Plate as Affected by Organic Acid Food Components. J. Agric. Food Chem., Vol. 24, p. 344-348 (1976).
13. K. H. Logan. Underground Corrosion. U.S. Department of Commerce. National Bureau of Standards. Circular C-450, p. 312 (1945).
14. G. H. Booth, A. W. Cooper, and A. K. Tiller. Corrosion of Mild Steel in Tidal Waters of the Thames Estuary. I. Results of Six Months and One Year's Immersion. J. Appl. Chem., Vol. 13, p. 211-220 (1963).
15. G. H. Booth, A. W. Cooper, and A. K. Tiller. Corrosion of Mild Steel in Tidal Waters of the Thames Estuary. II. Results of Two Year's Immersion. J. Appl. Chem., Vol. 15, p. 250-256 (1965).
16. G. H. Booth, A. K. Tiller, and F. Wormwell. Laboratory Study of Well Preserved Iron Nails from Apparently Corrosive Soils. Corr. Soc., Vol. 2, p. 197-202 (1962).
17. C. O. Obuekwe, D. W. S. Westlake, F. D. Cook, and J. W. Costerton. Surface Changes in Mild Steel Coupons from the Action of Corrosion-Causing Bacteria. Applied and Environmental Microbiology, Vol. 41, p. 766-774 (1981).

Corrosion of mild steel by nitrate reducing bacteria

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The anaerobic corrosion of ferrous metals in a neutral environment is usually attributed to the activities of sulphate reducing bacteria. Hydrogenase positive strains of these bacteria depolarise the metal surface by removing cathodic hydrogen.[1,2] Hydrogenase systems are known not to be confined to the sulphate reducers, but are possessed by many bacteria and microalgae. Von Wolzogen Kuhr[1] recognised that the oxidation of cathodic hydrogen by nitrate reducing bacteria might cause corrosion by a mechanism similar to that which he proposed for the sulphate reducers. Cook[3] showed that steel wool and nails were severely corroded by hydrogen utilising strains of chemo-metatrophic denitrifying bacteria growing in batch culture. Caldwell and Ackerman[4] reported the anaerobic corrosion of iron pipes which, on the evidence of chemical analysis and because chlorination of the pipe water arrested corrosion, was probably caused by nitrate reducing bacteria. We have recent evidence that hydrogenase positive heterotrophic nitrate reducing bacteria corrode ferrous metals anaerobically in accordance with the equation given by Caldwell and Ackerman:[4]

$$Fe + H_2O + NO_3^- \rightarrow Fe(OH)_2 + NO_2^-$$

Using a strain of *Escherichia coli* (NCIB 8666), an organism which possesses a hydrogenase system and is able to utilise nitrate as a hydrogen acceptor under anaerobic conditions, we have performed corrosion experiments in which mild steel specimens (composition: C 0·05 per cent, S 0·02 per cent, P 0·009 per cent, Mn 0·27 per cent, Sn 0·01 per cent, Cn 0·085 per cent, Ni 0·04 per cent, Cr 0·02 per cent and Mo 0·01 per cent) were suspended in a vessel in which the organism was maintained under nitrogen in semicontinuous culture at 25°c. The growth media were: medium A, containing 13·0g nutrient broth (Oxoid No 1, batch No 7005) 1·00g KNO_3, 0·01g $FeSO_4.7H_2O$, 0·1g Na thioglycollate, 1000ml distilled water, and medium B, which was similar but contained only 6·5g nutrient broth.

The steel specimens (6cm × 2·5cm × 20 s.w.g. with three 1cm diameter holes to facilitate suspension and mixing) were abraded (320 aluminium oxide grit), degreased (benzene), washed (ethanol), dried, weighed to 0·1mg and sterilised (ultraviolet radiation). Triplicate specimens were aseptically suspended in a corrosion vessel, which was filled with 140ml of medium at pH 7 and inoculated with 10ml of a 24h culture grown at 37° in simple nutrient broth. Immediately after inoculation oxygen-free nitrogen was passed through the whole culture unit until the end of the experiment. On the day after inoculation the daily refeeding procedure was started: 50ml of culture was withdrawn and an equal volume of fresh sterile medium replaced. The reaction of the culture was maintained at pH 7·2±0·2 by acidifying the medium in the supply reservoir to pH 4·5 for medium A and pH 5·0 for medium B. After 35 days the specimens were removed, rinsed with 10 per cent formalin, washed (tap water), immersed in inhibited HCl for a few seconds (to remove any trace of corrosion products), rewashed (tap water and ethanol), dried and reweighed. Uninoculated control experiments were run under identical conditions except that the reaction of the supply media was at pH 7·3. In order to determine the amount of nitrate used to oxidise the organic matter present in the media, inoculated control experiments without specimens were also run, the pH of medium A in the supply reservoir being 5·5 and that of medium B 6·0. Effluent samples, taken weekly from all experiments, were checked for pH and purity, and analysed for nitrite concentration by the Greiss-Ilosvay method; hydrogenase activity of the cultures was demonstrated by the Thunberg technique. A final control experiment was performed using a strain of *Pseudomonas stuzeri* (NCIB 9040), a hydrogenase negative denitrifying organism.

The specimens removed from the vessels inoculated with *E. coli* were found to be covered with a loose flocculent film of $Fe(OH)_2$ which was almost completely removed by

Table I

Weight losses and corrosion rates of mild steel specimens

Organism	*Medium*	*Weight loss (mg/specimen)*	*Mean weight loss (mg/specimen)*	*Corrosion rate (mg/dm²/d)*
None (sterile control)	A	13·2 14·1 13·7	13·7	1·5
None (sterile control)	B	13·6 13·5 14·4	13·8	1·5
Ps. stuzeri	A	17·2 15·4 14·0	15·5	1·7
E. coli	A	90·9 109·8 96·4	99·0	10·6
E. coli	A	99·4 95·3 82·7	92·5	9·9
E. coli	B	60·8 57·1 55·9	57·9	6·2

washing with tap water. The weight loss results and calculated corrosion rates (Table I) show that *Ps. stuzeri* caused negligible corrosion, whereas *E. coli* gave corrosion rates comparable to those obtained with sulphate reducing bacteria growing in a medium containing minimal ferrous salts.[5] When the total weight loss in each inoculated (*E. coli*) experiment is compared with the total amount of nitrate reduced (Table II), a clearer insight into the corrosion mechanism is obtained. In each of the three runs good agreement is recorded between the amount of nitrate used for the oxidation of cathodic hydrogen and the quantity of Fe corroded by bacterial action. These results indicate that the organism preferentially utilises nitrate for the oxidation of organics, and also that, when the cells are in a resting state, incidental dissimilatory nitrate reduction occurs with concomitant corrosion if ferrous metals are present. Furthermore the extent of this corrosion can be considered as following stoichiometrically the equation given above. These findings provide further evidence for the original theory of von Wolzogen Kuhr. In view of the diverse natures of hydrogenase positive nitrate reducing bacteria and of their high numbers in soils, the corrosion caused by these organisms should be investigated in greater detail.

Table II

Experimental data on the equivalence of iron corroded and nitrate reduced by E. coli

Medium	*A*	*A*	*B*
Total NO_3^- reduced (steel present)	16·3*	16·6	8·5
Total NO_3^- reduced (steel absent)	12·1	12·1	5·7
NO_3^- reduced in corrosion process	4·2	4·5	2·8
Fe corroded (bacteria present)	5·3	5·0	3·1
Fe corroded (bacteria absent)	0·7	0·7	0·7
Fe corroded by bacterial action	4·6	4·3	2·4

* All quantities expressed in millimoles

References

1 Von Wolzogen Kuhr, C. A. H., *Corrosion*, 1961, 17, 119
2 Booth, G. H. & Tiller, A. K., *Corrosion Sci.*, 1968, 8, 583
3 Cook, F. D., PhD Thesis, Edinburgh, 1961
4 Caldwell, D. H. & Ackerman, J. B., *J. Am. Water Works Assoc.*, 1946, 38, 61
5 Booth, G. H., *J. Appl. Bact.*, 1964, 27, 174

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THE ROLE OF METAL-BINDING BACTERIAL EXOPOLYMERS IN CORROSION PROCESSES

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ABSTRACT

Corrosion is frequently accelerated in the presence of bacterial biofilms on metal surfaces. Microbially produced exopolymers have been implicated in these corrosion processes. However, the mechanisms are not fully understood. We have examined the binding of four different metals to microbially produced exopolymer and have established considerable differences in maximum binding abilities and stability constants. These results have important implications for corrosion under biofilms, and are discussed in terms of specific ion concentration cells and galvanic coupling.

INTRODUCTION

Microbially induced corrosion (MIC) is now widely acknowleged as a major economic concern. However, our knowledge of the mechanisms of MIC are largely limited to speculation, with considerable emphasis on a descriptive rather than a mechanistic approach.

Recently the importance of microbially produced exopolymer/metal interactions has received attention[1,2]. These exopolymers comprise key structural components of biofilms and may mediate in corrosion processes by providing sites for aeration cells[3], ion concentration cells[4] and anaerobic zones for sulfate reducing bacteria[5]. They are also important in providing a protective matrix for the microorganisms, conveying biocide tolerance[3] and even protection against cleaning techniques[6].

Most recent work has examined the role of bacterial exopolymers in metal binding[7]. The ability of an exopolymer to bind specific metal ions will strongly influence its adhesion to metal surfaces and its ability to concentrate metal ions both from those surfaces and from the bulk media. Work by Mittelman and Geesey[4,7] indicates that exopolymer produced by a river sediment bacterium binds copper ions in solution. They suggest that when this bacterium is attached to copper metal surfaces its exopolymer has the capacity to form copper concentration cells. We have measured copper binding by exopolymers

produced by a thermophilic bacterium isolated from corroded nickel tubing[8]. We have shown that this themophile accelerates corrosion of various metals[9] and have obtained evidence that copper concentration cells are responsible for the corrosion[3].

Given the ability of microbial exopolymers to bind metal ions, then it is of interest from the corrosion aspect to look at the ability of these exopolymers to bind diferent metal ions in order to establish potential galvanic cells, both in terms of metal ions within the metal lattice and free ions in solution. There are a number of recent reports in the literature describing metal ion complex formation with exopolymeric material resulting from heavy metal/ microbial interactions in activated sludge systems[10,11,12,13]. These systems are organically very rich, and complexation under these conditions is not neccessarily comparable to processes in biofilms on metal surfaces, often in nutrient poor water systems.

This paper describes the results of binding studies of exopolymer produced by a marine bacterium, Deleya marina, originally isolated from the littoral zone[14]. This bacterium is known to produce exopolymer[15] and has been shown to assimilate trace metal ions against a gradient[16]. These trace elements were assimilated in the following order:

$$Fe > Cd > Cu > Zn > Pb = Ni = Mn$$

In order to establish the importance of exopolymer in this assimilation process and relate metal binding to potential corrosion questions, we investigated metal binding by D. marina exopolymer for iron, copper, nickel and manganese. These metal ions are all constituents of industrially important alloys and can be expected to be present in the solutions in contact with metal surfaces.

MATERIALS AND METHODS

The bacterium and culture methods

Deleya (=Pseudomonas) marina (ATCC 25374) was obtained from the American Type Culture Collection (Rockville, MD) and cultures for experiments were maintained on slants of Marine Agar 2216 (Difco, Detroit, MI). In order to preserve the integrity of the culture, it was stored frozen (-20° C) in glycerol. Periodically, fresh slants of the bacterium were prepared from the frozen cultures. For exopolymer extraction, bacteria in batch cultures were grown to stationary phase (A_{660} = 2.7) in a defined minimal carbon medium[17] with glucose as the carbon source.

Polymer isolation

Batch cultures that had reached stationary phase were centrifuged (20,000 x g for 20 minutes) to separate cells from extracellular material followed by filtration of the supernatant through sterile 0.2 um pore size filters[10]. The supernatant was concentrated in a stirred ultrafiltration cell (Amicon, Danvers, MA) with a nominal MW cut-off of 5000. The concentrated supernatant was then precipitated with 3 volumes of 95% (v/v) ethanol and stored at 4°C for 24 hours. The precipitate was collected through centrifugation, and, after decanting the ethanol, was dissolved in distilled water and dialyzed extensively with distilled water to remove any remaining ethanol. The solution containing the exopolymer was then lyophilized. This lyophilized product was considered crude exopolymer.

Further purification of the exopolymer involved an ether extraction to remove lipids, followed by extensive dialysis with distilled water. The exopolymer was deproteinated several times using chloroform/isopentanol[18] again followed by extensive dialysis. This purified exopolymer was then lyophilized and stored in a desicator.

Polymer characterization

After filtration of the supernatant and after each lyophilization, the extent of contamination of the exopolymer by intracellular and cell wall components was estimated by measuring DNA by the diphenylamine method[19], and heptose sugars by the cysteine-H_2SO_4 reaction[20]. Other routine assays were for total protein, using Coomassie blue[21], hexose sugars

using phenol/H_2SO_4[22], and uronic acids using the m-hydroxydiphenyl method[23].

Metal binding studies

Binding experiments were conducted using the protocol of Mittleman and Geesey[1]. 0.1 mg/ml solutions of exopolymer were prepared at pH 6 and dialysed against iron, copper, nickel and manganese solutions also adjusted to pH 6 ranging from 50 to 8000 ug/l. Exopolymer solutions were dialysed for at least 36 hours against metal solutions using a teflon multichamber equilibrium dialysis apparatus based on the design of Furlong et al[24]. Exopolymer solutions were separated from the metal solutions by Spectrapor 4 dialysis tubing, molecular weight cutoff, 12000 to 14000. Metal binding by each sample was determined by subtracting the final free metal concentration from the final metal concentration in the chamber containing exopolymer solution. Binding constants were calculated from the Langmuir linear expression $c/x = 1/k_1k_c + c/k_1$ (c is the free metal concentration, x is moles of metal bound per unit weight of metal-binding component, k_1 is the maximum binding ability (MBA), and k_c is the conditional stability constant).

Metal analysis

Metals were analysed on a Perkin-Elmer (3030) Atomic absorption spectrophotometer equipped with a graphite furnace.

RESULTS

Exopolysaccharide material

Figure 1 is a transmission electron micrograph of the bacterium Deleya marina showing the presence of exopolymeric material. The results of chemical analysis of both crude and purified exopolymer preparations are presented in Table 1. The absence of detectable levels of DNA suggests that there was negligible intracellular contamination of the exopolymer. However, the presence of heptose sugers in the crude exopolymer indicated that some cell wall material was present. The heptose assay for the purified exopolymer was judged to be unreliable, because of interference from contaminated reagents. Insufficient exopolymer was available to repeat this assay so the degree of cell wall contamination in the purified exopolymer is uncertain. Extractions used to create the purified exopolymer resulted in a decrease in all the components measured.

Binding studies

The results of the metal binding studies with crude D. marina exopolymer are given in Table 2. Maximum binding affinity and conditional stability constants were derived from the Langmuir linear relationship. Metal binding to D. marina exopolymer was considerably greater than metal binding we have measured for exopolymer from a thermophilic bacterium[8]. It was also far greater than metal binding reported by Mittelmen and Geesey[1]. The binding we obtained for all metals except iron was in the initial part of the binding curve. As a result we have generated idealized binding curves for the four metals by extrapolating back from the Langmuir relationship. These curves are presented in Figure 2. The order of maximum binding ability was as follows:

$$Mn > Cu > Ni > Fe$$

In contrast the order of conditional stability constants was:

$$Ni > Cu > Mn > Fe$$

When these binding experiments were repeated on the purified exopolymer no binding was obtained.

Discussion

The results of these binding studies indicate that D. marina crude exopolymer exhibits extremely strong binding to four different metals. The maximum binding abilities and the conditional stability constants of these exopolymer/metal complexes do not follow the reported order of trace metal assimilation by the microorganism[16]. This result suggests that the intracellular concentration of trace metals by D. marina is not influenced by

the ability of its exopolymer to bind them. However, there may in fact be a relationship between the inability of the exopolymer to strongly bind iron and its preferential concentration within the cell. Tightly bound metal ions are less available to the bacterial cell; a hypothesis that has been suggested for the development of copper tolerance by bacteria attached to copper surfaces[8].

The fact that purified exopolymer did not bind the metal ions suggests that the purification procedure removed the binding moieties. The purification was primarilly designed to remove proteinaceous material, however, considerable quantities of other material was also lost. It is reasonable to suppose that metal binding may be determined by 'reactive' functional groups such as amino groups. Protein is an important part of a biofilm matrix and therefore its importance should not be dismissed.

In terms of corrosion, the order of standard electrode potentials for the four metals are $E^{o}_{Cu/Cu++} > E^{o}_{Ni/Ni++} > E^{o}_{Fe/Fe++} > E^{o}_{Mn/Mn++}$. This means that the most readily bound metal ion, manganese, is also the least noble of the four metals and would therefore be oxidized relative to the other three. Manganese in solution might therefore be considered to act as a corrosion suppressant if it is preferentially bound by bacterial exopolymers over other metals. By the same token a manganese rich alloy would be particularly prone to oxidation reactions in the presence of exopolymer with a particularly strong affinity for this metal. However, two factors need to be considered before this kind of generalization can be made:

(1) Many different microorganisms are present in a biofilm, all of which may produce exopolymer with differing metal binding affinities. In fact the Irving-Williams series of stabilities of bivalent metal ion complexes[25] suggests the following order of stabilty for the four metals:

Cu > Ni > Fe > Mn

Therefore with most complexing agents we would expect that manganese would form the least stable complex.

(2) These experiments were run using the four different metals individually. Inter-metal reactions may strongly influence the affinity of microbial exopolymers for different metals. Some work has been done on the capsular exopolymer of an activated sludge bacterium Klebsiella aerogenes[2]. This showed that addition of metals separately and together did not affect the relative positions of the metals in an affinity series. However, quantity of bound metals relative to each other was strongly affected. Since concentration of metals is likely to be an important aspect of formation of ion concentration cells, it is obviously important to look at consortia of metal ions.

We have presented data in this report from a preliminary study designed to show not only the importance of exopolymers in metal binding but also the differential binding ability for different metals. If we can understand these differential binding abilities, particularly in the presence of many different metal ions, then we may be able to estimate the effect of particular microorganisms and their polymers on corrosion of specific alloys. It may be possible to inhibit biocorrosion by supplying 'sacrificial' metal ions that are readily bound by those polymers.

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REFERENCES

1. M.W. Mittelman, G.G. Geesey, Applied and Environmental Microbiology, Vol. 49, p. 846 (1985).
2. T. Rudd, R.M. Sterritt, J.N. Lester, Water Research, Vol. 18, p. 379 (1984).
3. B.J. Little, P.A. Wagner, S.M. Gerchakov, M. Walch, R. Mitchell, "The Involvement of a Thermophilic Bacteria in Corrosion Processes," Corrosion (in press).
4. G.G. Geesey, M.W. Mittelman, "The Role of High-Affinity, Metal-Binding Exopolymers of Adherent Bacteria in Microbial-Enhanced Corrosion," CORROSION/85, Paper No. 297,

National Association of Corrosion Engineers, Houston, Texas, 1985.
5. J.S. Nickels, R.J. Bobbie, D.F. Lott, R.F. Martz, P.H. Benson, D.C. White, Applied and Environmental Microbiology, Vol. 41, p. 1442 (1981).
6. D.H. Pope, D.J. Duquette, A.H. Johannes, P.C. Wayner, "Microbiologically Influenced Corrosion of Industrial Alloys," CORROSION/83, Paper No. 247, National Association of Corrosion Engineers, Houston, Texas, 1983.
7. G.G. Geesey, M.W. Mittelman, T. Iwaoka, P.R. Griffiths, Materials Performance, Vol, 25, No. 2, p. 37 (1986).
8. T.E. Ford, J.S. Maki, J.P. Black, R. Mitchell, "Interaction of Thermus with Metal Surfaces," presented at the American Society for Microbiology annual meeting, Washington, DC, 1986.
9. T.E. Ford, M. Walch, R. Mitchell, "Corrosion of Metals by Thermophilic Microorganisms," CORROSION/86, Paper No. 123, National Association of Corrosion Engineers, Houston, Texas, 1986.
10. M.S. Gould, E.J. Genetelli, Water Research, Vol. 12, p. 505 (1978).
11. S. Stoveland; J.N. Lester, The Science of the Total Environment, Vol. 16, p. 37 (1980).
12. M.J. Brown, J.N. Lester, Water Research, Vol. 16, p. 1539 (1982).
13. J.N. Lester, R.M. Sterritt, T. Rudd, M.J. Brown, In: J.M. Grainger, J.M. Lynch (eds), "Microbiological Methods for Environmental Biotechnology," Academic Press, London, p. 197, 1984.
14. A.B. Cobet, C. Wirsen, G.E. Jones, Journal of General Microbiology, Vol. 62, p. 159 (1970).
15. S.A. Fazio, D.J. Uhlinger, J.H. Parker, D.C. White, Applied and Environmental Microbiology, Vol. 43, p. 1151 (1982).
16. G.E. Jones, L.G. Royle, L. Murray, In: J.M. Sharpley, A.M. Kaplan, "Proceedings of the Third International Biodegradation Symposium," Applied Science Publishers, p.889, 1975.
17. P. Baumann, L. Baumann, M. Mandel, Journal of Bacteriology, Vol. 107, p. 268 (1971).
18. A.M. Staub, Methods of Carbohydrate Chemistry, Vol. 5, p. 5 (1965).
19. K.W. Giles, A. Myers, Nature, Vol. 206, p. 93 (1965).
20. M.J. Osborn, Proceedings of the National Academy of Sciences, USA, Vol. 50, p. 499 (1963).
21. M.M. Bradford, Analytical Biochemistry, Vol. 72, p. 248 (1976).
22. R.S. Hanson, J.A. Phillips, In: P. Gerhardt et al (eds), "Manual of Methods for General Bacteriology, American Society for Microbiology, Washington, DC, p. 328, 1981.
23. N. Blumenkrantz, G. Asboe-Hansen, Analytical Biochemistry, Vol. 54, p. 484 (1973).
24. C.E. Furlong, R.G. Morris, M. Kandrach, B.P. Rosen, Analytical Biochemistry, Vol. 47, p. 514 (1972).
25. M. Irving, R.J.P. Williams, Nature, 162, p. 746 (1948).

TABLE 1
THE QUANTITIES OF THE VARIOUS COMPONENTS OF THE EXTRACELLULAR POLYMER OF STATIONARY PHASE DELEYA MARINA (in ug mg^{-1})

Component	Crude Exopolymer	Purified Exopolymer
Weight of lyophilized material (mg)	87.7	46.3
Hexose Sugars	368.5	244.6
Uronic Acids	84.8	104.4
Protein	10.4	14.4
DNA	ND*	ND
Heptose Sugars	48.3	-

*ND = not detected

TABLE 2
MAXIMUM BINDING ABILITIES (MBA) AND CONDITIONAL STABILITY CONSTANTS (Kc) OF THE COMPLEXES FORMED BETWEEN DELEYA MARINA CRUDE EXOPOLYMER AND COPPER, IRON, MANGANESE AND NICKEL

Metal Ion	MBA (nmoles mg^{-1} exopolymer)	K_c ($\times 10^9$)
Copper	263	2.4
Manganese	556	0.9
Iron	39	0.1
Nickel	435	1.4

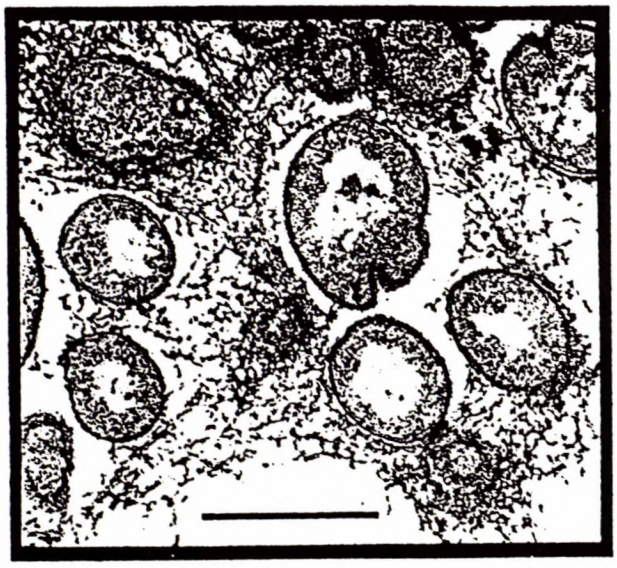


Figure 1. Transmission electron micrograph of Deleya marina stained with ruthenium red to demonstrate the presence of exopolymer. Bar = 1 um.

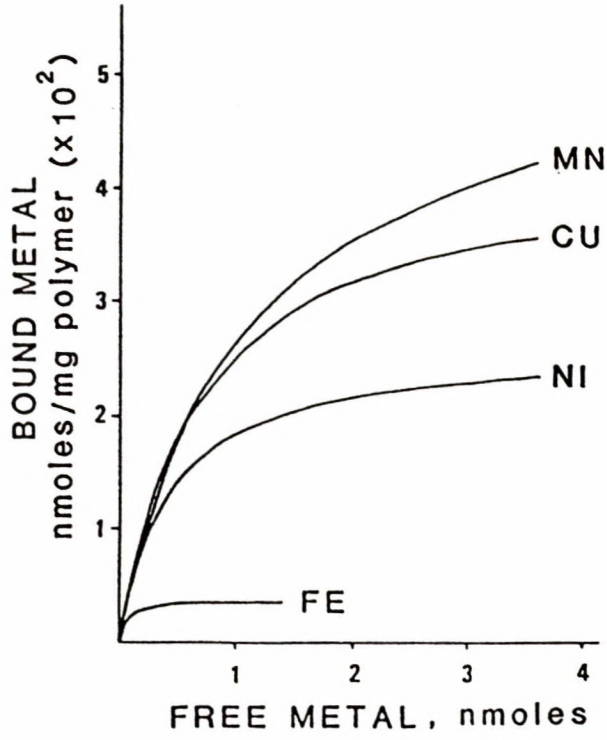


Figure 2. Binding curves for Deleya marina crude exopolymer with copper, manganese, iron and nickel. These curves have been extrapolated from the Langmuir relationship. With the exception of iron, data was obtained for only the lower sections of the curves.

What marine organisms are doing to our metals

By John J. Obrzut

Marine organisms attack metals in ways reminiscent of wolverines in a camper's tent. But research is learning to cope with them.

Heavy bio-fouling of test specimens at Inco's Francis L. LaQue Corrosion Laboratory gives some ideas of the loading placed on structures.

Metals' Fouling Resistance in Quiet Sea Water*

Arbitrary Rating Scale of Fouling Resistance		Materials
90-100	Best	Copper 90/10 copper-nickel alloy
70-90	Good	Brass and bronze
50	Fair	70/30 copper-nickel alloy, aluminum bronzes, zinc
10	Very slight	Nickel-copper alloy 400
0	Least	Carbon and low-alloy steels, stainless steels

*Above 3 fps continuous velocity (about 1.8 knots), fouling organisms have more difficulty in attaching and clinging to surfaces unless they are already securely attached
Source: International Nickel Co., Inc.

Marine bio-fouling is marine corrosion's partner in crime on the high seas. They both rob materials of their vital properties. And they both influence each other.

Marine bio-fouling is another major reason why the oceans are one of the most severe testing grounds for metals and other materials. As tiny as some of these marine organisms are, all they need is time to do their dirty work.

These creatures chew their way through resistant paint films, contribute to deterioration of steel structures, foul up and plug up water intake pipes, bore through all kinds of plastic materials, slow down our ocean liners and often accelerate the destruction of various metals and alloys. Unprotected wood appears as though it had been at the mercy of giant marine termites.

That's the bad part. But the part that's worse yet is that this marine bio-fouling extracts an enormous economic penalty—so huge that no estimates can be found as to what the annual destruction is. But it's safe to say that it runs into many hundreds of millions of dollars.

Moreover, the annual toll increases as the number and complexity of off-shore structures increase, and ocean-going vessels grow larger and more costly. On-shore structures, depending on sea water for cooling, are also equipped with expensive installations that are becoming more and more expensive with technology.

The brighter side of all this havoc is that there are companies and organizations that are doing something about this enormous problem. They're learning more about these creatures and their habits so that

they can isolate the organisms from the engineering materials that must be used in marine environments.

Who are these culprits? Some would be readily recognized in a lineup. Others sound as though they're performing their insideous work under assumed names.

These are some of the more common pests: Limnoria, algae, teredindae, sponges, hydroids, serpula, barnacles, encrusting bryozoa, filamentous bryozoa, anomia, mussels and tunicates. You might also add oysters and sea squirts.

If these organisms are to be categorized, they would fall essentially into two main types—the soft organisms that appear as plant-like slime, such as algae; and the second type are the hard or shell-like organisms, such as the barnacles, mussels and oysters.

Once a metal or other material is immersed in sea water, it isn't long afterwards that a bio-slime or micro-organisms settle on the surfaces. This initial layer then has a tendency to attract other fouling organisms.

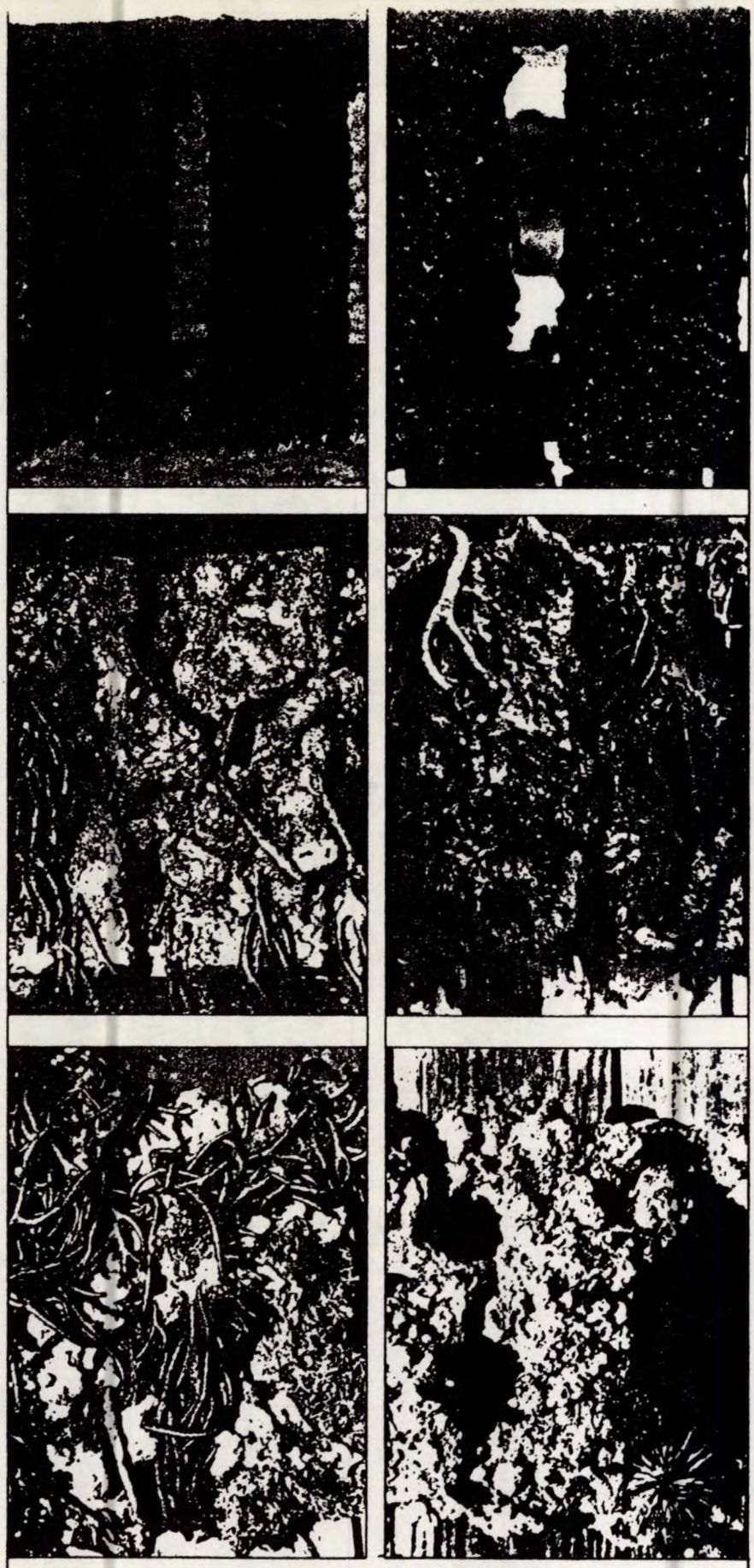
The effects of fouling isn't anything new. "Deterioration of wood ship hulls by marine borers," says W. W. Kirk, manager, Francis L. LaQue Corrosion Laboratory, International Nickel Co., Inc., Wrightsville Beach, N.C., "was noted long ago to the extent that owners found ways to sheath the hulls with lead or copper sheet.

"Still today," he continues, "the fouled steel hulls of ocean-going vessels increase fuel consumption to an alarming extent. Structures in sea water tidal zones can often be overloaded simply by the extra weight of fouling."

On cooling water intakes for industrial plants, the fouling products accumulate in thick layers. The layers, in fact, are so thick and reduce water flow to the point that the plants must be shut down to remove the encrustation in the line.

Fouling organisms can accelerate attack on some alloys by creating local shielding of the metal surfaces from the access of oxygen that's required to preserve passivity," notes Kirk. This is something that's common to stainless steels.

The hard-shelled organisms can penetrate and destroy soft coatings over structural materials. They'll



These carbon steel panels are typical of the fouling that occurs on the corrodible metals. These specimens were exposed to sea water at Inco's Francis L. LaQue Laboratory for 3, 9, 18, 36, 48 and 60 months.

even lift and work their way under lapped edges of harder protective wrappings. Certain members of the teredo family have penetrated coatings and some non-metallics, Kirk notes.

Metals and coatings aren't the only materials to suffer from the ravages of marine organisms—the borers in particular. Untreated wood piling, and especially in the tidal zone, will last only six months to a year or so. Treatment with creosote or coal tar under pressure will extend piling life many times over, but even then, it's destined for early replacement.

Kirk says: "The economics of prevention of fouling versus the replacement of marine structures should be considered. . . . Several alternatives are usually available to the design engineer. These include materials selection, isolation of the structure from its environment, or treatment of the environment or the metal/environment interface to prevent the settlement of marine organisms."

What are the materials that will resist fouling? "It is well known that copper and a few copper alloys are able to develop corrosion-product films that are toxic to most marine organisms and suppress the attachment of fouling organisms," Kirk comments.

"More recently, organic tin and lead compounds have been found to be effective agents in anti-fouling coatings. But some critical rate of release of the toxic ion in the corrosion products or from the paint film must be exceeded if the attachment and growth of fouling organisms is to be suppressed."

