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AUTOTROPHIC LEACHING

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.

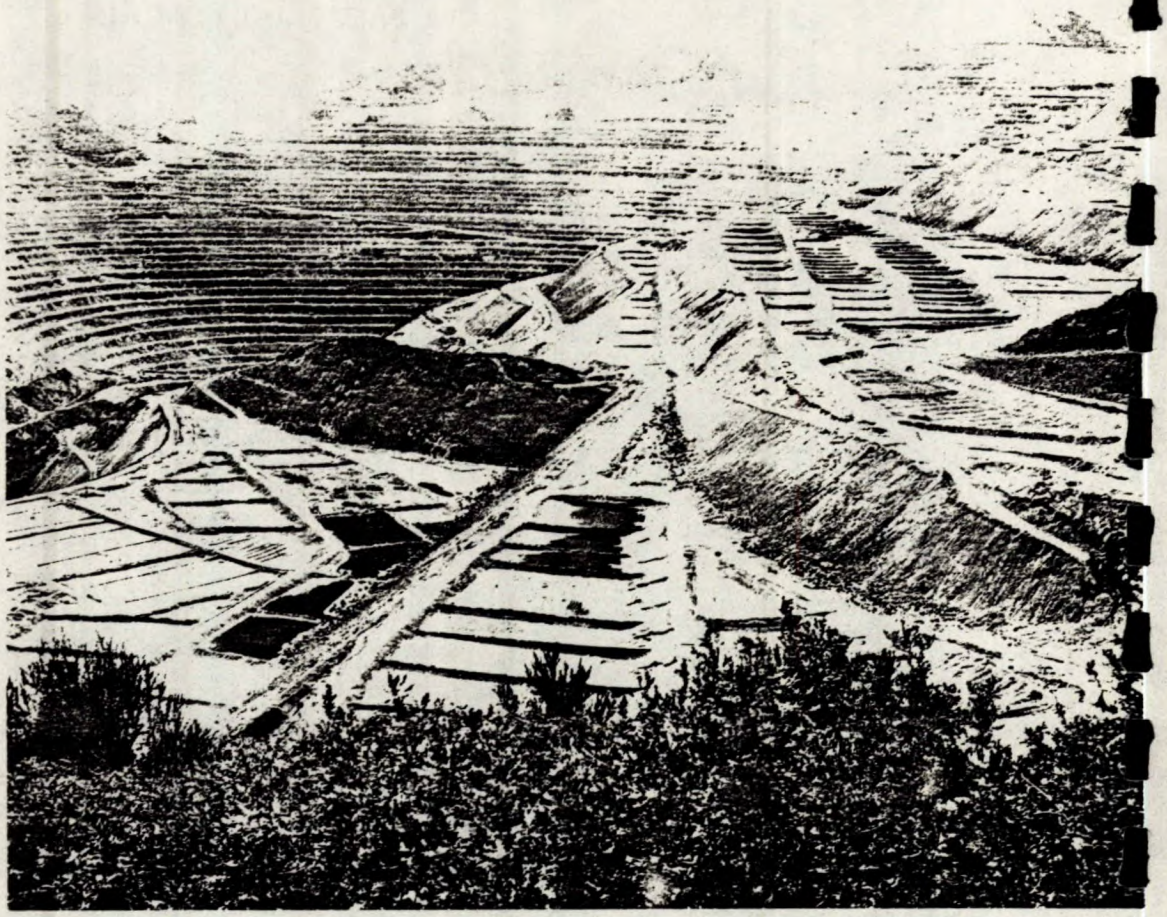

FIGURE 1. Copper leach dumps at Bingham Canyon, Utah, showing lifts for aeration. (Courtesy of Don Green, Sta Photographer and the Kennecott Copper Corporation).

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 impervio 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 cludes boulders weighing many tons and mate-rial of fine particle size, but most is less than 2 ft in diameter. Some dumps in the western U are constructed on 50- or 100-ft lifts, up to 1 ft high. The largest dump, located at Bingham Canyon, Utah, contains 4 billion tons of mate-rial (see Figure 1).

Leach solutions are usually introduced on the top surfaces of dumps by flooding or sprin-kling. The solutions then percolate through 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

TABLE 1

Minerals of Increasing Recalcitrance[11]

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

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 mM (1 g/l) does not increase the rate of leaching. Acid consumption by gangue materials 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.

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

$$MS + 2Fe^{3+} \longrightarrow M^{2+} + 2Fe^{2+} + S^0$$

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

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

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

$$S^0 + 3/2O_2 + H_2O \longrightarrow H_2SO_4 \quad (5)$$

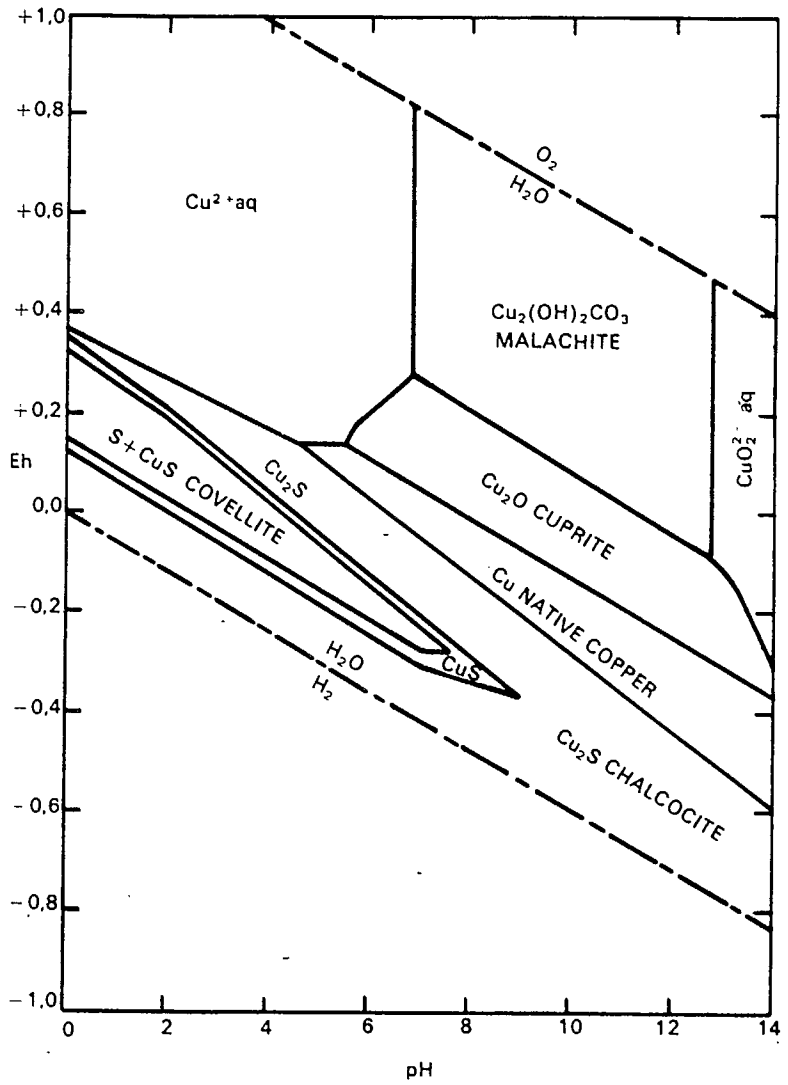

FIGURE 2. Stability relations among some copper compounds in the system Cu-H_2O-O_2-S-CO_2 at 25°C and 1 atm total pressure. $P_{CO_2} = 10^{-3.5}$, total dissolved sulfur species $= 10^{-1}$. (From Garrels, R. M. and Christ, C. L., *Solution, Minerals, and Equilibria*, Freeman, Cooper, and Co., San 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 in the Milliken Mine of Rio Algom Mines, Ltd., Elliot Lake,[48] Ontario.

Solution mining for uranium was initiated in 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 flow 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, particularily 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 sandstones 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

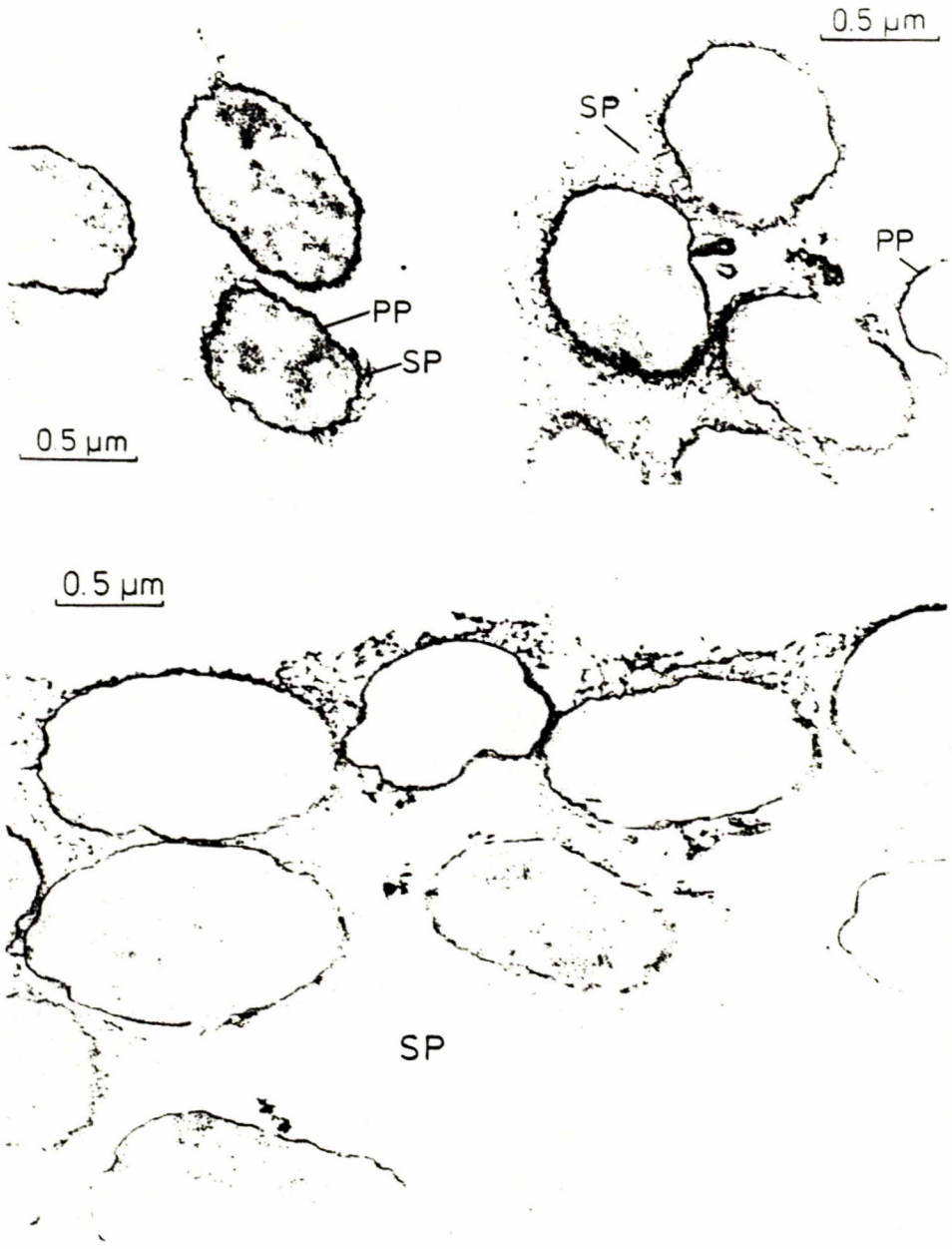

FIGURE 5. Sections from bacterial films showing *Pseudomonas* and their associated primary (PP) and secondary (SP5 polysaccharides. Top: The increase in the amount of secondary polysaccharide is apparently related to an increase in cell number. Bottom: Primary polysaccharide is eventually replaced by 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 th economics. The process will be viable if 70% o 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 i the solvent extraction circuit for uranium recovery were tested on *T. ferrooxidans*. A though Alamine 336, a tertiary amine, ad versely affected ferrous iron oxidation by the bacteria, it was suggested that dilution of the solvent encountered in the overall circuit wa enough to minimize the effects.

An in-plant process which uses bacterial leaching to treat ores has been described b Derry et al.[101] In this process, pyritic uraniu 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 lix viant, pumped into the base of one bed, is a lowed 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 leach ing 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 countercu rent wash procedure, and a portion of the was liquor is used for leaching. The water balance is maintained. The uranium is removed by io exchange, and the barren liquor passes to th 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% o the ferrous iron is oxidized and the Eh is + 45 mv. The lixiviant contains 0.22 *M* (12 g/l) ferric iron, but acid must be added to the regene ated lixiviant to avoid precipitation of jarosit Additional iron is also generated by the reaction of ferric iron with pyrite, but some iron is lost due to jarosite precipitation. At a pil scale level, 95% of the uranium can be ex tracted from ore containing 0.12% U_3O_8. The process is economical because the lixiviant bacterially generated and acid consumption low. The ore can be treated while having a fairly coarse particle size, thus avoiding fin grinding. The separation of leach liquor fro ore is facilitated because of the coarse ore size which avoids the conventional filtration or sedimentation step. Derry et al.[101] estimate a co savings of $2/kg as compared with conve tional 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

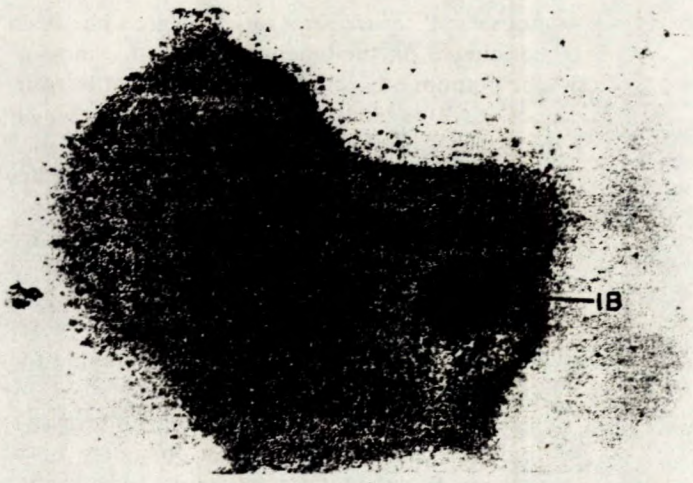

FIGURE 7. Thin section of *Sulfolobus*-like organism with intracellular body (IB). (Reproduced by permission of the National Research Council of Canada from Brierley, C. L. and Brierley, J. A., *Can. J. Microbiol.* 19, 183, 1973.)

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]speculate 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. 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* cel is dependent upon the cell concentration. A though iron oxidation occurs over the pH range 1 to 4.5, the pH optimum is 2.0. The enzym for iron oxidation are located in the cell env lope, and the optimum pH for iron oxidatio by cell envelopes is 3.5.

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

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

Schnaitman et al.[147] found that adding s fate in concentrations of 3.6 to 50 m*M* (0.2 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 sti latory at low concentrations, but they found that iron cannot be oxidized if sulfate is absent. Since the K_m of the reaction is not tered by adding different sulfate concen tions, these workers did not conform to Dugan and Lundgren's[153] theory that binding of iron requires sulfate. They[147] did not exclude e possibility that sulfate exposes more enzym ic sites. It was discovered that dibasic phosphate (HPO_4^{2-}) and diabasic arsenate ($HAsO_4^{2-}$) substitute for sulfate. Since these anions pre itate with ferric iron, they cannot totally replace sulfate in the reaction. Borate (BO_3^-) is ineffective, and nitrate (NO_3^-) and molybd (MoO_4^{2-}) are inhibitory. It was hypothesized Schnaitman and co-workers[147] that the divalent anions, such as dibasic phosphate and dib arsenate, may be able to replace sulf whereas chloride and nitrate cannot, owing to an unsuitable charge. The divalent anions may depolarize positively charged sites and en 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/

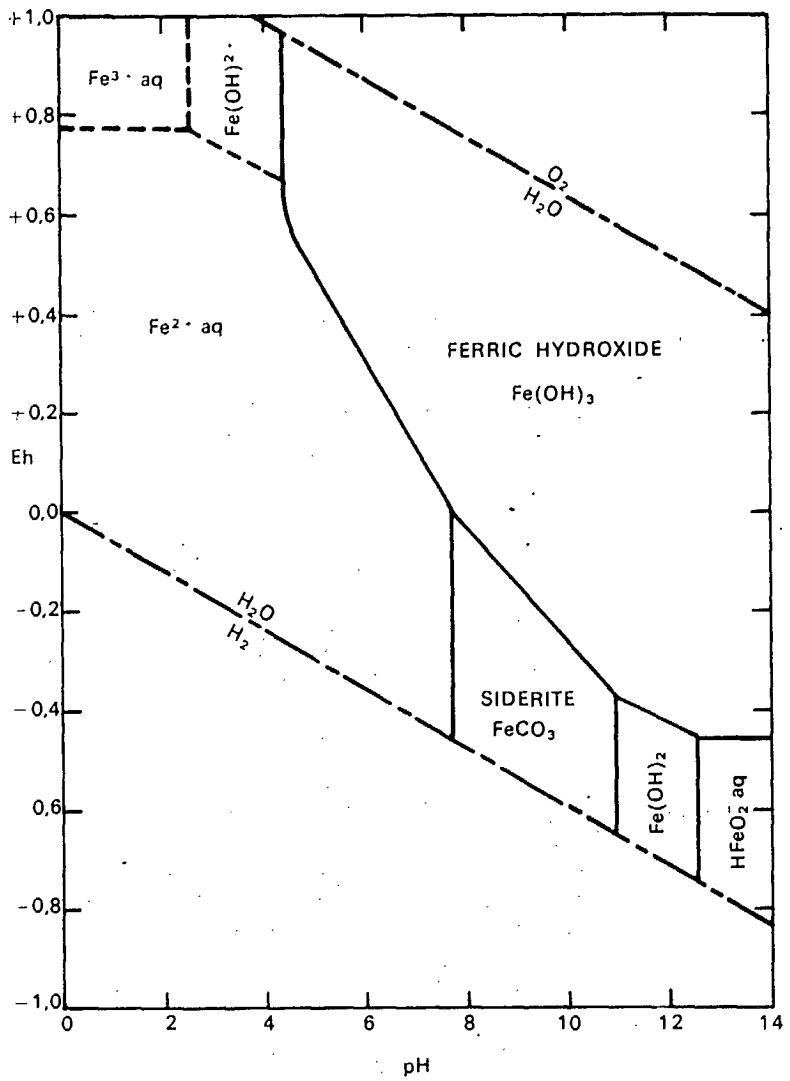

FIGURE 8. Diagram showing the relations among the metastable iron hydroxides and siderite at 25°C and 1 atm total pressure. Boundary between solids and ions at total activity of dissolved species = 10^{-6}. Total dissolved carbonate species = 10^{-1}. Dashed lines are boundaries between fields dominated 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

$$CaCO_3 + 2H^+ + SO_4^{2-} + H_2O \longrightarrow CaSO_4 \cdot 2H_2O + CO_2 \quad (12)$$

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

$$FeS_2 + 3/4\ O_2 + 3H^+ \longrightarrow Fe^{3+} + 2S^0 + 3/2\ H_2 \quad (13$$

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 potassium transport. Both divalent[27] and monovalent cations[162] reverse the toxic effects by having a greater affinity than uranium for the 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.