Anti-fouling paints, however, have a relatively short life. On ship hulls, for example, they have a useful life of a year or two. And recoating the hull can be quite costly on a large ship. This is especially so if bottom painting is involved.

For the long-term fouling protection, a 90-10 copper-nickel alloy has shown the most useful combination of properties, considering that this alloy also has excellent resistance to corrosion in sea water.

This had been known, but the extensive testing at the Francis L. LaQue Corrosion Laboratory confirmed many of the mechanisms that took place. This work was reported just two years ago by K. D. Efird at the NACE Corrosion Conference in Toronto.

For convenience and understanding, the study broke down metals into three classes: The corrodible metals such as carbon and low-alloy steels; the passive metals of which 5086-0 aluminum is one; and the so-called toxic film formers which includes copper, 90-10 copper-nickel and 70-30 copper nickel.

In other words, the fouling properties of metals are influenced by their corrosion characteristics.

What the study showed was that the steels, which are tremendously important in ocean-engineering structures, foul rather rapidly. But because they are also highly corrodible, the loose corrosion product on the surface sloughs off, removing the fouling with it.

The passive metals also foul quite readily. The fouling products, in this case, adhere very tightly. In addition to the 5086-0 aluminum alloy used in the tests, this class includes the stainless steels, titanium, a number of nickel-base alloys such as Inconel 625 and other aluminum alloys in the 5000-series.

The fouling products accumulated very heavily on the passive metal specimens and in many respects resembled the type of fouling that can be found on a non-toxic inorganic material such as slate. The sequence is that of one fouling product replacing another until such time that there's an overgrowth.

It is this kind of tenacious attachment that tends to restrict oxygen to the surface, accelerating local corrosion on certain alloys that are very susceptible to oxygen concentration cells. The crevice corrosion of stainless steels that occurs under barnacles is an excellent example of this effect.

The third class of metals—the toxic film-formers—offers the best possibility as ocean-engineering materials. Their protection against fouling is inherent in the material itself without the need of coatings, sea water treatment, or any other aid.

In this class of metals, it's expected that lead and beryllium would be included among the toxic forming metals that deter fouling. However, the concept of fouling resistance by toxicity doesn't always hold up. The fact of the matter is that only those metals based on the elements zinc, silver and copper actually have the needed inherent fouling resistance.

It should be pointed out that the adherent cuprous oxide corrosion product that forms on the surfaces of copper-base alloys is toxic only to the organisms. The toxic property is restricted to the surface layer and is not due to any release of poisonous ions into the sea water. Also, this toxic or anti-fouling property does not affect adjacent materials. In other words, there's no leaching or "throwing power."

The Efird report drew two other important conclusions: One, that pure copper, 90-10 copper-nickel and 70-30 copper-nickel resisted fouling equally well over a five-year period in sea water; and two, that these copper-base alloys aren't too susceptible to fouling at the waterline.

The practical significance of the anti-fouling properties of copper-alloys has been clearly spelled out in the construction of the so-called barnacle-resisting 90-10 copper-nickel hulls for shrimp boats. They replaced steel hulls, otherwise the boats were of standard design and construction. The boats should retain their original design efficiency for 30 years or more. The capital cost recovery period is less than seven years.

The first of this fleet, the Copper Mariner, effected a fuel saving of 45 to 48 pct over the steel-hulled sister shrimp boats—and without hull cleaning. The concept is being developed further for larger ships, using 90-10 copper-nickel clad plate.

A sailing vessel, the 52-ft ketch Asperida, also has a copper-nickel hull but of the 70-30 alloy. It, too, has effectively resisted fouling. In addition to the hull being built of copper-nickel, the keel, deck plates, lockers, cockpit and framing are also constructed of the same alloy.

The use of the copper-nickel alloys has certainly not been restricted to vessels. They have proved to be invaluable for screens around sea water intakes, cages and fencing used for fish farming, marine instrumentation, platform grating and ladders, for the intake piping itself as well as for liners in larger piping.

And the list of applications keeps growing.

The use of 90-10 copper-nickel sheathing over wood piling and power poles has, likewise, proven highly effective by extending their life from 7 to 10 years to an estimated 25 to 30 years.

The sheathing is wrapped around the pile or pole snugly so that it extends from one foot below the mud line to two feet above mean low tide. The sheathing is overlapped, then fastened with Monel anchorfast slating nails about two inches apart. If more than a 10-ft length of sheathing is required, the second sheet will overlap the first by two inches and that, too, is nailed in place.

Bio-fouling in the circulating systems using sea water has persistently plagued power plants. Again, the usual solution had been to simply shut the plant down and remove the fouling mechanically. The problem was generally twofold: Fouling of the intake piping, restricting flow to the point that it affected pumping efficiency; and, buildup of slime in the condenser tubing which affected heat transfer.

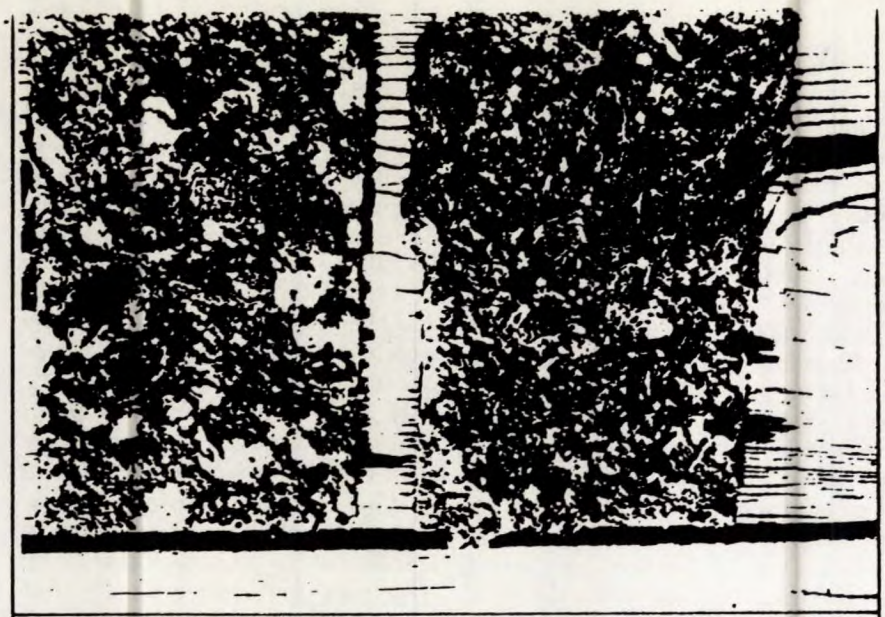
The problem was such that finding a solution to one was not necessarily a solution for the other. Removal of slime from the condenser tubing was generally the easier of the two.

In such cases, chlorination proved very effective in controlling both problems—sliming and mussel buildup. But the degree of chlorination was unpredictable in controlling a given amount of fouling in a particular plant.

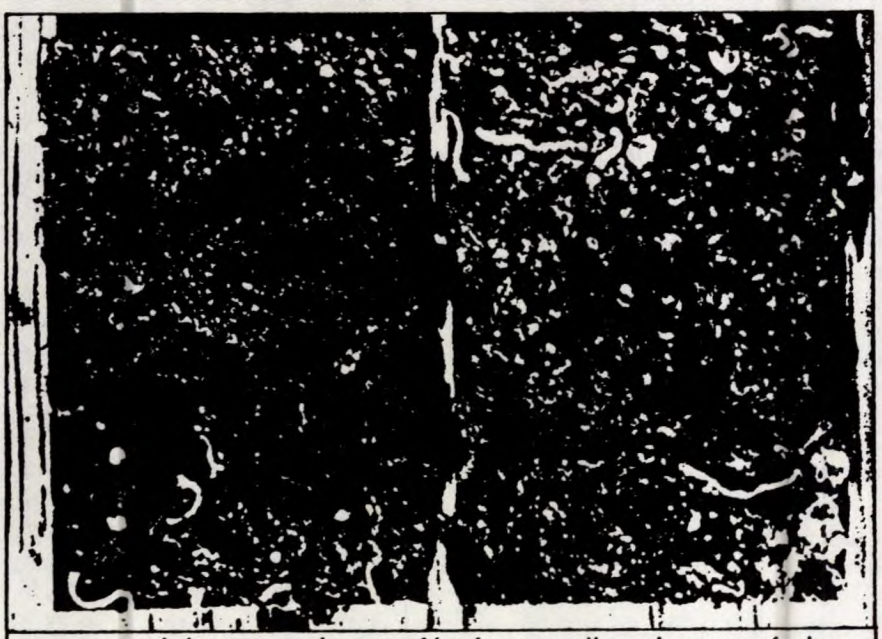
Heating the sea water to above 110°F has also been effective in controlling mussel fouling. But here, too, was the problem of determining the optimum time interval for heating the circulating water without getting into a detailed study.

As for chlorination, intermittent treatment is satisfactory for slime and algae removal. However, continuous chlorination is regarded more effective when fouling settling is expected. When chlorination is used, the system should have some way to monitor residual chlorine.

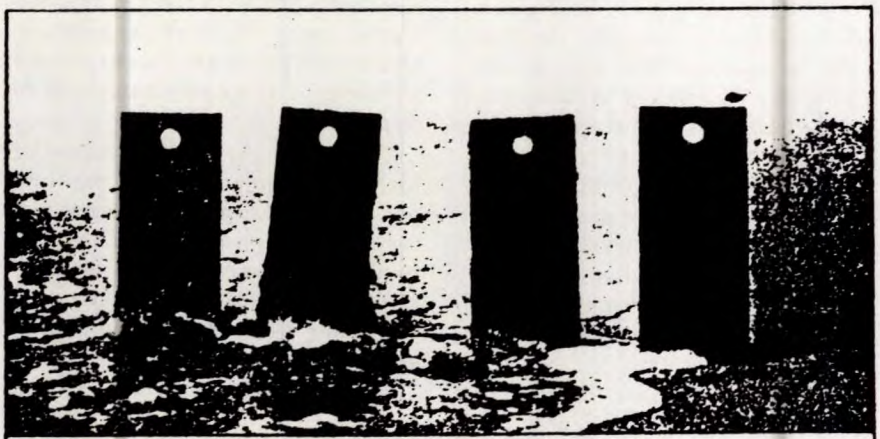
Another important factor that must be considered in any problem dealing with fouling is that the con-



Passive metals, such as these titanium specimens, accumulate a tightly adherent film that builds up with time. These panels were exposed for six months.



Copper-nickel is among the toxic film-forming alloys that resist fouling. These 70-30 copper-nickel panels were exposed to sea water for 14 years.



The sea water itself and fouling progression are continually monitored at Wrightsville Beach, N.C.

ditions of time, location, water velocity, temperature, water analysis and others all have a bearing on the type and severity of the fouling.

In some relatively recent studies at the Woods Hole Oceanographic Institution in Massachusetts, Stephen C. Dexter, a marine research biologist, determined that microbiological fouling occurs very slowly on surfaces immersed at depths below 500 meters.

Some tests that spanned a period of 132 days showed virtually no fouling on any of the samples. In fact, there had been very little more fouling activity at depths between 500 and 5000 meters in the open ocean even after 408 days of exposure. Some occasional slime stringers were found but without any associated organisms.

Dexter also drew the conclusion from these tests that none of the toxic anti-fouling coating formulations used were able to completely ward off slime formation. The alloys with more than 90 pct copper, on the other hand, reduce the rate of slime formation.

Non-toxic materials, including glass, polystyrene and high-nickel alloys failed in their mission. None was capable of discouraging fouling by the hard-shelled organisms such as barnacles.

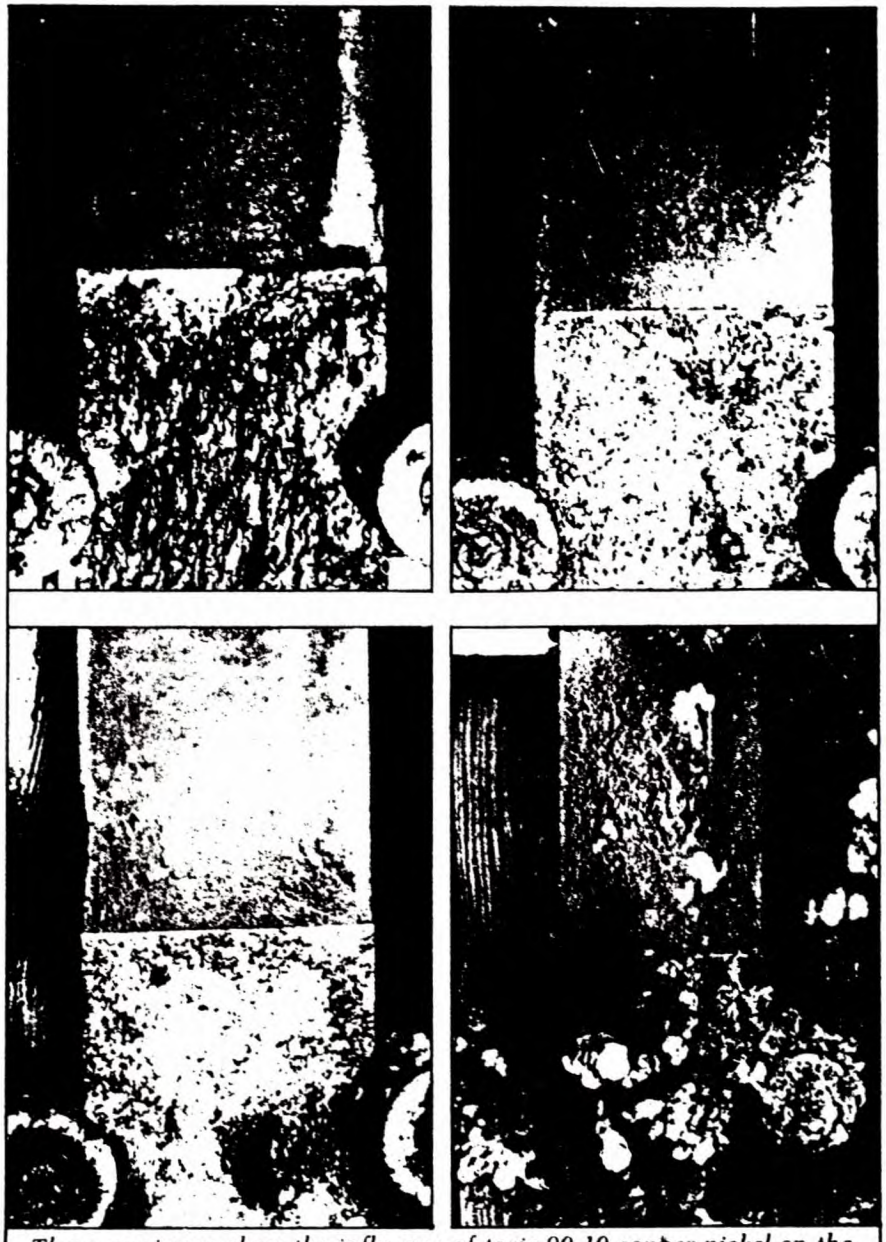
Of the paints that Dexter tested in the North Atlantic environment, the 90-10 copper-nickel in a phenolic matrix served best.

"Surface energy," says Dexter, "appears to play as large a role as toxicity in determining the rate of attachment of micro-organisms to solid surfaces immersed in a marine environment. Surface energy, however, has no effect on the attachment of macrofouling organisms.

A perfectly nonfouling surface," he continues, "therefore, must be both toxic and have the proper surface energy."

In various tests performed at the Francis L. LaQue Corrosion Laboratory at Wrightsville, N.C., the surface finish of a material had little or no bearing on its capability to resist fouling. Glass, smooth plastics, polished metal specimens—all reacted in a manner similar to that of the same materials with rougher surfaces.

The inherent capability of a material to resist fouling has more to do with its composition than its finish.



These specimens show the influence of toxic 90-10 copper-nickel on the adjacent non-toxic vinyl paint surfaces after exposure to sea water during the summer fouling season. Exposure was 3, 7, 10 and 24 weeks.

Anti-fouling paints abound by the scores. They're formulated from vinyls, polyesters, rubber-based, oil-based, plus many other types of ingredients. Other than ocean-going liners, off-shore installations and on-shore facilities, one of the larger markets for anti-fouling paints is the weekend boating enthusiast.

One of his chief disappointments in his battle with barnacles and teredos has been the inconsistency of results in the protection he purchases, often with the same paint that he had used previously.

The difference is not so much with any changes in paint formulation as it is in the ever-changing conditions under which the anti-fouling paints are used. In some years, the boat owner will get a full year's service. The following year, he may get far less than a full season's use.

Differing performance can be attributed to many factors. For one, there's boat location from one season to another. Another is the specific usage and speeds.

BEHAVIOR OF METALLIC AND OXIDE COATINGS DURING DEVELOPMENT OF MICROBIOLOGICAL CORROSION IN THE ATMOSPHERE

A. A. Gerasimenko

UDC 620.198

Metallic materials may suffer microbiochemical damage under conditions of high humidity and restricted access or exchange of air and when organic contaminants are present on the surface. Microorganisms of plant origin, especially mycelial fungi, cause the greatest damage to structural elements [1]. The products of their vital activity may reduce the pH and intensify metal corrosion processes. There is no information on the protective capacity of metallic and oxide coatings under conditions of microbiological corrosion. The following types of protective and protective-decorative coating were tested in the present work: zinc chromate, cadmium chromate, and copper, produced from cyanide, polyethylenepolyamine, and acid electrolytes; lustrous chromium, combined nickel — chromium and copper — nickel — chromium, chemical nickel — phosphorus, and tin and oxide coatings with a chromate filler. Testpieces measuring 30 × 30 × 1-2 mm with pre-applied coatings were used. The mixture of fungi was isolated from the most resistant cultures found on the surfaces of structures in operational use (Table 1).

One ml of suspension contained 1-2 million spores. Preliminary tests on the selected mixture of fungi and the collection of biocultures suggested by the International Electrical Engineering Commission showed that the fungi found in conditions of structure operation exhibited great vitality and dynamic rates of development (Fig. 1). The appearance of the growing fungi included in the mixture used for testing and a brief description of them appear in Fig. 2 and in Table 2. The coating material is not directly assimilated by the microorganisms; the bioculture mixture was therefore applied to the testpiece surfaces with addition of nutrient in the form of mineralized agar (2% leached agar with the following mineral salts, %): sodium nitrate 0.3, dipotassium orthophosphate 0.03, magnesium sulfate 0.05, potassium chloride 0.05, monopotassium orthophosphate 0.07, divalent iron sulfate 0.001. The pH of the medium was 5.8 (2-ml nutrient to 10-ml suspension). The testpieces were placed in Petri dishes, and these in turn were placed in desiccator chambers in which 95-98% relative humidity and a temperature of 20-25° were maintained. The tests were repeated 3-6 times, the surfaces being examined after 1, 3, 6, and 12 months. The protective powers of the coatings were evaluated quantitatively by a points system, taking account of changes in the condition of the coating due to microbiological and corrosion factors (Table 3).

TABLE 1. Cultures of Mycelial Fungi from Prints from Surfaces of Operational Structures: Number of Cases in Inspections Using Special Method [2, 3]

Genus of fungus	Region			Genus of fungus	Region		
	Urals	Volga	Amur		Urals	Volga	Amur
Penicillium sp.	39	29	29	Aspergillus sp.	–	6	13
Cephalosporium sp.	21	6	4	Torula	5	2	–
Trichoderma sp.	16	35	29	Pecilomyces	3	6	–
Cladosporium sp.	4	–	–	Sterile mycelium	2	6	–
Verticillium	9	2	–	Sporotrichum	–	4	–
Helminthosporium	2	–	5	Acremonium	–	3	–

Translated from Zashchita Metallov, Vol. 12, No. 1, pp. 99-105, January-February, 1976. Original article submitted August 23, 1974.

TABLE 2. Brief Description of Microorganisms Encountered in Prints from Structures

Microorganism	Optimum conditions for development	Brief description in taxonomic terms	Main possible products of vital activity
1. Penicillium. sp.	t = 25–28°, pH 5.5, total saturation with moisture	Imperfect fungi of more than 120 species. Conidiophores branched bearing sterigmata (often two rows) at the tip from which spring long chains of spherical conidia	Acids: oxalic, citric, gluconic. Ethyl acetate. Fats. Enzymes: saccharase, amylase, cellulase, protease. Pigments, quinone derivatives. Antibiotics, including penicillin
2. Cladosporium sp.	t = 18–25°, pH slightly acid, total saturation with moisture	Imperfect fungi of more than 15 species. Olive and light brown conidia in chains on the tip and the lateral projections of the conidiophores. At the base of the chain the conidia are larger and have transverse septa and may bud	Organic acids. Enzymes: cellulase, lipase, saccharase, Pigments.
3. Cephalosporium sp.	t = 25–28°, pH slightly acid, humidity close to saturation	Imperfect fungi of about 10 species. Colorless creeping mycelium. Simple erect conidiophores, not distended. Conidia oval, oviform, at the tip or crown, stuck together with mucus	Organic acids. Enzymes: cellulase, protease, saccharase. Toxins
4. Trichoderma sp.	t = 25–26°, pH slightly acid, humidity close to saturation	Imperfect fungi of several species. Creeping colorless mycelium, forming fluffly accumulations about 1 mm in diameter within the colony. These cushions are white initially and then become green and are rich in conidiophores with conidia. The conidiophores have opposite branching and bear bottle-shaped sterigmata. The conidia are green, spherical or oval, and gathered into heads on the ends of the sterigma	Organic acids. Enzymes: cellulase, saccharase, amylase. Antibiotics, antibacterial and antifungal agents

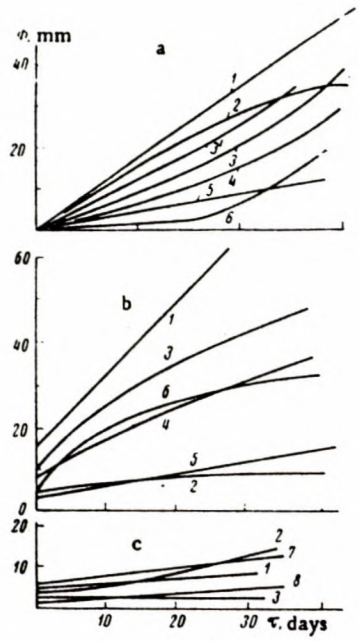


Fig. 1. Growth of cultures of fungi isolated under operational conditions: a) at 6°; b) at 6° after preliminary cultivation at 20°; c) growth of cultures of International Electrical Engineering Commission fungi at 6° after preliminary cultivation at 20°: 1) Trichoderma sp.; 2) Cladosporium sp.; 3) Penicillium sp.; 3') Penicillium (FLT); 4) Cephalosporium sp.; 5) Cladosporium sp. (white colonies); 6) Trichoderma sp. (FLT); 7) Aurocobsidium; 8) Scopulariopsis.

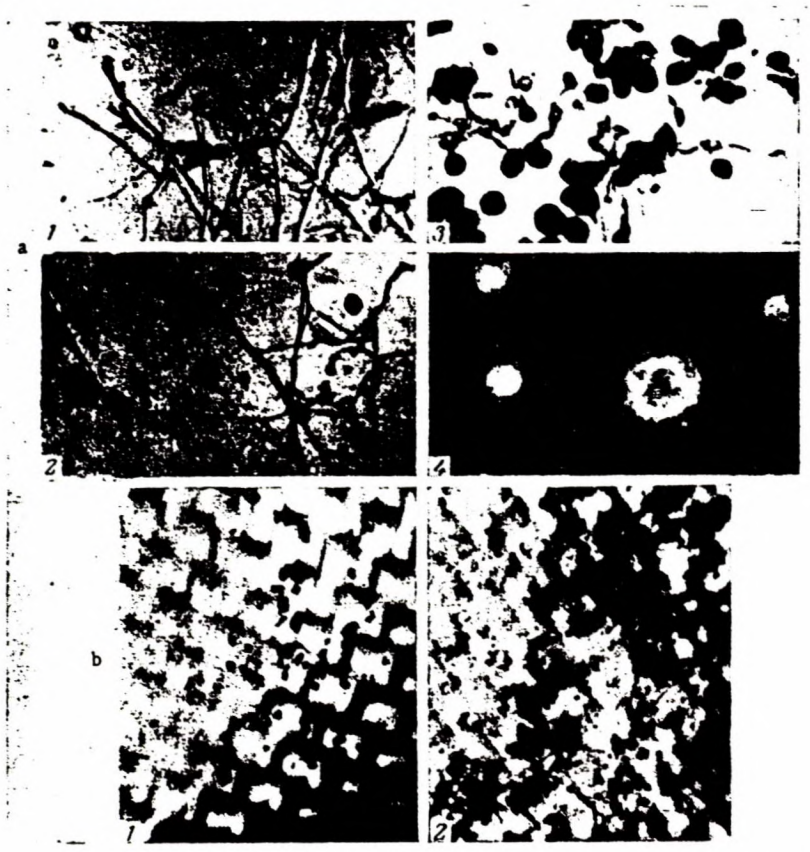


Fig. 2. a) Appearance of colonies of mycelial fungi on surfaces of structural elements in operational use (×300): 1) Penicillium sp.; 2) Cephalosporium sp.; 3) Trichosporium sp.; 4) Aspergillus sp. (colonies); b) development of Aspergillus sp. colonies: 1) after 3 days; 2) after 15 days.

The results of the tests are given in Table 4. Analysis of the data given in Table 4 shows that the coatings tested have sufficient protective power under conditions in which microbiological corrosion can develop. Weak microscopic growth of mycelial fungi, mainly Cephalosporium, with spore germination

TABLE 3. Microbiological Resistance and Fungicidal Characteristics of Materials and Coatings

Points	Microbiological resistance of coating	Fungal growth corresponding to points level	Condition of metallic (inorganic) coating	Fungicidal characteristics of material
0	Total resistance	No fungi present (×56)	No change	Fungicidal
1	Resistance	Microscopic growth of fungi, germination of spores, slight development of mycelium in the form of short unbranched hyphae without sporophores (·56)	Slight changes in color and luster, superficial points and patches of corrosion on up to 1% of surface	Slightly fungicidal
2	Satisfactory resistance	Microscopic growth of fungi, well developed mycelium in the form of branching hyphae with sporophores	Appearance of corrosion products, points and patches of corrosion on 1 to 5% of surface	Includes constituents with weak fungicidal properties
3	Reduced resistance	Weak fungal growth visible with the naked eye, intensive development of mycelium	Continuous corrosion on up to 10% of surface, small bulges in coating (up to 5%)	Not fungicidal
4	Low resistance	Moderate fungal growth, mycelium in the form of an unbroken network visible with the naked eye	Continuous corrosion on from 10 to 50% of surface, bulges and flaking off 5-20%, centers of corrosion in up to 10% of underlying metal	Includes constituents that can be assimilated by fungi
5	No resistance	Intensive fungal growth, abundant mycelium over the entire surface	Coating flaking off and centers of corrosion in excess of 10%	Not fungus-resistant

TABLE 4. Results of Experiments to Assess the Microbiological Resistance of Coatings*

Backing material and coating		Assessment by months, points: microbiological resistance 1	3	6	12	total corrosion effect 1	3	6	12
Steel 08KP	Zn15 (cyanide), chromate	0	1	1	1	0	0	1	2
"	Zn15 (pepa) chromate	0	1	1	2	0	0	0	1
"	Cd12 (cyanide), chromate	0	0	0	0	—	—	—	—
	Cd12 (pep)chromate	0	0	0	0	0	0	1	1
	Cd9 (acid), chromate	0	0	0	0	0	1	1	2
Steel 3	Cu15 (acid)	0	0	0	0	0	0	0	1
"	Cu15 (cyanide)	0	0	0	0	0	0	0	1
"	Cu15 (pepa)	0	0	0	0	0	0	0	1
"	Chem. Ni18	0	0	0	0	—	—	—	—
"	Lustrous Cr12	0	0	0	1	—	—	—	—
"	Ni_6Cr_3	0	0	0	0	—	—	—	—
	$Cu_4Ni_2Cr0.5$	0	0	0	0	0	0	0	1
L13	09	0	0	0	0	—	—	—	—
D16AT	AN oxide, chromated	0	0	0	0	—	—	—	—
AD1M	AN oxide, chromated	0	0	0	0	—	—	—	—

* The preliminary biological activity testing of biocultures was done in collaboration with I. S. Vostrov at the Institute of Microbiology, Academy of Sciences of the USSR.

and slight development of mycelium in the form of short unbranched hyphae without sporophores is observed on zinc coatings, irrespective of their method of production, after a three-month exposure. The total effect of corrosion and microbiological damage is greater on zinc coatings produced from a cyanide electrolyte. The pattern with lustrous chromium coatings is similar. Mostly Cladosporium germinates after a twelve-month exposure. Zinc, cadmium, copper, and combined copper — nickel — chromium

TABLE 5. Fungicidal Power of Certain Substances

Substance	Solvent	Concentration, %			
		0.1		1	
		paper	FLT fabric	paper	FLT fabric
Salicylanilide	Alcohol	–	4	–	3
Dibutyltinlauric acid	Water	2	1	2	1
Polyethylenepolyamine	Alcohol	4	2	3	1
Ammonium fluoride	Water	2	1	0	0
Copper-2,4-dichlorophenoxyacetic acid	Alcohol	3	1	1	1
Benzotriazole	"	0	0	0	0
Iodoallylurotropin	Water	1	0	0	0
K-17 lubricant	–	0	0	0	0
K-17N lubricant	Water	3	2	2	2
Dichloraminochloriminochloromethane	Carbon tetrachloride	0	0	0	0
Propolis	Alcohol	2	1	2	2
Polyethylenimine	Water	0	0	0	0
Benzaldehyde	Alcohol	0	0	0	0
o-Oxybenzaldehyde	"	0	0	0	0
p-Methyl o-benzaldehyde	"	0	0	0	0
m-Methyl p-oxybenzaldehyde	"	0	0	0	0
7-Nitrotetrahydroquinoline	"	4	2	0	0
6,8-Dinitro-1-formyltetrahydroquinoline	"	2	1	2	1

coatings were subject to slight corrosive changes (surface darkening, formation of a film of corrosion products). Thus the coatings studied retain their protective powers in operation at increased humidity and with exposure to microorganisms over a period of one year. The protective powers of zinc, cadmium, and copper coatings can be increased when the anticipated total period of exposure to these factors is more than one year and for more severe operational conditions by applying water-repellent compositions which incorporate effective fungicides and corrosion inhibitors. The substances given in Table 5 were studied with this in view. The method used to assess the fungicidal power of the substances was as follows: 0.1-1% solutions of the substances chosen as proposed fungicides were placed on backings (paper, FLT-42 fabric), then 6-10 drops of a suspension of fungal spores in nutrient were placed on the treated surface. The backings were put into Petri dishes and kept at 95-98% humidity and 20-25° for 45 days. The fungicidal power was assessed from the extent to which the surfaces were overgrown by the fungi.

The fungicides used industrially [4] as additives to paints and lacquers and polymer materials proved to be either highly toxic (mercury — lead — zinc — copper — organic compounds) or relatively ineffective (salicylanilide, Trilan, etc.). Salicylanilide, e.g., suppresses fungal growth only in concentrations of over 4%. Substances well known as fairly effective bactericides, corrosion inhibitors or ligands were tested, as well as new heteroorganic compounds synthesized at Moscow State University by E. G. Rukhadze. Of the substances studied, the following can be recommended as supplementary means of protecting metallic and inorganic coatings: iodoallylurotropin, benzotriazole, polyethylenimine, and benzaldehyde and its derivatives. These substances are sprayed onto the surfaces of structures as 0.1-1% solutions in water or alcohol and dried. The structural elements may contain polymer materials and metals protected by paint and lacquer.

The evaluation of the resistance of metallic and oxide coatings to the action of microbiological agents (mycelial fungi) holds good only for atmospheric conditions of operation.

Microbiological corrosion of metals and coatings on contact with aqueous solutions of electrolytes, with nonelectrolytes (petroleum, organic fuels, and oils), with greases, and with the soil leads to substantial damage [5, 6]. However, these matters were outside the scope of the present investigation.

CONCLUSIONS

1. A study was made of the protective properties of a number of metallic and oxide coatings on exposure to mycelial fungi during their vital activity on the surface of testpieces under atmospheric conditions.

2. The coatings studied retain a fairly high level of protective properties under combined exposure to increased humidity and the most persistent fungi over a period of one year.

3. Fungicide formulas including chemical compounds with inhibitor properties have been developed which can be used for supplementary protection of coatings under more severe operating conditions.

LITERATURE CITED

1. B. Blagnik and V. Zanova, Microbiological Corrosion [in Russian], Khimiya, Moscow (1965).
2. D. G. Zvyagintsev, Reactions Between Microorganisms and Hard Surfaces [in Russian], Izd. MGU (1973).
3. N. V. Kalakutskii, I. S. Vostrov, and R. V. Zaitsev, Zh. Prikl. Biokhim. i Mikrobiol., 9, 186 (1973).
4. Data from the 2nd All-Union Symposium on Biological Damage and Growth on Materials, Components, and Installations [in Russian], Nauka, Moscow (1972).
5. P. A. Vinogradov, Various Ways and Means of Preserving Machines, Instruments, and Components Depending on Conditions of Storage [in Russian], Leningrad. Dom Nauchn.-Tekhn. Propagandy (1967).
6. N. A. Zaikina, M. P. Blinov, M. A. Vinogradov, and G. G. Golovanenko, Zashchita Metal., 8, 232 (1972).

THE ROLE OF SULPHATE-REDUCING AND SULPHUR-O.')IZING BACTERIA IN THE LOCALIZED CORROSION OF IRON-BASE ALLOYS — A REVIEW

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Abstract: Chemical and microbiological interactions of inorganic sulphur compounds, which result in the formation of various aggressive sulphoanions such as sulphide, sulphite, thiosulphate, and tetrathionate are reviewed with emphasis on the catalytic role played in these transformations by exposed metal surfaces and traces of metal ions in solution. The effects of these sulphur anions on the electrochemical conditions leading to pitting, crevice corrosion and stress corrosion cracking of iron-base alloys in chloride- and non-chloride-containing environments are also reviewed, as an appropriate background for discussing the role of sulphate-reducing and sulphur-oxidizing bacteria in localized corrosion processes.

Introduction

Examination of the literature on corrosion induced by bacteria indicates that localized corrosion can occur. Especially the presence of sulphate-reducing bacteria in corroded pipelines and other industrial installations has been reported. The chemistry of this type of corrosion can involve a number of entities, particularly metastable sulphur compounds. This review discusses the various metabolic pathways associated with sulphur-oxidizing thiobacilli and sulphate-reducing bacteria. Both groups of microorganisms are ubiquitous in soils, sediments, and waters and their ability to utilize sulphur compounds is well documented. Metastable sulphur species, such as thiosulphate and polythionates are involved in both the oxidative and reductive biological transformations. These compounds are known to interact with each other, often generating or involving ionic species that are extremely aggressive towards iron, carbon steels and stainless steels at ambient temperatures. Possible effects of thiosulphate, tetrathionate, hydrogen sulphide, and other inorganic compounds of sulphur are discussed in relation to pitting corrosion, crevice corrosion, and stress corrosion cracking. In the latter case, attention is drawn to the selective chemistry which will encourage this type of corrosion at ambient temperature, particularly where the environment is conducive to bacterial growth and biofouling. Although no case histories have so far been reported on microbiologically induced stress corrosion cracking, the potential for these problems to occur should not be overlooked.