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 interaction 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 five-fold 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. 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. 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 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 this 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 *MM*/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 + [illegible] 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, Beckstead et al.[205] found that 90% of the copper from chalcopyrite can be extracted in 3 [illegible] 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 the 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 0.5 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 the 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 ore 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 reasons 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 condition 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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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.

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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.
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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

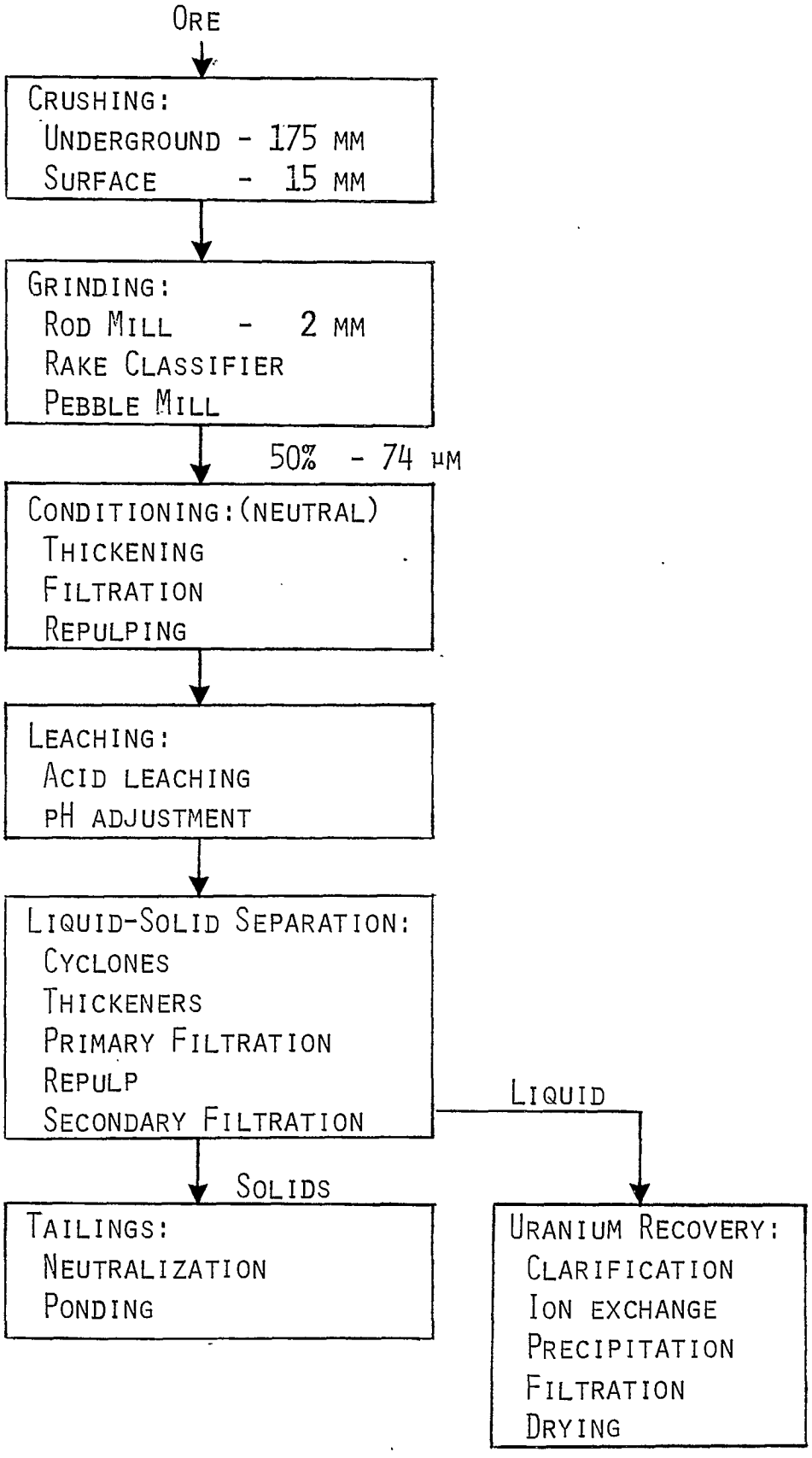
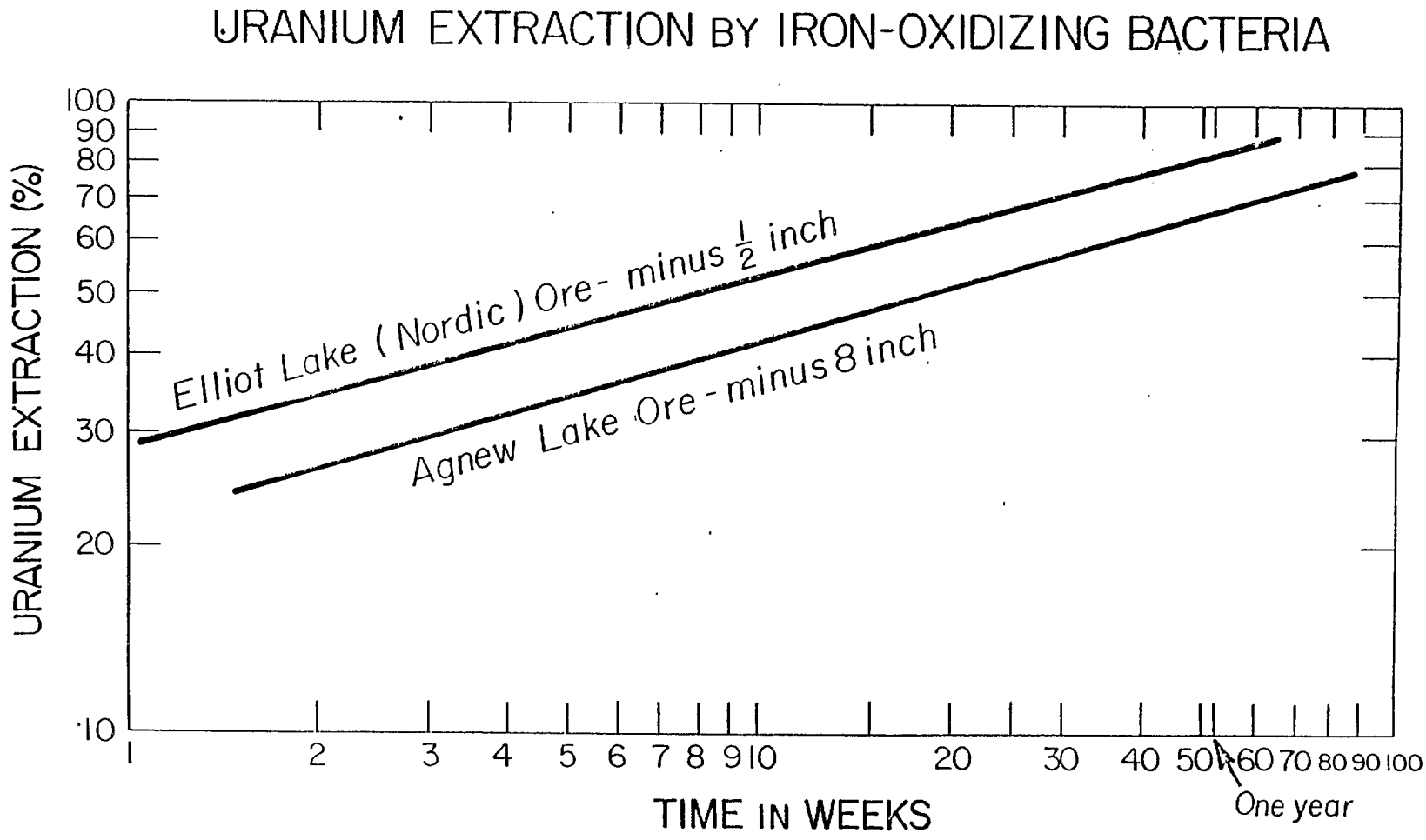


FIGURE 2


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

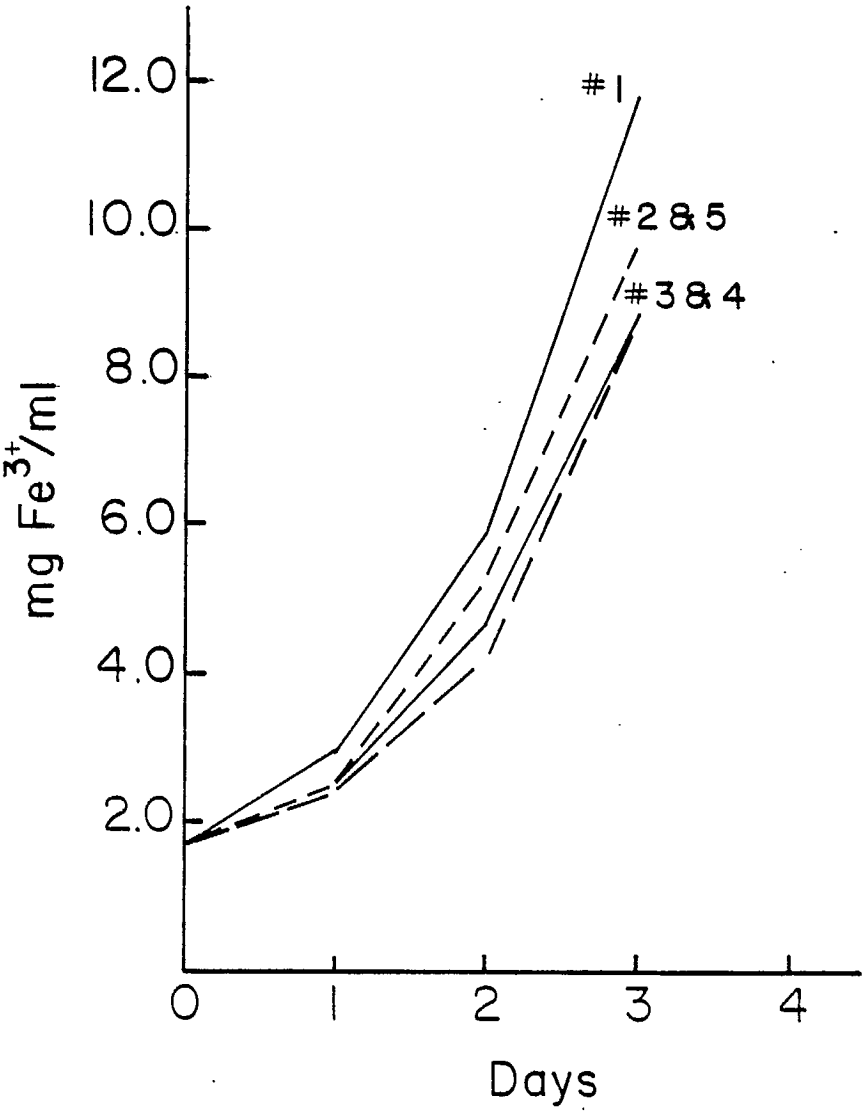


FIG. 3. GROWTH OF DENISON MINE ISOLATES AT 28° ON LOW PHOSPHATE MEDIUM (6)

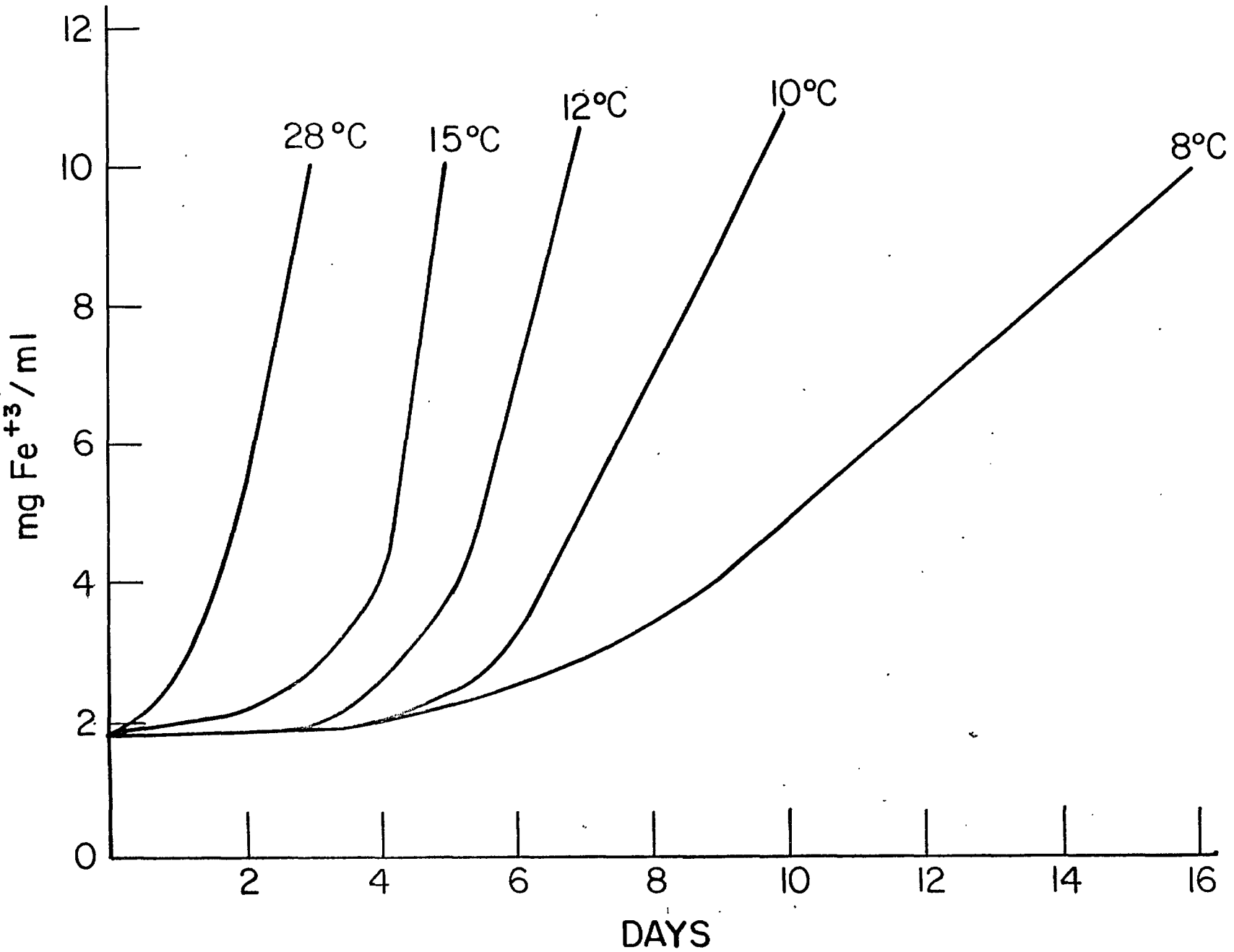


FIG. 4. GROWTH OF T. FERROOXIDANS E.L. #5 AT VARIOUS TEMPERATURES ON LOW PO_4 MEDIUM AT 150 RPM. (AVERAGE OF TRIPLICATE CULTURES) 10 PPM PO_4, 10 PPM MG & 10 PPM NH_4^+ AT PH 2.3

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	% Extraction
			days	
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

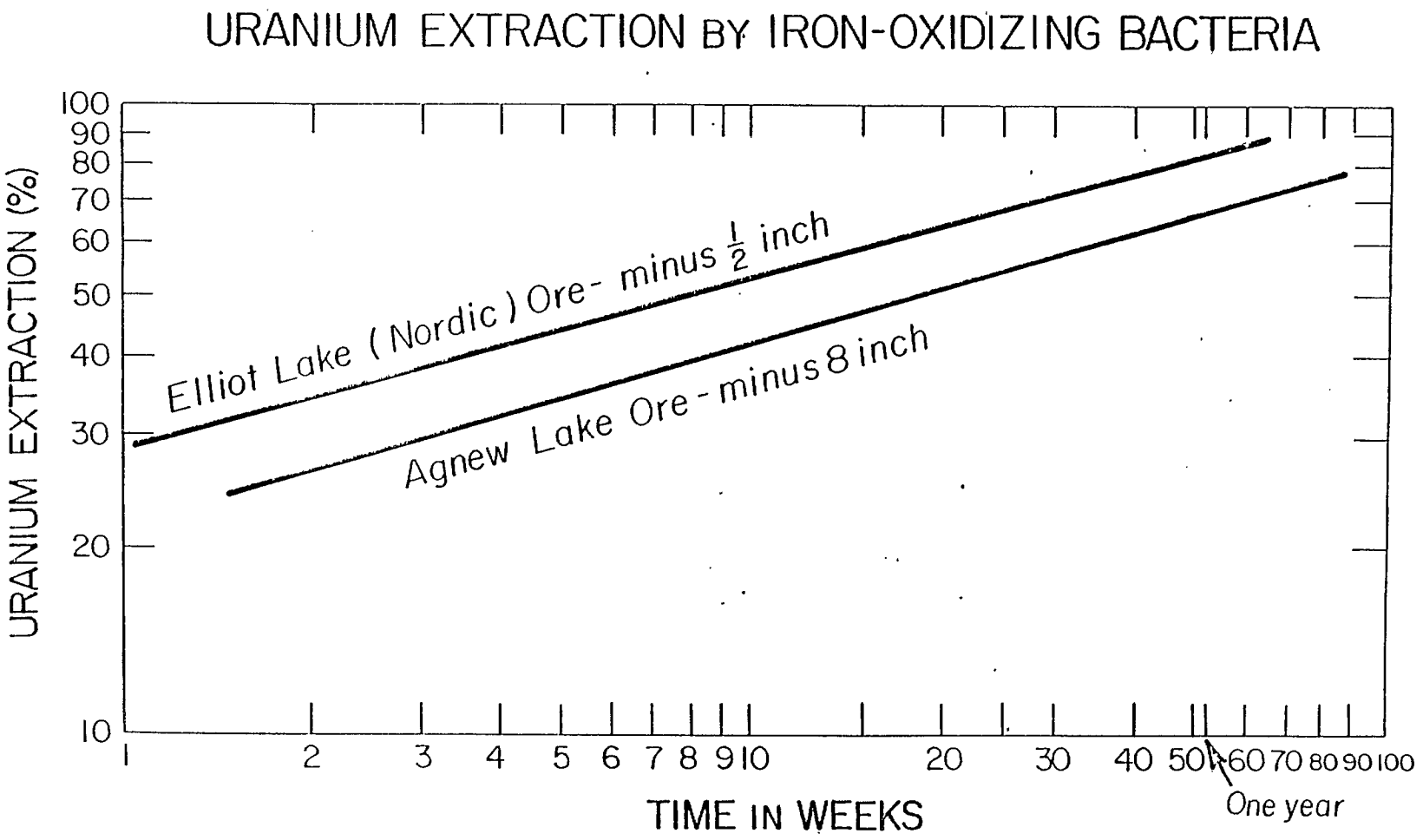
Block	Leach Method	Comment	Time Elapsed	% Extraction
46072/73	Flood	Inoculated	125	32.2
46074/75	Flood	Control	135	23.1

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

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 ½ 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
TIME IN WEEKS
One year

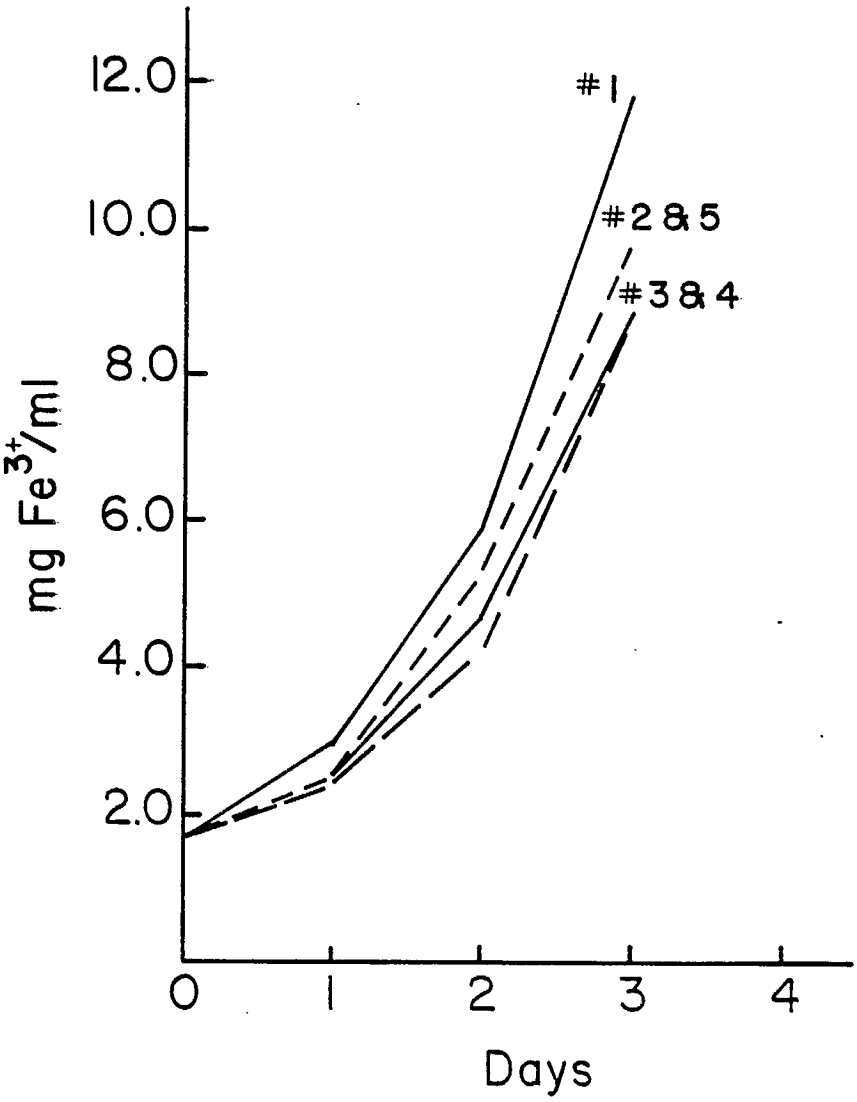


FIG. 3. GROWTH OF DENISON MINE ISOLATES AT 28° ON LOW PHOSPHATE MEDIUM (6)

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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Ontario Research Foundation

February 1986

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.

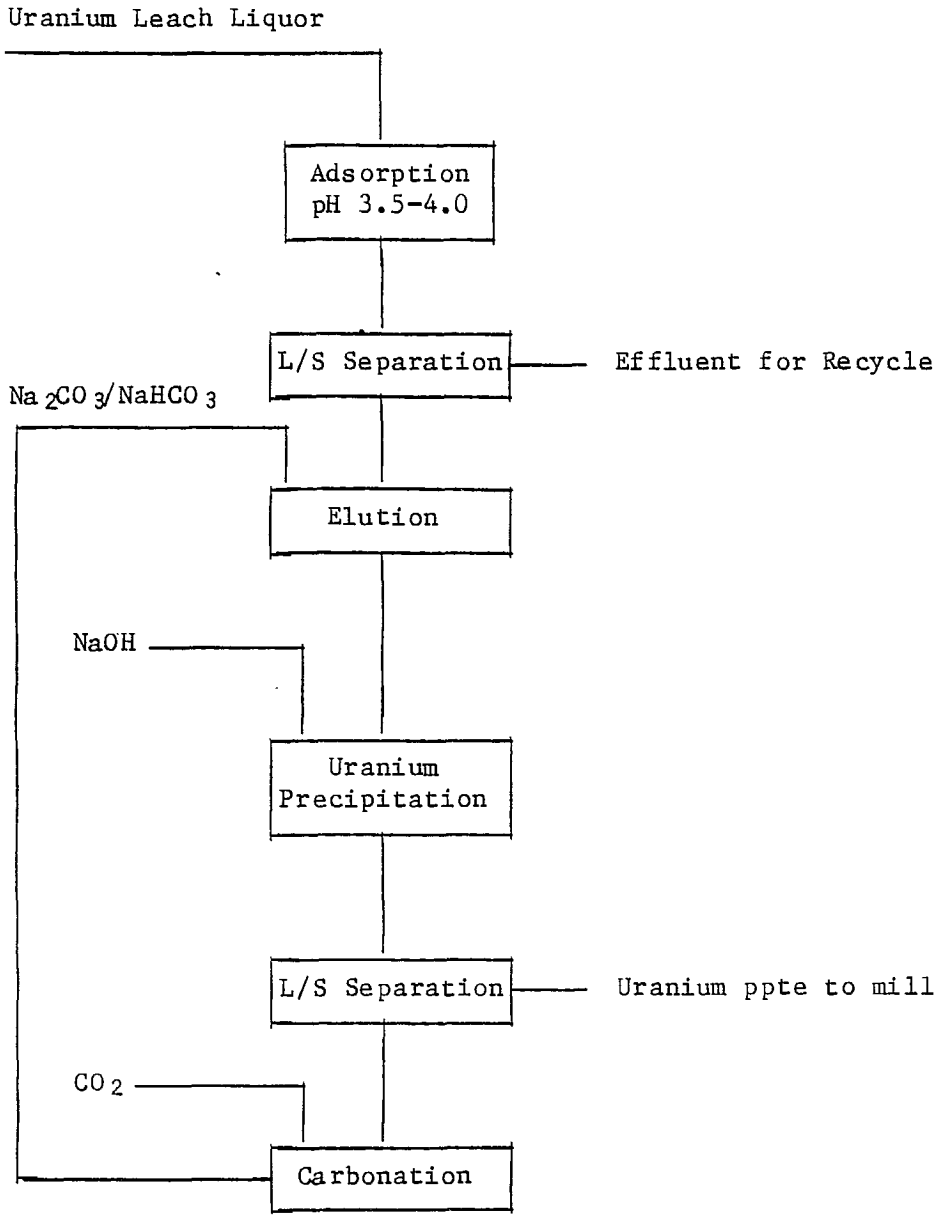
Table 3. Relative cost comparison of ion exchange, bioadsorption and reverse osmosis for uranium recovery

	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.

Figure 1

Conceptual Flowsheet to Recover Uranium From Dilute Leach Liquor



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.

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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.

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.

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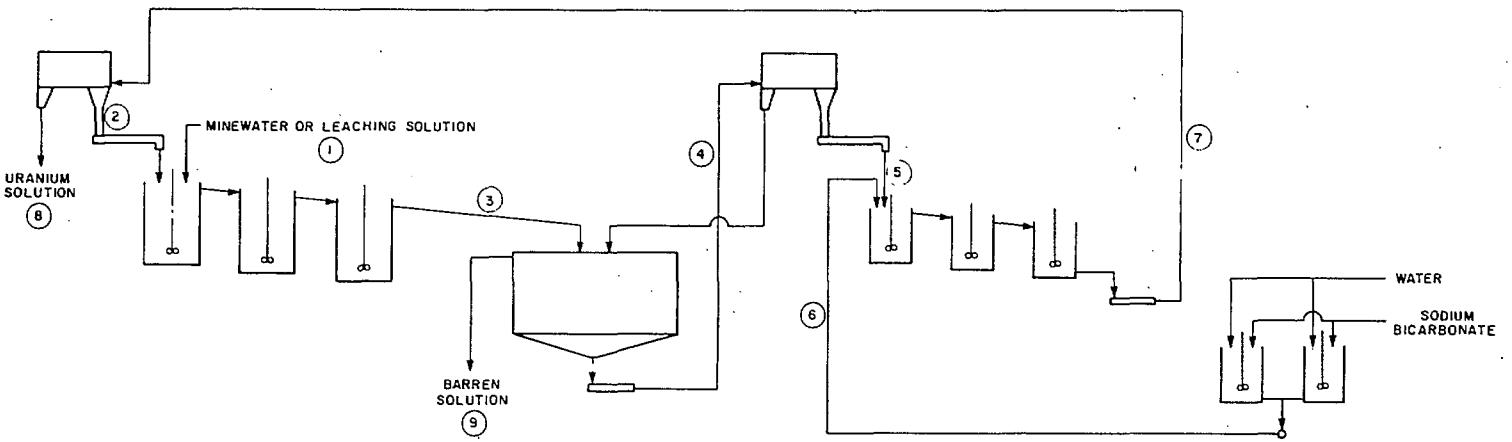
FIGURES



	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

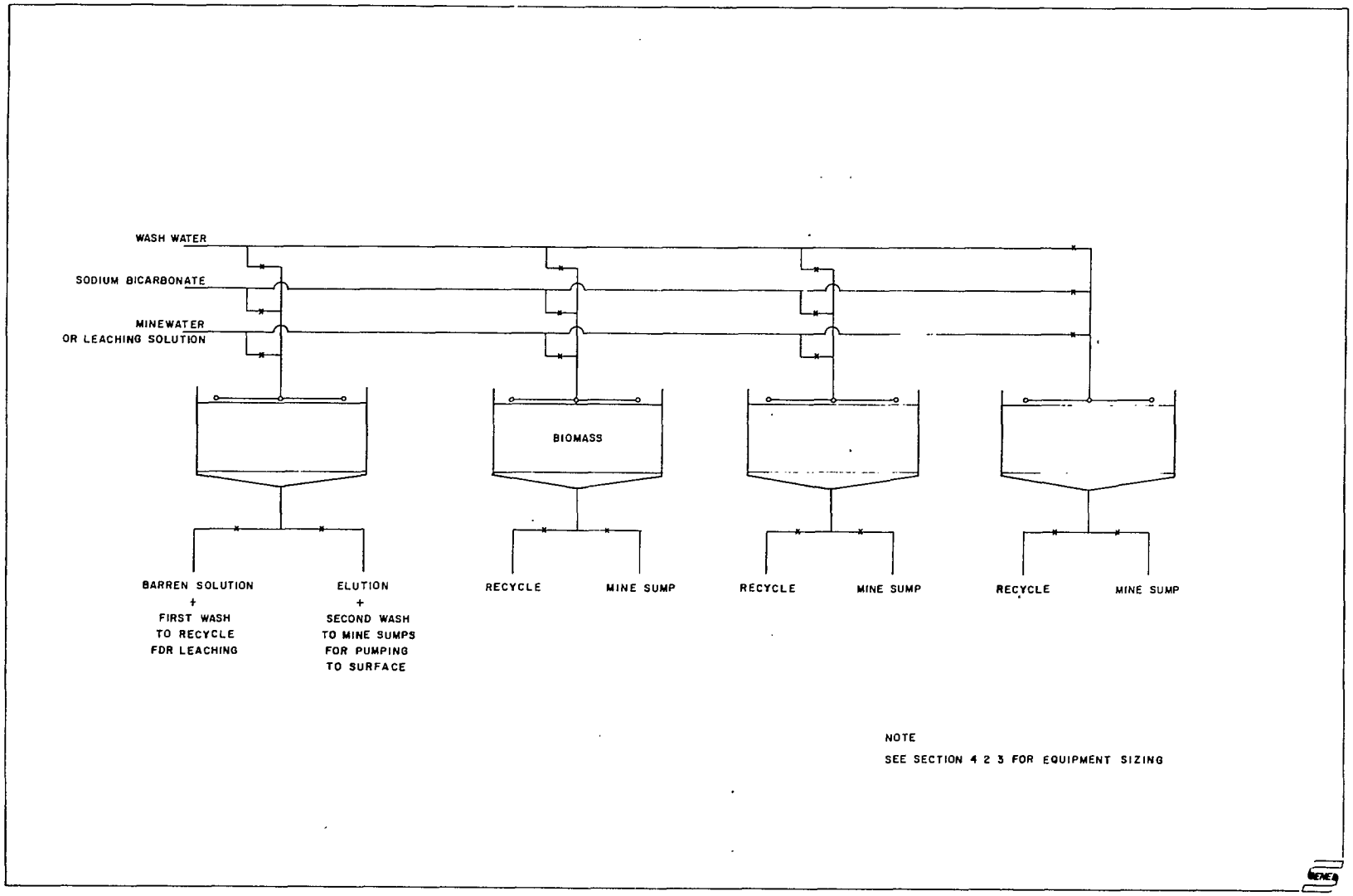


Fig. 2 - Preliminary flowsheet for uranium recovery from minewater using biomass and columns

BIOFOULING

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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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Iron Ore Processing

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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

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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

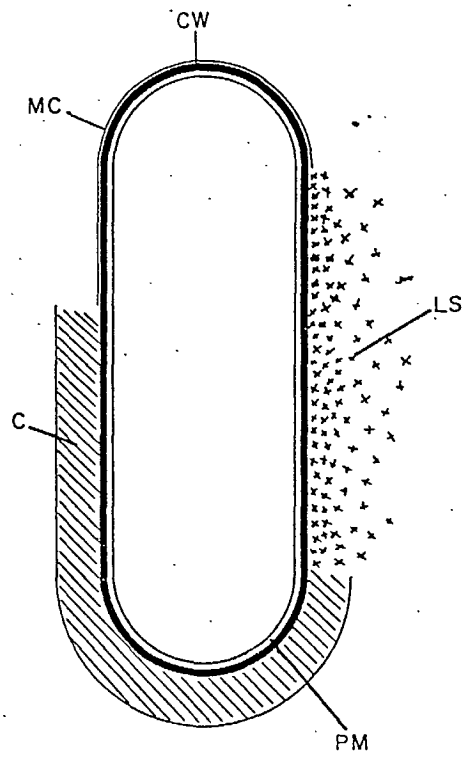

FIG. 1. A diagrammatic representation of the relationship between the capsules, slime, and microcapsular layers to the cell wall and protoplast membrane. CW = cell wall; PM = protoplasmic membrane; C = capsule; MC = microcapsule; LS = loose slime.