Chemical Interactions of Inorganic Compounds of Sulphur

Several excellent reviews have been published on the chemistry of metastable ionic sulphur species (Schmidt 1972, Schmidt & Siebert 1973, Nriagu & Hem 1978). Particular emphasis has been placed on inorganic sulphur compounds regarding the environmental cycling and the methods of their determination (Szekeres 1974, Granat *et al.* 1976, Brown 1982). Though the role of sulphur anions has been recognized in laboratory studies of pitting and stress corrosion cracking, these compounds have received relatively little attention in corrosion related studies of installation failures.

Some of the inorganic sulphur compounds are poorly characterized and defined. An example is colloidal sulphur prepared by sulphdolysis of tetrathionate. The sulphur thus produced is extremely metastable and probably a complex mixture of S_8, polythionates, and polysulphides. Even the relatively stable S_8 ring structure is susceptible to nucleophilic attack by a variety of reductants including sulphide and cyanide. According to the potential-pH diagram for the sulphur-water system at 25°C (Valensi *et al.* 1974), all sulphur species between the oxidation numbers of −2 (sulphide) and +6 (sulphate) are thermodynamically metastable with the exception of elemental sulphur. Thiosulphate ($S_2O_3^{2-}$) and polythionates ($S_xO_6^{2-}$; x = 3, 4, 5, 6) all tend to decompose in aqueous solutions. A simplified Pourbaix diagram for some of these metastable sulphur ions is shown in Fig. 1 (Valensi 1973) in which the stability domains of S, dissolved H_2S, HS^-, and S^{2-} are also included. A complete series of polysulphides can coexist according to the following equilibrium:

$$2S_x^{2-} = S_{x+1}^{2-} + S_{x-1}^{2-}$$
$$(x=2,\ 3,\ 4,\ 5)$$

but they would appear in the diagram only at a higher total sulphur concentration than that presented in Fig. 1, S_5^{2-} being the predominant species. The diagram yields the potentials and pH values at which disproportionation reactions occur. For example, thiosulphate is decomposed in acid solutions as follows (Johnston & McAmish 1973):

$$S_2O_3^{2-} + H^+ \rightarrow S\ (\text{colloidal}) + HSO_3^-$$

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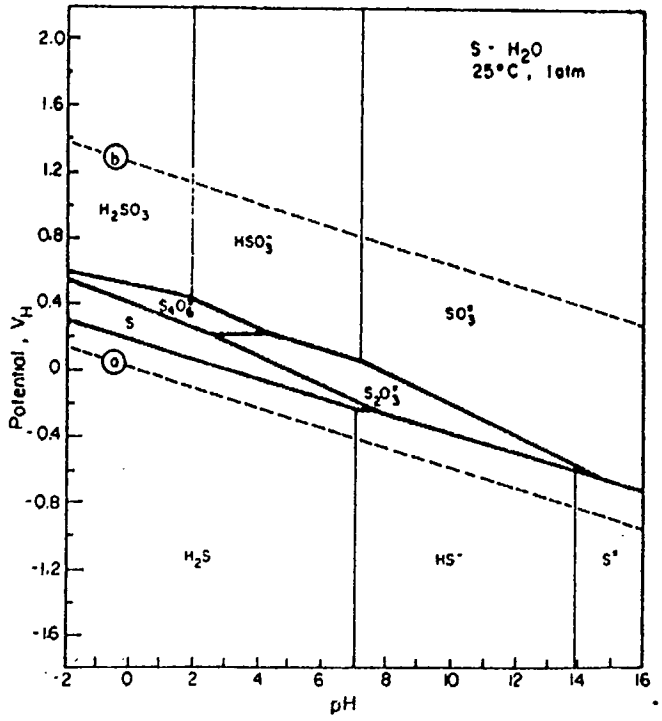

Fig. 1. Potential-pH metastable equilibrium diagram for the system S-H_2O at 25°C and 1 atm. Dithionite ($S_2O_4^{2-}$), dithionate ($S_2O_6^{2-}$), trithionate ($S_3O_6^{2-}$), and sulphate (SO_4^{2-}) are not included. Total concentration = 0·2 g S/l H_2O (Valensi 1973). With permission of CEBELCOR.

but neither one of the products is stable. Colloidal sulphur may readily be transformed to polysulphides through a nucleophilic attack of the S_8 ring by HS^- or S^{2-} particularly in neutral and alkaline solutions (Schmidt & Siebert 1973).

Although the potential-pH diagram for the thermodynamically stable species of the sulphur-water system (Valensi *et al.* 1974) indicates that sulphate can be reduced to sulphur or sulphide in aqueous solutions, the reduction of sulphate is a highly irreversible process. Therefore, sulphate is electrochemically inactive even at very low potentials. On the other hand, sulphate can be easily reduced to polythionates, thiosulphate, and sulphide by anaerobic bacteria.

The chemistry of metastable sulphur compounds is extremely complex as illustrated by the following examples. Polythionates are able to react with sulphite (Blasius & Münch 1972, Wagner & Schreier 1978, Tuovinen 1978):

$$S_4O_6^{2-} + SO_3^{2-} \rightarrow S_2O_3^{2-} + S_3O_6^{2-}$$

$$S_xO_6^{2-} + (x-3)SO_3^{2-} \rightarrow S_3O_6^{2-} + (x-3)S_2O_3^{2-}$$

In alkaline solutions polythionates are hydrolyzed:

$$S_4O_6^{2-} + OH^- \rightarrow S_2O_3^{2-} + S^0 + HSO_4^-$$

They are also powerful oxidizing agents for sulphide:

$$S_4O_6^{2-} + S^{2-} \rightarrow 2S_2O_3^{2-} + S^0$$

$$S_4O_6^{2-} + S^{2-} + SO_3^{2-} \rightarrow 3S_2O_3^{2-}$$

Oxidants such as Fe(III) and MnO_4^- are able to oxidize thiosulphate to tetrathionate in a manner similar to that by iodine:

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^-$$

Ferrous, ferric, manganous and manganic ions, and other transition metal ions (CuII, NiII) are known to have catalytic effects on sulphur transformations such as polythionate and thiosulphate degradation. Their reduced forms (Fe^{2+}, Mn^{2+}) can also be oxidized by aerobic micro-organisms. Thus, in oxygenated environments not only the metastable sulphur species as discussed in the following section, but also some transition metal cations are biologically active.

It should be emphasized that sulphide-covered surfaces and metal cations produced by the dissolution of iron base alloys can catalyze chemical transformations of sulphur compounds without the direct involvement of sulphate-reducing or sulphur-oxidizing microorganisms. In industrial installations, and even in synthetic solutions, trace level contamination by metal ions is to be expected and therefore, the distribution profiles of reactive sulphur species need to be determined in order to predict the chemical pathways and to identify the species responsible for localized corrosion processes.

Microbiological Reactions of Inorganic Compounds of Sulphur

Aerobic sulphur-oxidizing thiobacilli

Bacteria capable of deriving energy for growth from the oxidation of inorganic sulphur compounds are ubiquitous in the nature. Thiobacilli are the best-known group of sulphur-oxidizers and they have been implicated in microbiological corrosion phenomena because of their ability to produce sulphuric acid (Table 1). Several oxidation reactions have been presented by Roy & Trudinger (1970) and Kuenen & Tuovinen (1981).

Table 1. *Oxidation reactions of thiobacilli.*

$S_2O_3^{2-} + 2O_2 + H_2O$	$\longrightarrow 2SO_4^{2-} + 2H^+$
$2S + 3O_2 + 2H_2O$	$\longrightarrow 2SO_4^{2-} + 4H^+$
$4S_2O_3^{2-} + O_2 + 2H_2O$	$\longrightarrow 2S_4O_6^{2-} + 4OH^-$
$2S_4O_6^{2-} + 7O_2 + 6H_2O$	$\longrightarrow 8SO_4^{2-} + 12H^+$
$2SCN^- + 4O_2 + 4H_2O$	$\longrightarrow 2SO_4^{2-} + 2CO_2 + 2NH_4^+$
$H_2S + 2O_2$	$\longrightarrow SO_4^{2-} + 2H^+$
$2H_2S + O_2$	$\longrightarrow 2S + 2H_2O$
$2S_3O_6^{2-} + 4O_2 + 4H_2O$	$\longrightarrow 6SO_4^{2-} + 8H^+$
$5H_2S + 8NO_3^-$	$\longrightarrow 5SO_4^{2-} + 4N_2 + 4H_2O$
$5S + 6NO_3^- + 2H_2O$	$\longrightarrow 5SO_4^{2-} + 3N_2 + 4H^+$
$5S_2O_3^{2-} + 8NO_3^- + H_2O$	$\longrightarrow 10SO_4^{2-} + 4N_2 + 2H^+$

As the reactions in Table 1 indicate, many inorganic sulphur compounds are susceptible to microbiological oxidation. In the biological pathway of oxidation, the various oxidative steps are mediated by specific enzymes with coupling to an electron transport system where oxygen is reduced to water as the terminal reaction:

$$1/2O_2 + 2H+ + 2e^- \rightarrow H_2O$$

Fig. 2 outlines the various steps involved in the biological oxidation of sulphide, elemental sulphur, and sulpho-oxyanions. The current knowledge on sulphur oxidation pathways in thiobacilli has been discussed by Kelly (1982). Little information is available on the microbiological oxidation of polythionates other than $S_4O_6^{2-}$.

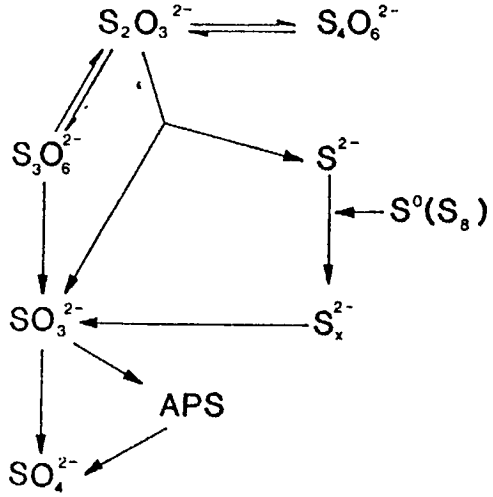

Fig. 2. A schematic, non-stoichiometric presentation of the microbiological oxidation of inorganic sulphur compounds. Several enzymatic reactions need to be characterized, especially those associated with the oxidation of sulphide, polysulphide, and elemental sulphur. Several other pathways have been presented in the literature (Kelly 1982). APS = Adenosine 5'-phosphosulphate.

The role of thiobacilli in producing sulphur oxyanions as metastable intermediates has not been associated with aerobic corrosion problems of microbiological origin. These intermediates do not persist in oxidative, bacteria-containing environments. However, some intermediates such as $S_4O_6^{2-}$ may accumulate until the substrate is virtually completely oxidized, but the ensuing oxidation leads to the formation of sulphuric acid (Murphy *et al.* 1972, Tuovinen & Kelly 1974). Sulphur may accumulate as a result of $S_2O_3^{2-}$ disproportionation by thiobacilli if a stress factor is imposed on the bacteria; e.g. inadequate aeration, toxic metal ions, or excessive acidity (Tuovinen 1973, Tuovinen & Kelly 1974).

Some thiobicalli, namely *T. denitrificans* and *T. thioparus,* are able to oxidize sulphur compounds under anaerobic conditions if sufficient nitrate or nitrite is present to substitute for oxygen as an electron acceptor as illustrated in Fig. 3.

The sequential oxidative pathway of sulphur is coupled via specific enzymes and electron carriers to the reduction of nitrate as shown in Fig. 4.

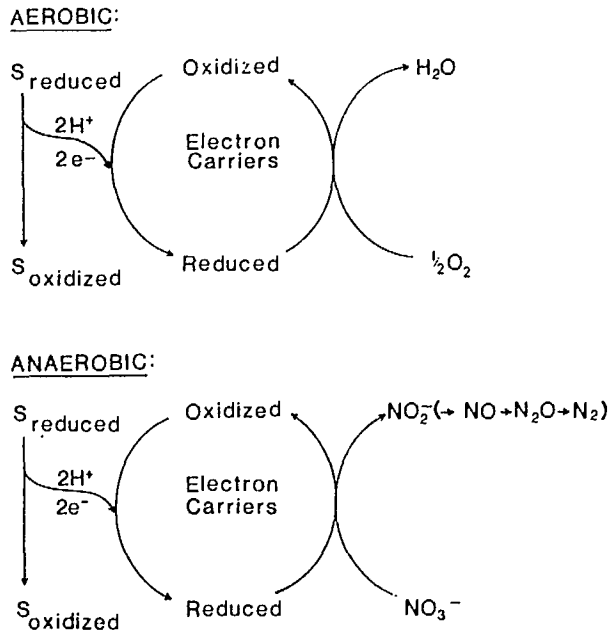

Fig. 3. Acrobic and anaerobic oxidation of sulphur compounds by thiobacilli.

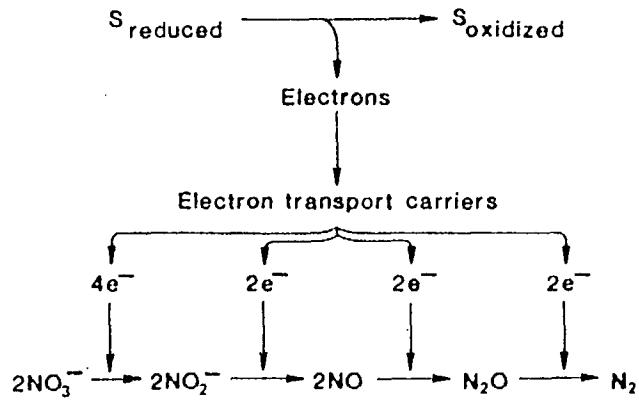

Fig. 4. Microbiological oxidation of sulphur compounds coupled to denitrification.

In biological systems electrons have a higher affinity towards oxygen as compared with other electron acceptors (e.g., NO_3^-, SO_4^{2-}) and therefore denitrification usually occurs only in the absence of O_2. This has been demonstrated by several techniques, including a gradual transition from aerobic to anaerobic conditions: the concentration of nitrate did not decrease during thiosulphate oxidation by *T. denitrificans* until oxygen was depleted below the level of detection (Justin & Kelly 1977).

Thiobacilli comprise a rather heterogeneous group of bacteria some of which have only a few common characteristics (Kuenen & Tuovinen 1981). All thiobacilli are able to oxidize some inorganic compounds of sulphur. For example, *T. acidophilus*, an acid-tolerant bacterium, can grow with elemental sulphur as a substrate, but not with thiosulphate according to its original description (Guay & Silver 1975). Such a limited capacity to oxidize sulphur compounds has also been reported for a few other thiobacilli and may indicate either the lack of certain enzymes of the sulphur metabolism or the lack of appropriate conditions for testing the microbiological oxidizability of sulphur compounds.

The oxidation rates of sulphur by thiobacilli vary depending on the particular sulphur compound. Both freshwater and marine strains of thiobacilli have been isolated and characterized from a variety of sources. The marine strains have an obligate requirement of chloride ion (as NaCl) in the growth medium (Tilton *et al.* 1967, Adair & Gundersen 1969) and therefore, their activities are not hindered in high-chloride (0.5 M) environments. In contrast, the freshwater isolates of thiobacilli are inhibited at high chloride concentrations.

Thermophilic sulphur-oxidizing thiobacilli have been described which tolerate temperatures up to 55-60°C (Brierley *et al.* 1980). With the exception of one disputable and poorly characterized isolate, none of the thiobacilli form spores. Therefore, these bacteria are relatively more sensitive than the spore-forming sulphate-reducers to chemical disinfection agents and heat treatment.

Some thiobacilli require a low pH environment; these include *T. thiooxidans*, *T. ferrooxidans*, *T. kabobis*, and *T. acidophilus*. It is not uncommon to find these acidophilic thiobacilli associated with sulphur waste piles and acid mine drainage effluents where the biological production of sulphuric acid is difficult to curtail.

Anaerobic sulphate-reducing bacteria

Sulphate-reducing bacteria have often been detected in corrosion deposits and their catalytic effect on accelerating the corrosion of cast iron and steels has been demonstrated in many laboratory studies. These bacteria are obligately anaerobic and become inactivated upon exposure to air (oxygen). The inhibition by aerobiosis may reverse upon resumption of anaerobic conditions.

The microbiological reduction of sulphate is a respiratory activity (Thauer & Badziong 1980) in which sulphate substitutes for oxygen as the terminal electron acceptor (Fig. 5). Intermediates of the microbiological sulphate reduction are indicated in Fig. 6. All intermediates of the reductive pathway are metastable and susceptible to microbiological oxidation under suitable conditions.

Both thiosulphate and tetrathionate can be detected in transient concentrations in culture solutions. The enzymes mediating the intermediate reactions vary in different sulphate-reducing bacteria. Work published during recent years indicates that sulphate reducers represent a heterogeneous bacterial group capable of using many different acids and

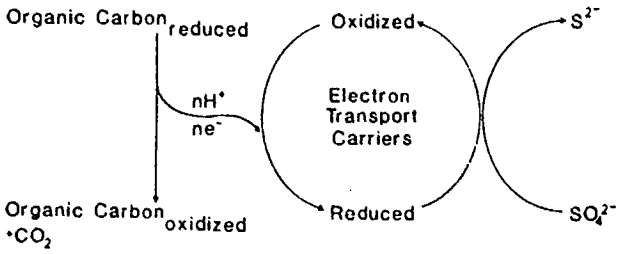

Fig. 5. Organic carbon as an electron donor for the microbiological reduction of sulphate.

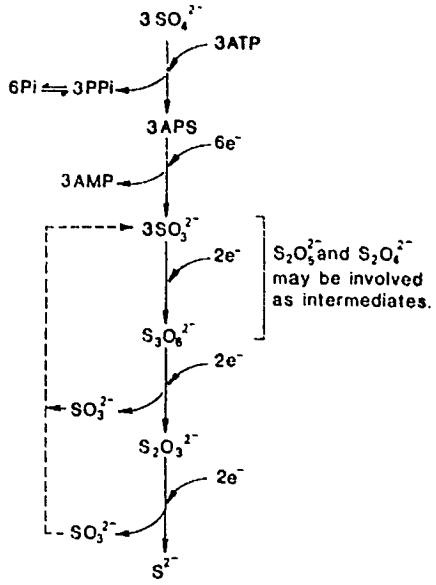

Fig. 6. Pathway of the microbiological reduction of sulphate (modified from Thauer & Badziong 1980). APS = Adenosine 5'-phosphosulphate.

sugars as substrates (Postgate 1979). New descriptions include *Desulfobacter* (Widdel & Pfennig 1981), *Desulfomonas* (Moore *et al.* 1976), and *Desulfurococcus* (Zillig *et al.* 1982) whose carbon metabolism seems to be distinctly different from that of *Desulfovibrio* and the spore-forming *Desulfotomaculum*. The suitability of organic compounds for supporting growth of sulphate reducers is an important consideration since these bacteria are not able to satisfy their carbon requirement by the fixation of carbon dioxide. In marine systems, for example, it is the concentration of organic substrate rather than that of sulphate that may be a limiting factor for their development.

Elemental sulphur is not an intermediate in the biological reduction of sulphate to sulphide. Colloidal sulphur and elemental sulphur (S_8) can both be reduced by some species (Pfennig & Biebl 1976, Biebl & Pfennig 1977, Fauque *et al.* 1979) including sulphur-respiring anaerobic archaebacteria (Zillig *et al.* 1983a, b), but this activity has not been well characterized.

Both freshwater and marine species have been described in the literature (Postgate 1979). Thermophilic sulphate and sulphur reducers have also been isolated from thermally influenced environments (e.g. hot springs, pipe lines, sewage digestors).

Biological sulphate reduction can also be coupled to the oxidation of H_2:

$$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O$$

The role of H_2 as a reductant for sulphate reduction has evoked interesting ideas about the significance of the hydrogen oxidation system in the surface events of microbiological corrosion. Hydrogenase, the enzyme mediating the electron transfer, is found in some sulphate-reducing micro-organisms (e.g., *Desulfovibrio)*. The reaction can be expressed with the following equations:

Hydrogenase:

$$H_2 = 2H^+ + 2e^-$$

Hydrogenase coupled with the reduction of cytochrome c_3:

$$H_2 + \text{cytochrome } c_3 \text{ (oxid)} \rightarrow \text{cytochrome } c_3 \text{ (red)} + 2H^+$$

Many sulphate-reducing bacteria appear to have at least two hydrogenase enzymes, one cytoplasmic and the other located in the periplasmic space of the cell wall (Le Gall *et al.* 1982). The presence of hydrogenase in sulphate-reducers has lent support to the cathodic depolarization hypothesis which will be discussed in a later section of this paper.

None of the sulphate-reducers are known to tolerate low pH values (pH < 3-4) for prolonged periods. It would not be surprising, however, to detect these bacteria in low pH environments because corrosion deposits and other precipitates may exhibit pH gradients, thereby rendering the interior more suitable for sulphate-reducers as opposed to the low pH of the bulk solution.

In laboratory cultures the redox potential needs to be lowered to < -100 mV_{SHE} before the sulphate-reducers are able to grow. Ascorbic acid, sulphide, and thioglycollate can be used to poise the redox potential. In corrosion deposits the interior redox potential is typically low and provides the reducing environment for these bacteria, even if the bulk solution is oxygenated as is the case for tubercles in water distribution systems (Tuovinen *et al.* 1980, Tuovinen & Hsu 1982).

Effect of Sulphur Compounds on Localized Corrosion

It is well known that some sulphur compounds, such as H_2S and SO_2, as well as their respective anions enhance the dissolution rate of iron and steels in acidic solutions. Iofa (1970, 1980) suggested that the catalytic effect of sulphide ions on the anodic reaction can be explained by the formation of intermediate adsorbed sulphide species as opposed to hydroxide species as follows:

$$Fe + HS^- = Fe(HS^-)_{ads}$$

$$Fe(HS^-)_{ads} \rightarrow Fe(HS)_{ads} + e^-$$

$$Fe(HS)_{ads} \rightarrow FeHS^+ + e^-$$

$$FeHS^+ + H_3O^+ \rightarrow Fe^{2+} + H_2S + H_2O$$

The acceleration of the cathodic reaction in the presence of H_2S has been explained by the following equations (Iofa 1970, 1980):

$$Fe + HS^- = Fe(HS^-)_{ads}$$

$$Fe(HS^-)_{ads} + H_3O^+ \rightarrow Fe(H\text{-}S\text{-}H)_{ads} + H_2O$$

$$Fe(H\text{-}S\text{-}H)_{ads} + e^- \rightarrow Fe(HS^-)_{ads} + H_{ads}$$

in which the catalytic active species $Fe(H\text{-}S\text{-}H)_{ads}$ is readily reduced to adsorbed H atoms. More detailed discussions on the effect of sulphide concentration, pH and other environmental variables on the general corrosion of iron and carbon steel have been presented (Süry 1976, Foroulis 1980). The anodic behaviour in alkaline sulphide solutions was investigated by Shoesmith *et al.* (1978a,b), taking into consideration the nucleation and growth of iron sulphides (i.e. mackinawite) accompanied by the formation of sulphur, polysulphides and eventually thiosulphate. More recently the anodic behaviour has been interpreted (Salvarezza *et al.* 1982) in terms of competitive adsorption of H_2O, OH^-, and HS^-. Processes like the formation of mackinawite, development of pitting, or passivation of the metal surface by an oxide layer are suggested to depend on the HS^-/OH^- concentration ratio.

Even in the case of more corrosion resistant alloys, such as stainless steels, the anodic dissolution rate in acidic solutions can be enhanced by several orders of magnitude in the presence of H_2S or SO_2, as reported by several authors (Herbsleb & Schwenk 1966, Greene & Wilde 1970, Crolet *et al.* 1976, Masuo *et al.* 1978). Relatively low concentrations of either species have a pronounced effect on the anodic behaviour, shifting the corrosion potential to more negative values and the passivation potential to more positive values, thereby enlarging the active range as illustrated in Fig. 7 for the case of SO_2 additions. While the effect of sulphur compounds on uniform corrosion of steels and stainless steels has been extensively studied due to their widespread applications in the chemical and petrochemical industries, the role of metastable sulphur compounds in promoting or accelerating localized corrosion processes has not been properly recognized in many circumstances. Therefore, we will briefly review the influence of a variety of sulphur species on three types of localized corrosion phenomena: pitting, crevice corrosion and stress corrosion cracking which are usually identified as the main cause of failure in the use of steels, particularly stainless steels, and other corrosion resistant alloys in industrial installations. At the same time the advantages of using electrochemical techniques for studying localized corrosion processes will be emphasized.

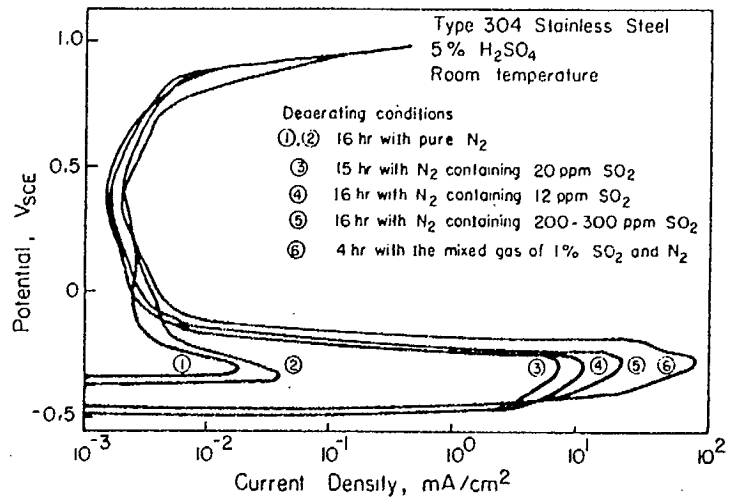

Fig. 7. Anodic polarization curves of Type 304 stainless steel in 5% H_2SO_4 solution showing the influence of SO_2 addition to nitrogen gas used for deaeration (curve 2 was obtained after a cathodic pretreatment for 10 min. at -0.7 V_{SCE}) (Masuo *et al.* 1978). With permission of the Japan Society of Corrosion Engineers.

Pitting corrosion

It is commonly accepted that for a variety of metal/solution systems, in which the metal surface is covered by a protective passive layer, pitting occurs only above a certain critical potential, which can be measured by anodic polarization of the metal or alloy in the test environment. Using this technique as well as other electrochemical methods (Pessall & Liu 1971, Smialowska & Czachor 1974) pitting corrosion of stainless steels in chloride solutions has been extensively investigated (Smialowska 1974, Hisamatsu 1976, Okamoto & Shibata 1978). The pitting potential decreases with increasing chloride concentration. Different anions, e.g., OH^-, NO_3^-, ClO_4^-, and SO_4^{2-}, inhibit pitting as evaluated from the increasing values of the pitting potentials in the presence of increasing concentrations of the inhibiting anions.

Except for sulphate no studies were available until recently on the effect of sulphur compounds on the pitting potential of austenitic stainless steels. The effect of sulphate should be clearly distinguished from that of other sulphur species. Leckie & Uhlig (1966) found that sulphate concentrations ranging from 0·0125 to 0·15 M monotonically increased the pitting potential of Type 304 stainless steel in 0·1 M NaCl. Competitive electromigration of SO_4^{2-} (passivating anion) with respect to Cl^- (aggressive anion) was suggested as a reasonable explanation for that inhibiting effect (Galvele 1976). On the other hand, Newman *et al.* (1982a) demonstrated that sulphur species such as H_2S, $S_2O_3^{2-}$, $S_4O_6^{2-}$ and SCN^- within certain concentrations ranges decrease the pitting potential of Type 304 stainless steel in neutral or slightly acidic solutions containing 0·25 M NaCl. The behaviour is clearly illustrated in Fig. 8. It seems that additions of $S_2O_3^{2-}$ ranging from 0·01 M to 0·02 M lowered the pitting potential by more than 300 mV, while additions of more than 0·5 M inhibit pitting. SCN^- showed similar but less marked effects, while increasing Na_2S additions up to 0.1 M (present as H_2S and HS^- at neutral pH) caused a monotonic decrease in the pitting potential. The effect of $S_4O_6^{2-}$ is similar to that of $S_2O_3^{2-}$, but the minimum value of the pitting potential is displaced to higher concentrations. Newman *et al.* (1982a) noted that pits formed in the presence of sulphide, thiosulphate or tetrathionate contained metal sulphide as a black deposit on the pit bottom. Since thiosulphate alone did not promote pitting, Newman *et al.* (1982a) suggested that the main role of the active sulphur species is to impede the repassivation of the bare metal surface following chloride induced film breakdown, thereby enhancing the dissolution via the presence of adsorbed sulphide or sulphur.

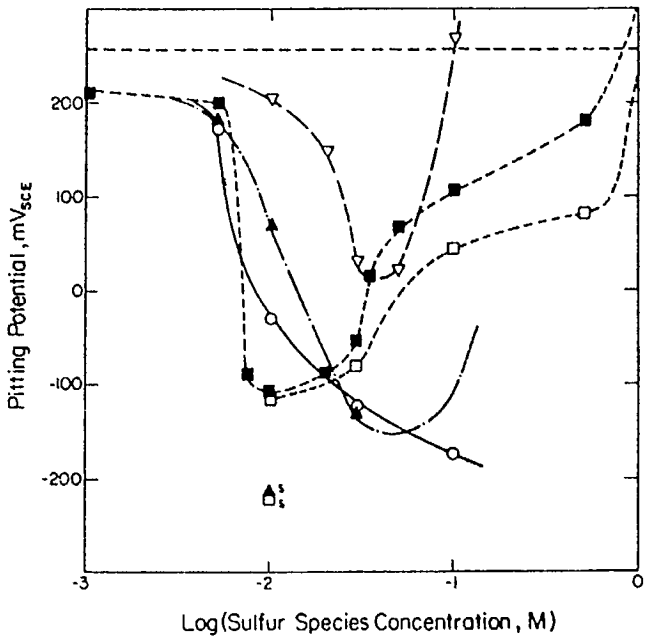

Fig. 8. Pitting potential data for Type 304 stainless steel in 0·25 M NaCl with additions of sulphur compounds. The dotted line at 260 mV represents the pitting potential with no additions. Pitting potentials are shown for $Na_2S_2O_3$ (■) and $Na_2S_4O_6$ (▲) with no pH adjustment and for the following with 5·05 < pH < 6·5: $Na_2S_2O_3$ (□), KSCN (▽), H_2 (○). Pitting potentials indicated by "s" were measured by using a scratching technique (Newman *et al.* 1982a). With permission of the National Association of Corrosion Engineers.

Newman *et al.* (1982a) also noted that the addition of 0·01 M Na_2SO_3 to the chloride solution had no effect on the pitting potential. This result agrees with previous work reported by Luffkin (1973), who did not observe a specific effect of sulphite at pH values of 8 to 10. He carried out potentiodynamic polarization tests in 0·017 M NaCl solutions containing additions of Na_2SO_3 ranging from 0 to 0·32 M and claimed that the anodic behaviour was dominated by the oxidation of sulphite to sulphate. However, at more acidic pH values, such as those obtained by adding various amounts of aqueous SO_2 (30 l SO_2/l H_2O) to 0·017 M NaCl, a decrease of about 100 mV in the pitting potential was observed over the range of concentrations studied (Luffkin 1973), as shown in Fig. 9. The pitting potentials for Type 316 and Type 430 stainless steels are included in Fig. 9 and they indicate that the addition of SO_2 has no effect on the pitting potential of Type 316 whereas the pitting potential of Type 430 is significantly affected. The pitting potential values

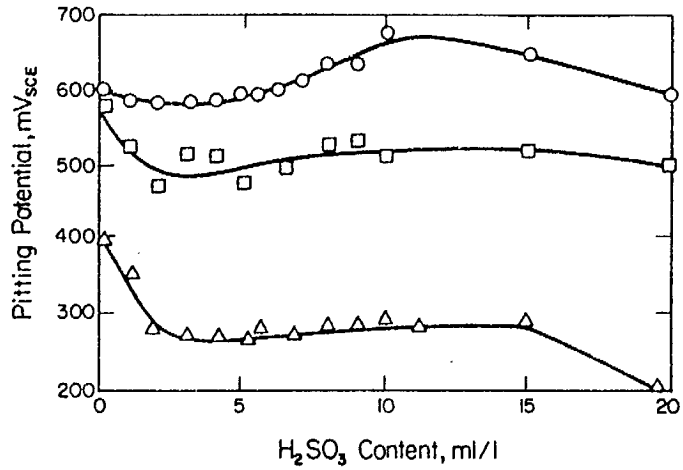

Fig. 9. Pitting potentials of Types 316 (○), 304 (□), and 430 (△) stainless steels in 0·017 M NaCl solution containing increasing concentrations (0 to 20 ml/l) of a sulphurous solution containing 30 l SO_2/l H_2O (Luffkin 1973). With permission of the Anti-Corrosion Methods and Materials.

given in Fig. 9 cannot be directly compared with those provided by Newman *et al.* (1982a) because different potential scanning rates were used in both studies. It is known that pitting potentials for stainless steels are extremely dependent on the potential scanning rate employed for anodic polarization. Luffkin (1973) observed that the enhanced effect of sulphur compounds on the pitting corrosion in chloride solutions was not confined to SO_2-containing solutions. A decrease in the pitting potential was found in solutions containing either $Na_2S_2O_3$, $Na_2S_4O_6$, or $Na_2S_2O_5$. Luffkin (1973) suggested that the reduction of SO_2 or HSO_3^- leads to the formation of metastable species, such as $S_2O_4^{2-}$, $S_2O_3^{2-}$ or even S and S^{2-} that may be responsible for the decrease in the pitting potential. A significant decrease in the pitting potential of Type 304 stainless steel in acidified (pH < 3) 3·5% NaCl solutions was also observed by Masuo *et al.* (1978) when Na_2S (7·3 ppm) was added to the solution. A similar effect was noted when nitrogen gas contaminated with up to 1% SO_2 was bubbled into the solution. The lowering of the pitting potential was attributed (Masuo *et al.* 1978) to the specific action of H_2S which was formed by reduction of SO_2 combined with the decrease in pH resulting from the addition of SO_2.

Herbsleb (1982) studied the effect of H_2S and SO_2 on the pitting potential for a series of Cr-Ni and Cr-Ni-Mo stainless steels exposed to 1 M NaCl solution. These results are summarized in Fig. 10, where the pitting potentials in N_2-deaerated chloride solutions are compared with those measured in chloride solutions saturated with either SO_2 (pH 0·7) or H_2S (pH 3·96). Pitting potentials are plotted as a function of an efficacy factor W(%) = Cr(%) + 3·3 (Mo(%) in order to rank the pitting resistance of the various alloys. For all alloys studied, H_2S induced a larger decrease of the pitting potential than SO_2, but both sulphur species were conducive to pitting corrosion at lower potentials than those for plain chloride solutions. The favourable effect of increasing Mo content in plain chloride solutions was significantly attenuated in the presence of H_2S or SO_2. Only the alloy with the highest Mo content showed a pitting potential in SO_2-containing solutions higher than that in plain chloride solution (Fig. 10). Hersleb (1982) discussed redox and

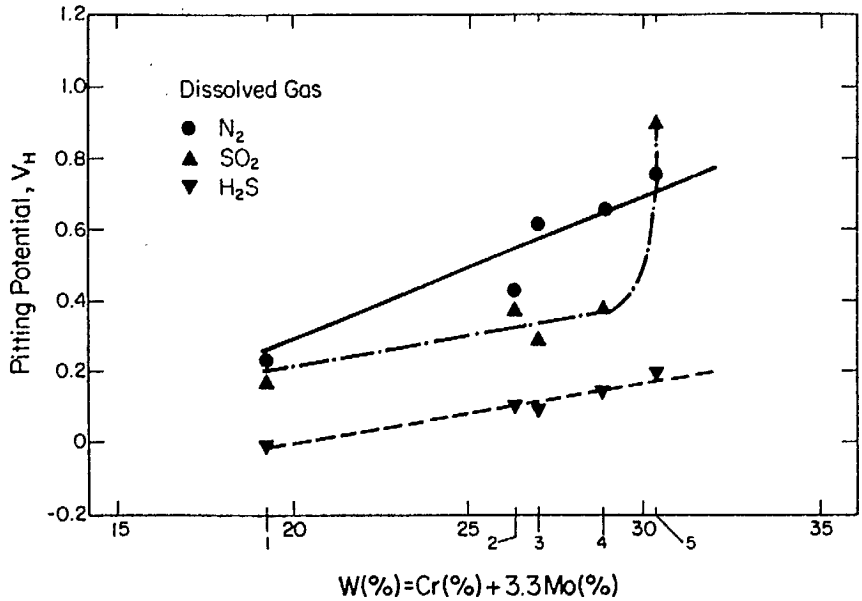

Fig. 10. Effect of dissolved SO_2 and H_2S on the pitting potential of austenitic CrNi and CrNiMo stainless steels in 1 M NaCl solution. 1-Type 304; 2-Type 316; 3-Type 316L; 4-Type 317; 5-Type 317L (Herbsleb 1982). With permission of Werkstoffe und Korrosion.

disproportionation reactions of SO_2 and its anions in aqueous solutions, but he concluded that it is difficult to identify the specific species responsible for the stimulation of pitting.

Except for SO_4^{2-}, most of the metastable sulphur oxyanions, as well as H_2S and its related anions (HS^-), decrease the pitting potential of stainless steel in chloride solutions. The effect seems to be due to a delayed repassivation of pit initiation sites in the presence of adsorbed hydrosulphide species formed by the reduction of sulphur oxyanions.

Crevice corrosion

Pitting and crevice corrosion are considered (Rozenfeld 1974, Sedriks 1979, Ijsseling 1980) closely related phenomena, having the same propagation mechanism particularly in the case of stainless steel in chloride solutions. The main difference between these corrosion processes is exhibited during the initiation stage. Crevice corrosion occurs within crevices or other shielded areas where a stagnant solution is present, whereas pitting takes place on smooth metal surfaces. A wide variety of macro-organisms (*i.e.*. barnacles, algae) can lead to the formation of crevices as a consequence of marine fouling. Micro-organisms that grow in a coherent colony or mass of 'slime' on damp or immersed metal usually lead to the establishment of occluded cells.

Crevice corrosion has been extensively studied on corrosion resistant alloys exposed to chloride solutions. However, the effect of sulphur species has not been investigated, even though sulphur compounds are prevalent in polluted sea water. Electrochemical techniques (Rozenfeld 1974, Ijsseling 1980) have lead to considerable progress in the understanding of the mechanisms of crevice corrosion. Differential aeration seems to be the initial stage in the development of crevice corrosion but a sustained attack is only possible as a consequence of local acidification produced by hydrolysis of metal cations within the crevice. Coupled to this process a local build-up of chloride anions is required for maintaining electroneutrality, but the generation of an environment rich in chloride and metal ions induces a further decrease in the pH leading to enhanced anodic dissolution.

The propagation of the attack within the crevice area can be in the form of general corrosion or localized pitting. Oldfield & Sutton (1978a,b) presented a detailed mathematical model, in which the various stages of crevice corrosion are simulated, taking into consideration several factors involved. Four stages were distinguished, namely: (a) deoxygenation, (b) increase of salt and hydrogen ion concentration, (c) depassivation, and (d) propagation. Based on the pitting studies summarized in the previous section, it seems that the presence of metastable sulphur oxyanions or sulphide may decrease the incubation time associated to the generation of an aggressive solution within the crevice responsible of the depassivation stage.

Stress corrosion cracking

Stress corrosion cracking (SCC) is an insidious form of metal failure because its occurrence is difficult to predict. The presence of a tensile stress (applied and/or residual) and the existence of a susceptible metallurgical microstructure, coupled with the simultaneous action of a specific environment are the requirements for the occurrence of SCC. Although the environmental requirements are highly specific, the list of environments identified as causing cracking for a given alloy continues to grow with time. Apart from the well known effect of H_2S on causing hydrogen embrittlement of high strength steels, a variety of sulphur species are able to induce intergranular stress corrosion cracking (IGSCC) of sensitized austenitic stainless steels at ambient temperature (Cragnolino & Macdonald 1982). In the early 1950s intergranular cracking of catalytic reformers used in the petroleum industry was observed. Dravnieks & Samans (1957) demonstrated that polythionic acids, formed by the reaction of oxygen and water with an iron sulphide scale, were the species responsible for cracking. Since then "polythionic acid cracking" of sensitized austenitic stainless steel has been extensively studied. The subject has been reviewed recently (Cragnolino & Macdonald 1982), covering the effect of pH, solution composition, potential, sensitization, and alloy composition, as well as the influence of stress on the cracking behaviour. The following reaction has been proposed (Brophy 1974) to account for the interaction between iron sulphide and aerated water:

$$8FeS + 11O_2 + 2H_2O \rightarrow 4Fe_2O_3 + 2H_2S_4O^6$$

in which, for simplicity, only the formation of tetrathionic acid is indicated. However, it was found recently (Horowitz 1983) that the oxidation of FeS in oxygenated water should be expressed by the equation:

$$FeS + 3/4O_2 \rightarrow 1/2Fe_2O_3 + S$$

in accordance to the yields for the various reaction products given in Table 2. In addition, tetrathionate and thiosulphate were detected polarographically as soluble reaction products. The remaining sulphur species was considered to be sulphate, which is polarographically inactive.

Intergranular cracking has also been observed at room temperature in water saturated with SO_2 (Pichl 1964), H_2S (Ryabchenkov & Nikiforova 1962, Heller & Prescott 1965), in aqueous solutions of $Na_2S_2O_3$ (Isaacs *et al.* 1982, Dhawale *et al.* 1982, Newman *et al.* 1982c), and KSCN (Isaacs 1980). As an example, Fig. 11 shows the elongation to failure as a measure of SCC susceptibility, plotted against the sulphur concentration for sensitized Type 304 stainless steel tested under slow straining conditions in air saturated 0·21 M boric acid solution containing various concentrations of either $Na_2S_2O_3$ or $Na_2S_4O_6$. The data show that a threshold concentration exists for both sulphur oxyanions, below

Table 2. *Products of* FeS-O_2 *reaction* (Horowitz 1983)[a].

Substance	Concentration (mM)	% Yield
Fe (in Fe_2O_3)	In solid	89·1
Elemental S	In solid	81·1
Soluble Fe[b,c]	16·7	10·1
S_2O^{2-} [c]	0·6	0·7
S_4O^{2-} [c]	0·9	2·1
S[c]	1·4	0·8

[a] 5 moles FeS suspended in 30 cc water
[b] 38% Ferric ion, 62% Ferrous
[c] Identified and roughly estimated by differential pulse polarography

which IGSCC was not observed. The threshold concentration is an order of magnitude lower in thiosulphate than in tetrathionate solutions, but it is also evident that very low concentrations of either anion were sufficient to induce severe IGSCC. The effect of potential on the cracking susceptibility in thiosulphate-containing solution is depicted in Fig. 12. The potential range of maximum susceptibility corresponds to the corrosion potential measured in air saturated solutions.

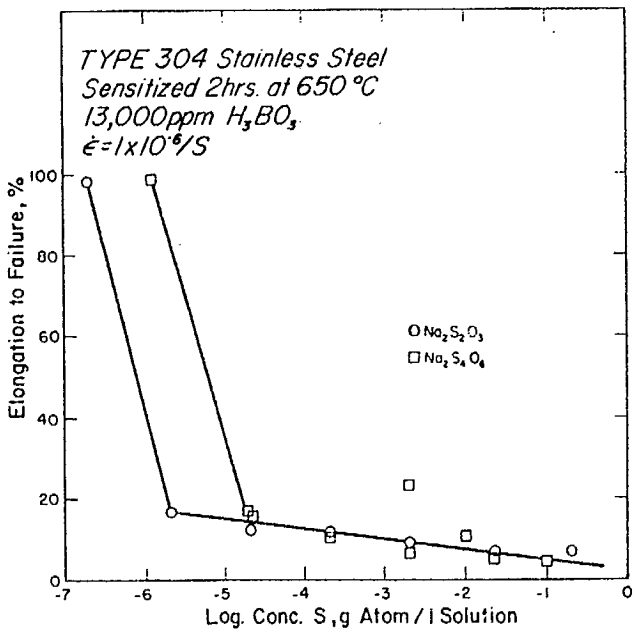

Fig. 11. Elongation to failure vs. concentration of sulphur for sensitized Type 304 stainless steel in air saturated boric acid solution containing $S_2O_3^{2-}$ and $S_4O_6^{2-}$ at room temperature (Dhawale *et al.* 1982). With permission of the Electric Power Research Institute.

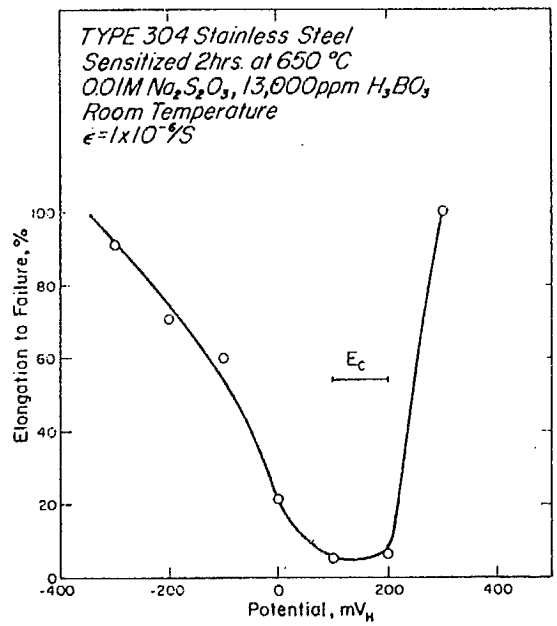

Fig. 12. Elongation to failure vs. potential curve for sensitized Type 304 stainless steel in deaerated boric acid solution containing 0·01 M $Na_2S_2O_3$ at room temperature. The corrosion potential range attained in air saturated solutions is also indicated (Dhawale *et al.* 1982). With permission of the Electric Power Research Institute.

Sulphate is not able to induce IGSCC (Cragnolino & Macdonald 1982), whereas the interaction of SO_2 or H_2S with oxygenated water in the presence of suspended iron sulphide or an iron base surface covered with a sulphide layer leads to the formation of tetrathionic acid (Ahmad *et al.* 1981).

A detailed discussion of the cracking mechanism is beyond the scope of this review. However, it should be noted that some authors (Cragnolino & Macdonald 1982, Dhawale *et al.* 1982, Newman *et al.* 1982c) have claimed that the main role of the metastable sulphur oxyanions is to release atomic sulphur by a disproportionation reaction to the acidified crack tip, thereby enhancing anodic dissolution of the chromium depleted grain boundaries and therefore the propagation of intergranular cracks according to the film-rupture mechanism for SCC. The thermodynamic basis of this interpretation can be visualized on the composite potential-pH diagram shown in Fig. 13. In this diagram only the metastable oxyanions of sulphur are included with the stability field for FeS. Potential ranges for severe IGSCC in polythionic and thiosulphate solutions are included on the basis that there is no essential distinction between either types of environments aside from pH effects. The correlation with the stability domain for Fe^{2+} + S is apparent and therefore, the formation of atomic sulphur may be important in promoting intergranular cracking. Chemiadsorbed sulphur enhances significantly the rate of active dissolution of Fe and Ni in acidified solutions (Lacombe 1962, Oudar & Marcus 1979), inhibiting the passivation for sulphur coverages slightly lower than a complete monolayer. Even wet

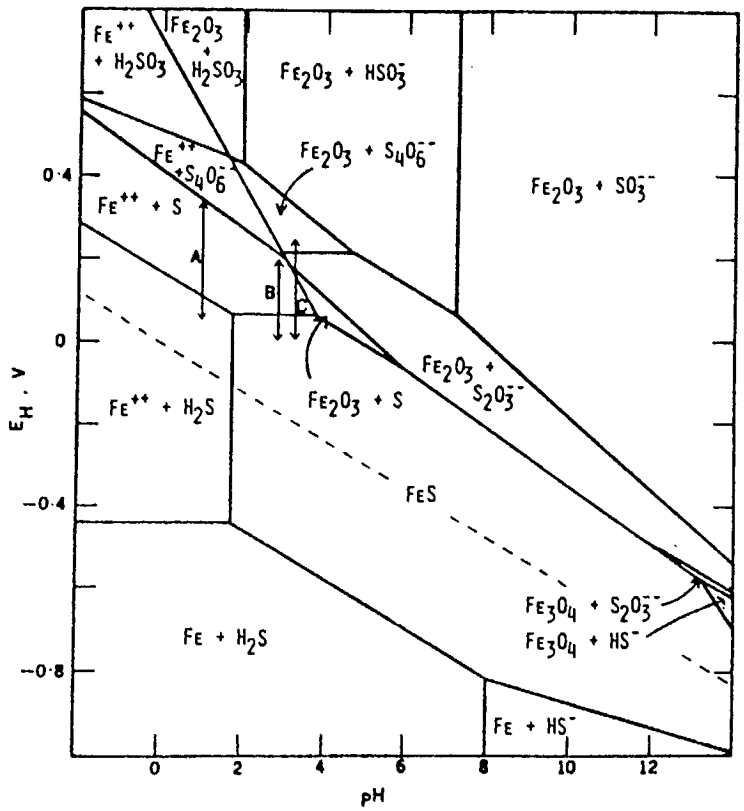

Fig. 13. Potential-pH diagram for Fe-S-H_2O at 25°C (excluding SO_4^{2-}). Severe SCC ranges obtained by different authors are indicated by arrows (Newman *et al.* 1982c). With permission of the Metallurgical Transactions.

elemental sulphur produces a significant increase in the corrosion rate of iron and mild steel (Farrer & Wormwell 1953, Macdonald *et al.* 1978).

The above data indicate that metastable sulphur oxyanions may induce severe IGSCC on sensitized stainless steel, even at very low concentrations and in almost neutral solutions. The effect is by no means confined to stainless steels, since nickel base alloys such as Incoloy 800 and Inconel 600, heat treated under conditions leading to carbide precipitation and concurrent chromium depletion, are also extremely susceptible to IGSCC in tetrathionate and thiosulphate solution (Scarberry *et al.* 1976, Cowan & Gordon 1978, Lee *et al.* 1981, Newman *et al.* 1982b). With the exception of sulphate, which is electrochemically inert even at very low potentials, almost all metastable sulphur oxyanions or compounds are able to induce cracking under appropriate conditions. However, the complexity of the sulphur compound chemistry makes difficult to establish unequivocally the nature of the specific species responsible for cracking.

General remarks

From the previous discussion it is apparent that a variety of sulphur compounds are able to accelerate or promote localized corrosin of corrosion resistant materials (stainless steels and nickel base alloys) in chloride- and non-chloride containing environments. The role of these compounds seems to be associated with their ability, in many cases via the formation of other sulphur species by redox or disproportionation reactions, to delay repassivation of bare metal surfaces in competition with oxygen-containing passivating species. In the case of pitting corrosion it seems that their effect can be exercised only in the presence of an aggressive anion such as chloride, which is able to induce film breakdown. Although there are no data to ascertain this hypothesis, a similar consideration may be valid for crevice corrosion. On the other hand, the presence of chloride anions is not required under sustained stress, indicating that the localized mechanical breakdown of a protective film is the unique requirement for the initiation of intergranular cracks in materials possessing a pre-existing active path (chromium depletion along grain boundaries). In the case of transgranular cracking of quench-annealed austenitic stainless steels in chloride-containing environments, the possible synergistic effect of sulphur compounds should be investigated.

Effect of Sulphate-Reducing and Sulphur-Oxidizing Bacteria on Localized Corrosion

The effects of sulphate-reducing and sulphur-oxidizing bacteria on the corrosion of ferrous materials have been studied extensively. Very useful reviews have been published during the last decade (Miller & Tiller 1970, Booth 1971, Miller

1981, Iverson 1972, 1974, 1981). However, most of the available information as well as the discussion of the mechanisms involved are confined to the corrosion behaviour of cast iron and carbon steels. Historically, these materials were used in buried pipeline constructions under conditions promoting the growth of the aforementioned bacteria. With the development of the chemical, petrochemical, and pulp and paper industries more corrosion resistant-alloys were required. As a consequence many failures have been reported in the literature in which sulphate-reducing bacteria were implicated in the localized corrosion of stainless steels and nickel base alloys. Several examples can be cited. Kobrin (1976) reported intense microbial activity, including sulphate reduction, associated with extensive pitting of nickel and nickel-base alloys such as Monel 400 and Hastelloy B in heat exchangers cooled by river water. Tatnall (1981a) described severe crevice corrosion of Type 304 stainless steel in and around gasketed joints in a cooling tower system which was fed with river water; the effluent contained a high concentration of chloride and microorganisms such as iron bacteria and sulphate-reducing bacteria (*Desulfovibrio*). Crevice corrosion was always observed in the presence of bulky deposits. Another case, in which Type 304 stainless steel was involved (Tatnall 1981a), revealed the development of deep pits under voluminous, mound-like deposits on an air distribution pipe located inside a waste water treatment tank and covered by sludge in which high counts of sulphate-reducing and iron bacteria were determined. A pump propeller and a screen, both made of Type 304 stainless steel, showed severe localized corrosion induced by bacteria in the clarifier of a paper mill closed water system carrying "white water" (Tatnall 1981a). The presence of *Desulfovibrio* and *Desulfotomaculum* was suspected under slime deposits. Tatnall (1981a) also described two cases of pitting of galvanized steel in a cooling tower basin. High counts of both aerobic sulphur-oxidizing thiobacilli and anaerobic sulphate-reducers were observed.

In a review of corrosion problems in the pulp and paper industry, Chakrapani & Czyzewski (1978) discussed the occurrence of localized corrosion in the form of pits under slime and fibrous deposits on Type 304 and Type 316 stainless steels used in the fabrication of head boxes employed in the paper making stages. They attributed the damage to sulphate-reducing bacteria, present in an environment of pH 4·9 containing 140 ppm SO_4^{2-} and 8 ppm Cl^-, because energy dispersive X-ray analysis of the deposits found inside pits revealed the presence of metallic sulphides. Charlton (1978) has also shown the occurrence of pitting on Type 316 stainless steel used as liner in a paper machine head box, attributing to sulphate-reducing bacteria the localized corrosion, but no other details were provided. Soimajärvi *et al.* (1978) have conclusively proved the presence of sulphate-reducing bacteria belonging to the genus *Desulfovibrio* in paper machine waters and in plugged perforations of a suction roll used in the paper-making stage.

Thus, there is no doubt that sulphate-reducers are present in many circumstances leading to localized corrosion in diverse industrial installations. Experimental observations have focused particularly on sulphate-reducing micro-organisms; however, in many failure cases extremely heterogeneous microbial populations, including both aerobes and anaerobes, are likely to be present but rarely determined. It should also be noted that the mere presence of sulphate-reducing bacteria, determined mostly by enumeration of viable cells, is not a sufficient demonstration of the causative relationship with respect to the corrosion problem. An environment may support relatively high numbers of sulphate-reducers but their *in-situ* activity, which could be expressed by sulphate reduction, sulphide production, hydrogen uptake, or organic carbon utilization as a function of time, has never been determined in field studies of corrosion problems. Laboratory studies provide little insight into this relationship because they employ high nutrient concentrations under non-steady conditions. In the field, sulphate-reducers are influenced by numerous factors, including competition for nutrients and synergistic interactions, and thus their enumeration cannot be used as a reliable measure of their activity.

Except for the case of buried pipelines made of cast-iron and carbon steels (Miller 1981), very little is known about corrosion problems of other alloys attributable to thiobacilli. Since sulphuric acid is the main product of the activity of these bacteria, it seems that more acid-resistant alloys, such as stainless steels, are not so adversely affected. However, since these bacteria are able to form metastable sulphur species in both aerobic and anaerobic environments, their role in localized corrosion phenomena deserves to be further investigated. A recent study with *T. thiooxidans* indicates that, in addition to sulphuric acid formation, other metabolic products (which were not identified) may have an accelerating effect on the corrosion of a low alloy steel (Baru *et al.* 1982).

Sulphate-reducing bacteria: proposed mechanisms

The role of sulphate-reducing bacteria in inducing or accelerating electrochemical corrosion processes has been interpreted on the basis of different mechanisms, which can be conveniently classified as follows:

(1) Stimulation of the cathodic reaction in the absence of oxygen by sulphate-reducing bacteria, either directly by removal of atomic hydrogen or indirectly by the formation of iron sulphides or hydrogen sulphide.

(2) Acceleration of the anodic reaction by the action of sulphide ions or other sulphur species produced by the sulphate-reducing bacteria.

(3) A combination of both effects.

Von Wolzogen Kühr & van der Vlugt (1934) provided an explanation for the underground corrosion of cast iron by the sulphate-reducing bacteria in electrochemical terms according to the so-called cathodic depolarization theory. They proposed that the bacteria could remove hydrogen from a cathodic area on the iron surface by the hydrogenase enzyme coupled to the reduction of sulphate to sulphide. This causes the depolarization of iron, thereby enhancing its

dissolution. The overall mechanism has been usually described as follows:

Anodic reaction $4Fe \rightarrow 4Fe^{2+} + 8e^-$

Dissociation of water $8H_2O = 8H^+ + 8OH^-$

Cathodic reaction $8H^+ + 8e^- \rightarrow 8H$

Cathodic depolarization by bacteria $SO_4^{2-} + 8H \rightarrow S^{2-} + 4H_2O$

Corrosion products formation $Fe^{2+} + S^{2-} \rightarrow FeS$

$3Fe^{2+} + 6OH^- \rightarrow 3Fe(OH)_2$

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

The cathodic depolarization step was based on the findings of Stephenson & Stickland (1931) who first suggested the biological activation of H and termed the enzyme hydrogenase.

It should be noted, however, that the charge transfer step for the cathodic reaction leads to the formation of adsorbed hydrogen atoms:

$$M + H^+ + e^- \rightarrow M\text{-}H_{ads}$$

Therefore, it is by no means clear, as pointed out by Miller (1981), how an intact bacterium can remove adsorbed hydrogen atoms from the metal surface as distinct from the uptake of molecular hydrogen dissolved in water. Furthermore, the two alternative steps for completion of the hydrogen evolution reaction are (1) the chemical recombination

$$M\text{-}H_{ads} + M\text{-}H_{ads} \rightarrow H_2 + 2M$$

and (2) the electrochemical desorption

$$M\text{-}H_{ads} + H^+ + e^- \rightarrow H_2 + M$$

For transition metals there are conflicting views about the rate determining step (Bockris & Reddy 1973, Subramanyan 1981). In addition, the presence of SH^- modifies the path of the charge transfer reaction. It is doubtful that the bacterial uptake of H_2, as related to the hydrogenase activity, would accelerate these reactions because the desorption of molecular H_2 does not control the reaction rate.

Many years later, Horvath & Solti (1959) and Booth & Tiller (1960) used polarization curves as a measure of cathodic depolarization of iron in culture media inoculated with different species of *Desulfovibrio*. Booth & Tiller (1960) found that only *D. vulgaris*, a hydrogenase-positive species, caused a marked decrease in cathodic polarization, while *Desulfotomaculum orientis*, a hydrogenase-negative sulphate-reducer, did not affect the cathodic polarization curve. Both organisms induced the formation of a partially protective film of iron sulphide after one week exposure. At that time these results were interpreted (Horvath & Solti 1959, Booth & Tiller 1960) as a confirmation of the cathodic depolarization theory, even though the corrosion rates obtained were significantly lower than those measured in the field. Booth *et al.* (1965) found later on that the semiprotective sulphide films became detached after 20-30 weeks exposure to bacterial action and the corrosion rates increased significantly, even in the case of *Dt. orientis*. The direct correlation between corrosion and hydrogenase activity, which is one of the basic assumptions of the cathodic depolarization theory, became doubtful. Booth *et al.* (1967) also observed that the addition of high Fe^{2+} concentrations to the culture medium gave rise to very high corrosion rates, comparable to those measured in the field, because ferrous iron reacted with sulphide produced by bacterial action and thus prevented the formation of a semi-protective sulphide film. Under such conditions a completely unprotective and loose mass of corrosion products, consisting of iron sulphide and ferrous carbonate, was formed. Booth *et al.* (1968) were able to demonstrate that chemically-produced suspensions of ferrous sulphide added to 1% NaCl solutions caused considerable cathodic depolarization of mild steel. It was shown (King & Wakerley 1973, King *et al.* 1973a,b) that different iron sulphides can lead to accelerated corrosion of mild steel, indicating that the action of sulphate-reducing bacteria is exercised through the formation of fresh iron sulphides. The properties of different iron sulphides (mackinawite, pyrrhotite, greigite, marcasite, etc.) and their corrosive effects were reviewed by Smith & Miller (1975). On the basis of work conducted at the Corrosion and Protection Centre, UMIST (UK), Miller (1981) suggested that all iron sulphides are cathodic towards iron. Recognizing the fact that in bacteria-free systems they do not act as permanent cathodes, he claimed that the role of bacteria could be either to "regenerate" (or depolarize) the iron sulphide enabling it to remain cathodic, to produce "fresh" iron sulphide by their growth reaction or even to bring fresh iron sulphides surfaces constantly into contact with the steel by cell movement.

A different point of view is held by Costello (1974). He measured cathodic polarization curves of mild steel in cultures of *D. vulgaris* and two other strains of the genus *Desulfovibrio* at pH 6·5 and compared the results with those obtained in the presence of 0·01 M H_2S at the same pH. He concluded that cathodic depolarization in cultures of sulphate-reducing bacteria may be attributed to the cathodic activity of dissolved hydrogen sulphide produced by the microorganisms. The specific role of biogenic hydrogen sulphide was clearly demonstrated by Togano *et al.* (1975). The authors measured corrosion rates of mild steel as a function of time by lineal polarization methods, accompanied by simultaneous measurements of the corrosion potential, concentration and rate of formation of H_2S, and viable numbers

of sulphate-reducing bacteria. A correlation was established indicating that the corrosion rate was proportional to the instantaneous concentration of H_2S produced by the bacteria, although the influence of H_2S became complicated because of the formation of sulphide films. Togano *et al.* (1975) also claimed that the accelerating effect of the H_2S was greater on the anodic reaction than on the cathodic reaction. The same opinion was expressed in the early 1950s by Wanklyn & Spruit (1952). There is no doubt, as noted in the section devoted to the effect of sulphur compounds on a localized corrosion, that H_2S can accelerate both the anodic and cathodic reaction. The complexity of the systems associated with the growth of sulphate-reducers makes it more difficult, compared with sterile environments, to define the precise role of H_2S in the corrosion kinetics. The participation of other metastable sulphur species in the acceleration of the anodic reaction cannot be excluded. Iverson (1981, 1983) suggested, on the basis of experiments in which high corrosion rates were observed, that in addition to hydrogen sulphide sulphate-reducing bacteria produce a highly corrosive substance, possibly a soluble compound containing phosphorus, which enhances the dissolution of iron under anaerobic conditions at a neutral pH. He emphasized that enhanced corrosion can be expected only when the substance comes in contact with iron before sulphide film formation takes place. Otherwise corrosion is stifled, although the subsequent breakdown of the film could result in a further increase of the corrosion rate,

On the other hand, Schaschl (1980) showed that elemental sulphur, dissolved in the presence of sulphide ions, promotes the accelerated corrosion of mild steel in contaminated brines by a concentration cell mechanism similar to that of differential aeration. He claimed that dissolved sulphur acts as a cathodic reactant, indicating that bacteria may provide the shielding action needed to promote concentration cell action. Bates (1981) reinterpreted the action of sulphur, proposing that polysulphides (S_x^{2-}) are the cathodic reactants.

Until now, we have considered the role of the sulphate-reducing bacteria independently of the morphological development of the attack. In the case of localized corrosion, a basic requirement is the physical separation of anodic and cathodic sites. Uniform or general corrosion takes place when such physical separation does not occur. A condition frequently found for the localization of anodic sites arises from the existence of areas of the metal surface occluded by some means and hence less oxygenated than others. All types of microbes can colonize surfaces and produce a mass of "slime", thereby establishing a differential aeration cell. In such cases active growth of the micro-organisms decreases the concentration of oxygen to very low levels. Even the death of the organisms in the interior of the colony will not inhibit the development of the electrochemical cell because a physical barrier prevents the ingress of oxygen. Such anaerobic conditions are conducive to the development of sulphate-reducing bacteria. In the case of cast iron the preferential dissolution of iron leads to the well known phenomenon of graphitization. For carbon steels, the attack manifests itself in the form of pitting, but due to the relative poor protective properties of the passive film the attack tends to be shallow, but extended over a large area. In more corrosion resistant alloys covered by protective films, pits are usually small in diameter, but can be extremely deep. In this context, it is appropriate to indicate the concern (Tatnall 1981b) that the replacement of carbon steels by stainless steel may lead to even greater corrosion problems if the mechanisms of the bacterial action are not well understood. The use of appropriate electrochemical methods, coupled with a careful characterization of the type of bacteria and evaluation of the effect of their metabolic products can lead to a better understanding of the mechanisms involved.

As an example of the type of approach that seems to be fruitful in the study of localized corrosion processes, we can mention the studies of Salvarezza & Videla (1980). They studied the effect of sulphate-reducing bacteria on the anodic

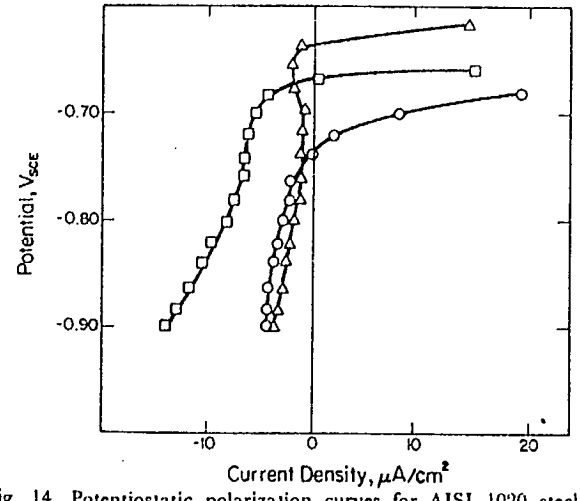

Fig. 14. Potentiostatic polarization curves for AISI 1020 steel in artificial sea water contaminated with sulphate-reducing bacteria for various incubation periods. 96 hr of incubation (total sulphides 10^{-3} M, pH 7·8, redox potential −510 mV) (○); 72 hr of incubation (total sulphides $1{\cdot}4 \times 10^{-4}$ M, pH 7·5, redox potential −500 mV) (△); 240 hr of incubation (total sulphides 8×10^{-4} M, pH 7·2, redox potential −510 mV) (□) (Salvarezza & Videla 1980). With permission of the National Association of Corrosion Engineers.

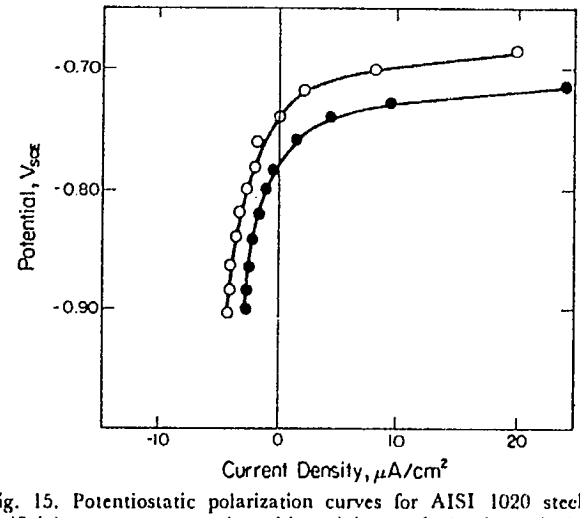

Fig. 15. Potentiostatic polarization curves for AISI 1020 steel in artificial sea water contaminated by sulphate-reducing bacteria (total sulphides 10^{-3} M, pH 7·8, redox potential −510 mV) (○) and in sea water with the addition of 10^{-3} M Na_2S (pH 8·0) (●) (Salvarezza & Videla 1980). With permission of the National Association of Corrosion Engineers.

behaviour of mild steel in deaerated artificial sea water using potentiostatic polarization methods. A significant decrease in the pitting potential (>150 mV) was found when artificial sea water was contaminated with cultures of sulphate-reducing bacteria (*Desulfovibrio*). Fig. 14 shows the effect of various incubation times on the polarization curves, revealing that the decrease in the pitting potential is associated with the bacterial formation of sulphide. This relationship is further illustrated in Fig. 15, where the anodic behaviour in the presence of bacteria is compared with that observed in a sterile environment containing an equivalent concentration of sulphide. Salvarezza & Videla (1980) observed the development of pits in the samples, but the attack seemed to be shallow and extended over large areas.