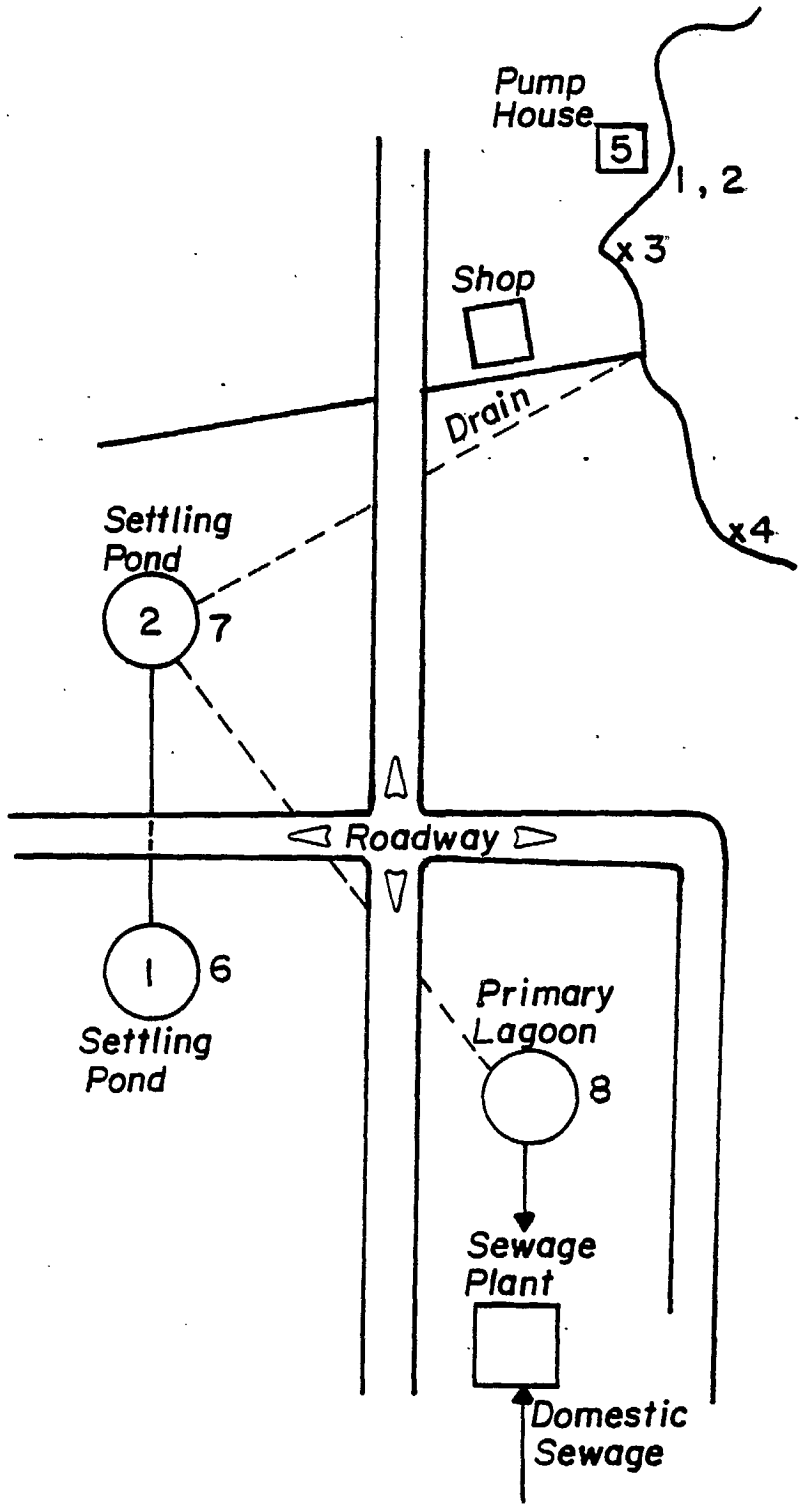

Figure 2. Sampling Locations 1 to 8

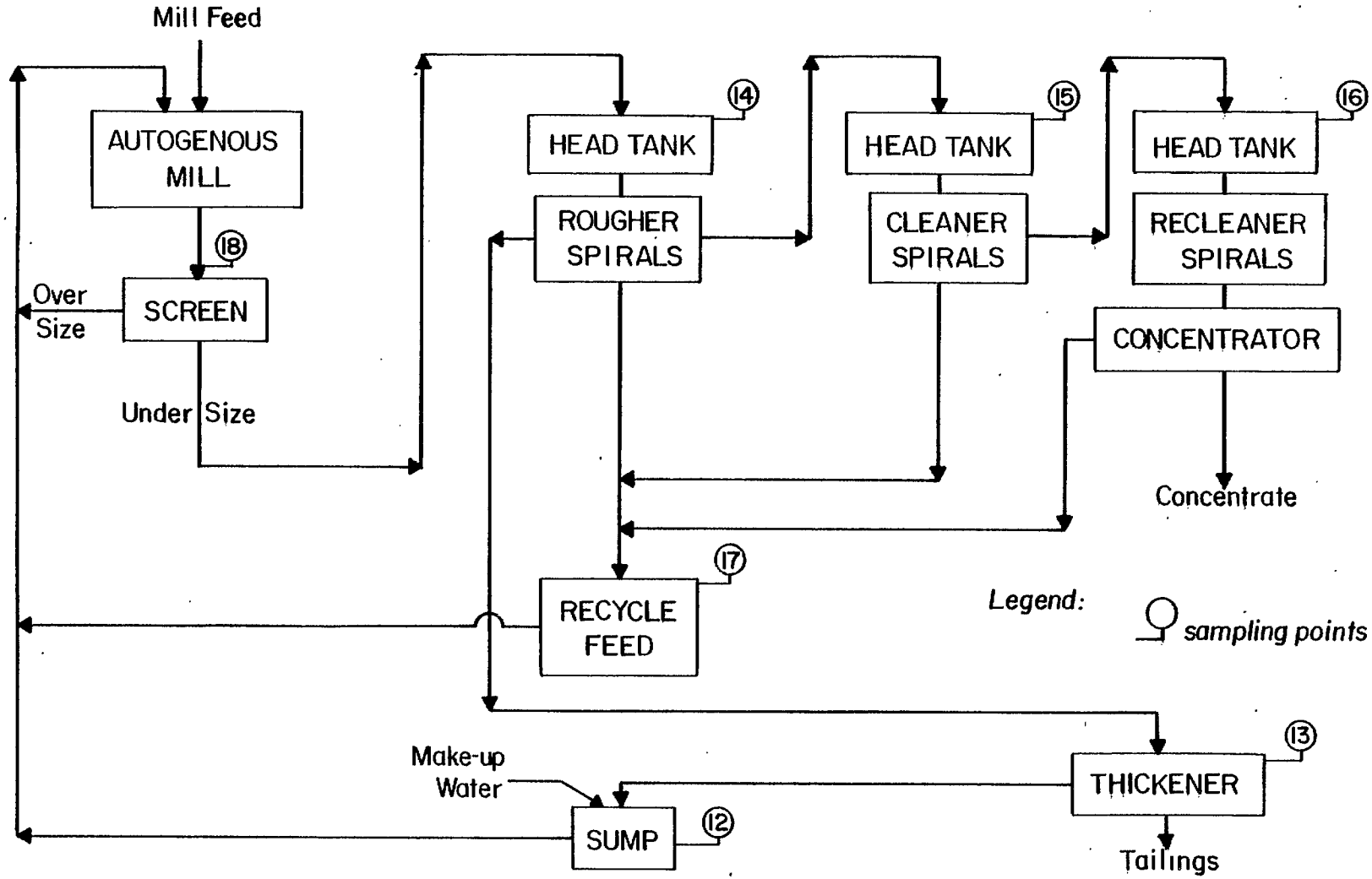

Figure 3 Schematic Block Diagram Showing Sampling Locations 12 to 18.

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 ability 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.

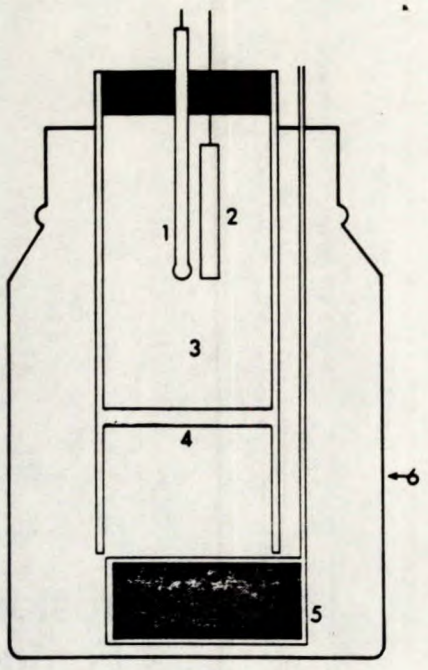

FIGURE 1 — Polarization cell. 1—Reference electrode (SCE); 2—working electrode (mild steel); 3—corrosion chamber; 4—porous seal; 5—auxiliary electrode (platinum); 6—auxiliary electrode chamber (medium reservoir).

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

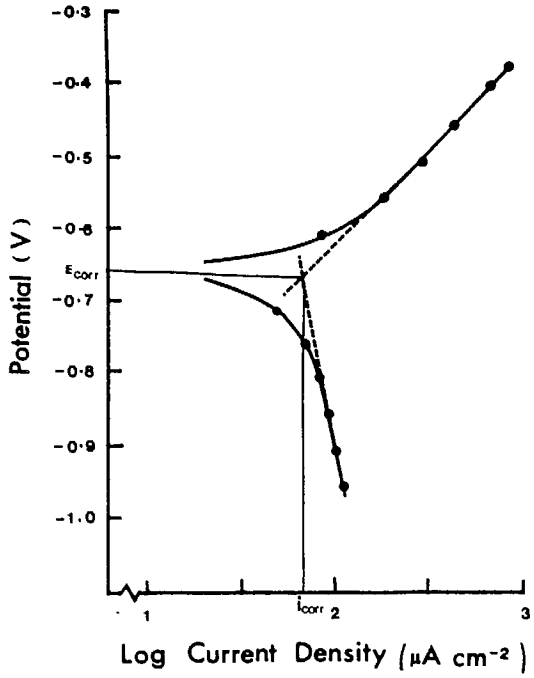

FIGURE 2 — Tafel plot constructed from data obtained during polarization of mild steel coupon in sterile B_{10} medium in the newly constructed electrochemical cell.

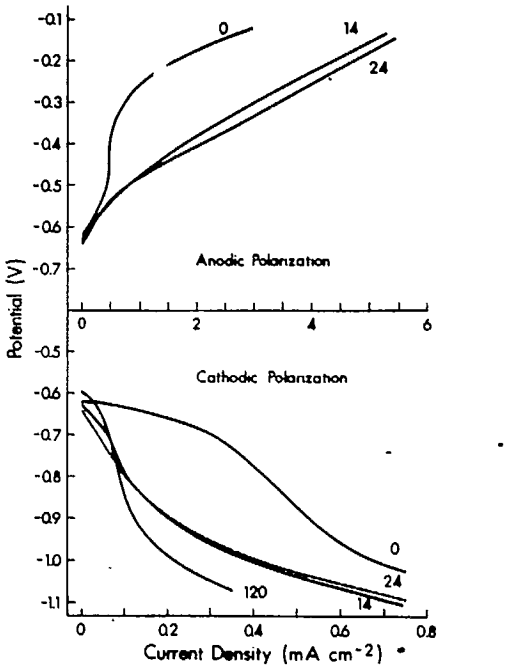

FIGURE 3 — Polarization curves for mild steel in B_{10} medium inoculated with isolate # 200. Dashed lines are used for clarity only in distinguishing intersecting curves. (0, 14, 24, 120 denote incubation time (hours) at 25 ± 2 C).

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.

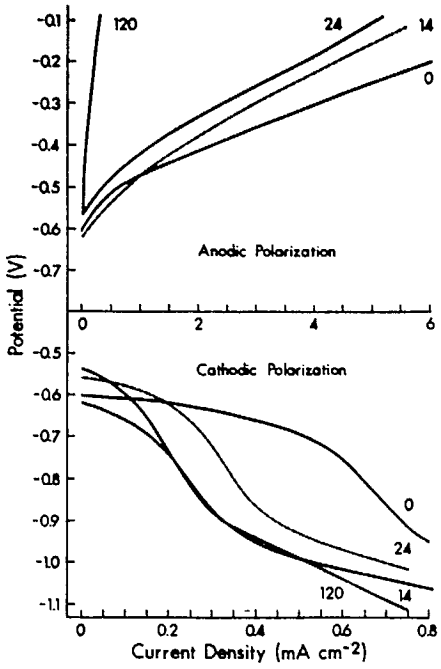

FIGURE 4 — Polarization curves for mild steel in uninoculated (control) B_{10} medium. Dashed lines are used for clarity only in distinguishing intersecting curves. (0, 14, 24, 120 denote incubation time (hours) at 25 ± 2 C).

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).

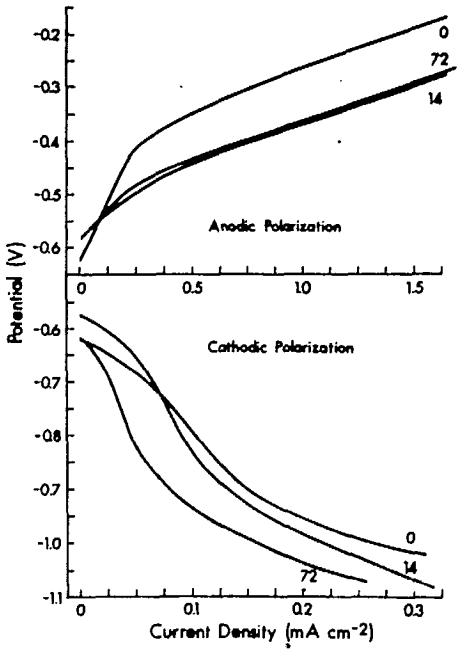

FIGURE 5 — Polarization curves for mild steel in Butlin's medium inoculated with isolate #200. (0, 14, 72 denote incubation time (hours) at 25 ± 2 C).

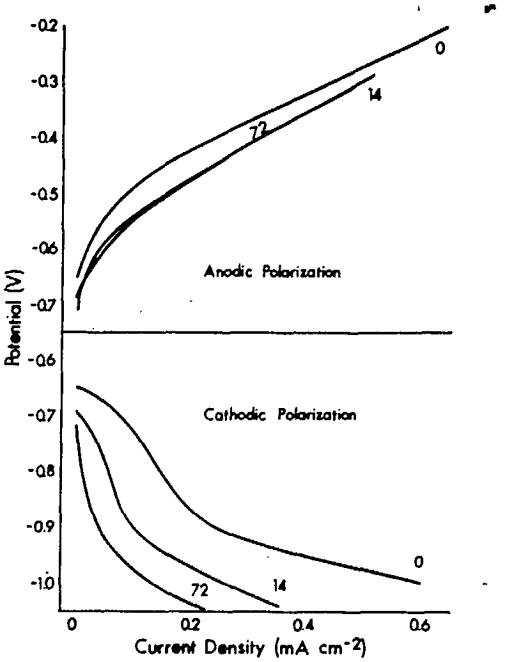

FIGURE 7 — Polarization curves for mild steel in synthetic medium (containing 1800 mg sodium lactate/1) inoculated with isolate #200. (0, 14, 72 denote incubation time (hours) at 25 ± 2 C).

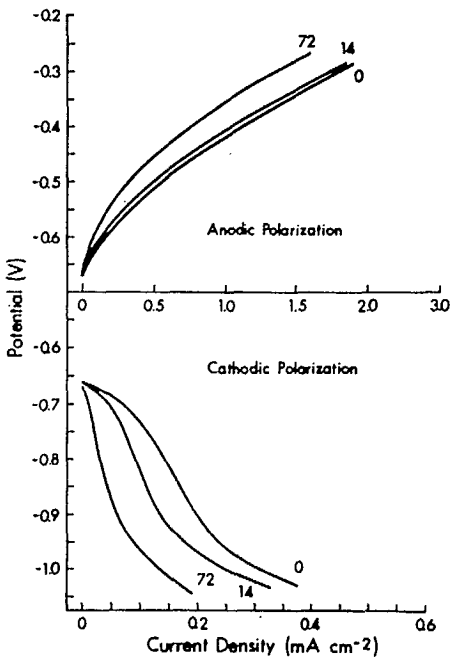

FIGURE 6 — Polarization curves of mild steel in uninoculated Butlin's medium (control). (0, 14, 72 denote incubation time (hours) at 25 ± 2 C).

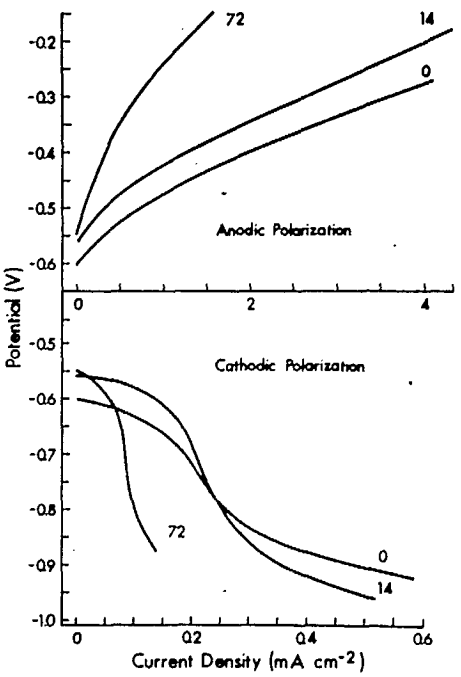

FIGURE 8 — Polarization curves for mild steel in uninoculated (control) synthetic medium (containing 1800 mg sodium lactate/1). (0, 12, 72 denote incubation time (hours) at 25 ± 2 C).

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 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

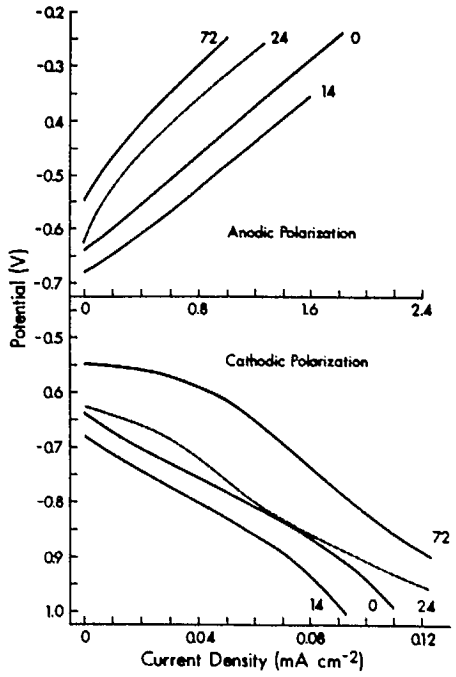

FIGURE 9 — Polarization curves for mild steel in unautoclaved, uninoculated (*i.e.*, with natural flora) produced water. (0, 14, 24, 72 denote incubation time (hours) at 25 ± 2 C).