In the case of stainless steels, as mentioned in the section devoted to the effect of sulphur compounds on localized corrosion, a significant decrease in the pitting potential with respect to that in plain chloride solutions can also be expected because of the action of biogenic hydrogen sulphide or other sulphur species produced by the sulphate-reducing bacteria. The development of crevice corrosion in the presence of bacteria may be also explainable in terms of the effects exercised by their metabolic products. On the other hand, no cases of stress corrosion cracking in relation to sulphate-reducers have been reported, but indirect evidence indicates that their metabolic products may also induce cracking failures under appropriate stress conditions.

Concluding Remarks

A survey conducted in the UK (Wakerley 1979) indicated that microbiological corrosion problems are widely distributed in the industry. In many cases there is no precise documentation on the chemical or microbiological characterization of failure cases. A causative relationship has not clearly emerged from the examination of corrosion failures in the industry, but is well established in laboratory studies. In future studies it would be helpful to analyze ionic sulphur species present in the corrosive environment because there is no doubt about their aggressive role in the stimulation of corrosion. In the laboratory almost any living (and dead) micro-organisms populations can at least partially attach on metal surfaces, and by extrapolating this phenomenon to industrial failures it is not surprising that diverse micro-organisms are found in corroded specimens. In view of the ubiquity of micro-organisms in the nature at ambient temperatures, it seems that we cannot rely on qualitative descriptions of micro-organisms detected in failure cases. In future endeavours, microbial activities should be estimated together with the chemical speciation of major elements in order to better understand the dynamic character of microbiologically induced corrosion problems. In many cases, however, the presence of micro-organisms determined, for example, by enumeration of viable cells serves to indicate potential problems. Perhaps even more importantly, microbial counts indicate the efficiency of protection measures (e.g. disinfection) adopted to curtail the microbiologically mediated deterioration of installations. More intensive surveillance programmes are warranted in industries that use materials susceptible to corrosion. It is obvious that better predictions and effective counter-measures can be offered once the microbiological and chemical conditions and interactions are better elucidated.

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References

Adair, F. W.; Gundersen, K. (1969) Chemoautotrophic sulphur bacteria in the marine environment. I. Isolation, cultivation and distribution. *Canadian Journal of Microbiology*. **15**, 345-353.

Ahmad, S.; Mehta, M. L.; Saraf, S. K.; Saraswat, I. P. (1981) Stress corrosion cracking of sensitized 304 austenitic stainless steel in sulphurous acid. *Corrosion* **37**, 412-415.

Baru, R. L.; Zinevich, A. M.; Mogilnitskii, G. M.; Sapozhnikova, G. A.; Starosvetskaya, Zh. O.; Timonin, V. A. (1982) Role of metabolic products of the bacterium *Thiobacillus thiooxidans* in the corrosion of steel 10KhSND. *Protection of Metals* **18**, 605-606 [English translation.]

Bates, J. F. (1981) Elemental sulphur as a corrodent in deaerated, neutral aqueous solutions (Discussion). *Materials Performance*. **20(2)**, 56.

Biebl, H.; Pfennig, N. (1977) Growth of sulphate-reducing bacteria with sulphur as electron acceptor. *Archives of Microbiology* **112**, 115-117.

Blasius, E.; Münch, J. (1972) Schnelle coulometrische Mikrobestimmung einzelner Polythionate. *Zeitschrift für Analytische Chemie* **261**, 198-203.

Bockris, J. O'M.; Reddy, A. K. N. (1973) *Modern Electrochemistry*, Vol. 2. New York; Plenum Press.

Booth, G. H. (1971) *Microbiological Corrosion*. London; Mills & Boon.

Booth, G. H.; Tiller, A. K. (1960) Polarization studies of mild steel in cultures of sulphate-reducing bacteria. *Transactions of the Faraday Society* **56**, 1689-1696.

Booth, G. H.; Shinn, P. M.; Wakerley, D. S. (1965) The influence of various strains of actively growing sulphate-reducing bacteria on the anaerobic corrosion of mild steel. *Comptes Rendus du Congres Internationale Corrosion Marine et des Salissures*, 363-371. Paris; CREO.

Booth, G. H.; Cooper, A. W.; Cooper, P. M. (1967) Rates of microbial corrosion in continuous culture. *Chemistry and Industry* **49**, 2084-2085.

Booth, G. H.; Elford, L.; Wakerley, D. S. (1968) Corrosion of mild steel by sulphate-reducing bacteria: an alternative mechanism. *British Corrosion Journal* **3**, 242-245.

Brierley, C. L.; Brierley, J. A.; Norris, P. R.; Kelly, D. P. (1980) Metal-tolerant micro-organisms of hot, acid environments. In *Microbial Growth and Survival in Extremes of Environment* (G. W. Gould; J. E. L. Corry, eds), 39-51. London; Academic Press.

Brophy, A. J. (1974) Stress corrosion cracking of austenitic stainless steels in refinery environments. *Materials Performance* **15**(5), 9-15.

Brown, K. A. (1982) Sulphur in the environment: a review. *Environmental Pollution* **3**, 47-80.

Chakrapani, D. G.; Czyzeaski, H. (1978) Corrosion problems in the pulp and paper industry: a few case histories. *Corrosion* **78**, paper no. 19. Houston; NACE.

Charlton, R. S. (1978) Materials and corrosion problems in the pulp and paper industry. *Corrosion* **78**, paper no. 17. Houston; NACE.

Costello, J. A. (1974) Cathodic depolarization by sulphate-reducing bacteria. *South African Journal of Science* **70**, 202-204.

Cowan, R. L.; Gordon, G. M. (1978) Intergranular stress corrosion cracking and grain boundary composition of Fe-Ni-Cr alloys. In *Stress Corrosion Cracking and Hydrogen Embrittlement of Iron Base Alloys* (J. Hochmann; R. W. Staehle, eds), 1023-1070. Houston; NACE.

Cragnolino, G.; Macdonald, D. D. (1982) Intergranular stress corrosion cracking of austenitic stainless steel at temperatures below 100C: a review. *Corrosion* **38**, 406-424.

Crolet, J. L.; Seraphin, L.; Tricot, R. (1976) Mecanisme d'action de soufre sur la resistance a la corrosion generale des aciers inoxydables. *Metaux-Corrosion-Industrie* **616** (Dec.), 1-11.

Dhawale, S.; Cragnolino, G.; Macdonald, D. D. (1982) Stress corrosion cracking of sensitized austenitic stainless steel in boric acid solutions containing sulphur species. In *Intergrannular Stress Corrosion Cracking of Austenitic Stainless Steels in PWR Boric Acid Storage Systems.* [EPRI NP-2531, 41-4-28.] Palo Alto; Energy Power Research Institute.

Dravnieks, A.; Samans, C. H. (1957) Corrosion control in ultraforming. *Proceedings of the American Petroleum Institute* **37(3)**, 100-115.

Farrer, T. W.; Wormwell, F. (1953) Corrosion of iron and steel by aqueous suspensions of sulphur. *Chemistry and Industry* **5**, 196.

Fauque, G.; Herve, D.; Le Gall, J. (1979) Structure-function relationship in hemoproteins: the role of cytochrome c_3 in the reduction of colloidal sulphur by sulphate-reducing bacteria. *Archives of Microbiology* **121**, 261-264.

Foroulis, Z. A. (1980) Electrochemical behaviour and corrosion of iron in aqueous sulphidic solution. *Werkstoffe und Korrosion* **31**, 463-470.

Galvele, J. R. (1976) Transport processes and the mechanism of pitting of metals. *Journal of the Electrochemical Society* **123**, 464-474.

Greene, N. D.; Wilde, B. E. (1970) Variable corrosion resistance of 18 Cr-8 Ni stainless steels: influence of environmental and metallurgical factors. *Corrosion* **26**, 533-538.

Granat, L.; Rodhe, H.; Hallberg, R. O. (1976) The global sulphur cycle. In *Nitrogen, Phosphorus and Sulphur—Global Cycles* (B. H. Svensson; R. Söderlund, eds), 89-134. [SCOPE Report no. 7.] Stockholm; Swedish Natural Science Research Council.

Guay, R.; Silver, M. (1975) *Thiobacillus acidophilus* sp. nov.; isolation and some physiological characteristics. *Canadian Journal of Microbiology* **21**, 281-288.

Heller, J. J.; Prescott, G. R. (1965) Cracking of stainless steels in wet sulphidic environments in refinery units. *Materials Protection* **4**(4), 14-18.

Herbsleb, G. (1982) The influence of SO_2, H_2S and CO on pitting corrosion of austenitic chromium-nickel stainless steels with up to 4 wt% molybdenum in 1 M NaCl. *Werkstoffe und Korrosion* **33**, 334-340.

Herbsleb, G.; Schwenk, W. (1966) Investigations into the influence of hydrogen sulphide and sulphur dioxide on the corrosion of chemically resistant chromium nickel steels in a sulphuric acid solution of sodium sulphate. *Werkstoffe und Korrosion* **17**, 745-750.

Hisamatsu, Y. (1976) Pitting corrosion of stainless steels in chloride solutions. In *Passivity and its Breakdown on Iron and Iron Base Alloys* (R. W. Staehle; H. Okada, eds), 99-110. Houston; NACE.

Horowitz, H. H. (1983) Chemical studies of polythionic acid stress corrosion cracking. *Corrosion Science* **23**, 353-362.

Horvath, J.; Solti, M. (1959) Mechanism of anaerobic microbiological corrosion of metals in soil. *Werkstoffe und Korrosion* **10**, 621-630.

Ijsseling, F. P. (1980) Electrochemical methods in crevice corrosion testing. *British Corrosion Journal* **15**, 51-69.

Iofa, Z. A. (1970) Effect of hydrogen sulphide on iron corrosion and on inhibitor adsorption in acidic solutions. *Protection of Metals* **6**, 445-450. [English translation.].

Iofa, Z. A. (1980) Mechanism of the effect of hydrogen sulphide and inhibitors on iron corrosion in acid solutions *Protection of Metals* **16**, 220-225. [English translation.]

Isaacs, H. S. (1980) Intergranular stress corrosion of sensitized stainless steels. In *BWR Water Chemistry Projects Review Meeting*. Palo Alto; Energy Power Research Institute.

Isaacs, H. S.; Vyas, B.; Kendig, M. W. (1982) The stress corrosion cracking of sensitized stainless steel in thiosulphate solutions. *Corrosion* **38**, 130-136.

Iverson, W. P. (1972) Biological corrosion. *Advances in Corrosion Science and Technology* **2**, 1-42.

Iverson, W. P. (1974) Microbial corrosion of iron. In *Microbial Iron Metabolism* (J. B. Neilands, ed.), 475-513. New York; Academic Press.

Iverson, W. P. (1981) An overview of the anaerobic corrosion of underground metallic structures, evidence for a new mechanism. In *Underground corrosion* (E. Escalante, ed.), 33-52. Philadelphia; American Society for Testing and Materials.

Iverson, W. P. (1983) Anaerobic corrosion mechanisms. *Corrosion* **83**, paper no. 243. Houston; NACE.

Johnston, F.; McAmish, L. (1973) A study of the rates of sulphur production in acid thiosulphate solutions using S-35. *Journal of Colloid and Interface Science* **42**, 112-119.

Justin, P.; Kelly, D. P. (1977) Metabolic changes accompanying transition of chemostat cultures of *Thiobacillus denitrificans* from aerobic to anaerobic growth. *Proceedings of the Society for General Microbiology* **5**, 18.

Kelly, D. P. (1982) Biochemistry of the chemolithotrophic oxidation of inorganic sulphur. *Philosophical Transactions of the Royal Society of London* **B298**, 499-527.

King, R. A.; Miller, J. D. A.; Smith J. S. (1973a) Corrosion of mild steel by iron sulphides. *British Corrosion Journal* **8**, 137-141.

King, R. A.; Miller, J. D. A.; Wakerley, D. S. (1973b) Corrosion of mild steel in cultures of sulphate-reducing bacteria: effect of changing the soluble iron concentration during growth. *British Corrosion Journal* **8**, 89-93.

King, R. A.; Wakerley, D. S. (1973) Corrosion of mild steel by ferrous sulphide. *British Corrosion Journal* **8**, 41-45.

Kobrin, G. (1976) Corrosion by microbiological organisms in natural waters. *Materials Performance* **15**(7), 38-43.

Kuenen, J. G.; Tuovinen, O. H. (1981) The genera *Thiobacillus* and *Thiomicrospira*. In *The Prokaryotes, a Handbook of Habitats, Isolation, and Identification of Bacteria* (M. P. Starr *et al.*, eds), 1023-1036. New York; Springer.

Lacombe, P. (1962) Radio-tracers as applied to the study of metallic corrosion. In *Proceedings of the First International Congress on Metallic Corrosion*, 21-35. London; Butterworths.

Leckie, H. P.; Uhlig, H. H. (1966) Environmental factors affecting the critical potential for pitting in 18-8 stainless steel. *Journal of the Electrochemical Society* **113**, 1262-1267.

Lee, K. H.; Cragnolino, G; Macdonald, D. D. (1981) Effect of heat treatment and applied potential on the caustic stress corrosion cracking of Inconel 600. In *Corrosion and Corrosion Cracking of Materials for Water Cooled Reactors*, 2·20-2·32. [EPRI Progress Report, FCC 1808.] Columbus; Ohio State University.

LeGall, J.; Moura, J. J. G.; Peck, H. D.; Xavier, A. V. (1982) Hydrogenase and other iron-sulphur proteins from sulphate-reducing and methane-forming bacteria. In *Iron-Sulphur Proteins* (T. G. Spiro, ed.), 177-248. New York; John Wiley & Sons.

Luffkin, A. (1973) Influence of SO_3^{2-} ions on pitting corrosion of stainless steels, *Anti-Corrosion Methods and Materials* **20**(7), 3-8.

Macdonald, D. D.; Roberts, B.; Hyne, J. B. (1978) The corrosion of carbon steel by wet elemental sulphur. *Corrosion Science* **18**, 411-425.

Masuo, M.; Tanigawa, T.; Ono, Y.; Ohashi, N. (1978) [Influence of SO_2 and H_2S on the anodic polarization behaviour of stainless steels in 5% sulphuric acid and 3·5% sodium chloride solutions.] *Corrosion Engineering* **27**, 565-572. [In Japanese.]

Miller, J. D. A. (1981) Metals. In *Economic Microbiology* (A. H. Rose, ed.), **6**, 149-202. London; Academic Press.

Miller, J. D. A.; Tiller, A. K. (1970) Microbial corrosion of buried and immersed metals. In *Microbial Aspects of Metallurgy* (J. D. A. Miller, ed.), 61-105. New York; American Elsevier.

Moore, W. E. C.; Johnson, J. L.; Holdeman, L. V. (1976) Emendation of *Bacteroidaceae* and *Butyrivibrio* and descriptions of *Desulfomonas* gen. nov. and ten new species in the genera *Desulfomonas, Butyrivibrio, Eubacterium, Clostridium*, and *Ruminococcus*. *International Journal of Systematic Bacteriology* **26**, 238-253.

Murphy, J. R.; Kornfield, J. M.; Tilton, R. C. (1973) Effect of tetrathionate on the growth of a marine thiobacillus. *Journal of General Microbiology* **73**, 339-343.

Newman, R. C.; Isaacs, H. S.; Alman, B. (1982a) Effects of sulphur compounds on the pitting behaviour of type 304 stainless steel in near-neutral chloride solutions. *Corrosion* **38**, 261-265.

Newman, R. C.; Roberge, R.; Bandy, R. (1982b) Evaluation of SCC test methods for Inconel 600 in low temperature aqueous solutions. In *Symposium on Environment-Sensitive Fracture: Evaluation and Comparison of Test Methods*. Gaithesburg, Maryland; National Bureau of Standards.

Newman, R. C.; Sieradzki, K.; Isaacs, H. S. (1982c) Stress corrosion cracking of sensitized type 304 stainless steel in thiosulphate solutions. *Metallurgical Transactions, A*, **13**, 2015-2026.

Nriagu, J. O.; Hem, J. D. (1978) Chemistry of pollutant sulphur in natural waters. In *Sulphur in the Environment* (J. O. Nriagu, ed.). Part II: *Ecological Impacts*, 211-270. New York; John Wiley & Sons.

Okamoto, G.; Shibata, T. (1978) Passivity and the breakdown of passivity of stainless steel. In *Passivity of Metals* (R. P. Frankenthal; J. Kruger, eds), 646-677. Princeton; The Electrochemical Society.

Oldfield, J. W.; Sutton, W. H. (1978a) Crevice in corrosion of stainless steels. I. A mathematical model. *British Corrosion Journal* **13**, 13-22.

Oldfield, J. W.; Sutton, W. H. (1978b) Crevice corrosin of stainless steels. II. Experimental studies. *British Corrosion Journal* **13**, 104-111.

Oudar, J.; Marcus, P. (1979) Role of adsorbed sulphur in the dissolution and passivation of nickel and nickel-sulphur alloys. *Applications of Surface Sciences* **3**, 48-67.

Pessall, N.; Liu, C. (1971) Determination of critical pitting potentials of stainless steels in aqueous chloride environments. *Electrochimica Acta* **16**, 1987-2003.

Pfennig, N.; Biebl, H. (1976) *Desulfuromonas acetoxidans* gen. nov. and sp. nov., a new anaerobic, sulphur-reducing acetate-oxidizing bacterium. *Archives of Microbiology* **110**, 3-12.

Piehl, R. L. (1964) Stress corrosion cracking by sulphur acids. *Proceedings of the American Petroleum Institute* **44**(3), 189-197.

Postgate, J. R. (1979) *The Sulphate-Reducing Bacteria*. Cambridge; Cambridge University Press.

Roy, A. B.; Trudinger, P. A. (1970) *The Biochemistry of Inorganic Compounds of Sulphur*. Cambridge; Cambridge University Press.

Rozenfeld, I. L. (1974) Crevice corrosion of metals and alloys. In *Localized Corrosion* (R. W. Staehle *et al.*, eds), 373-398. Houston; NACE.

Ryabchenkov, A. V.; Nikiforova, R. M. (1962) Role of electrochemical factors in the process of corrosion cracking of austenitic steels. In *Intercrystalline Corrosion and Corrosion of Metals Under Stress* (I. A. Levin, ed.), 161-178. New York; Consultants Bureau.

Salvarezza, R. C.; Videla, H. A. (1980) Passivity breakdown of mild steel in sea water in the presence of sulphate reducing bacteria. *Corrosion* **36**, 550-554.

Salvarezza, R. C.; Videla, H. A.; Arvia, A. J. (1982) The electrodissolution and passivation of mild steel in alkaline sulphide solutions. *Corrosion Science* **22**, 815-829.

Scarberry, R. C.; Pearman, S. C.; Crum, J. R. (1976) Precipitation reactions in Inconel alloy 600 and their effect on corrosion behaviour. *Corrosion* **32**, 401-406.

Schaschl, E. (1980) Elemental sulphur as a corrodent in deaerated, neutral aqueous solutions. *Materials Performance* **19**(7), 9-12.

Schmidt, M. (1972) Oxyacids of sulphur. In *Sulphur in Organic and Inorganic Chemistry* (A. Senning, ed.), 71-112. New York; Marcel Dekker.

Schmidt, M.; Siebert, W. (1973) The chemistry of sulphur. In *Comprehensive Inorganic Chemistry* (J. C. Bailar *et al.*, eds), **15**, 795-932. New York; Pergamon Press.

Shoesmith, D. W.; Bailey, M. G.; Ikeda, B. (1978a) Electrochemical formation of mackinawite in alkaline sulphide solutions. *Electrochimica Acta* **23**, 1329-1339.

Shoesmith, D. W.; Taylor, P.; Bailey, M. G.; Ikeda, B. (1978b) Electrochemical behaviour of iron in alkaline sulphide solutions. *Electrochimica Acta* **23**, 903-916.

Sedriks, A. J. (1979) *Corrosion of Stainless Steel*. New York; John Wiley & Sons.
Smialowska, S. (1974) The pitting of iron-chromium-nickel alloys. In *Localized Corrosion* (R. W. Staehle *et al.*, eds), 312-341. Houston: NACE.
Smialowska, S.; Czachor, M. (1974) The anaysis of electrochemical methods for the determination of characteristic potentials of pitting corrosion. In: *Localized Corrosion* (R. W. Staehle *et al.*, eds), 353-362. Houston: NACE.
Smith, J. S.; Miller, J. D. A. (1975) Nature of sulphides and their corrosive effects on ferrous metals: a review. *British Corrosion Journal* **10**, 136-143.
Soimajärvi, J.; Pursiainen, M.; Korhonen, J. (1978) Sulphate-reducing bacteria in paper machine waters and in suction roll preparations. *European Journal of Applied Microbiology and Biotechnology* **5**, 87-93.
Stephenson, M.; Stickland, L. H. (1931) Hydrogenase. II. The reduction of sulphate to sulphide by molecular hydrogen. *Biochemical Journal* **25**, 215-220.
Subramanyan, P. K. (1981) Electrochemical aspects of hydrogen in metals. In *Comprehensive Treatise of Electrochemistry*. Vol. 4. *Electrochemical Materials Science* (J. O'M. Bockris *et al.*, eds), 411-462. New York; Plenum Press.
Süry, P. (1976) Similarities in the corrosion behaviour of iron, cobalt and nickel in acid solutions. A review with special reference to the sulphide adsorption. *Corrosion Science* **16**, 879-901.
Szekeres, L. (1974) Analytical chemistry of the sulphur acids. *Talanta* **21**, 1-44.
Tatnall, R. E. (1981a) Case histories: bacteria induced corrosion. *Materials Performance* **20**(8), 41-48.
Tatnall, R. E. (1981b) Fundamentals of bacteria induced corrosion. *Materials Performance* **20**(9), 32-38.
Thauer, R. K.; Badziong, W. (1980) Respiration with sulphate as electron acceptor. In *Diversity of Bacterial Respiratory Systems* (C. J. Knowles, ed.), **2**, 65-85. Boca Raton, Florida: CRC Press.
Tilton, R. C.; Stewart, G. J.; Jones, G. E. (1967) Marine thiobacilli. II. Culture and ultrastructure. *Canadian Journal of Microbiology* **13**, 1529-1534.
Togano, H.; Sasaki, H.; Nakahara, T.; Nakauchi, H.; Kauda, Y.; Osato, K. (1975) The influence of sulphate-reducing bacteria on the corrosion of mild steel in artificial sea water. In *Proceedings of the Sixth International Congress on Metallic Corrosion*, 1258-1269. Victoria: Australian Corrosion Association.
Tuovinen, O. H. (1973) Thiobacillus ferrooxidans: *autotrophic growth on ferrous iron and inorganic sulphur compounds with special reference to the toxicity of metal ions and the development of tolerance to uranium*. PhD thesis, University of London.
Tuovinen, O. H. (1978) Interactions of tetrathionate with sulphite and sulphide. *Talanta* **25**, 408-409.
Tuovinen, O. H.; Kelly, D. P. (1974) Studies on the growth of *Thiobacillus ferrooxidans*. V. Factors affecting growth in liquid culture and development of colonies on solid media containing inorganic sulphur compounds. *Archives of Microbiology* **98**, 351-364.
Tuovinen, O. H.; Button, K. S.; Vuorinen, A.; Carlson, L.; Mair, D. M.; Yut, L. A. (1980) Bacterial, chemical, and mineralogical characteristics of tubercles in distribution pipelines. *Journal of the American Water Works Association* **72**, 626-635.
Tuovinen, O. H.; Hsu, J. C. (1982) Aerobic and anaerobic micro-organisms in tubercles of the Columbus, Ohio, water distribution system. *Applied and Environmental Microbiology* **44**, 761-764.
Valensi, G. (1973) *Comportement electrochimique du soufre. Diagrammes d'equilibres tension-pH du système S-H_2O a 25°C. 1 atms. [Rapport Techniques* vol. 121, RT 207.] Bruxelles; CEBELCOR.
Valensi, G.; van Muylder, J.; Pourbaix, M. (1974) Sulphur. In *Atlas of Electrochemical Equilibria in Aqueous Solutions* (M. Pourbaix, ed.), 545-553. Houston, NACE. Bruxelles: CEBELCOR.
von Wolzogen Kühr, C. A. H.; van der Klugt, L. S. (1934) Graphitization of cast iron as an electro-biochemical process in anaerobic soils. *Water* (The Hague) **18**, 147-165.
Wagner, H.; Schreier, H. (1978) Investigations on the sulphite degradation of polythionates. *Phosphorus and Sulphur* **4**, 285-286.
Wakerley, D. S. (1979) Microbial corrosion in UK industry; a preliminary survey of the problem. *Chemistry and Industry* **19**, 657-658.
Wanklyn, J. N.; Spruit, C. J. P. (1952) Influence of sulphate-reducing bacteria on the corrosion potential of iron. *Nature, London* **169**, 928-929.
Widdel, F.; Pfennig, N. (1981) Studies on dissimilatory sulphate-reducing bacteria that decompose fatty acids. I. Isolation of new sulphate-reducing bacteria enriched with acetate from saline environments. Description of *Desulfobacter postgatei* gen. nov., sp. nov. *Archives of Microbiology* **129**, 395-400.
Zillig, W.; Stetter, K. O.; Prangishvilli, D.; Schäfer, W.; Wunderl, S.; Janekovic, D.; Holz, I.; Palm, P. (1982) *Desulfurococcaceae*, the second family of the extremely thermophilic, anaerobic, sulphur-respiring *Thermoproteales*. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene*. I Abt. C3, 304-317.
Zillig, W.; Gierl, A.; Schreiber, G.; Wunderl, S.; Janekovic, D.; Stetter, K. O.; Klenk, H. P. (1983a) The archaebacterium *Thermofilum pendens* represents a novel genus of the thermophilic, anaerobic sulphur respiring *Thermoproteales*. *Systematic and Applied Microbiology* **4**, 79-87.
Zillig, W.; Holz, I.; Janekovic, D.; Schäfer, W.; Reiter, W. D. (1983b) The archaebacterium *Thermococcus celer* represents a novel genus with the thermophilic branch of the archaebacteria. *Systematic and Applied Microbiology* **4**, 88-94.

CHAPTER THREE: J. D. A. MILLER & A. K. TILLER

Microbial Corrosion of Buried and Immersed Metal

Background to the Problem

Introduction

That unprotected iron or steel objects in the ground or immersed in water tend to corrode is a truism; the electrochemical mechanisms involved are well understood, and are described in Chapter 2. Nevertheless it is still perhaps not generally realized that a large proportion of this corrosion is brought about, or assisted, by the action of microbes.

For the proper understanding of microbial processes leading to corrosion in these situations, some basic knowledge of the range of physical and chemical conditions in natural environments is necessary. Soil, in particular, is a complex and extremely variable environment; thus it is often difficult to trace the mechanism of underground corrosion, or to separate the purely electrochemical processes from those caused by microbes. This is especially true in situations where cyclical changes in conditions enable first one process and then another to predominate and where, therefore, different types of corrosion product are superimposed on one another or existing ones are altered and become unrecognizable. It has been estimated, though, that in the UK at least 50% of failures of underground pipes are due to microbial action[1], and that as long ago as 1955 the maintenance and replacement of underground pipes because of corrosion cost about £20 million.[2]

The Soil

"Soil", for the purposes of this article, can be taken to mean all types of substratum from sand to clay, from highly acidic and almost purely organic peat with great water retention to the topsoil of downland, consisting sometimes of little more than lumps of chalk and hence retaining scarcely any moisture.

Pure peat apart, soils owe their basic structure to inorganic particles that first arose by the weathering of the rock of the earth's mantle. These particles have been sorted out by the action of wind and water, and according to their situation and their chemical nature have been colonized to a greater or lesser extent by pioneer plants such as lichens. With the death and decay of these early colonizers, and transport and deposition of other plant and animal remains, the primitive soils have acquired a variable amount of organic material (least so in the constantly washed sand on the shores of seas and rivers) which has provided the nutriment for an often rich and varied microbial population; and some type of covering of "higher" vegetation has developed, characteristic of the water content, pH, fertility etc.

There are thus various possible ways of classifying soils according to mineral particle size, content of organic material, type of vegetation they support, and so on; a convenient classification for our purposes is on the basis of particle size, since this determines some properties of soil which bear upon corrosion.

The mineral particles in soil range from gravel (1–2 mm or more in diameter), through coarse and fine sand (the finest usually being called silt) to clay, the particles of which are generally less than 5 μ in diameter and approach the colloidal range. Soil types derived from these particles range from pure gravel or sand, well-drained and with no organic content, through sandy loam, loams (light and heavy) with high organic content, to clay loam and at the end of the scale pure clay, which if the particles are small enough will be impermeable to water and relatively free from organic matter.

Although the treatment of electrochemical corrosion unassisted by microbial action is outside the scope of this chapter, it should be mentioned in passing – indeed it is clear from the preceding chapter – that this type of corrosion is prevalent in acid soils such as peat, and also in more neutral situations such as coastal and estuarine soils which have a high content of ions. Moreover, all except markedly alkaline soils could be expected to be aggressive to some degree towards buried metal objects provided that both oxygen and moisture are present.

The degree of oxygenation and the water-holding capacity of a soil are interdependent, and are determined by the particle size, larger particles giving a soil with low water-holding capacity and large air capacity. It follows that clays and clay loams, which abound over much of the UK owing to the Pleistocene glaciation, have a high water-holding capacity and readily become waterlogged. If there is a significant organic content in such clayey soils, supporting the growth of a microbial population, oxygen (solubility in water about 10 parts per million) will rapidly be removed from the water in the soil since the rate of consumption by the microflora and microfauna will almost always exceed the rate of dissolution of oxygen at the surface and transport downwards by diffusion. Oxygen will only return to this soil by its drying out or by mass movement of oxygenated water into it. The process of deoxygenation is also described in Chapter 1 under *Biochemical Oxygen Demand.*

Clay soils are frequently near neutral in reaction (pH about 6–8), favouring the growth of micro-organisms. The common presence of water in these soils might also be thought to favour microbial growth; though in fact the well-drained soils are usually quite damp enough, at least in temperate countries, to support an active microbial population.

Water

A similar situation to the waterlogged clay soils can exist at the bottom of rivers and lakes. In the upper reaches of rivers, the bed is scoured free of loose material by the rapid current of water which is itself well aerated by its churning motion; but in the lower reaches the current is slower and deposition of this scoured material begins to occur. This deposited material may remain scarcely disturbed, especially at and near estuaries. The same applies, of course, to the bottom mud of lakes and ponds. Oxygen-free conditions can

then very easily arise in these bottom muds by the action of aerobic and micro-aerophilic microbes; and frequently the situation is exacerbated by the water itself having a high BOD (see Chapter 1) because of pollution, and supporting heavy microbial growth.

Types of Microbial Corrosion

In this chapter we are going to consider microbial corrosion of metals in a variety of natural conditions. For micro-organisms to produce their characteristic chemical effects they usually must be growing, not merely lying dormant (though secondary, physical effects can be produced merely by the *presence* of a thick clump of micro-organisms, whether growing or not). This would suggest that, in general, damp or wet environments of near-neutral pH, well aerated and with a supply of organic nutrients, would most favour the growth of any microbes involved in corrosion. Though this is partly true, the small group of bacteria which cause by far the worst havoc in this respect – the sulphate-reducing bacteria – have different requirements from the above in one important respect: they are anaerobic, and indeed are quite unable to grow in the presence of any but the most minute traces of oxygen. Thus, ironically, the soil conditions which an electrochemist might believe were the ideal ones in which, for example, to lay a pipeline – a neutral clay soil, water-logged and hence oxygen-free for much of the year, so that polarization of the cathodic areas of the buried metal should prevent anodic dissolution – are in fact the ones that most favour the growth of the anaerobic sulphate-reducing bacteria.

It should be pointed out here that the *immediate* cause of corrosion is electrochemical in all the cases we are about to consider. The sulphate-reducing bacteria appear to allow a stifled electrochemical reaction to be resumed, while initiating another; a different group of microbes produces acids, mineral and organic, with well-known corrosive effects; a third forms thick clumps which allow differential aeration and concentration cells to arise; while a fourth, already described very briefly in the legend of Fig. 1.11 in Chapter 1, breaks down protective coatings and thus allows electrochemical corrosion (pure, or microbially-induced) to occur.

Corrosion by the Sulphate-Reducing Bacteria

Historical

It was suspected even before the turn of the century that microbes might have a role in the corrosion of metals immersed in water, and in 1910 Gaines[3] suggested that iron bacteria and sulphur bacteria were responsible in part for the corrosion of buried ferrous metals. By 1924 it was considered[4] that hydrogen sulphide production by sulphate-reducing bacteria may play an

important part in this type of corrosion. Von Wolzogen Kühr noticed in Holland that corrosion associated with sulphide occurred in wet, anaerobic soil in which polarization of cathodic areas would be expected to inhibit corrosion. Realizing that there may therefore be other depolarizing agents, that is, hydrogen acceptors, present in the soil besides atmospheric oxygen, he and Van der Vlugt made the first attempt[5] to provide a detailed explanation of underground corrosion by sulphate-reducing bacteria in electrochemical terms. It was already known[6] that sulphate-reducing bacteria were able to take up molecular hydrogen while reducing sulphate according to the equation:

$$SO_4^{2-} + 4H_2 \rightarrow S^{2-} + 4H_2O \qquad (1)$$

They proposed that these bacteria could similarly remove the *atomic* hydrogen from polarized cathodes, oxidizing it to protons and electrons, and utilizing the reducing power so obtained for the reduction of sulphate. Full details of their theory are given further on.

It will now be helpful to look at the properties of the sulphate-reducing bacteria in a little detail.

Characteristics of the Sulphate-Reducing Bacteria

The sulphate-reducing bacteria (or more correctly the anaerobic dissimilatory sulphate-reducing bacteria, since numerous microbes reduce small amounts of sulphate for the synthesis of sulphur-containing organic substances) are abundant in nature, and probably ubiquitous. They have been found in Europe, Asia, Africa, North and South America, Australasia and Antarctica; and they have been encountered at a depth of 71m in clay. In the UK it is a rare occurrence to take a soil sample in which they cannot be demonstrated. In this connexion it is difficult to tell whether a soil is oxygen-free *in situ*, because disturbance by taking a sample or even by pushing an electrode into it to measure redox potential inevitably leads to some oxygenation; but nevertheless open-textured dry soils which must undoubtedly be oxygenated will almost always contain demonstrable sulphate-reducing bacteria. The organisms cannot be active in these situations, but they are nevertheless viable, so that the onset of favourable conditions in the soil will soon result in their growth.

Water, both fresh and salt, often contains sulphate reducers, especially if the BOD is high. Whereas water-living organisms usually tend to be physiologically adapted either to fresh-water or salt-water habitats but not both, most strains of the sulphate-reducing bacteria hitherto isolated are readily adaptable between NaCl-free medium and that containing the sea-water concentration, with the exception of a few strains of marine origin (members of the species *Desulfovibrio salexigens*) which appear to have an absolute requirement for the chloride ion. There is some confusion in the terminology used by microbiologists here: microbes isolated from a marine environment

(whether or not they can be adapted to salt-free conditions) have often been described as "halophilic", while some microbiologists would reserve this term for organisms found growing in more strongly saline habitats such as salt lakes in desert regions and would describe isolates from the sea as "marine" or "salt-water" forms. In this review, in common with many of the authors we have cited, we use the term *halophile* for any laboratory strain that originated from salt water.

Certain features of their metabolism suggest that the sulphate-reducing bacteria are very ancient, or "primitive" inasmuch as they may be an ancestral form that has given rise to many of the species of living things existing today: they certainly must have found the early conditions on this planet, such as the reducing atmosphere, highly conducive to active growth.

It is doubtful whether until fairly recently many bacteriologists had pure cultures of sulphate-reducing bacteria; this is borne out by certain activities attributed to these organisms in the earlier literature which cannot now be demonstrated. There are, though, a number of distinct species of sulphate reducers (eight are now recognized, some of these having aberrant strains or distinct varieties), so that some of the conflicting results of earlier days may be explained by the unawareness of workers in different laboratories that they were handling different types. Even today, much work remains to be done in comparing the nutrition, physiology and biochemistry of these newly recognized species. Moreover, even with pure cultures and refined techniques at our disposal, we are still perhaps not aware of, or do not fully understand, all the chemical processes involved in corrosion by these bacteria, and are certainly not yet able to assess the relative contributions of the various known processes to the overall reaction.

Not the least of the desulfovibriologist's problems is merely that of growing the bacteria: even experienced workers sometimes encounter complete failures of growth. In addition, laboratory studies are rendered difficult by certain peculiarities of these organisms. Examples are their production of sulphide ions, which react with the ferrous ions that must be incorporated in the growth medium, forming a precipitate or fine suspension of ferrous sulphide; and their excretion, especially in later stages of growth and more especially by halophiles, of a viscous mucin in which a considerable number of the bacteria become embedded. Population densities and growth rates are thus impossible to determine accurately, and uniform or standardized suspensions of the bacteria are difficult to prepare; while in electrochemical studies the electrodes may be poisoned by the sulphide so that readings are unreliable.

* * *

A table showing the modern classification of the sulphate-reducing bacteria, with synonyms, is given below (Table 3.1); it will be seen that all

Table 3.1 *List of the sulphate-reducing bacteria, with some of their more important characteristics*

Species	Synonyms (old names)	Characteristics
Genus *Desulfovibrio*	*Vibrio, Sporovibrio, Spirillum, Microspira*	Single flagellum, or a tuft. Do not form spores. Hydrogenase usually present
Dv. desulfuricans	*Desulfovibrio desulfuricans*	Curved rods (vibrios); sometimes spirilloid, occasionally straight. Typical size 3–5 μ × 0·5–1 μ. Single flagellum. — Growth on pyruvate or choline in absence of sulphate.
Dv. vulgaris		—
Dv. salexigens		Obligate salt-water species (requires Cl^-).
Dv. gigas		Large curved rods or spirilla, 5–10 μ × 1·2–1·5 μ. Tuft of flagella.
Dv. africanus		Long, slender, sigmoid rods, 5–10 μ × 0·5 μ. Tuft of flagella.
Genus *Desulfotomaculum*		Peritrichous flagella (*i.e.*, distributed all over the organism). Spores formed.
Dt. nigrificans	*Vibrio thermodesulfuricans, Clostridium nigrificans*	The only thermophilic species (optimum temperature 55°C). Rods, 3–6 μ × 0·3–0·5 μ. Hydrogenase activity variable; not coupled to sulphate reduction. Growth on pyruvate in sulphate-free media.
Dt. orientis	*Desulfovibrio orientis*	Fat curved rods, 5 μ × 1·5 μ. Hydrogenase apparently absent.
Dt. ruminis		Rods, 3–6 μ × 0·5 μ. Growth on pyruvate in sulphate-free media. Found in rumen of sheep.

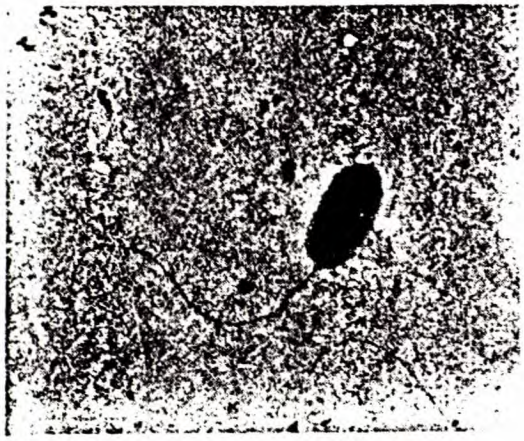


Fig. 3.1 *Electron micrograph of* Desulfovibrio desulfuricans, *showing single flagellum* (× 5000)

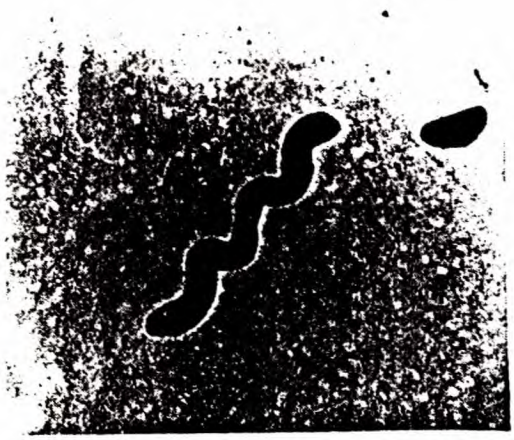


Fig. 3.2 *Electron micrograph of a spirillar form and a swollen form of* Desulfovibrio desulfuricans (× 3000)

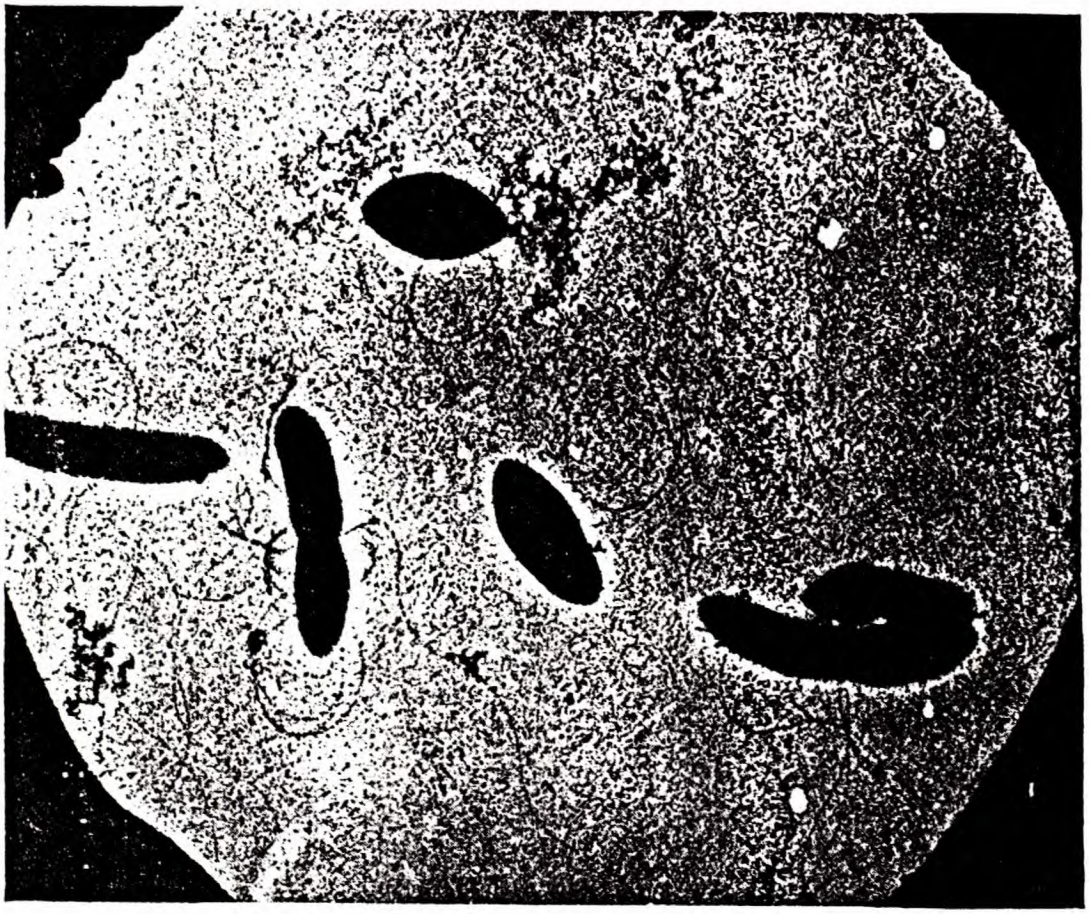


Fig. 3.3 *Electron micrograph of* Desulfotomaculum nigrificans, *showing numerous flagella and swollen forms* (× 10 000)

species except one are mesophilic, that is, their optimum temperature for growth lies within the normal limits of temperate and tropical climates (say 10–40°C). Some electron micrographs of sulphate reducers are shown in Figs. 3.1–3.3.

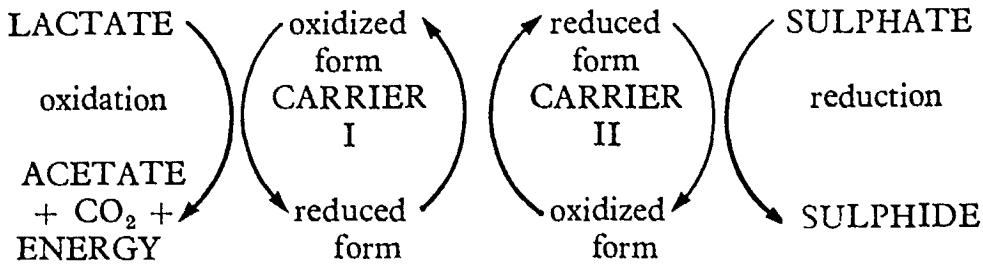
Table 3.1 also shows that there are marked dissimilarities between the genera *Desulfovibrio* and *Desulfotomaculum*. The difference in the number and arrangement of the flagella in the two genera would alone be enough to make many bacterial taxonomists separate them widely, and there is no doubt that they are biochemically very different though, as already stated, many more studies need to be made. We shall be concerned principally with members of the genus *Desulfovibrio* in this article.

Desulfovibrio spp. in pure culture in the laboratory can use sulphate, sulphite and a few other inorganic sulphur-containing anions as terminal electron acceptors for energy-yielding reaction;[7] that is, they carry out a form of anaerobic respiration. They are chemo-organotrophic heterotrophs, though they appear to grow well on relatively few organic carbon-and-energy sources. Salts of certain carboxylic acids (lactic, pyruvic, malic, fumaric) support the most vigorous growth, while markedly poorer growth is obtained with a few sugars, alcohols etc. One strain utilizes oxamate; certain others will grow on pyruvate, fumarate or choline in sulphate-free medium by a fermentative type of reaction (see Chapter 1). All the strains that have been examined appear to grow normally under nitrogen, so that hydrogen is not essential for growth reactions. Some strains can obtain energy by the oxidation of certain simple organic substances such as oxamate, mentioned above, which they cannot use as carbon sources. Earlier workers claimed that *Desulfovibrio* is chemolithotrophic, using the oxidation of hydrogen to provide energy for the autotrophic assimilation of CO_2; but recent reports, and unpublished work in the Manchester laboratories, cast strong doubts on the existence of autotrophy and chemolithotrophy respectively. The nutrition of the sulphate reducers, and many other aspects, have been dealt with by Postgate in a very valuable review.[8]

In most of the recent laboratory studies, sulphate reducers have been grown in media containing sodium lactate as a carbon source. Yeast extract is usually added, since this results in more rapid and abundant growth, though the few strains that have yet been critically examined can grow in the absence of yeast extract in a mineral salts + sodium lactate medium. Acetate and CO_2 are the major end-products of the growth reaction, the overall equation for which can be written:

$$2CH_3.CHOH.COONa + SO_4^{2-} \rightarrow 2CH_3.COONa + 2CO_2 + 2H_2O + S^{2-} \quad (2)$$

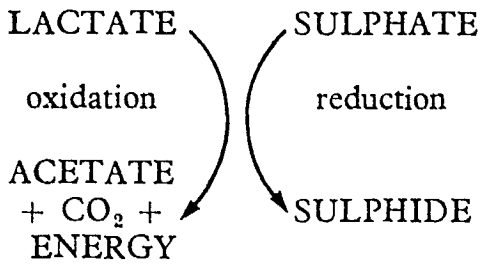
This can be expressed in a convenient pictorial form often used by biochemists, to illustrate the linked cell reactions:



(3)

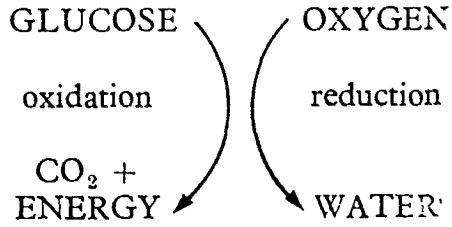
There are thus several intermediate stages in this process, involving the cyclical reduction and re-oxidation of intermediate electron carriers (two are shown participating in the reaction), but these need not concern us here. The important point is that the E'_0 for the initial oxidation (the lactate system) must, just as in any non-biological electrochemical coupled reactions, be more negative than that for the final (sulphide) system.

We can therefore simplify scheme (3) to the following:



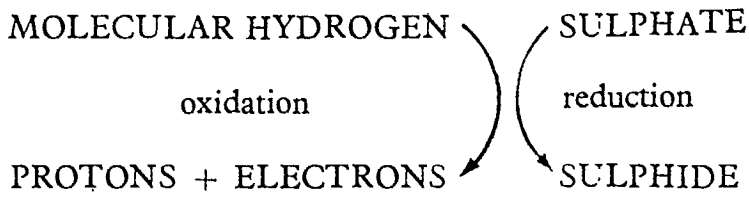
(4)

The analogy with aerobic respiration – the standard type of oxidation of a sugar by the majority of living things, using atmospheric oxygen as the terminal electron acceptor – can now easily be shown:



(5)

Equation (1) can of course similarly be written:



(6)

(the oxygen atoms from the sulphate radicals combining of course with the

hydrogen to form water: hence there is no evolution of oxygen, although this misconception has occasionally found its way into print and been offered as a mechanism of corrosion by these bacteria). This bacterial oxidation of hydrogen requires the presence of the enzyme *hydrogenase*, which is an unusual enzyme in that it appears to catalyse two reversible reactions:

$$H_2 \rightleftharpoons 2H \rightleftharpoons 2H^+ + 2e \qquad (7)$$

Hydrogenase activity towards *molecular* hydrogen can easily be demonstrated in non-growing suspensions of sulphate-reducing bacteria in phosphate or other buffer at about neutral pH and under oxygen-free hydrogen, either by adding sodium sulphate solution and measuring the hydrogen uptake manometrically or adding an oxidation-reduction dye such as methylene blue or benzyl viologen and observing its colour change. The hydrogen uptake in μl per standard amount of bacteria (1 mg dry weight) per hour determined manometrically is called the hydrogen absorption coefficient, $-Q_{H_2}$, and it should always be made clear which electron acceptor was used, *e.g.*, $-Q_{H_2}^{SO_4^{2-}}$ or $-Q_{H_2}^{BV}$, (= benzyl viologen: sulphate reducers frequently show much greater hydrogenase activity with the redox dyes as electron acceptors than with sulphate).

Sulphate reduction to sulphide is a stepwise process catalysed by a number of enzymes that tend to be collectively referred to as the *sulphate reductase system.*

* * *

The extreme sensitivity of sulphate-reducing bacteria to oxygen means that growth tests designed to establish whether these bacteria are present in a sample of soil, water, corrosion product etc., or to obtain a most probable number, can be unreliable in inexperienced hands. In rich media, or in the presence of a large amount of natural material such as a pellet of soil, growth will often occur in a screw-capped vessel completely filled with freshly boiled-out medium, though it is doubtful whether this growth originates from more than a few per cent of the sulphate reducers initially present. In media used for counts, and indeed in all media for laboratory work with these bacteria, it is standard practice to incorporate reducing agents such as sodium sulphide, or neutralized cysteine hydrochloride, thioglycollic acid or ascorbic acid, to ensure that all or most of the viable organisms in the inoculum will grow.

Actual tests for the presence of sulphate-reducing bacteria are given in the appendix *Microbiological Examination of Soil, Water etc. for Sulphate-Reducing Bacteria* at the end of this chapter.

Mechanism of Anaerobic Corrosion

Support for the Cathodic Depolarization Theory

As we have already seen, the sulphate-reducing bacteria had long been known

to reduce sulphate to sulphide using molecular hydrogen (equation (1)). Von Wolzogen Kühr and Van der Vlugt[5], studying the corrosion of buried pipes in the polder districts of Holland – low-lying areas of clayey soil, rich in organic matter and often waterlogged – noticed that iron sulphide occurred, not only as an adherent corrosion product on the pipes themselves but also in the soil in the vicinity of these corroding pipes. From its presence they deduced, and duly confirmed, the presence of sulphate-reducing bacteria; and they suggested that the bacteria acted as a cathodic depolarizing agent, stimulating the corrosion process by the removal of the (atomic) cathodic hydrogen which was then utilized for the reduction of sulphate to sulphide. The overall mechanism they postulated is as below.

anodic reaction	$4Fe \rightarrow 4Fe^{2+} + 8e$	(8)
electrolytic dissociation of water	$8H_2O \rightarrow 8H^+ + 8OH^-$	(9)
cathodic reaction	$8H^+ + 8e \rightarrow 8H$	(10)
cathodic depolarization by bacteria (compare equation (1))	$SO_4^{2-} + 8H \rightarrow S^{2-} + 4H_2O$	(11)
corrosion product	$Fe^{2+} + S^{2-} \rightarrow FeS$	(12)
corrosion product	$3Fe^{2+} + 6OH^- \rightarrow 3Fe(OH)_2$	(13)
overall reaction	$4Fe + SO_4^{2-} + 4H_2O \rightarrow 3Fe(OH)_2 + FeS + 2OH^-$	(14)

It follows from these equations that the ratio of iron corroded to FeS produced (mole:mole) should be 4:1, but in fact values from 0·9 to 48 have been recorded.

Other evidence has been presented, both for and against the "Dutch theory". Wanklyn and Spruit[9], who observed high ratios of iron corroded/FeS produced, reported an *anodic* depolarization. They concluded that corrosion was due to anodic stimulation by sulphide produced by bacteria utilizing organic electron donors, and considered that the consequent fall in the anode potential would increase the e.m.f. for hydrogen evolution at the cathode. Other work has broadly supported these findings[10,11]. On the other hand, early attempts to confirm the Dutch theory experimentally by demonstrating cathodic depolarization by sulphate-reducing bacteria were inconclusive.

Eventually, in 1959, Horváth and Solti[12] studied the polarization curves for mild steel electrodes in cultures of sulphate reducers and showed that the strain they used did bring about cathodic depolarization. Although this provided strong evidence in favour of the Dutch theory it lost some of its force because of inadequate characterization of the organism.

An independent study by Booth and Tiller[13] using pure cultures of sulphate-reducing bacteria also showed quite conclusively that cathodic depolarization by the Hildenborough strain of *Desulfovibrio vulgaris* could be effected, but only when the culture was in active growth. This organism is hydrogenase-

positive; a hydrogenase-negative organism (*Desulfotomaculum orientis*) did not cause cathodic depolarization. However, both organisms brought about an initial anodic stimulation, followed by an inhibitory effect due to the presence of a partially protective film of ferrous sulphide that gradually formed over the surface. The polarization cell they used is shown in Fig. 3.4.

Supporting these results were those of Booth and Wormwell[14] who studied the rate of corrosion of mild steel in batch cultures of various pure strains of non-halophilic sulphate reducers of different $-Q_{H_2}^{SO_4^{2-}}$. They found a linear relationship between corrosion rate and hydrogenase activity towards sulphate, and concluded that the ability to bring about cathodic depolarization was the most important factor in the stimulation of corrosion by sulphate-reducing bacteria. (As will be seen below, these results were not confirmed when halophiles were examined, nor in semicontinuous and continuous culture.)

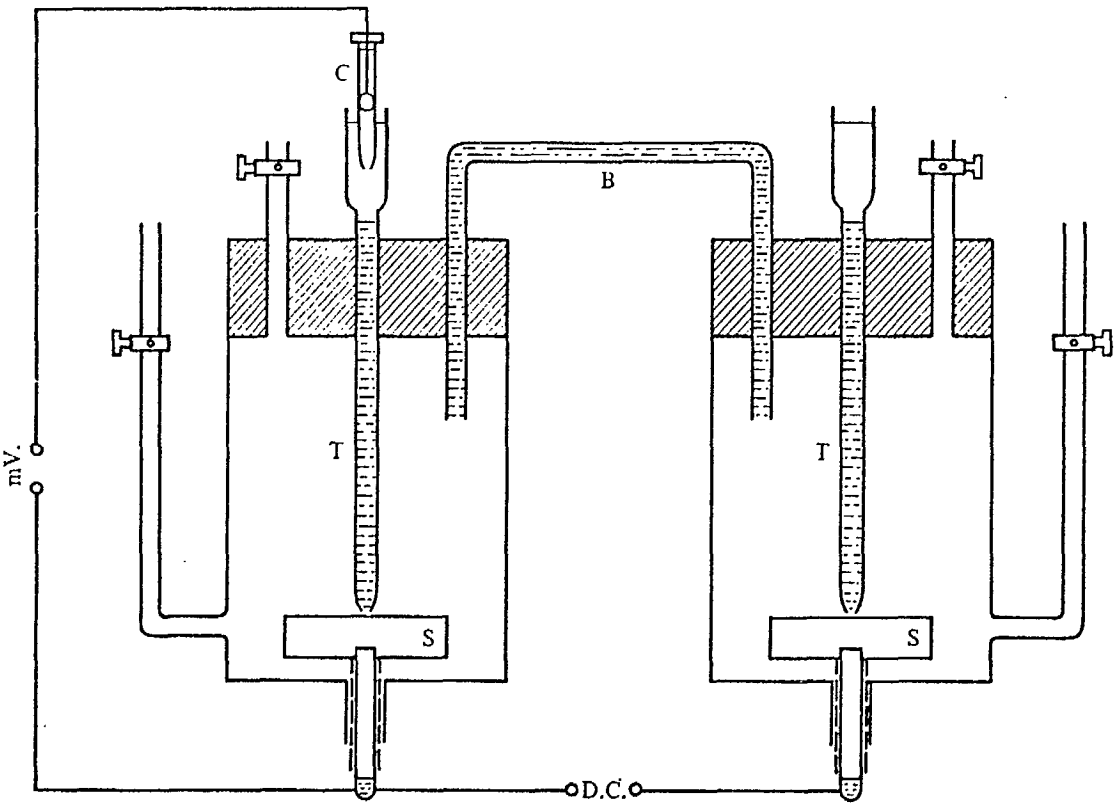


Fig. 3.4 *Divided polarization cell.* The electrodes (mild steel specimens, S) are mounted horizontally in glass vessels which have provision for deaeration by a stream of nitrogen. Electrical contact between the solutions in the vessels is *via* a KCl solution agar bridge (B); the mild steel electrodes are connected to the galvanostatic circuit. The potential of an electrode is determined with respect to a saturated calomel electrode (C), using a KCl/agar tubulus (T) as a Luggin probe. (By courtesy H.M.S.O. Crown Copyright)

Extension of the polarization studies to include thermophilic strains

enabled a fresh assessment of the mechanism of depolarization to be made[15]. Two strains of *Desulfotomaculum nigrificans*, both hydrogenase-negative towards sulphate, behaved differently in polarization cells: one, which was hydrogenase-*positive* towards benzyl viologen, caused cathodic depolarization, while the other, hydrogenase-negative towards the redox dye, did not depolarize. Both strains caused ennoblement of the anode by formation of a sulphide film.

The strain possessing the enzyme hydrogenase was apparently unable to couple its action to that of the sulphate reductase system, suggesting the absence of an essential electron carrier (see linked-reaction scheme (3)). Such a carrier could be the red pigment cytochrome c_3, present in *Desulfovibrio* but absent from *Desulfotomaculum*. The reduction of a redox dye by hydrogenase-catalysed oxidation of hydrogen needs no intermediate electron carrier. Hydrogenase activity was assumed, though, to be occurring in the polarization cell in which there was cathodic depolarization but in which of course no redox dye was present. It was therefore considered that either some organic intermediate metabolite was serving as electron acceptor for hydrogen oxidation, or that hydrogenase was performing only the first reaction in equation (7) and in the direction $2H \rightarrow H_2$.

Four halophilic types were next studied, in saline medium[16]. One, El Agheila Z, was a strain of the species *Desulfovibrio* (*Dv.*) *desulfuricans* (in the new sense – see Table 3.1) which was hydrogenase-negative towards both sulphate and benzyl viologen. A hydrogenase-positive variant of this strain towards both electron acceptors had been produced by growing it under hydrogen with repeated subculturing for a year; this was also studied for depolarizing effects. In addition, two obligate halophiles of the species *Dv. salexigens* were examined.

The results of these experiments, and those on *Dv. vulgaris*, *Desulfotomaculum* (*Dt.*) *orientis* and the thermophiles, are all presented for comparison in Table 3.2.

Anodic and cathodic depolarization curves for mild steel in sterile saline medium are shown in Fig. 3.5, and in presence of the actively growing strains El Agheila Z and Z_1, and the *Dv. salexigens* strains, in Figs. 3.6–3.9.

Several interesting facts emerged. Firstly, the presence and absence of hydrogenase respectively in the two *Dv. desulfuricans* strains correlated with their power to depolarize the cathode, confirming the previous work. Secondly, though in all cases a black film of ferrous sulphide was laid down on the anodes during the course of the experiment, ennoblement of the anode potential did not occur in the case of *Dv. salexigens* strain California 43:63 (Fig. 3.9). In other words, the anodic sulphide film produced during the growth of this strain appeared to be relatively unprotective, though no chemical difference between this film and any of those produced by other strains could be detected. Thirdly, the extreme vigour with which strain California 43:63 caused cathodic depolarization correlated better, as in

Table 3.2 *Hydrogenase and depolarization activity of some sulphate-reducing bacteria*

Organism	N.C.I.B.* Number	Hydrogenase Activity with Sulphate	Hydrogenase Activity with Benzyl Viologen	Cathodic effect	Anodic effect	Reference
Fresh-water mesophiles						
Desulfovibrio vulgaris strain Hildenborough	8303	++	+†	Depolarization	Initial stimulation of anodic reaction, followed by a move to a more noble potential	13
Desulfotomaculum orientis strain Singapore 1	8382	—	—†	No depolarization		
Thermophiles						
Desulfotomaculum nigrificans strain Teddington Garden	8351	—	+	Depolarization	Formation of partially protective sulphide film	15
Desulfotomaculum nigrificans strain Staines Garden	8353	—	—	No depolarization		
Halophilic mesophiles						
Desulfovibrio desulfuricans strain El Agheila Z	8380	—	—	No depolarization	Initial stimulation of the anode reaction followed by a move to more noble potentials	16
Desulfovibrio desulfuricans strain El Agheila Z_1	8380/1	+	++	Depolarization		
Desulfovibrio salexigens strain El Agheila C	8308	+	+	Depolarization		
Desulfovibrio salexigens strain California 43:63	8364	+	++	Vigorous depolarization	No ennoblement: sulphide film evidently non-protective	

Divided polarization-cell technique (separate anode and cathode compartments).
Experiments lasted for varying periods of up to 11 days after inoculation.
Medium for halophiles contained 2·5% sodium chloride.
Thermophiles incubated at 55°C; mesophiles at 30°C.
*National Collection of Industrial Bacteria: see *Appendix*.
†Professor J. R. Postgate: unpublished results.

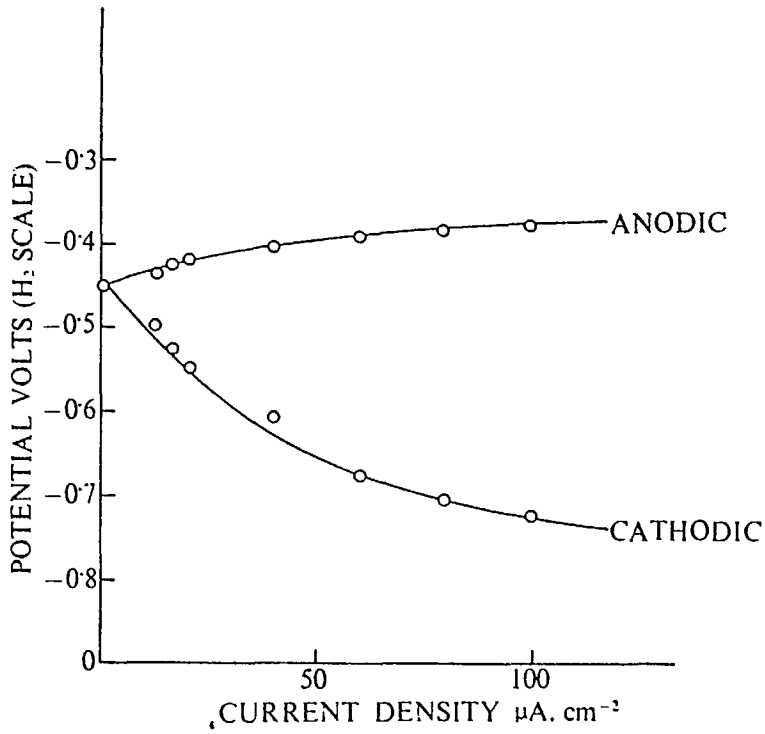


Fig. 3.5 *Polarization curves for mild steel at 30°C in sterile saline medium (Crown Copyright reserved)*

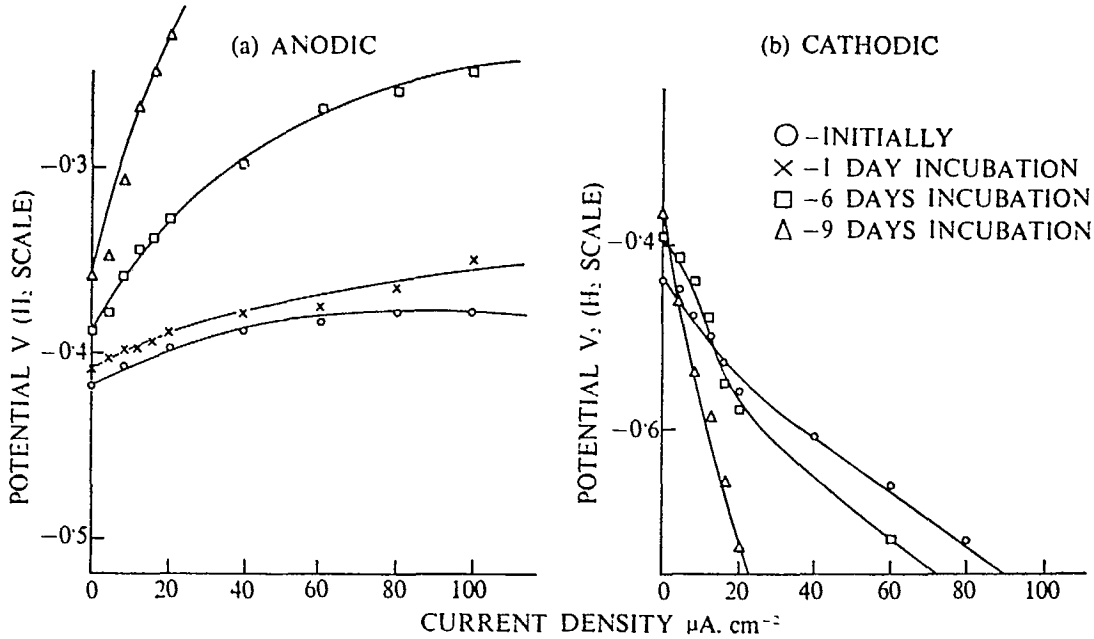


Fig. 3.6 *Polarization curves for mild steel at 30°C in cultures of* Desulfovibrio desulfuricans *(strain El Agheila Z, a hydrogenase-negative strain), indicating both the formation of a protective sulphide film and a lack of cathodic depolarization by the organisms (Crown Copyright reserved)*

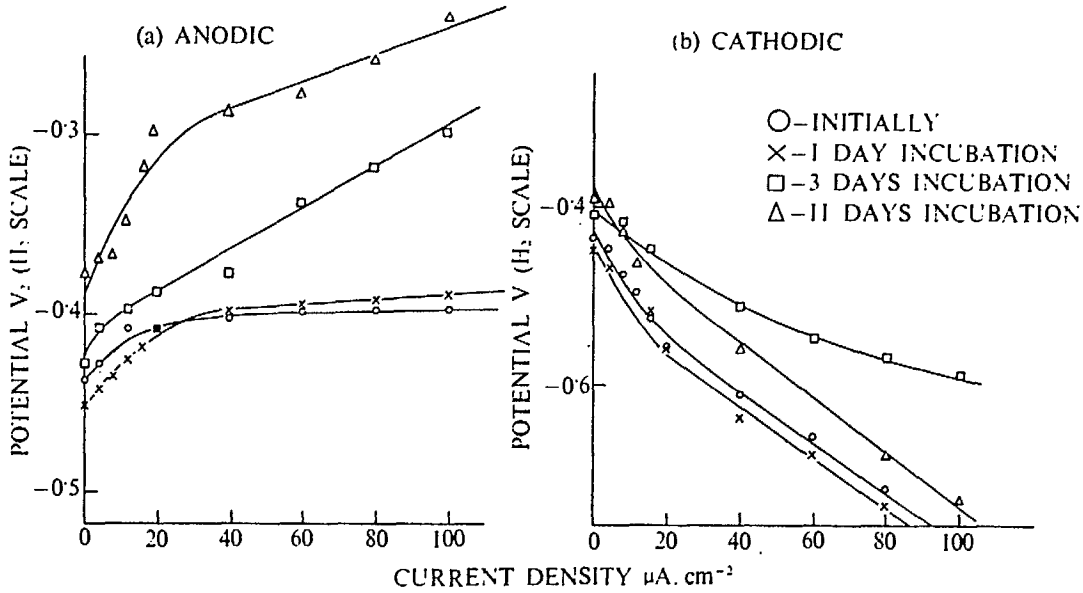


Fig 3.7 *Polarization curves for mild steel at 30°C in cultures of* Desulfovibrio desulfuricans (*strain El Agheila Z_1, a hydrogenase-positive strain*), *showing again the formation of a protective sulphide film, and cathodic depolarization during active growth* (*Crown Copyright reserved*)

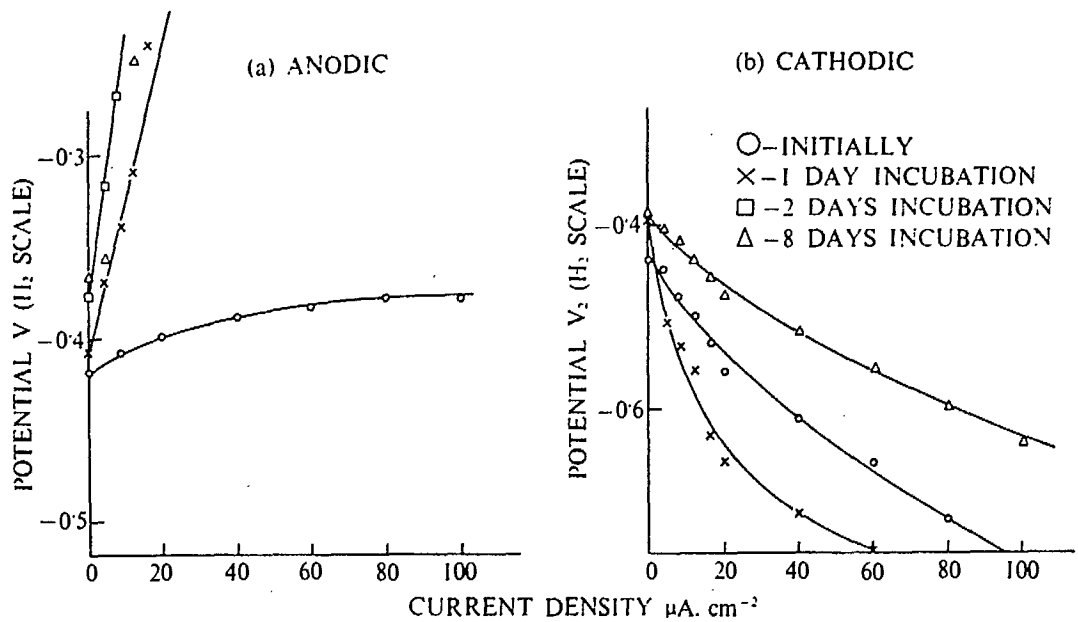


Fig. 3.8 *Polarization curves for mild steel at 30°C in cultures of* Desulfovibrio salexigens (*strain El Agheila C*) (*Crown Copyright reserved*)

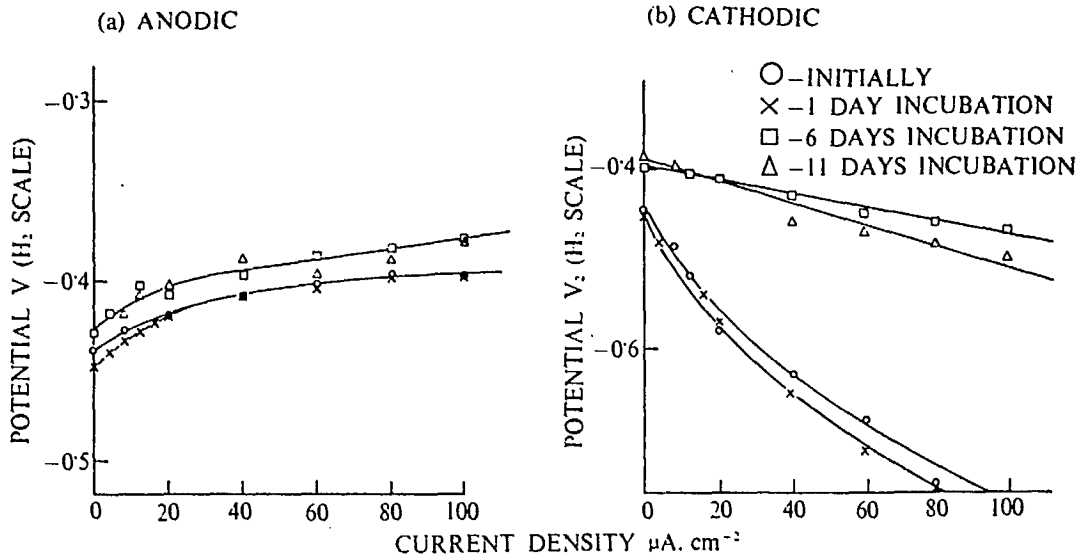


Fig. 3.9 *Polarization curves for mild steel at* 30°*C in cultures of* Desulfovibrio salexigens (*strain California* 43:63) (*Crown Copyright reserved*)

previously examined strains, with its $-Q_{H_2}^{BV}$ than its $-Q_{H_2}^{SO_4^{2-}}$; in fact, no hydrogenase activity by this strain towards sulphate has ever been demonstrated in the Manchester laboratories.