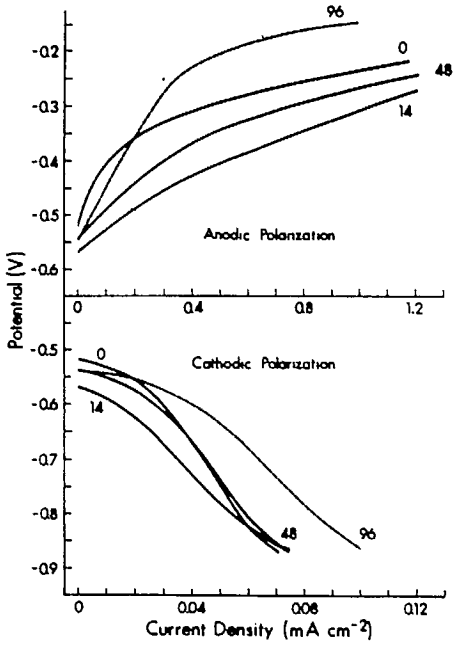

FIGURE 10 — Polarization curves for mild steel in autoclaved produced water inoculated with isolate # 200. (0, 14, 48, 96 denote incubation time (hour) at 25 ± 2 C).

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.

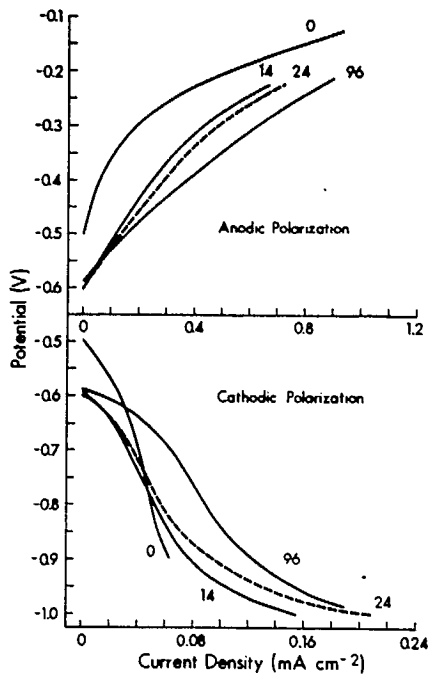

FIGURE 11 — Polarization of mild steel in produced water modified by the addition of 900 mg sodium lactate/l and inoculated with isolate # 200. (0, 14, 24, 96 denote incubation time (hours) at 25 ± 2 C).

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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Abstract

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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 ability 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 x 12 x 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.

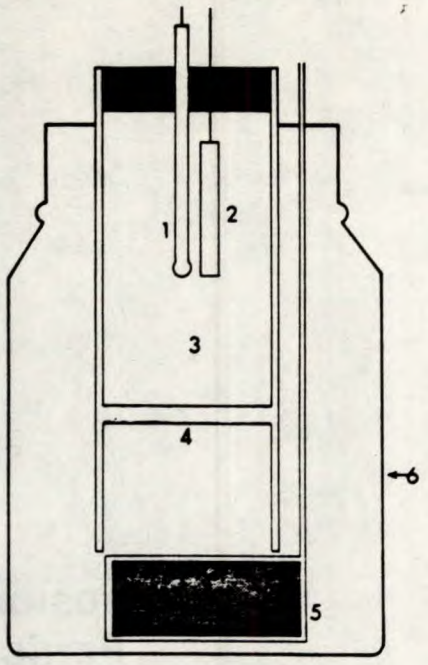

FIGURE 1 — Polarization cell. 1—Reference electrode (SCE); 2—working electrode (mild steel); 3—corrosion chamber; 4—porous seal; 5—auxiliary electrode (platinum); 6—auxiliary electrode chamber (medium reservoir).

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

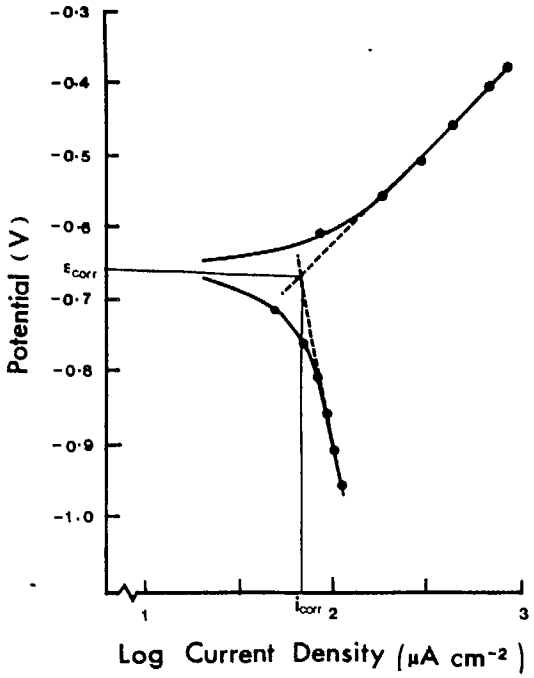

FIGURE 2 — Tafel plot constructed from data obtained during polarization of mild steel coupon in sterile B_{10} medium in the newly constructed electrochemical cell.

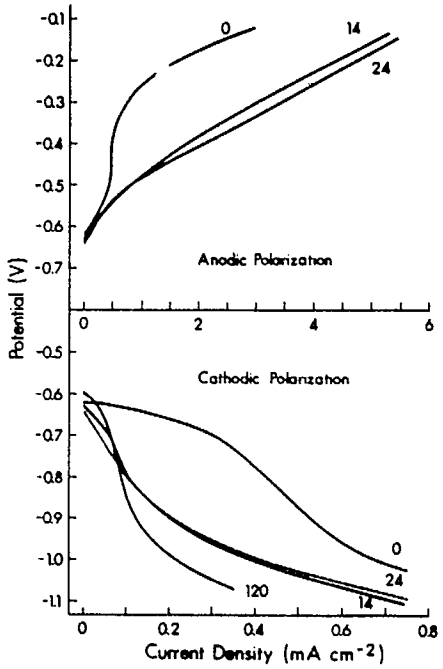

FIGURE 3 — Polarization curves for mild steel in B_{10} medium inoculated with isolate #200. Dashed lines are used for clarity only in distinguishing intersecting curves. (0, 14, 24, 120 denote incubation time (hours) at 25 ± 2 C).

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.

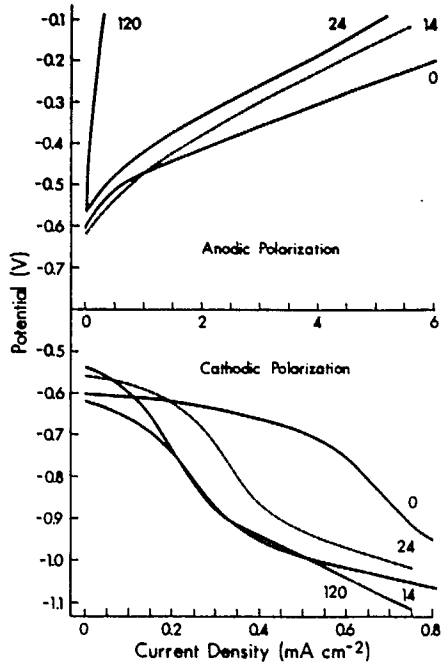

FIGURE 4 — Polarization curves for mild steel in uninoculated (control) B_{10} medium. Dashed lines are used for clarity only in distinguishing intersecting curves. (0, 14, 24, 120 denote incubation time (hours) at 25 ± 2 C).

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).

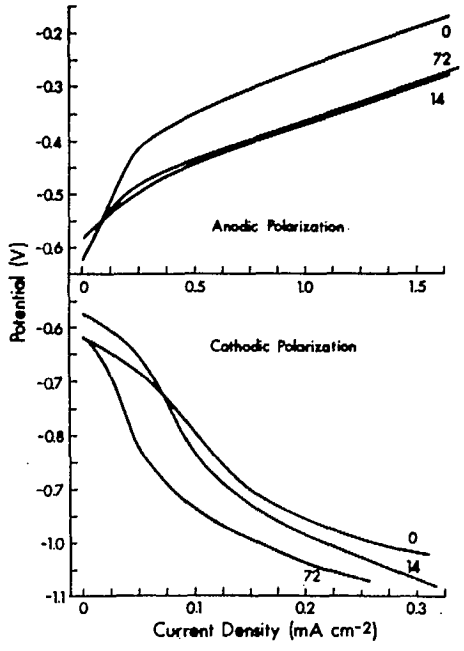

FIGURE 5 — Polarization curves for mild steel in Butlin's medium inoculated with isolate #200. (0, 14, 72 denote incubation time (hours) at 25 ± 2 C).

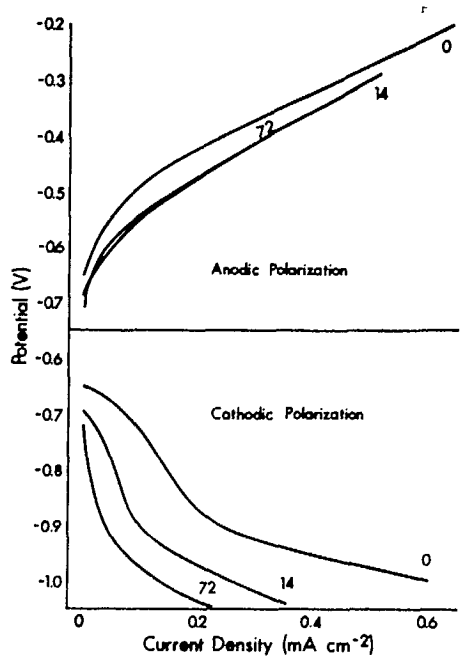

FIGURE 7 — Polarization curves for mild steel in synthetic medium (containing 1800 mg sodium lactate/1) inoculated with isolate #200. (0, 14, 72 denote incubation time (hours) at 25 ± 2 C).

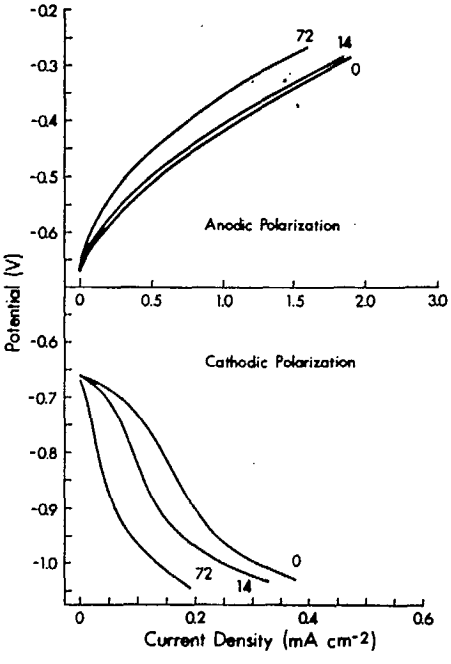

FIGURE 6 — Polarization curves of mild steel in uninoculated Butlin's medium (control). (0, 14, 72 denote incubation time (hours) at 25 ± 2 C).

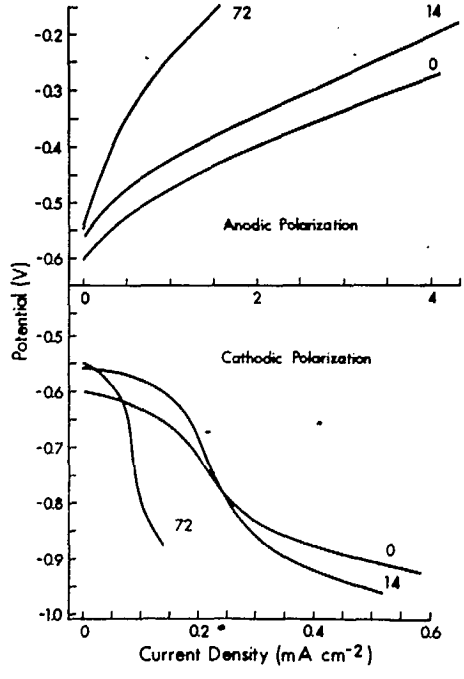

FIGURE 8 — Polarization curves for mild steel in uninoculated (control) synthetic medium (containing 1800 mg sodium lactate/1). (0, 12, 72 denote incubation time (hours) at 25 ± 2 C).

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 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

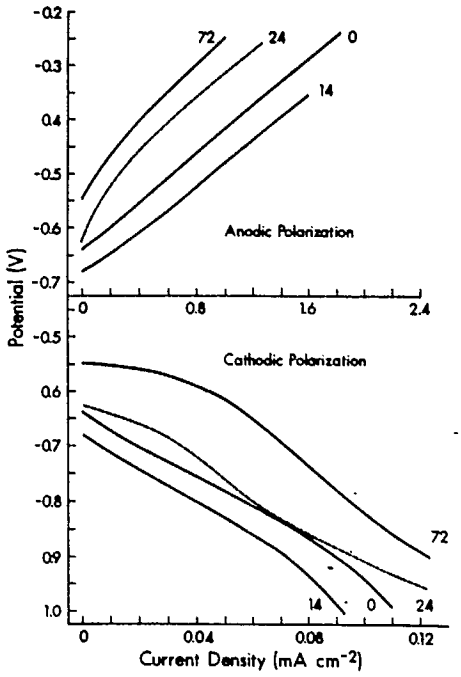

FIGURE 9 — Polarization curves for mild steel in unautoclaved, uninoculated (*i.e.*, with natural flora) produced water. (0, 14, 24, 72 denote incubation time (hours) at 25 ± 2 C).

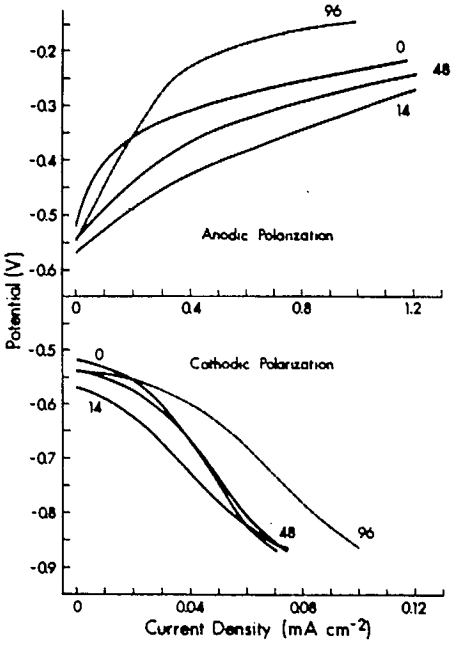

FIGURE 10 — Polarization curves for mild steel in autoclaved produced water inoculated with isolate # 200. (0, 14, 48, 96 denote incubation time (hour) at 25 ± 2 C).

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.

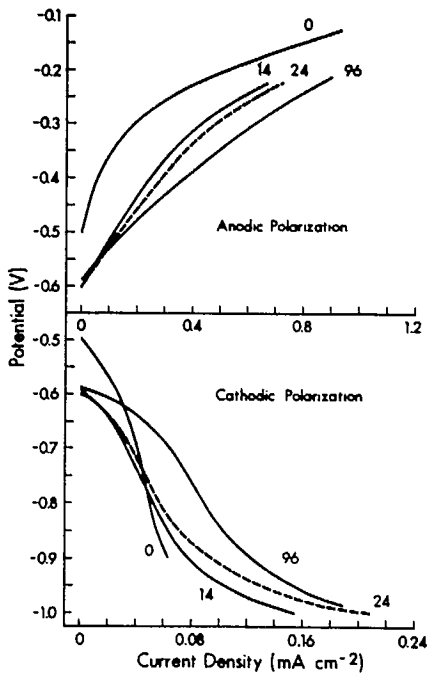

FIGURE 11 — Polarization of mild steel in produced water modified by the addition of 900 mg sodium lactate/1 and inoculated with isolate # 200. (0, 14, 24, 96 denote incubation time (hours) at 25 ± 2 C).

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 fleld 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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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.*, 9 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.

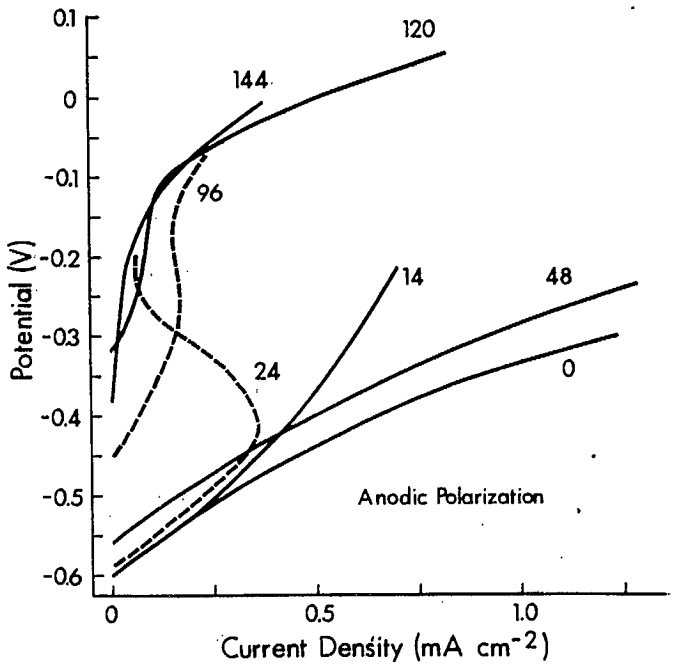

FIGURE 1 — Anodic polarization curves of mild steel in B_{10} medium containing KNO_3 (1 g/l) inoculated with isolate #200. (0, 14, 24, 48, 96, 120, and 144 denote the incubation time—hours at 25 ± 2 C).