Booth and Tiller concluded that the ability of a sulphate-reducing bacterium to utilize hydrogen, for whatever purpose, is the criterion for anaerobic microbial corrosion of ferrous metals, and that the earlier equation of corrosion with sulphate reduction is unwarranted.

Recent Developments

Although the work just described undoubtedly confirmed that the sulphate-reducing bacteria play an important role in the corrosion of ferrous metal, the *rate* of corrosion in all these experiments was low compared to those often reported in metal objects exhumed from natural situations; this was attributed to the small amount of chemical activity of the bacteria in batch culture experiments in which there is only a limited supply of nutrients. This objection was met to some extent by determining the rates of corrosion of mild steel in semicontinuous[17] and continuous[18] culture. (Semicontinuous culture is a technique in which a portion of a culture is run off aseptically at intervals and replaced by an equal volume of sterile growth medium. If this operation is designed to give a long mean residence time, for example by removing one-fifth of the culture daily and topping up[17], the organisms grow out of the exponential phase of growth into the stationary phase – even at the unusually slow rate at which the sulphate-reducing bacteria grow – and are subsequently "rejuvenated" by each addition of medium. In true continuous culture, on the other hand, the organisms rapidly reach a steady state).

In the early stages of semicontinuous culture experiments the corrosion rates were relatively low owing to the formation of a protective sulphide film, but after several weeks of exposure to the bacterial action these films fractured and became detached, and the corrosion rate then markedly increased. Once the sulphide film had become detached, either wholly or in fragments, no further film formation occurred. The time interval before the film became detached depended to some extent on the strain of organism present, but in general the detachment process began after 20–30 weeks. Thereafter the corrosion rated increased up to sixfold, but still tended to be lower than those encountered in natural conditions. In these semicontinuous experiments the direct correlation between corrosion and hydrogenase activity disappeared, although hydrogenase-positive strains were more corrosive than the single hydrogenase-negative organism (*Dt. orientis*) tested.

In the continuous culture experiments, Booth, Cooper and Cooper[18] found that if the culture medium contained high concentrations of ferrous iron all strains appeared to influence corrosion to the same degree, corrosion/time curves were linear, and there was no correlation again between corrosion rates and hydrogenase activity. The corrosion rates were high: values of the order of 220 mg dm^{-1} day^{-1} were obtained. Under these conditions no real film formation occurred on the metal, but instead a bulky black cocoon of corrosion product formed around the metal specimen. An interesting feature of this corrosion product is that it consisted of an equimolar mixture of ferrous sulphide and ferrous carbonate (siderite, $FeCO_3$). This has been discussed elsewhere[19].

Semicontinuous cultures in high-iron medium (0·5% crystalline ferrous ammonium sulphate)[20] gave broadly similar results to the high-iron continuous experiments.

At about the same time it was discovered that vigorous depolarization of cathodes was effected by ferrous sulphide itself, and that once such a bulky layer had formed in semicontinuous culture in high-iron medium its depolarizing activity continued for a long period after change-over to a low-iron medium[21]. In addition it has been shown that chemically prepared ferrous sulphide can act as a depolarizing agent in polarization experiments in the absence of bacteria[22], and stimulates the uptake of molecular hydrogen for bacterial sulphate reduction in manometric experiments[23]. That ferrous sulphide plays an important role in anaerobic corrosion is therefore certain.

Mara[24] recently found, using a continuous culture technique in minimal-iron medium, that the time for breakdown of the protective sulphide film to occur was dependent on the growth rate of the bacteria (governed by the dilution rate), as was the structure of the film. After film breakdown, the corrosion rate was high and apparently not dependent on growth rate. In high-iron medium, corrosion rate was independent of growth rate except when the latter was very low.

To attempt to establish that hydrogenase is an important contributory

factor in the corrosion process entailed avoiding the complications due to the presence of sulphide ion. Utilizing the discovery that certain strains of the sulphate-reducing bacteria can grow on fumarate in sulphate-free medium[25], it was shown that actively growing cultures of hydrogenase-positive strains did indeed cause depolarization, although in this system the corrosion rates for mild steel were very low[22]. In this connexion, it is interesting to note that several workers have tried to determine whether hydrogenase-positive organisms other than the sulphate-reducing bacteria can contribute towards anaerobic corrosion by causing depolarization. Findings have been inconsistent: Booth, Elford and Wakerley[26] concluded that neither hydrogen bacteria nor methane bacteria participate significantly in this process, while Mara[24] recorded active depolarization by suspensions of certain photosynthetic and non-photosynthetic microbes.

The available evidence, then, suggests that the factors controlling anaerobic microbiological corrosion are (*a*) the utilization of hydrogen by sulphate-reducing bacteria (and possibly by other microbes that possess a suitable enzyme system); (*b*) cathodic depolarization by precipitated ferrous sulphide; (*c*) the prevention of formation of protective sulphide films in the presence of excess ferrous ions; (*d*) anodic stimulation by the sulphide ions; and perhaps (*e*) the formation of local concentration cells. It would seem that (*a*) and (*b*) are of most importance, and are perhaps interrelated to some extent in a way that is not yet fully understood. Factor (*b*) is dependent upon the concentration of ferrous ions in the system, and so this latter may well have some bearing on the corrosion behaviour of buried metals and could therefore be a significant factor in determining the potential aggressiveness of a soil. Indeed the data presented in Tables 3.3 and 3.4 indicate that corrosion occurred in a soil having a mean ferrous ion content over a year of 333 μg/g soil, but not in a soil of mean content 59 μg/g.

Corrosion in Natural Environments

Corrosion in the Soil

As we have already seen, underground corrosion of unprotected ferrous metal objects is often most severe in wet clay or clayey soils of about neutral pH. Progressive pitting corrosion is common: cast-iron pipes of wall thickness $\frac{1}{4}$ inch (0·63 cm) have occasionally become perforated within a year of installation in these conditions, while perforation within four years is quite common. Such corroded objects, if examined immediately on removal from the soil, have a black corrosion product frequently smelling of H_2S (or which liberates H_2S on treatment with acid: this should be used on the site as a test to distinguish the corrosion product from black magnetite). The sulphide-containing corrosion product is often loose, and when lifted reveals pits lined

with bright metal (the anodic areas). A type of corrosion peculiar to cast iron is *graphitization*: in certain areas the iron has been leached out leaving a matrix of graphite, often indistinguishable from the rest of the iron pipe except on closer inspection, but soft enough to be pierced by sharp instruments or to give way under pressure. Typical examples of pipes that have corroded in the sort of conditions just described are shown in Figs. 3.10 and 3.11.

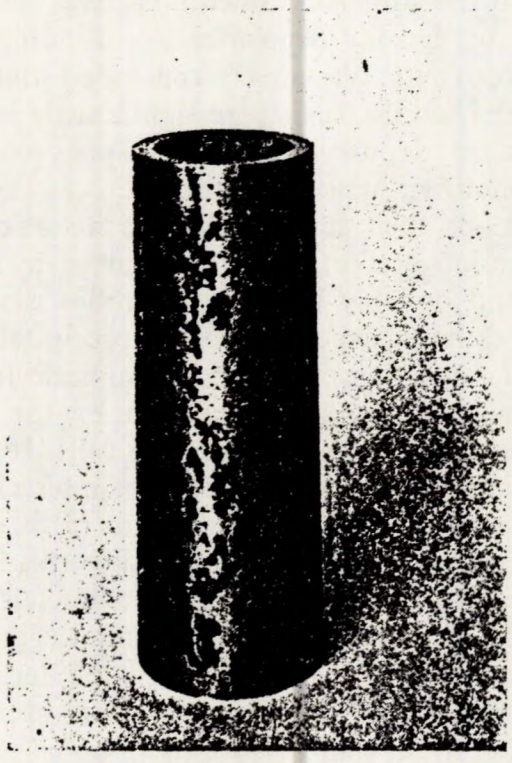


Fig. 3.10 *Cast iron pipe section turned to show pitting by bacterial corrosion (Crown Copyright reserved)*

When these symptoms are discovered in such environments, it can be assumed that the sulphate-reducing bacteria are probably responsible; and they can in fact usually be demonstrated in the corrosion product and the soil in the vicinity of the pipe in very much larger numbers than the average "background" count for that district. In other words, the presence of a ferrous object would appear to have rendered the local conditions more favourable for the growth of sulphate reducers, perhaps by providing a supply of cathodic hydrogen or of ferrous ions which render the redox potential more negative. Once the bacterial growth has received a slight stimulus in this way, it probably becomes a self-reinforcing process: a little sulphide ion will be produced which in turn further depresses the redox potential and stimulates more growth. This can only be regarded as conjectural, though, until more studies are made.

There have been several attempts to pinpoint the physical and chemical

Fig. 3.11 *Cast iron pipe showing penetration by bacterial corrosion (Crown Copyright reserved)*

factors that influence corrosion in soils. Butlin and Vernon[27] suggested that six main factors were responsible, and of these they considered that four were characteristics of the soil: anaerobic bacteria, differential aeration, the chemical nature of the soil (particularly the acidity and the salt content), and the formation of concentration cells. A different point of view was taken by Starkey and Wight[28], who considered that the activity of sulphate-reducing bacteria ought to reflect the redox potential of the soil. From their studies they compiled the following table:

Soil E_h (redox potential on hydrogen scale)	Corrosivity
$<$100 mV	severe
100–200 mV	moderate
200–400 mV	slight
$>$400 mV	non-corrosive

Romanoff[29], in a useful review of underground corrosion in 1957, regarded the important factors as being aeration, the nature of the electrolyte, electrical factors such as the number and arrangement of anodic areas, and miscellaneous in which he included the presence of sulphate reducers. According to Romanoff, soil resistivity correlates well with the corrosiveness of soils; other

workers have stressed the importance of pH or water content, while Stratfull[30] has shown a close correlation between soil aggressiveness, an "optimum" water content and a minimum resistivity.

Attempts have also been made to correlate soil aggressiveness with counts of viable sulphate-reducing bacteria. The relative merits of these criteria are discussed in the next section.

Evaluation of Criteria of Soil Aggressiveness

It became clear that insufficient data were available to establish the reliability of any one, or any combination, of the proposed criteria of soil aggressiveness. Nevertheless such reliable criteria would be extremely useful to a corrosion engineer, and therefore Booth and colleagues[31,32] devised a large-scale investigation from which it was hoped to determine the validity of various criteria. This study entailed periodic measurements of a number of factors at 87 sites distributed over England and Wales. The corrosivity of 59 of these sites was already documented by local authorities, gas and water boards etc., and of these 59 sites, 41 were known to be aggressive.

The following tests were made periodically over five years. At the sites, soil resistivity (indicative of the likelihood of oxidative corrosion) and soil redox potential (considered indicative of the risk of bacterial corrosion) were determined. The latter of course required pH determinations; these were found to be more reliable when made in the laboratory on soil samples collected in polystyrene jars with tightly fitting lids. The soil samples were also used for laboratory determinations of water content, soluble iron concentration and hydrogen uptake, and for qualitative and quantitative tests for sulphate-reducing bacteria.

Tables 3.3 and 3.4 show typical sets of measurements obtained from a corrosive and a non-corrosive site respectively; and Figs. 3.12 and 3.13 show resistivity and redox potential readings taken during the survey and plotted as histograms.

Consideration of all the data in relation to the history of the sites, when known, suggested that the aggressive sites were characterized by a mean soil resistivity of less than 2000 ohm.cm or a mean redox potential more negative than +400 mV with respect to the normal hydrogen electrode when corrected to pH 7 (+430 mV in the case of a predominantly clay soil). These criteria would predict that 38 sites were aggressive out of the 41 that were actually known to be. They were also used to predict that six of the 28 sites of unknown aggressiveness would prove aggressive; this prediction was subsequently confirmed.

It is interesting to note that in the two histograms (Figs. 3.12 and 3.13), in which the aggressive sites are subdivided according to whether the aggressiveness was attributed to low resistivity or to low redox potentials, the critical resistivity value of 2000 ohm.cm agrees exactly with the value below which

Table 3.3 *Results of tests on a soil known to be aggressive (Crown Copyright reserved)*

Name of site: Haverfordwest.
Description of soil: Heavy, black, waterlogged and marshy soil.
Corrosion history: Corrosion of pipelines recorded by Wales Gas Board

	Resistivity Ω-cm at 3 ft	Redox-potential, V at pH = 7 (N.H.E.)	Water content, %	'Bottle test', days to blacken		Viable count of sulphate-reducers, cells/g	H_2 uptake, ml	pH	Fe, μg/g
				Baars' medium	Baars' medium + cysteine				
January	2,400	0·060	53	9	3	10^5	—	6·0	420
February	3,260	0·040	66	9	3	10^4	—	6·0	1,000
March	1,630	0·040	59	9	2	10^6	—	6·1	104
April	2,300	0·070	52	5	6	10^4	—	5·9	250
May	2,010	0·132	61	4	4	10^4	—	4·3	420
June	1,680	0·065	49	13	4	10^5	41	5·5	166
July	1,150	0·150	46	6	3	10^6	—	6·5	86
August	1,680	0·070	51	11	4	10^5	55	6·0	325
September	2,400	0·070	44	7	2	10^4	—	6·2	390
October	1,150	0·160	49	8	3	10^7	40	6·7	42
November	2,300	0·100	54	7	3	10^6	—	6·6	120
December	3,350	0·070	53	12	4	10^3	51	6·0	720
Mean	2,110	0·064	53	8	4	10^5	47	5·9	333

Table 3.4 *Results of tests on a soil known to be non-aggressive (Crown Copyright reserved)*

Name of site: Heston.
Description of soil: Light brown loam with good crumb formation.
Corrosion history: No incidence of corrosion reported by Metropolitan Water Board.

	Resistivity, Ω-cm at 3 ft	Redox-potential, V at pH = 7 (N.H.E.)	Water content, %	'Bottle test', days to blacken		Viable count of sulphate-reducers, cells/g	H_2 uptake, ml	pH	Fe, μg/g
				Baars' medium	Baars' medium + cysteine				
January	4,790	0·528	13	6	3	10^5	—	5·8	40
February	9,580	0·350	14	8	4	10^5	—	5·3	40
March	4,790	0·504	18	10	3	10^4	—	5·9	130
April	9,580	0·466	16	neg.	32	10^3	0	5·3	0
May	4,790	0·550	16	7	3	10^4	—	7·4	54
June	7,660	0·530	14	5	4	10^5	—	5·3	50
July	9,580	0·540	10	6	3	10^4	—	6·0	50
August	26,820	0·548	10	8	3	10^4	0	5·7	30
September	8,620	0·510	14	9	2	10^4	25	7·3	66
October	14,370	0·550	16	5	2	10^4	—	5·8	71
November	9,580	0·520	18	6	3	10^4	—	6·5	45
December	4,790	0·560	15	7	2	10^4	0	6·3	134
Mean	9,560	0·513	15	7	5	10^4	6	6·0	59

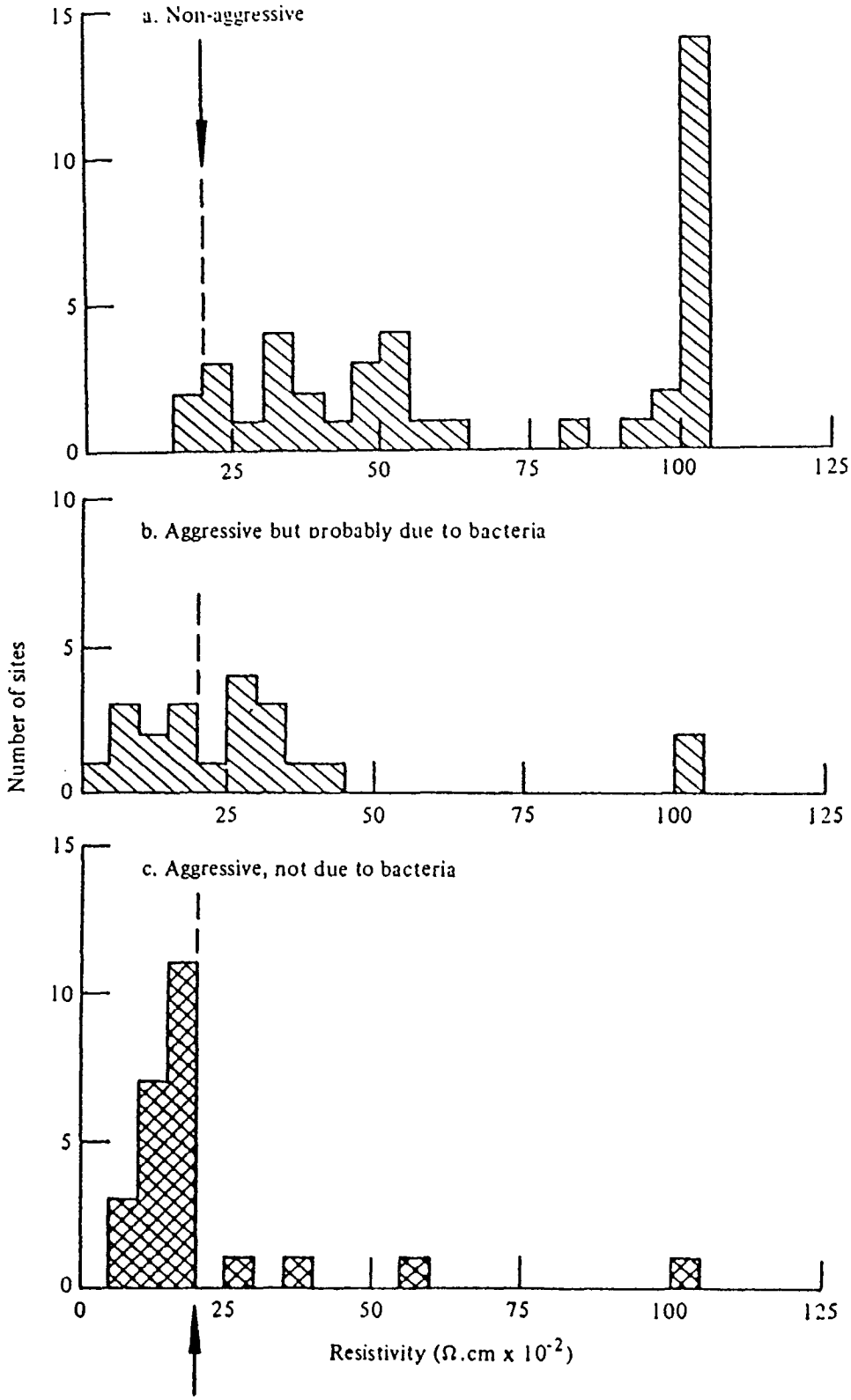


Fig. 3.12 *Electrical resistivity of soils (for explanation see text) (Crown Copyright reserved)*

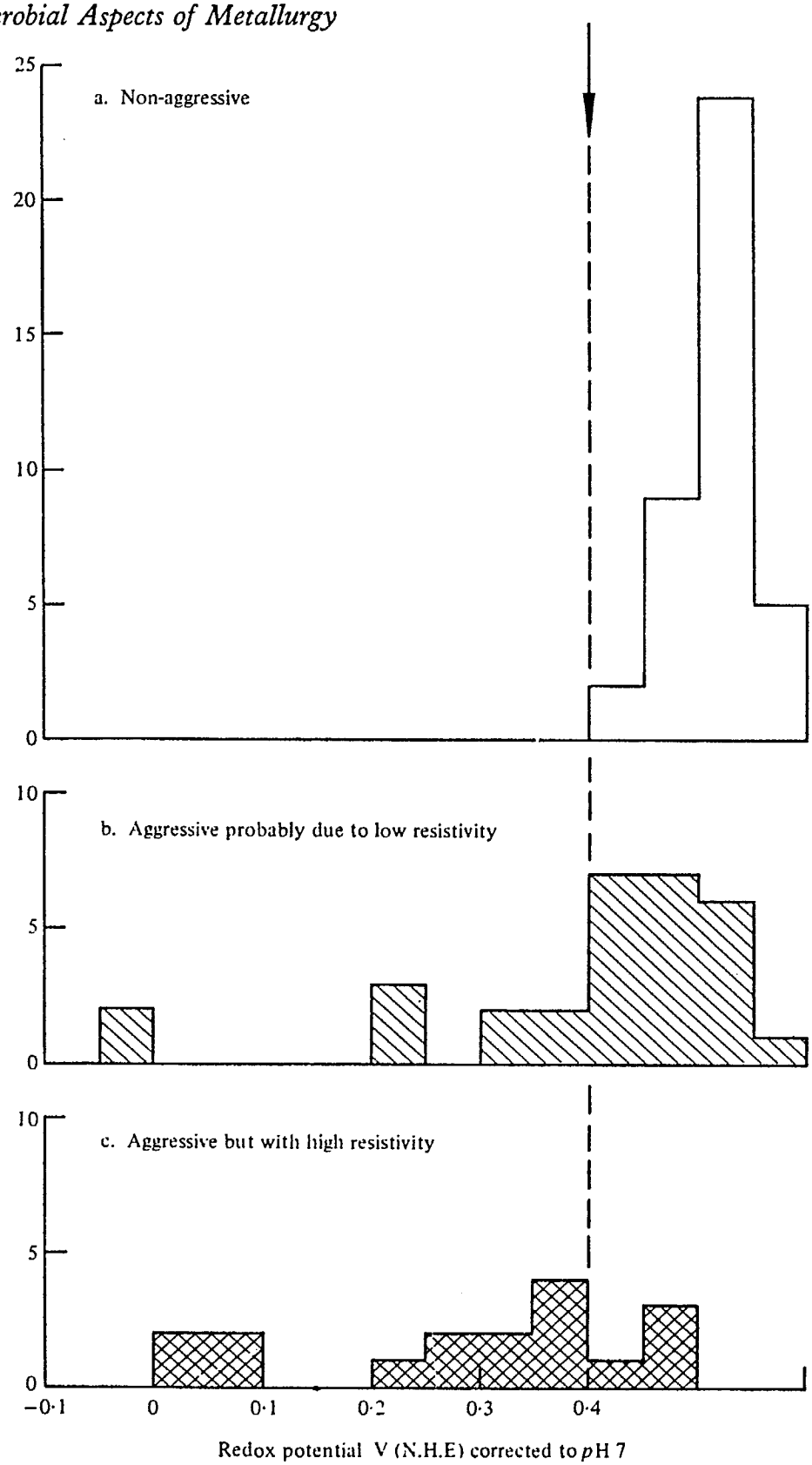


Fig. 3.13 *Redox potential of soils (Crown Copyright reserved)*

Schwerdtfeger[33] recommended protection, and that the value of 430 mV for critical redox potential is very close to the figure for non-aggressiveness given by Starkey and Wight[28] and quoted above.

When a soil was considered a "borderline" case because its resistivity was close to 2000 ohm.cm or its redox potential was within the range 400–430 mV, it was classified according to the water content: if this was greater than 20% the soil was regarded as aggressive, and if below this figure, non-aggressive. One borderline case, however, was not correctly resolved by this method, and it was considered that other factors were operating at this site which could not be detected.

Four sites did not conform at all. Of these, two were assessed as aggressive; since no industrial pipework was buried at one of them the prediction could not be confirmed, while the only pipework buried at the second site was situated on an embankment in made-up ground (a non-indigenous substratum composed of a mixture of soil and clay excavated from other sites in the district plus various kinds of rubbish, and often highly corrosive). The remaining two sites had been predicted as non-aggressive, but the local authorities reported several cases of corrosion occurring in buried pipelines; this was attributed to the fact that most of the breakdowns occurred on a housing estate where a great deal of made-up ground was interspersed with the virgin soil.

As regards hydrogen uptake by the soils, higher values were found in the more aggressive soils. Similarly, all soils with a mean soluble iron content greater than 120 μg/g were aggressive. Interesting though these results were because of their fulfilment of predictions from laboratory work, the estimations themselves were time-consuming and their use in diagnosing aggressive soils is not recommended.

Sulphate-reducing bacteria were present in all the 87 soils studied. The quantitative tests showed that the viable count was higher – often by several orders of magnitude – in soils of low redox potential. Since, however, it is much simpler to make an E_h measurement (though no satisfactory probes are available, and some practice is required with home-made ones constructed to the specifications in reference (31) to obtain meaningful results) than to carry out bacterial tests, the E_h measurement is recommended. In cases where resistivity and E_h measurements cannot be made *in situ* for various reasons, however, other methods such as bacteriological growth tests may have to be resorted to.

The reliability of the prognostic tests was confirmed by a programme of work[34] in which previously weighed plates of mild steel, aluminium, copper, zinc and lead were buried at 12 sites that had already had their aggressiveness predicted by resistivity, redox potential and water content measurements. Periodic measurements of metal-to-soil potential (with reference to a copper/copper sulphate electrode) and redox potential of both the bulk of the soil and of that within a few inches of each specimen were made, and the speci-

mens were exhumed after 1, 3 or 4 years for weight-loss determination.

The predictions based on the criteria previously recommended[32] were found to be valid for mild steel except in two cases where previously unnoticed local conditions had caused more corrosion than was anticipated. The method was also shown to be fairly satisfactory for lead and zinc, but of relatively little use for predicting aluminium corrosion. None of the soils was aggressive towards copper. The metal-to-soil potential measurements gave little information of value.

The relationship between soil type and corrosion was brought out clearly in these investigations, as is shown in Table 3.5.

Table 3.5 *Weight loss of metal plates during a year's burial in various types of soil (Crown Copyright reserved)*

Type of soil	Weight loss ($mg/dm^2/day$)				
	Mild steel	Al	Cu	Zn	Pb
Clay	9·7	0·1	0·7	13·1	1·2
Clay-loam	11·3	0·4	0·9	2·8	4·1
Loam	5·6	0	0·3	3·3	0·4
Chalk	5·3	0·03	0·3	7·7	0·3

Corrosion in Rivers

The tidal waters of the River Thames were tested for corrosiveness by exposure of pre-weighed mild steel plates for periods of up to five years, and attempts made to correlate corrosiveness with physical, chemical and biological factors[35,36,37]. The plates were mounted in such a way that they were buried in the bottom mud, permanently immersed or half immersed (by being supported in floating frames) or alternately immersed and exposed to the air by tidal action. When the plates were removed, weight loss was determined and the corrosion product analysed. Water samples were taken for analysis and for counts of sulphate-reducing bacteria.

Plates exposed for one or two years showed both aerobic and anaerobic (microbiological) corrosion. Maximum weight loss and depth of pitting occurred in those plates that had been immersed in the most polluted part of the river. X-ray diffraction patterns of the corrosion product revealed the presence of α-$Fe_2O_3.H_2O$, γ-$Fe_2O_3.H_2O$ and Fe_3O_4. FeS and $FeSO_4$ were also sometimes detectable. That they were not found more often was attributed to their complete removal after oxidation during the cyclical shifts to more oxygenated conditions in the river.

These coatings of corrosion product appeared to confer protection upon the metal specimens: the rate of weight loss gradually decreased over the next three years. During that period the river also became less polluted, and

the salinity of the estuarine water then proved to be the most important single factor governing corrosion.

The corrosion product on metal plates from the bottom mud was, in all cases but two, a mixture of FeS, $FeSO_4$ and Fe_3O_4, indicating that sulphate-reducing bacteria are highly active in such situations. The bacteria themselves were demonstrated in all the water samples taken, though the count ranged from about 10 organisms per ml at the nearest point to the open sea from which samples were taken (where pollution was low and dissolved oxygen concentration high) to about 10^5 per ml in highly polluted reaches of the river. In addition, sulphate reducers were demonstrated in the corrosion product from all plates.

It will be seen, then, that the corrosion picture is very complex in an environment such as the lower reaches of a river with its variation in salinity and level of pollution and seasonal variation in oxygen content. Nevertheless there is clearly a significant contribution by the sulphate-reducing bacteria, particularly in the bottom mud, while even in aerated waters there is evidence that sulphate reducers participate in corrosion, presumably by finding favourable micro-habitats in the corrosion product already present.

The Prevention of Corrosion of Buried and Immersed Metals

Introduction

There are various approaches to the problem of preventing underground and underwater corrosion, though all suffer from certain practical disadvantages so that there is no panacea. The following scheme for classifying these approaches is a modification of that of Hoar[38]; it was devised with underground installations in mind, but can be applied to aqueous situations where appropriate.

Methods of preventing or retarding underground corrosion:

1. Using non-corrodible materials.
2. Using corrodible materials
   - (*a*) in a non-aggressive environment
   - (*b*) in an aggressive environment
     - (i) giving the installation a non-aggressive surround
       1. by arranging that the environment contains nothing that will accept electrons or cations.
       2. by using a biocide in the environment to prevent the growth of sulphate-reducing bacteria.
     - (ii) making the metal sufficiently negative with respect to its environment to prevent cations escaping into it.

(iii) arranging a barrier, impervious to cations and/or electrons, between the metal and its environment

1. by application of protective coatings.
2. by the spontaneous or controlled development of conversion coatings or passive films on the metal.
3. by the spontaneous deposition on to the metal of various protective substances such as calcareous scale.

Most of these methods are dealt with in more detail below.

Use of Non-corrodible Materials (Method 1 in the above scheme)

Clearly the problem of underground corrosion could be avoided altogether by the use of chemically inert materials of adequate strength. High-silicon iron and austenitic chromium-nickel steels show high chemical resistance, but the former is too brittle for most purposes while the austenitic steels are very expensive. Copper also falls in the latter category. (Strictly speaking, stainless steel comes into category 2(b)(iii)2). Non-metallic materials that have proved satisfactory for pipes despite their low tensile strength include asbestos cement and plastics such as PVC and polyethylene. The plastics used should be free from plasticizers, since there is evidence that these can be microbially degraded.

Concrete is quite often used as an expensive alternative to steel for piles (a report by Romanoff[39] that steel piles suffer much less corrosion than pipelines should be treated with caution in view of catastrophic corrosion of piles in aggressive soil in Australia); but concrete suffers from attack in acid soils, in high sulphate ion concentration, and sometimes through the action of the sulphur-oxidizing bacteria.

No material can approach the tensile strength of mild steel on a cost basis, and hence it is likely to remain the most used material in the foreseeable future for the structures we are considering.

Construction of a Non-aggressive Surround (Method 2(b)(i)1)

Theoretically, Method 2(*b*)(i)1 demands that (*a*) no electron acceptors (the principal ones being molecular oxygen and hydrogen ions) should be present, and (*b*) water should be absent, to prevent cations forming from anodic metal. These conditions are obviously impossible to achieve in natural environments; and in fact, as we have already seen, the absence of oxygen will, if sulphate-reducing bacteria are present, itself result in severe corrosion. A fruitful approach is to aim for alkaline conditions and for good drainage – the latter to ensure oxygenation, and hence suppression of the activity of sulphate reducers, rather than to attempt to reduce anodic dissolution.

Such a non-aggressive surround for a pipeline can be made by completely surrounding it with at least 25 cm of chalk. Sometimes a chalk/sand mixture

is used for cheapness; or alkalinity may be dispensed with altogether by using gravel, rubble or clay-free sand. In all cases gradients must be controlled and soakaways provided at low points.

Use of Biocides (Method 2(b)(i)2)

In general, it would not be expected that a biocide, applied locally around a pipe before backfilling, would persist for long enough to be effective against sulphate-reducing bacteria for more than a fraction of the desired life-span of the pipeline, unless the substance were only slightly soluble and yet bacteriostatic at that low concentration even in the presence of organic matter etc. Nevertheless, a study reported in Chapter 4 under *Pipeline Corrosion* indicates that microbial corrosion could be inhibited by experimental treatment with a biocide. More studies could profitably be made on this aspect of corrosion control.

In closed (or relatively closed) industrial systems, of course, the control of sulphate-reducing bacteria with biocides is easier, though as will be seen in Chapter 4 the programme of treatment in these complex environments with mixed microfloras usually has to be determined empirically.

Cathodic Protection (Method 2(b)(ii))

The principle of this method is to make the metal-to-environment potential of the structure to be protected so negative that cations cannot pass into solution from the metal. The structure is therefore made cathodic with respect to another electrode installed for the purpose in the neighbourhood. Two methods are used. Sacrificial anodes of a baser (more electronegative) metal than iron – magnesium, aluminium or zinc – may be employed; the relatively low p.d. that can be obtained limits the effective range of a sacrificial anode, but there is the compensating advantage of minimal interference with other installations. The alternative method is to construct a large anode or ground-bed, often of graphite, fairly remote from the structure to be protected, and apply an e.m.f. (for example, from the mains *via* a rectifier) between the two. Theoretically a carbon ground-bed should be everlasting, but in practice occasional renewal is needed.

The choice between installation of sacrificial anodes and the use of an impressed e.m.f., then, usually depends on local conditions such as soil resistivity and availability of a source of cheap electric power. The correct application of cathodic protection requires an accurate knowledge of the potential of the structure relative to the corroding medium (soil or water) both before and after the application of the current. The standard way of measuring pipe potentials is by the copper/saturated copper sulphate electrode; and the standard practice in cathodic protection is to position the anodes or adjust the resistance of the circuits so as to depress the potential

of steel to about −0·85 V relative to that electrode, equivalent to −0·53 V (NHE).

Cathodic protection can also prevent corrosion by the sulphate-reducing bacteria, but here a more negative potential is required. Horváth and Novák[10] calculated the requirement in presence of sulphide; a value of −0·95 V ($Cu/CuSO_4$ electrode) is usually accepted, though these authors showed that in some cases an even more negative potential may be required.

The alkalinity of the catholyte probably assists by suppressing the growth of sulphate reducers; but on the other hand over-protection will result in over-alkalinity which in turn can damage certain types of protective coatings (of which more below), causing blistering and peeling.

Investigations of the cathodic characteristics of mild steel in the presence and absence of sulphate-reducing bacteria by Booth and Tiller[11] have provided evidence in favour of the existing practical criterion for cathodic protection in situations with bacterial activity. The relationships between hydrogenase activity, quantity of cathodic hydrogen liberated and utilized by the bacteria, and the amount of iron corroded at the anode, were studied in the following way. A mild steel electrode was maintained at a constant potential and the current supplied from the potentiostat to maintain the potential, and the loss of iron (determined by analysis of the anolyte) from a counter electrode, were recorded against time. The equivalence between anolyte iron and coulombs drawn from the potentiostat was determined.

Using washed non-growing suspensions of *Dv. vulgaris* strain Hildenborough (hydrogenase-positive) and *Dt. orientis* (hydrogenase-negative) in an electrochemically inert buffer of high conductivity with benzyl viologen as electron acceptor (thus avoiding complications due to growth, concomitant depletion of medium, and sulphide ion and FeS formation), the rate of change in potential due to reduction of the dye to reach the mid-point potential was recorded.

In the absence of the reducible substrate, the depolarization of the cathodes was independent of the hydrogenase activity of the bacteria whereas in its presence the cathodic depolarization was proportional to the hydrogenase activity. The rate of reduction of the substrate was also proportional to the hydrogenase activity. As an example, the curves obtained at a working potential of −0·85 V (NHE) are shown in Fig. 3.14. These results provide evidence in favour of the theory[5] that sulphate-reducing bacteria act as depolarizing agents.

If the logarithm of the extra current required to keep the cathode polarized in the presence of the bacteria over that required in buffer alone (log Δi) is plotted against enzymic activity of the organisms at each controlled potential, a straight-line relationship is found (Fig. 3.15) similar to that of a Tafel plot; this indicates that in the presence of a reducible substrate log Δi is proportional to the hydrogenase activity ($-Q_{H_2}^{BV}$). This is particularly striking at the lower controlled potentials of the electrode; and if the slopes of these lines

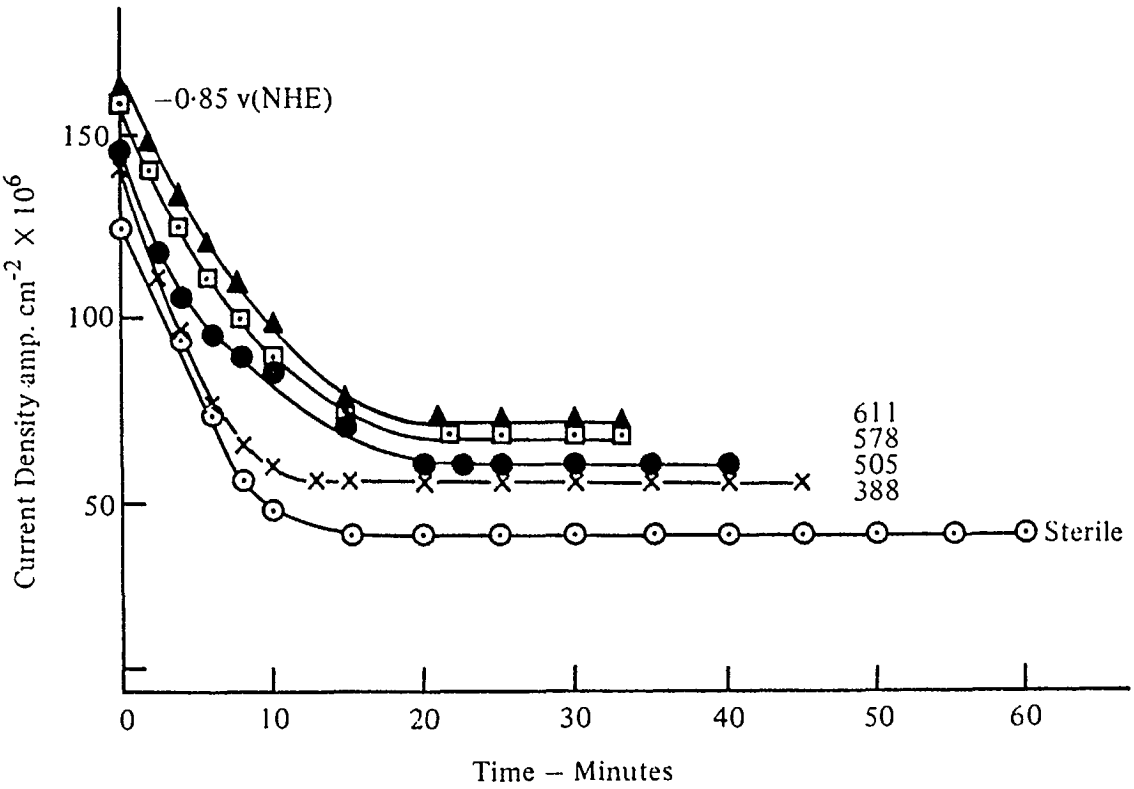


Fig. 3.14 *Current/time curves for* Dv. vulgaris *system with benzyl viologen as electron acceptor, at a controlled potential of* −0·85 *V. The numbers against the curves represent values of* $-Q_{H_2}^{BV}$ (μl/mg/h) *(Crown Copyright reserved)*

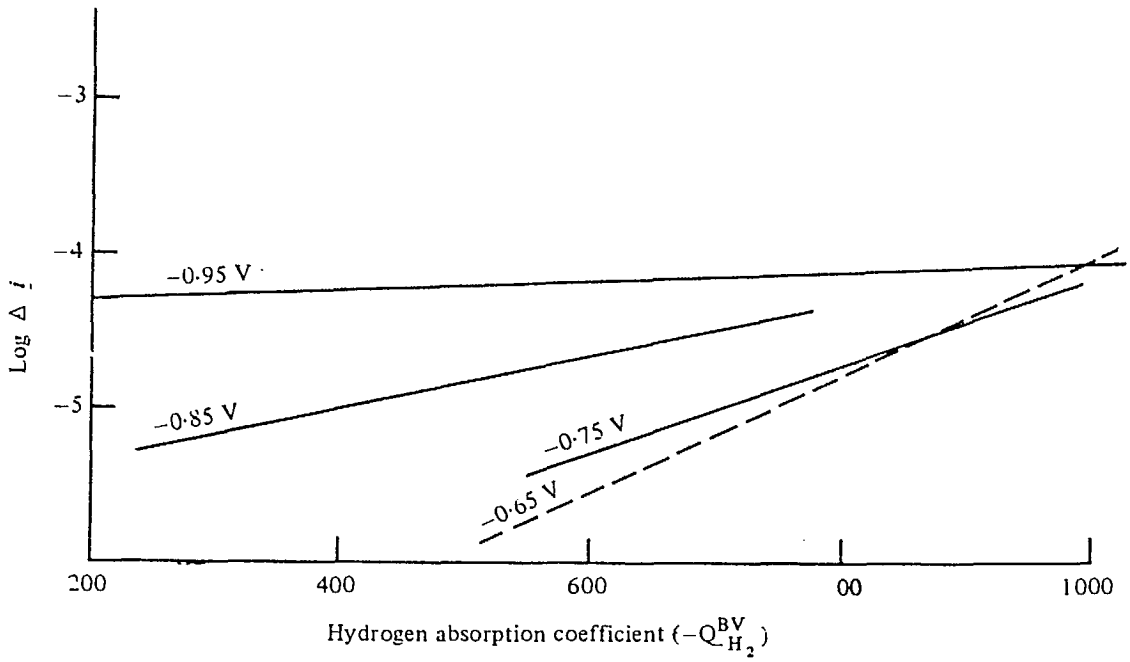


Fig. 3.15 *Relationship between 'bacterial current' and hydrogenase activity for* Dv. vulgaris *at various controlled potentials (Crown Copyright reserved)*

($d(\log \Delta i)/dQ_{H_2}^{BV}$) are plotted against the controlled potentials a straight line is obtained which intercepts the potential axis at −0·98 V (NHE), thus indicating that the extra current Δi is independent of the hydrogenase activity of the bacteria and that the rate of depolarization of a cathode becomes constant irrespective of the enzyme activity of the bacteria.

Dt. orientis, shown to be hydrogenase-negative by Warburg manometry, appeared completely inactive as a cathodic depolarizer in this system.

A significant feature of this work is the effect that the bacteria were found to have on local anodic action on the cathodic electrode, that is, on an incompletely cathodically-protected specimen. Table 3.6 illustrates this. A relationship between the corrosion rate and the controlled potential is also observed if the rate of corrosion of the cathode, W_c, (due to local action) is plotted against the controlled potential of the electrode (Fig. 3.16). Between −0·85 V and −0·65 V there is a linear relationship, and assuming that the linearity persists at more noble potentials, then by extrapolation the corrosion rate at −0·510 V (NHE) in buffer solution is the same as the corrosion rate at −0·618 V (NHE) in the presence of the bacteria. Therefore in the presence of the bacteria the potential must be depressed to −0·618 V (NHE) (= −0·958 V ($Cu/CuSO_4$ electrode)) in order to maintain the same degree of protection as that afforded by a potential 0·1 V more positive in situations free from active sulphate-reducing bacteria. These figures agree well with the generally-accepted values quoted above, and with those produced by other workers such as Whalley[42].

Table 3.6 *Catholyte iron analyses and corrosion rates of "cathode" at various controlled potentials* (*Crown Copyright reserved*)

For explanation see text.

Controlled potential of cathode V (NHE)	Catholyte iron μg/h		Corrosion rate of "cathode" mg/dm²/day	
	Buffer	Buffer + *Dv. vulgaris*	Buffer	Buffer + *Dv. vulgaris*
−0·95	0	0	0	0
−0·85	25·5	33	3·1	3·9
−0·75	52·0	82	6·3	9·8
−0·65	112·5	221	13·5	26·5

It will be observed from Table 3.6 that measurable corrosion occurs at as low a potential as −0·85 V (= −1·19 V ($Cu/CuSO_4$ electrode)) in this system, but such a low protective potential would in all probability be both uneconomic and a possible danger to the structure since hydrogen embrittlement of the metal could occur, while the effects of the alkali produced have already been mentioned.

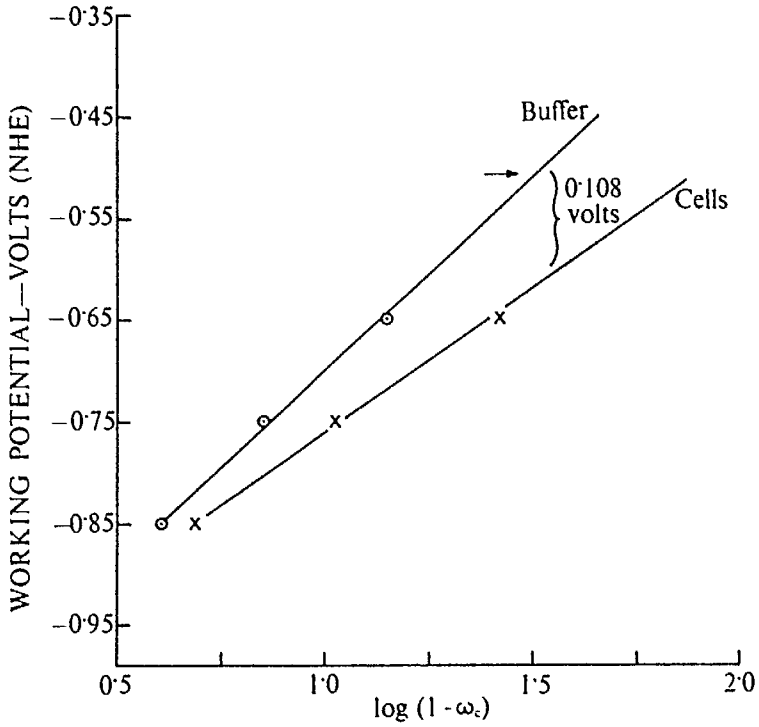


Fig. 3.16 *Relationship between potential and anaerobic corrosion (Crown Copyright reserved)*

The principles of cathodic protection have been dealt with at length by Morgan[43].

Protective Coatings (Method 2(b)(iii)1)

The concept of protective coatings for base metals is by no means new, and yet it is still doubtful whether there is an ideal coating substance that is adherent, coherent, completely non-porous, mechanically resistant to the hazards encountered during delivery, laying and backfilling and chemically resistant to prolonged contact with all kinds of natural environments.

Coal tar-based materials and asphaltic bitumen-based materials are frequently used; the former are said to be more stable and waterproof. It has long been suspected that organic reinforcements to these coatings, such as hessian, constitute if anything a weakness, and it was later demonstrated that they could be attacked and broken down by cellulose-decomposing microbes. A much better reinforcing material for bituminous coatings and linings is fibreglass. All joints, valves etc. in such protected pipelines must be coated with bitumen after assembly, and damaged parts of the original coating made good: corrosion is often extremely rapid at small discontinuities or "holidays" in protective coatings.

Concrete linings have sometimes been applied to pipes. Apart from their

alkalinity they have rather little to offer in the way of protection, being usually permeable to air, water and stray currents unless they are very thick – and hence expensive. Corrosion of the encased metal is particularly likely to occur at interfaces between different castings of the concrete.

The coating of steel pipes with zinc at a rate of 2·8 oz/ft^2 (854 g/m^2) has been stated to prevent appreciable corrosion for 10 years in neutral or alkaline soil. Zinc coatings are unsuited for acid conditions, however, and must not be used in electrolytic contact with a large area of uncoated steel. Spray-applied zinc-aluminium coatings show promise[38]. Lead-coated pipes have been tested; they performed well for a time, but once the coating failed rather rapid corrosion ensued.

Not surprisingly, plastic coatings have received a good deal of attention. The main problems seem to be the bonding of the plastic material to the pipe and the *in situ* coating of joints. Fibre-glass resin and epoxy resin coatings are stated to be highly protective, though expensive. A recent development dispenses with the property of adhesion, formerly considered essential for a coating: this is the "bagging" technique in which the pipe is put into a loose plastic sleeve. It is stated to be effective.

Logan and Parker[44] reviewed protective coating systems available in 1965.

The difficulty of achieving a completely unbroken, completely non-porous coating is so great – or, looked at from the other aspect, the expense of applying cathodic protection to a large metal installation is so high in terms of power or sacrificial anodes consumed – that for best results in an economic context a combination of these methods is often employed.

Other Types of Microbial Corrosion

Microbial Corrosion due to Acid Production

An economically important group of bacteria, the thiobacilli, are dealt with several times in this volume. They obtain energy from the oxidation of sulphur and its more reduced compounds including H_2S (see Chapter 1, and Chapter 4 under *Combined Action of the Sulphur Bacteria in Metallic Corrosion*), and in addition the species *Thiobacillus ferro-oxidans* can oxidize ferrous iron. The free sulphuric acid that certain species produce (or which, in the case of ferrous iron oxidation, arises as a secondary reaction as explained in Chapter 7) can naturally cause serious corrosion of metals underground: it is stated that they can produce, and remain viable in, solutions of 10–12% H_2SO_4[45].

These organisms are aerobes (with one exception), and in any case of severe corrosion in aerobic conditions, where the local pH is very low, sulphur-oxidizing bacterial activity should at once be suspected. A case recorded in the literature[46] concerns a gas main – actually bitumen-covered –

that corroded in a peaty soil within 2½ years. In the vicinity of the pipe a pH as low as 2·0 was recorded, and sulphuric acid demonstrated. At lower, oxygen-free levels in the soil were active sulphate-reducing bacteria, from which sulphide was diffusing upwards. This was then oxidized to H_2SO_4 by thiobacilli. Drainage and treatment with lime were recommended: thiobacilli will not grow in alkaline conditions.

Corrosion by acids is dealt with in Chapter 2, and will not be elaborated upon here. It is clearly necessary for protective coatings to be acid-resistant.

Organic acids are produced by a large number of microbes, and their effects may sometimes be serious. It has already been mentioned under *Protective Coatings* that cellulose-decomposing microbes damage hessian wraps. The hessian is broken down to give lower fatty acids such as acetic and butyric under anaerobic or partially anaerobic conditions. Pitting of the lead sheaths of buried cables protected with a bitumen and hessian wrap has occasionally occurred by this mechanism; it is known as "phenol corrosion" because it was thought to be due to phenols in the bitumen until its microbial origin was discovered.

Certain moulds produce organic acids in fully aerobic conditions and have been found to be responsible for cases of corrosion of iron, copper and aluminium protected by insulating material that was capable of being used as a growth substrate by the moulds.

Corrosion due to Heavy Microbial Growths and Deposits

Microbes that form large colonial masses or tufts on the substratum on which they grow (for example algae, fungi, protozoa, bacteria) are always potential agents of corrosion, because oxygen gradients and gradients of solutes present

Fig. 3.17. *Advanced stage of tuberculation in a section of cast iron pipe*

in the ambient water and of end-products of the organisms themselves all tend to establish concentration cells. The situation is obviously worse if the organism concerned produces an acid; and the microbiological picture is often very complex as other organisms than the clump-former, such as acid-producers, may become entangled in the growth, or indeed may find it provides good anchorage or a favourable microhabitat. Needless to say, the sulphate-reducing bacteria find suitable conditions in the depths of such a mass of growth, and can cause severe pitting corrosion there.

An example of massive growth is that produced by the iron bacteria, which, together with the ferric hydroxide they produce, can form extensive deposits called tubercles on the inside of iron water pipes. Fig. 3.17 shows an advanced stage of tuberculation in a section of cast iron pipe. Chlorination of the water helps to control tuberculation, confirming its biological origin, but in many cases regular internal cleaning of the pipework has to be resorted to.

APPENDIX

Microbiological Examination of Soil, Water etc. for Sulphate-Reducing Bacteria

Though it may be true that soil aggressiveness is accurately predictable merely from resistivity and redox potential readings, with resolution of border-line cases by determination of water content (and much more work is necessary before this can be finally established), qualitative and quantitative tests for sulphate-reducing bacteria are still an important part of corrosion detection and control. Unfortunately, a fair amount of expertise is necessary, especially for the quantitative tests. The following tests are given for those who have laboratory facilities and a little basic equipment such as an autoclave or pressure-cooker for sterilization.

Qualitative test

The medium in which sulphate-reducing bacteria may most easily be grown is that of Baars, as modified by Postgate[47]. The following is the formulation for 1 litre of this medium (it is best to start with almost a litre of distilled or tap water, adding and dissolving the various reagents, and finally making up to 1 litre): KH_2PO_4, 0·5 g; NH_4Cl, 1·0 g; $CaSO_4$, 1·0 g; $MgSO_4.7H_2O$, 2·0 g; sodium lactate, 3·5 g; yeast extract, 1·0 g; ascorbic acid, 1·0 g; thioglycollic acid, 1·0 g; $FeSO_4.7H_2O$, 0·5 g. Adjust to pH 7·0–7·5. Sterilize immediately after making up, by autoclaving at 15 p.s.i.g. (121 °C) for 15 min. An improvement is to autoclave the appropriate amount of thioglycollic acid separately and add it aseptically to the medium afterwards. This medium should be used immediately after it has cooled: the reducing agents become

oxidized fairly rapidly and thereafter the medium no longer has a redox potential negative enough (about −0·1 V (NHE)) to initiate growth reliably. This applies with even more force to the growth medium for quantitative tests (counts).

Small glass screw-capped bottles of around 10 ml capacity are sterilized, and samples (2 g or 0·2 g) of soil, corrosion product, water etc. placed in them. Sterilized medium is then poured in to fill the bottles completely (the medium has a precipitate, and so should be gently swirled before pouring to mix it thoroughly), and the caps are replaced so as not to trap any air. For examination for *Desulfovibrio* spp., the bottles are incubated at 30° or 37°C; for *Desulfotomaculum nigrificans*, 55°C. In either case blackening of the medium, which usually occurs within 2–3 days, indicates the growth of sulphate reducers. Blackening is due to the formation of ferrous sulphide in this high-iron medium. Note: if the sample is from a marine environment, 2·5% NaCl should be added to this and the following medium.

Quantitative test

This method depends on making serial dilutions of the sample (or of an extract, if it is soil etc.). This is done by preparing and sterilizing a number of cotton-wool plugged test tubes each containing 9 ml of saline (0·5% NaCl in water, or 2·5% for marine samples) or of the medium below with the agar, sodium lactate and yeast extract omitted. Details of this type of microbiological technique, and of the preparation of sterile pipettes, are given in the *Appendix* to Chapter 5.

1 g (+0·01 g because quantitative transfer is impossible) of the soil sample is ground in a sterilized mortar and 9 ml sterile saline gently "ground in" to give a fine suspension. This is the first dilution (1/10) of the original sample. 1 ml of this is taken up in a sterile pipette and run into a tube of 9 ml saline; this is labelled "1/100". The serial dilution is repeated down to say 1/1 000 000.

The medium to be used has the following formulation: KH_2PO_4, 0·5 g; NH_4Cl, 1·0 g; Na_2SO_4, 1·0 g; $CaCl_2.6H_2O$, 1·0 g; $MgSO_4.7H_2O$, 2·0 g; sodium lactate, 3·5 g; yeast extract, 1·0 g; ascorbic acid, 0·1 g; thioglycollic acid, 0·1 g; $FeSO_4.7H_2O$, 0·5 g; agar, 15 g. Distilled water to 1 litre. Adjust pH to 7·6 with NaOH. Autoclave as above; cool to about 42–43°C.

Aseptically pipette 1-ml portions of the 1/100 dilution into each of 3 or 5 sterile test tubes and label appropriately; repeat for each of the higher dilutions. Pour about 9–10 ml of the cooled (but still liquid) medium into each test tube. The pouring process should mix the saline and the nutrient medium fairly uniformly but a further mixing can be given if desired, by rolling the tube between the hands before the medium sets. After setting has occurred, another 1·5 cm depth of medium is added to each tube to reduce access of oxygen to the inoculated medium below. The tubes can then be incubated as described above.

Black colonies develop in 3–16 days. These are counted for each tube, if not too close together to render this impossible, and the most probable number (MPN) read from tables[18].

References

[1]BOOTH, G. H. '*Sulphur bacteria in relation to corrosion*'. J. Applied Bacteriol., **27**, 174–181 (1964).

[2]VERNON, W. H. J. '*Metallic corrosion and conservation*'. In *The Conservation of Natural Resources*, 105–133. London: Institution of Civil Engineers, 1957.

[3]GAINES, R. H. '*Bacterial activity as a corrosive influence in the soil*'. Industrial & Engineering Chem., **2**, 128–130 (1910).

[4]BENGOUGH, G. D. AND MAY, R. '*Seventh report to the Corrosion Research Committee of the Institute of Metals*'. J. Inst. Metals, **32**, 81–269 (1924).

[5]WOLZOGEN KÜHR, C. A. H. VON AND VAN DER VLUGT, L. S. '*De grafiteering van gietijzer als electrobiochemisch proces in anaerobe gronden*'. Water, Den Haag, **18** (16), 147–165 (1934).

[6]STEPHENSON, M. AND STICKLAND, L. H. '*Hydrogenase. II. The reduction of sulphate to sulphide by molecular hydrogen*'. Biochem. J., **25**, 215–220 (1931).

[7]POSTGATE, J. R. '*The reduction of sulphur compounds by* Desulphovibrio desulphuricans'. J. General Microbiol., **5**, 725–738 (1951).

[8]POSTGATE, J. R. '*Recent advances in the study of the sulfate-reducing bacteria*'. Bacteriol. Revs., **29**, 425–441 (1965).

[9]WANKLYN, J. N. AND SPRUIT, C. J. P. '*Influence of sulphate-reducing bacteria on the corrosion potential of iron*'. Nature, **169**, 928–929 (1952).

[10]WORMWELL, F. AND FARRER, T. W. '*Electrochemical studies of anaerobic corrosion in presence of sulphate-reducing bacteria*'. Chemistry & Industry, 108–109 (1952).

[11]HOAR, T. P. AND FARRER, T. W. '*The anodic characteristics of mild steel in dilute aqueous soil electrolytes*'. Corrosion Sci., **1**, 49–61 (1961).

[12]HORVÁTH, J. AND SOLTI, M. '*Beitrag zum Mechanismus der anaeroben mikrobiologischen Korrosion der Metalle im Boden*'. Werkstoffe und Korrosion, **10**, 624–630 (1959).

[13]BOOTH, G. H. AND TILLER, A. K. '*Polarization studies of mild steel in cultures of sulphate-reducing bacteria*'. Transactions Faraday Soc., **56**, 1689–1696 (1960).

[14]BOOTH, G. H. AND WORMWELL, F. '*Corrosion of mild steel by sulphate-reducing bacteria. Effect of different strains of organisms*'. In *First International Congress on Metallic Corrosion, London, 1961*, 341–344. London: Butterworth, 1962.

[15]TILLER, A. K. AND BOOTH, G. H. '*Polarization studies of mild steel in cultures of sulphate-reducing bacteria. Part 2: thermophilic organisms*'. Transactions Faraday Soc., **58**, 110–115 (1962).

[16]BOOTH, G. H. AND TILLER, A. K. '*Polarization studies of mild steel in cultures of sulphate-reducing bacteria. Part 3: halophilic organisms*'. Transactions Faraday Soc., **58**, 2510–2516 (1962).

[17]BOOTH, G. H., SHINN, P. M. AND WAKERLEY, D. S. '*The influence of various strains of actively growing sulphate-reducing bacteria on the anaerobic corrosion of mild steel*'. In *Comptes rendus du Congres International de la Corrosion Marine et des Salissures, Cannes, 1964*, 363–371. Paris: C.R.E.O., 1965.

[18]BOOTH, G. H., COOPER, A. W. AND COOPER, P. M. '*Rates of microbial corrosion in continuous culture*'. Chemistry & Industry, **86**, 2084–2085 (1967).

[19]BOOTH, G. H. AND TILLER, A. K. '*Some special features of the anaerobic corrosion of steel by organisms of estaurine origin*'. In *Second International Congress on Marine Corrosion and Fouling, Athens*, 1968, 1–3.

[20]BOOTH, G. H., COOPER, P. M. AND WAKERLEY, D. S. '*Corrosion of mild steel by actively growing cultures of sulphate-reducing bacteria. The influence of ferrous iron*'. Brit. Corrosion J., **1**, 345–349 (1966).

[21]BOOTH, G. H., ROBB, J. A. AND WAKERLEY, D. S. '*The influence of ferrous ions on the anaerobic corrosion of mild steel by actively growing cultures of sulphate-reducing bacteria*'. In *Third International Congress of Metallic Corrosion, Moscow, 1967*, vol. 2, section 5, 542–544.

[22]BOOTH, G. H., ELFORD, L. AND WAKERLEY, D. S. '*Corrosion of mild steel by sulphate-reducing bacteria: an alternative mechanism*'. Brit. Corrosion J., **3**, 242–245 (1968).

[23]KING, R. A. AND MILLER, J. D. A. Unpublished results.

[24]MARA, D. D. '*On the anaerobic corrosion of steel by microbes*'. Ph.D. thesis, University of Dundee, 1970.

[25]MILLER, J. D. A. AND WAKERLEY, D. S. '*Growth of sulphate-reducing bacteria by fumarate dismutation*'. J. General Microbiol., **43**, 101–107 (1966).

[26]BOOTH, G. H., ELFORD, L. AND WAKERLEY, D. S. '*Polarization of mild steel in the presence of hydrogen bacteria and methane bacteria*'. Brit. Corrosion J., **3**, 68–69 (1968).

[27]BUTLIN, K. R. AND VERNON, W. H. J. '*Underground corrosion of metals: causes and prevention*'. J. Inst. Water Engineers, **3**, 627–637 (1949).

[28]STARKEY, R. L. AND WIGHT, K. M. *Anaerobic Corrosion of Iron in Soil.* New York: American Gas Association, 1945. 108 pp.

[29]ROMANOFF, M. *Underground Corrosion.* Washington, D.C.: National Bureau of Standards Circular No. 579, 1957. 227 pp.

[30]STRATFULL, R. F. '*A new test for estimating soil corrosivity based on investigation of metal highway culverts*'. Corrosion, **17**, 493t–496t (1961).

[31]BOOTH, G. H., COOPER, A. W., COOPER, P. M. AND WAKERLEY, D. S. '*Criteria of soil aggressiveness towards buried metals. I. Experimental methods*'. Brit. Corrosion J., **2**, 104–108 (1967).

[32]BOOTH, G. H., COOPER, A. W. AND COOPER, P. M. '*Criteria of soil aggressiveness towards buried metals. II. Assessment of various soils*'. Brit. Corrosion J., **2**, 109–115 (1967).

[33]SCHWERDTFEGER, W. J. '*Soil resistivity as related to underground corrosion and cathodic protection*'. National Bureau of Standards J. of Research, **69C**, 71–77 (1965).

[34]BOOTH, G. H., COOPER, A. W. AND TILLER, A. K. '*Criteria of soil aggressiveness towards buried metals. III. Verification of predicted behaviour of selected soils*'. Brit. Corrosion J., **2**, 116–118 (1967).

[35]BOOTH, G. H., COOPER, A. W. AND TILLER, A. K. '*Corrosion of mild steel in the tidal waters of the Thames estuary. I. Results of six months' and one year's immersion*'. J. Applied Chem., **13**, 211–220 (1963).

[36]BOOTH, G. H., COOPER, A. W. AND TILLER, A. K. '*Corrosion of mild steel in the tidal waters of the Thames estuary. II. Results of two years' immersion*'. J. Applied Chem., **15**, 250–256 (1965).

[37]BOOTH, G. H., COOPER, A. W. AND TILLER, A. K. '*Corrosion of mild steel in the tidal waters of the Thames estuary. III. Results of three and five years' immersion*'. Brit. Corrosion J., **2**, 222–228 (1967).

[38]HOAR, T. P. '*Corrosion principles in pipe and pipeline protection*'. In *Proceedings of the 1968 Conference on the Corrosion and Protection of Pipes and Pipelines*, ed. by TRUMAN, W. F. AND MCLOUCHLIN, P., 7–11. London: Brintex Exhibitions Ltd., 1969.

[39]ROMANOFF, M. *Corrosion of Steel Pilings in Soils*. Washington, D.C.: National Bureau of Standards Monograph No. 58, 1962. 22 pp.

[40]HORVÁTH, J. AND NOVÁK, M. '*Potential/pH equilibrium diagrams of some M–S–H_2O ternary systems and their interpretation from the point of view of metallic corrosion*'. Corrosion Sci., **4**, 159–178 (1964).

[41]BOOTH, G. H. AND TILLER, A. K. '*Cathodic characteristics of mild steel in suspensions of sulphate-reducing bacteria*'. Corrosion Sci., **8**, 583–600 (1968).

[42]WHALLEY, W. C. R. '*Cathodic protection and control of corrosion in the Middle East*'. Chemistry & Industry, 140–147 (1954).

[43]MORGAN, J. H. *Cathodic Protection*. London: Leonard Hill Ltd., 1959.

[44]LOGAN, A. G. AND PARKER, W. D. '*Protective Coatings for buried pipes*'. Institution of Gas Engineers J., **5**, 351–366 (1965).

[45]POSTGATE, J. R. '*The microbiology of corrosion*'. In *Corrosion*, vol. 1, ed. by SHREIR, L. L. London: George Newnes Ltd., 1963, 2.51–2.64.

[46]CHEMISTRY RESEARCH 1956. London: Her Majesty's Stationery Office, 1957, p. 16.

[47]POSTGATE, J. R. '*Media for sulphur bacteria*'. Lab. Practice, **15**, 1239–1244 (1966).

[18]MINISTRY OF HEALTH. *The Bacteriological Examination of Water Supplies, 2nd edn.* (Reports on Public Health and Medical Subjects, No. 71). London: His Majesty's Stationery Office, 1939.

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