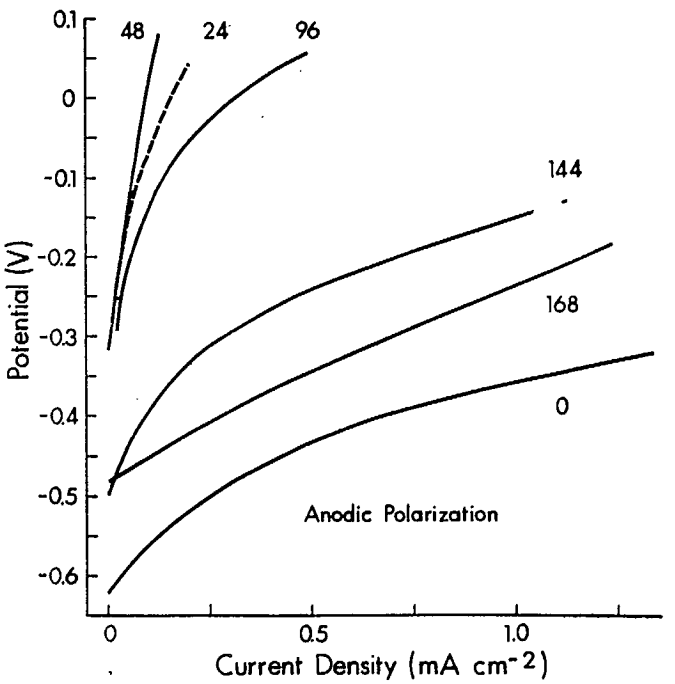

FIGURE 2 — Anodic polarization curves for mild steel in Butlin's medium containing KNO_3 (1 g/l) inoculated with isolate #200. (0, 24, 48, 96, 144, and 168 denotes incubation time—hours at 25 ± 2 C).

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,

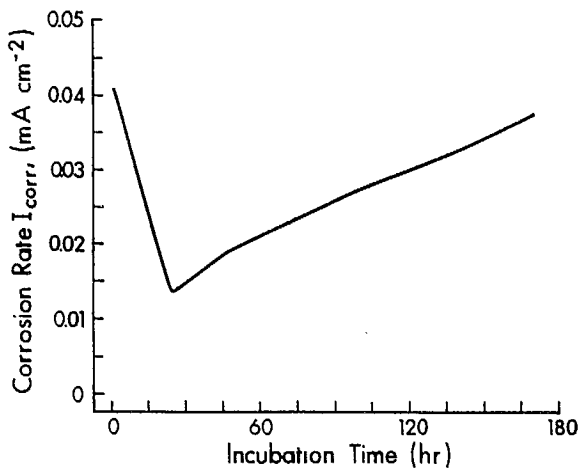

FIGURE 3 — Changes in corrosion rate of mild steel in Butlin's medium containing KNO_3 (1 g/l) inoculated with isolate #200. Corrosion current was estimated by the extrapolation of the Tafel slopes.

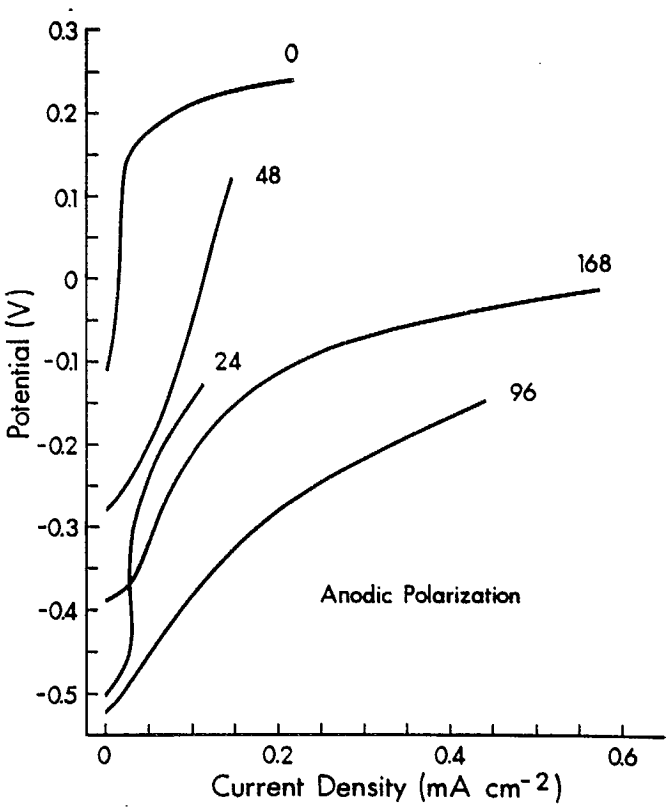

FIGURE 4a — Anodic polarization curves for mild steel in Butlin's medium containing 0.7 g/l sodium nitrite and inoculated with isolate #200. (0, 24, 48, 96, and 168 denote the incubation time—hours at 25 ± 2 C).

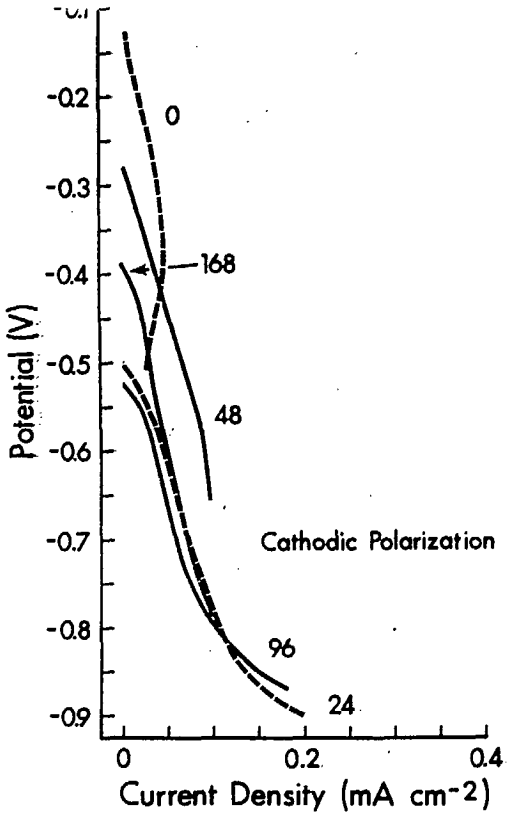

FIGURE 4b — Cathodic polarization curves for mild steel in Butlin's medium containing 0.7 g/l sodium nitrite inoculated with isolate #200. (0, 24, 48, 96, and 168 denote the incubation time—hours at 25 ± 2 C).

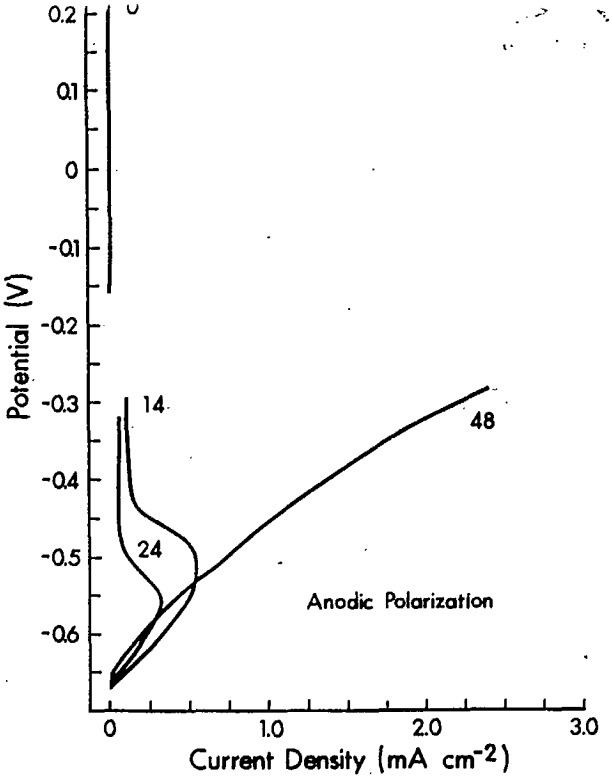

FIGURE 6 — Anodic polarization of mild steel in B_{10} medium containing 0.7 g/l sodium nitrite and inoculated in isolate #200. (0, 14, 24, and 48 denote incubation time—hours at 25 ± 2 C).

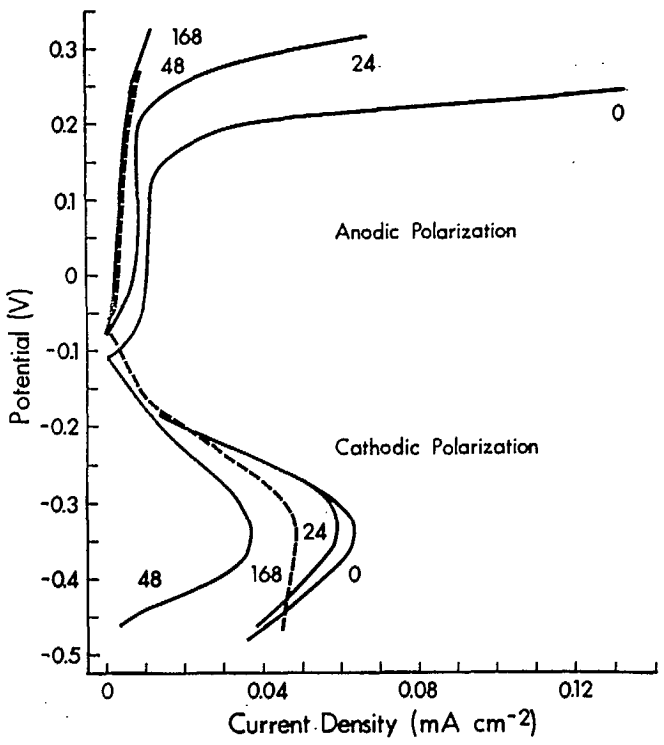

FIGURE 5 — Polarization curves of mild steeel in uninoculated (control) Butlin's medium containing 0.7 g/l sodium nitrite. (0, 24, 48, and 168 denote incubation time—hours at 25 ± 2 C).

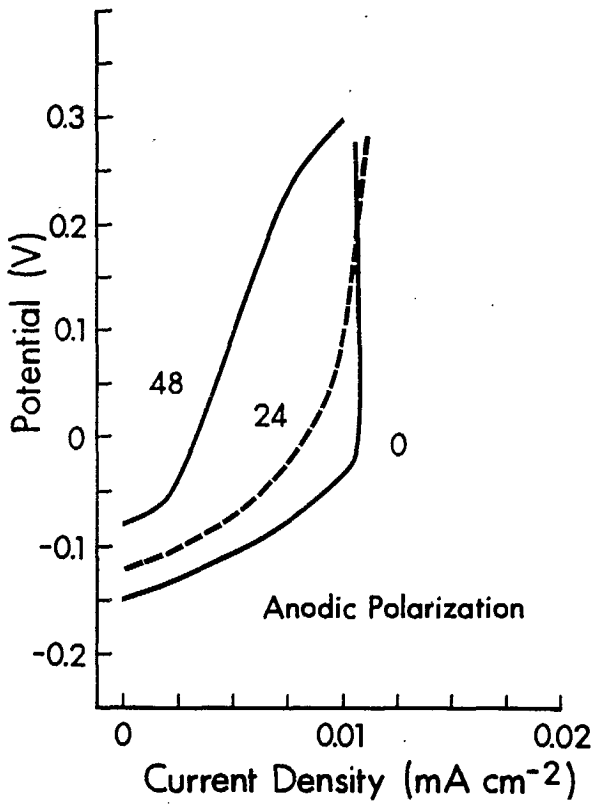

FIGURE 7 — Anodic polarization curves for mild steel in uninoculated (control) B_{10} medium containing 0.7 g/l sodium nitrite. (0, 24, and 48 denote incubation time—hours at 25 ± 2 C).

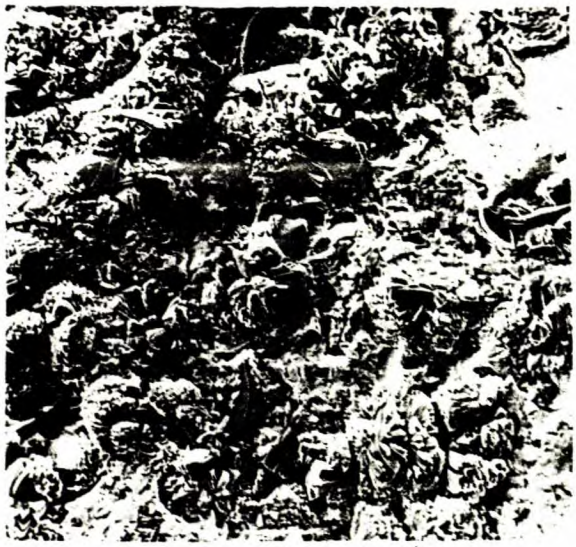

FIGURE 8 — Scanning electron micrograph (SEM) of mild steel coupon submerged in uninoculated (control) B_{10} medium for 48 hours. The coupon was first rinsed in distilled water and dehydrated in a regime of increasing ethanol concentration—30 to 100%. The micrograph shows formation of a protective densely 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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Corrosion of mild steel by nitrate reducing bacteria

by D. D. Mara and D. J. A. Williams

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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 Kühr[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

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Shirley Institute annual general meeting

At the recent annual general meeting of the Cotton Silk and Man-made Fibres Research Association the director of research, Mr L. A. Wiseman, spoke about the research policy of the Shirley Institute.

In 1966 the Ormerod Working Party recommended that the funds provided by the industry should be used exclusively for research on behalf of the industry; technical services for individual members should be charged; and the Institute should be authorised to carry out sponsored research, provided that such work did not damage British industry, particularly the textile industry. These recommendations were accepted by the Council and the Trade Associations.

Since the formation of the Shirley Institute, the structure of the textile industry has changed markedly. There is a persistent requirement to reduce costs, as the market is very competitive, and the market requirements are becoming increasingly stringent, because of the requirements of large buyers and of customers. It is therefore difficult to decide what research should be carried out by the research association.

The research necessary to support current technical services is complementary to that available from machinery producers, suppliers of textile products, and fibre producers. In supporting this aspect of the Institute's work, the industry supplies itself with an independent source of assistance which is independent of commercial pressures.

The current major research programme has been divided into a number of categories: research on new and potential technology, interface problems, automation and automatic control in finishing operations, relation of fibre properties to processing performance in spinning, project evaluation, engineering of end products, research relevant to clothing, and general services to the textile industry and the national economy.

The results of the Institute's research provide one source of information to members. Because of its world wide contacts, the Institute is able to supply a much wider range of information. It would be impossible for any organisation to be active in research in all the fields important to the textile industry.

Mr Wiseman praised the work done by the Institute's staff.

Mr J. H. Spencer, chairman of the Association, said that when he first took office in 1965 he did so because he believed strongly in the value of the central research and technical services provided to the textile industry by the Shirley Institute. Although his belief has increased during the intervening years, the support provided for central research has been reduced.

Between March 1966 and March 1971 cuts in the research levy of the Textile Council plus the effects of inflation resulted in a reduction of 35 per cent in the amount of central research undertaken for the industry. However, Shirley increased its sales of repayment and contract work from £38,000 in 1965-6 to £345,000 in 1969-70. The Institute cannot hope to continue increasing its sales and may not be able to maintain them.

Mr Spencer felt that Shirley needs a statutory levy to support its central researches on which to base its repayment and contract work. The alternative, voluntary contributions, has already proved a failure in various industries.

If the levy is not arranged the Institute will have to look after its own interests and much of the cooperation with Wira and Hatra will have to cease.

During the last few years the staff has been reduced from approximately 500 to 345 and it may have to be cut more.

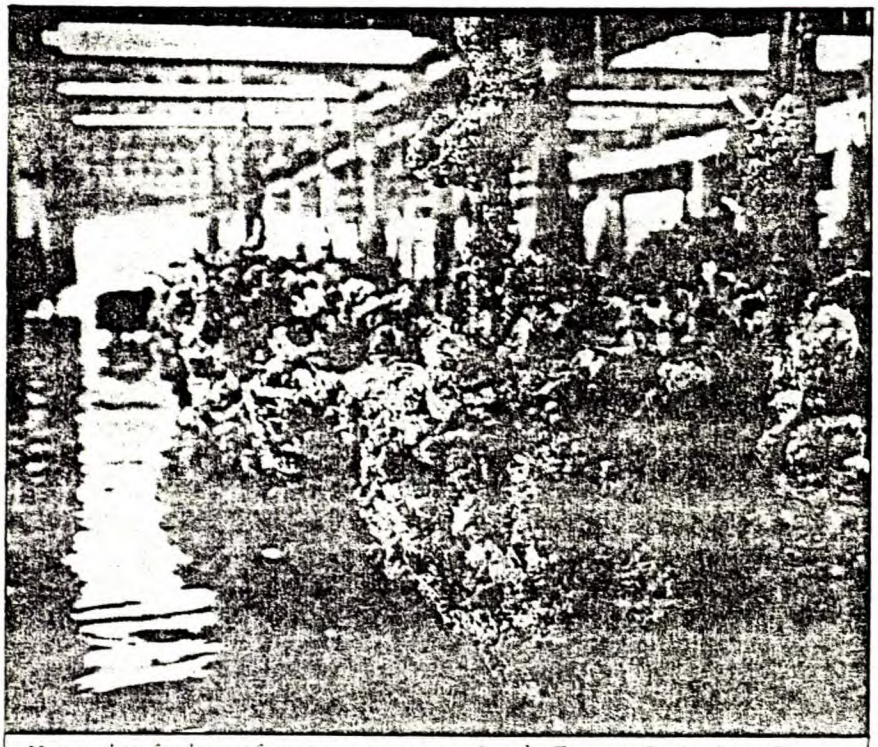
If Shirley did not exist the majority of firms would have no research assistance at all since only the larger firms could afford some of their own research.

Mr Spencer concluded by saying that the Shirley Institute is the servant of the industry and it is now for the industry, and the industry alone, to decide on the nature, the scope and the level of the Institute's future activities, researches and technical services.

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.

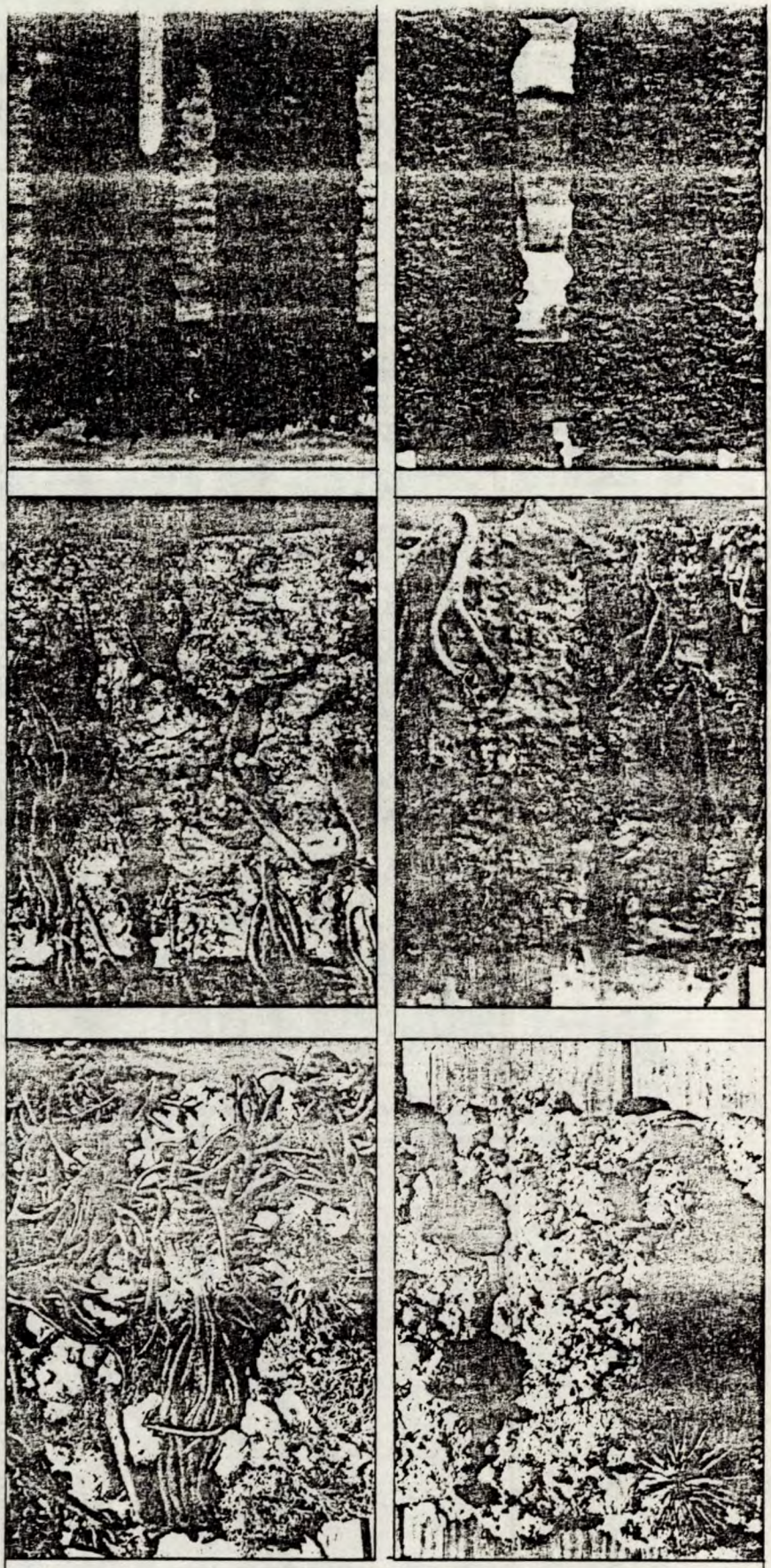
The effects of fouling isn't anything new. "Deterioration of wood ship hulls by marine borers," says W. W. Kirk, manager, Francis L. LaQue Corrosion Laboratory, International Nickel Co., Inc., Wrightsville Beach, N.C., "was noted long ago to the extent that owners found ways to sheath the hulls with 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.

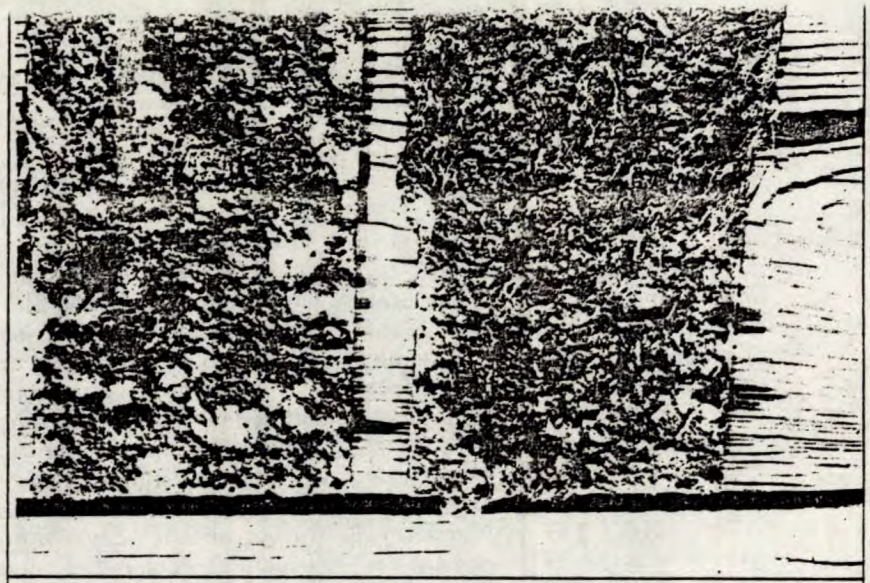
The problem was such that finding a solution to one was not necessarily a solution for the other. Removal of slime from the condenser tubing was generally the easier of the two.

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.

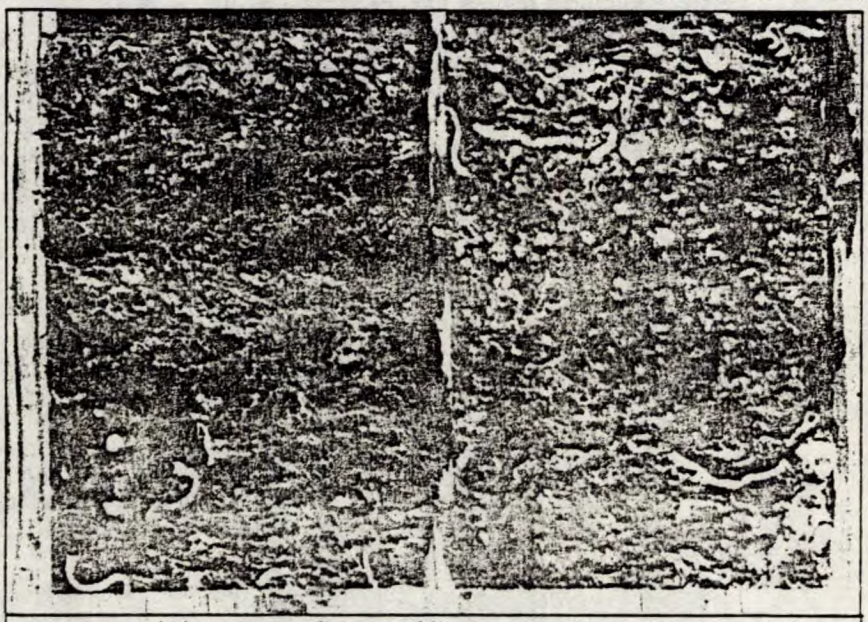
Heating the sea water to above 110°F has also been effective in controlling mussel fouling. But here, too, was the problem of determining the optimum time interval for heating the circulating water without getting into a detailed study.

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.

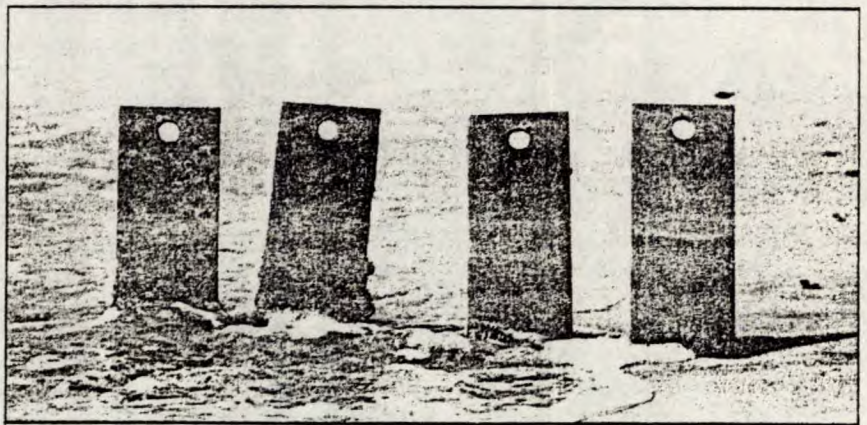
Another important factor that must be considered in any problem dealing with fouling is that the con-



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.

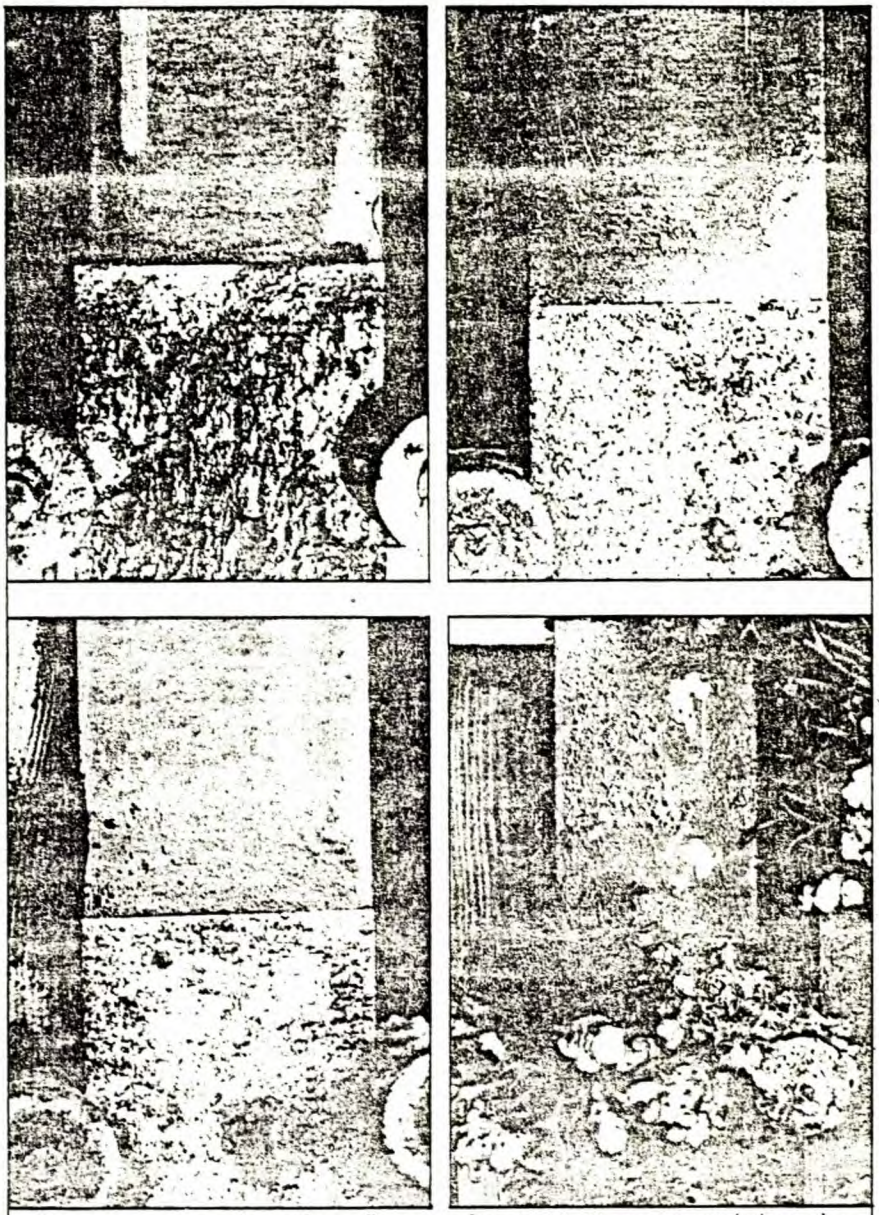
Of the paints that Dexter tested in the North Atlantic environment, the 90-10 copper-nickel in a phenolic matrix served best.

"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.

Fungal Corrosion

BEHAVIOR OF METALLIC AND OXIDE COATINGS DURING DEVELOPMENT OF MICROBIOLOGICAL CORROSION IN THE ATMOSPHERE

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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

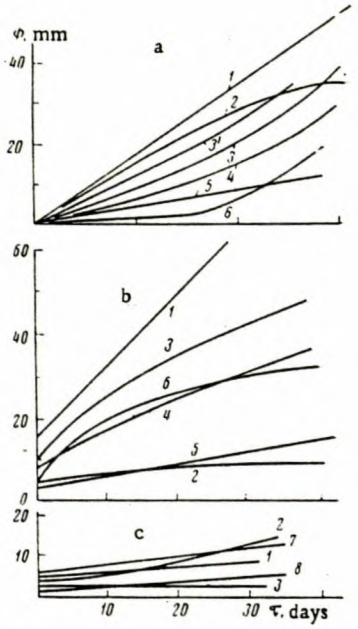

Fig. 1. Growth of cultures of fungi isolated under operational conditions: a) at 6°; b) at 6° after preliminary cultivation at 20°; c) growth of cultures of International Electrical Engineering Commission fungi at 6° after preliminary cultivation at 20°: 1) Trichoderma sp.; 2) Cladosporium sp.; 3) Penicillium sp.; 3') Penicillium (FLT); 4) Cephalosporium sp.; 5) Cladosporium sp. (white colonies); 6) Trichoderma sp. (FLT); 7) Aurocobsidium; 8) Scopulariopsis.

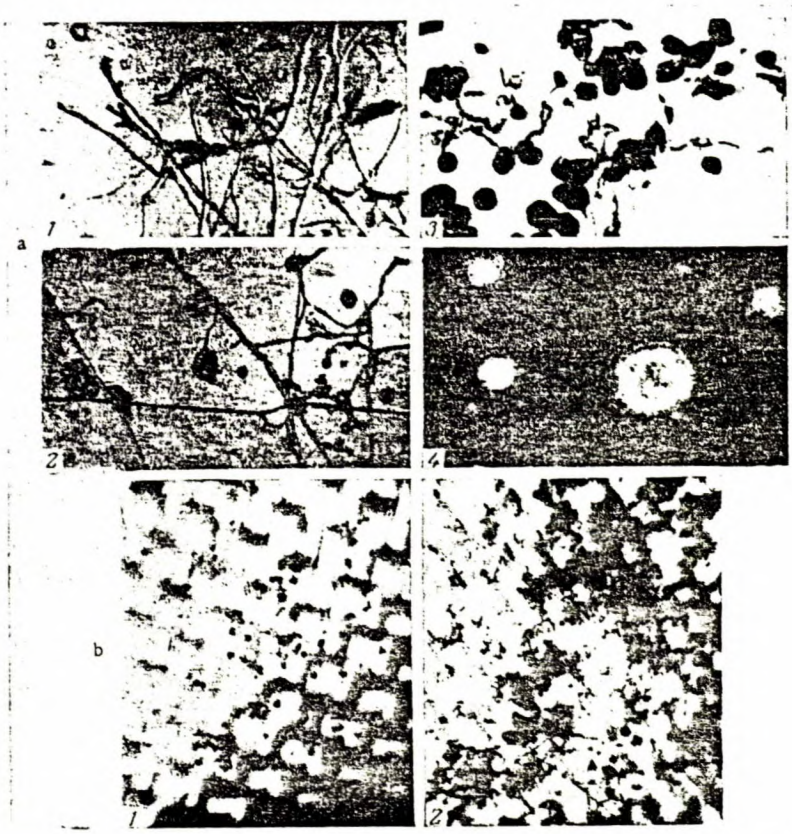

Fig. 2. a) Appearance of colonies of mycelial fungi on surfaces of structural elements in operational use (×300): 1) Penicillium sp.; 2) Cephalosporium sp.; 3) Trichosporium sp.; 4) Aspergillus sp. (colonies); b) development of Aspergillus sp. colonies: 1) after 3 days; 2) after 15 days.

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	—	—	—	—
	$Cu_6Ni_3Cr0.5$	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.

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THE ROLE OF SULPHATE-REDUCING AND SULPHUR-OXIDIZING BACTERIA IN THE LOCALIZED CORROSION OF IRON-BASE ALLOYS — A REVIEW

Gustavo CRAGNOLINO[1] and Olli H. TUOVINEN[2]

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:

$$2S_x^{2-} = S_{x+1}^{2-} + S_{x-1}^{2-} \quad (x=2,\ 3,\ 4,\ 5)$$

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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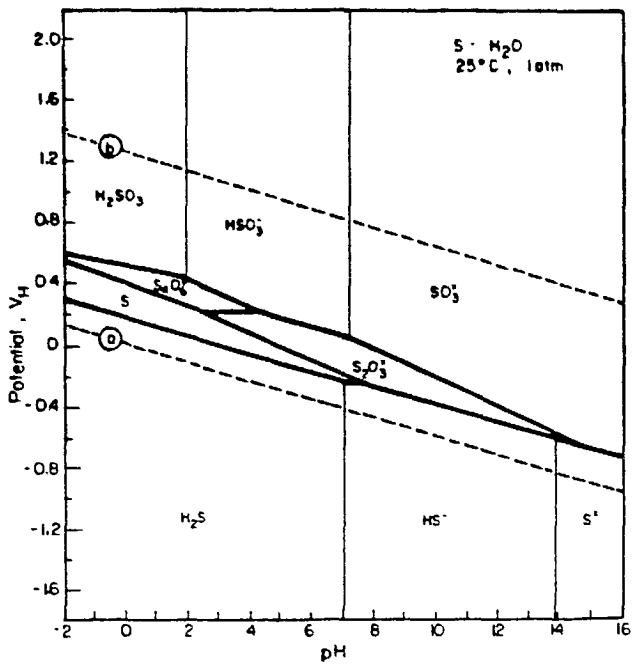

Fig. 1 Potential-pH metastable equilibrium diagram for the system S-H_2O at 25°C and 1 atm. Dithionite ($S_2O_4^{2-}$), dithionate ($S_2O_6^{2-}$), trithionate ($S_3O_6^{2-}$), and sulphate (SO_4^{2-}) are not included. Total concentration = 0·2 g S/l H_2O (Valensi 1973). With permission of CEBELCOR

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 $S_4O_6^{2-}$.

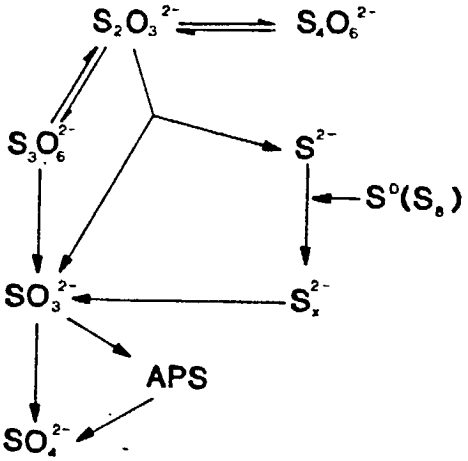

Fig 2. A schematic, non-stoichiometric presentation of the microbiological oxidation of inorganic sulphur compounds. Several enzymatic reactions need to be characterized, especially those associated with the oxidation of sulphide, polysulphide, and elemental sulphur. Several other pathways have been 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.

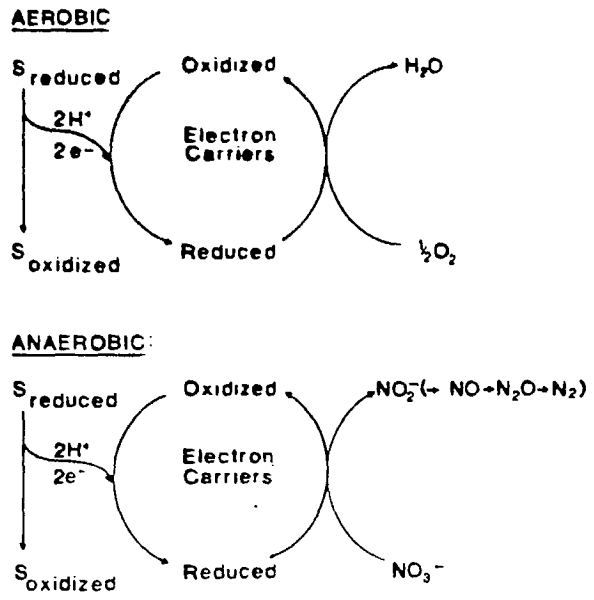

Fig 3 Aerobic and anaerobic oxidation of sulphur compounds by thiobacilli

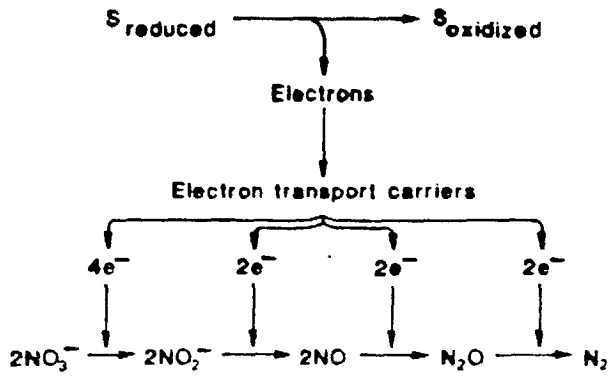

Fig 4. Microbiological oxidation of sulphur compounds coupled to denitrification

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

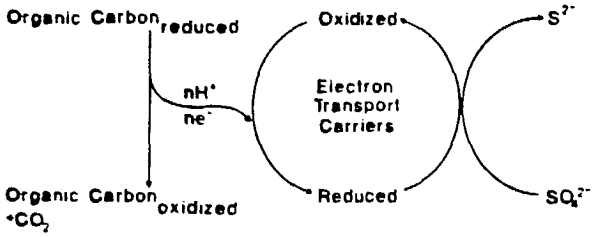

Fig 5 Organic carbon as an electron donor for the microbiological reduction of sulphate

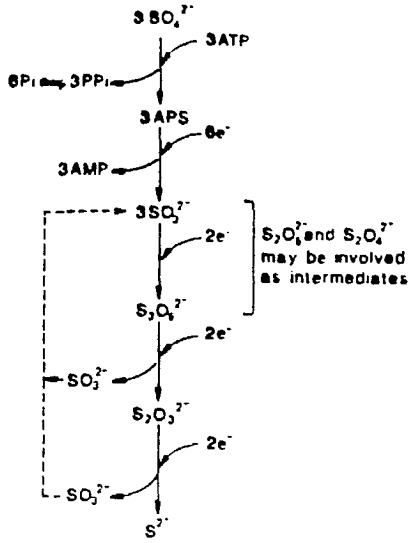

Fig 6. Pathway of the microbiological reduction of sulphate (modified from Thauer & Badziong 1980) APS = Adenosine 5'-phosphosulphate

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 (Sūry 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.

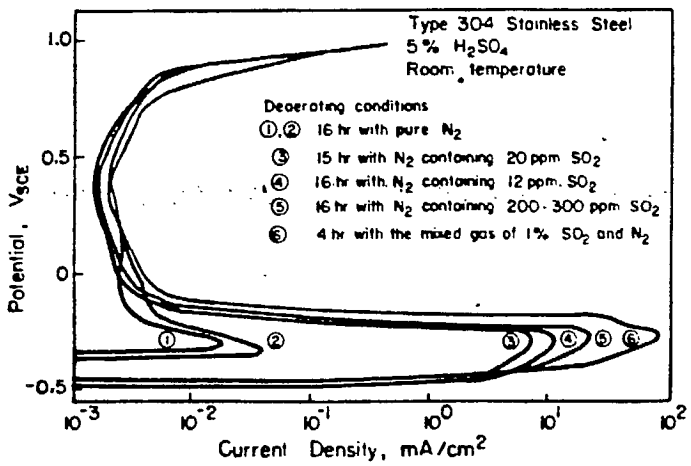

Fig. 7. Anodic polarization curves of Type 304 stainless steel in 5% H_2SO_4 solution showing the influence of SO_2 addition to nitrogen gas used for deaeration (curve 2 was obtained after a cathodic pretreatment for 10 min. at −0·7 V_{SCE}) (Masuo *et al.* 1978). With permission of the Japan Society 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.

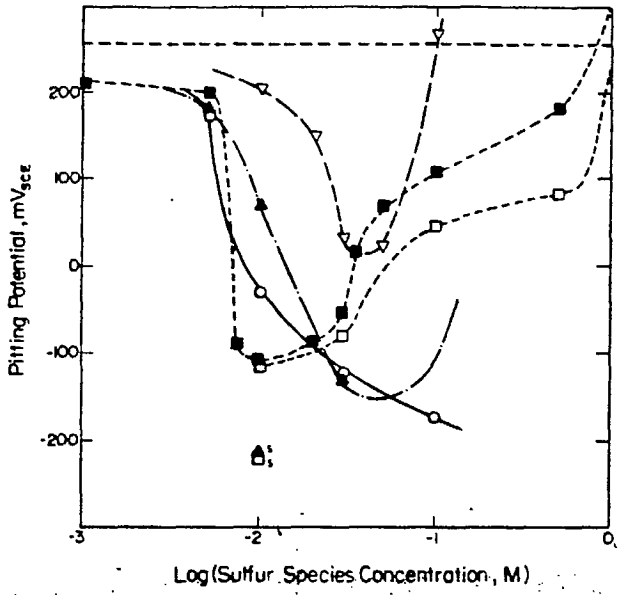

Fig. 8. Pitting potential data for Type 304 stainless steel in 0·25 M NaCl with additions of sulphur compounds. The dotted line at 260 mV represents the pitting potential with no additions. Pitting potentials are shown for $Na_2S_2O_3$ (■, and $Na_2S_4O_6$ (▲) with no pH adjustment and for the following with $5{\cdot}05 < pH < 6{\cdot}5$. $Na_2S_2O_3$ (□), 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

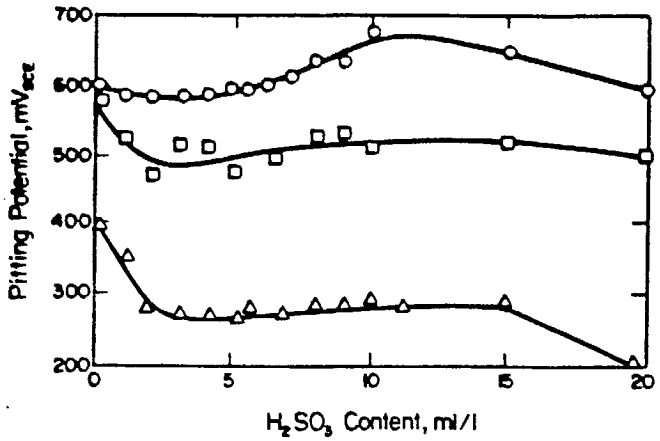

Fig 9 Pitting potentials of Types 316 (○), 304 (□), and 430 (△) stainless steels in 0·017 M NaCl solution containing increasing concentrations (0 to 20 ml/l) of a sulphurous solution containing 30 l SO_2/l H_2O (Luffkin 1973). With permission of the Anti-Corrosion Methods and Materials

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

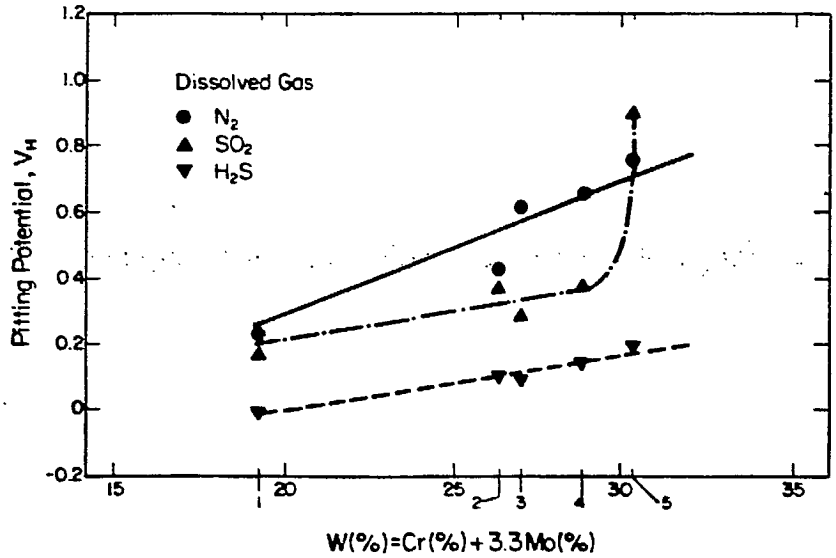

Fig. 10. Effect of dissolved SO_2 and H_2S on the pitting potential of austenitic CrNi and CrNiMo stainless steels in 1 M NaCl solution. 1-Type 304, 2-Type 316; 3-Type 316L; 4-Type 317; 5-Type 317L (Herbsleb 1982). With permission of Werkstoffe und Korrosion.

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 (Piehl 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.

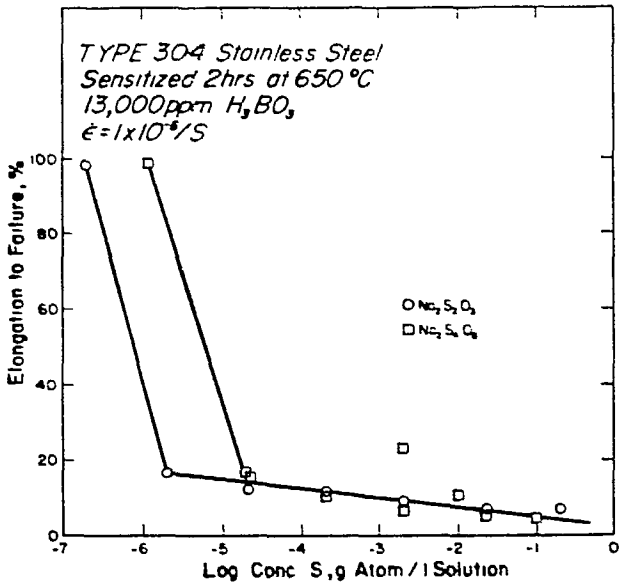

Fig 11. Elongation to failure vs. concentration of sulphur for sensitized Type 304 stainless steel in air saturated boric acid solution containing $S_2O_3^{2-}$ and $S_4O_6^{2-}$ at room temperature (Dhawale *et al.* 1982). With permission of the Electric Power Research Institute.

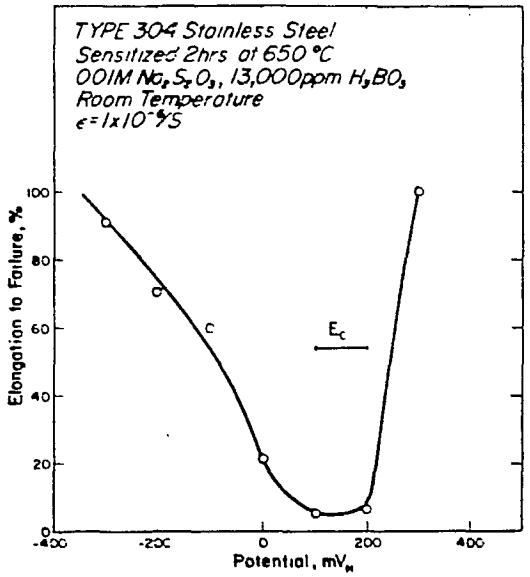

Fig. 12. Elongation to failure vs. potential curve for sensitized Type 304 stainless steel in deaerated boric acid solution containing 0·01 M $Na_2S_2O_3$ at room temperature. The corrosion potential range attained in air saturated solutions is also indicated (Dhawale *et al.* 1982). With permission 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

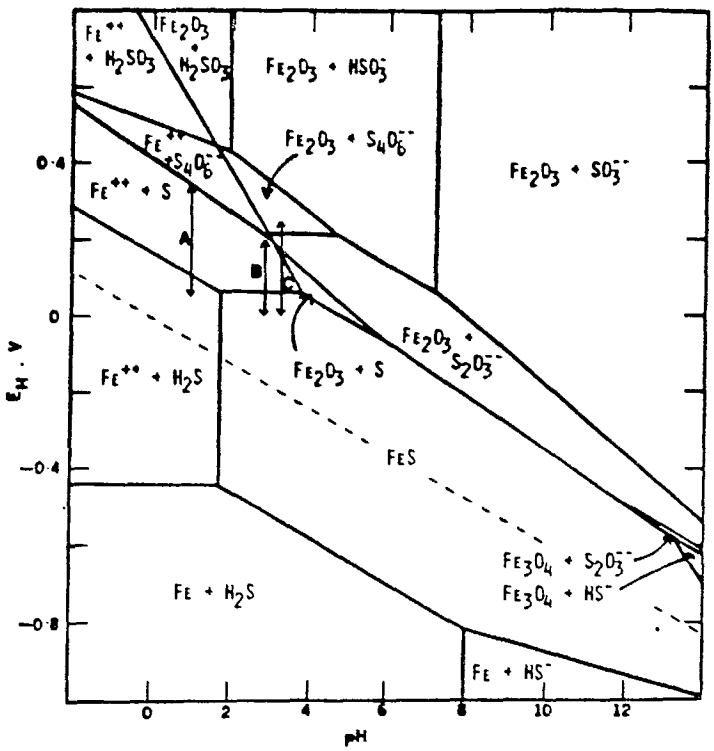

Fig. 13. Potential-pH diagram for Fe-S-H_2O at 25°C (excluding SO_4^{2-}). Severe SCC ranges obtained by different authors are indicated by arrows (Newman *et al* 1982c). With permission of the Metallurgical Transactions

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

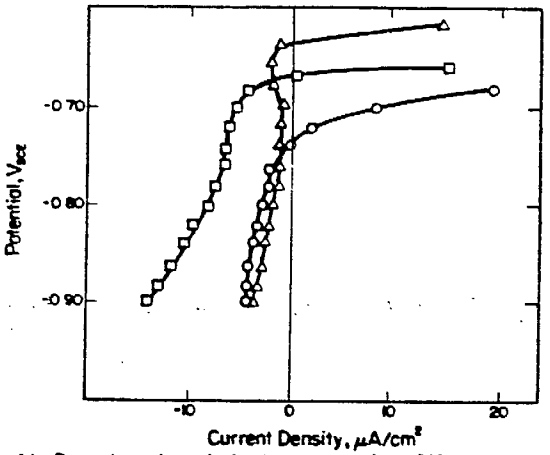

Fig. 14. Potentiostatic polarization curves for AISI 1020 steel in artificial sea water contaminated with sulphate-reducing bacteria for various incubation periods. 96 hr of incubation (total sulphides 10^{-3} M, pH 7·8, redox potential −510 mV) (○); 72 hr of incubation (total sulphides $1{\cdot}4 \times 10^{-4}$ 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.

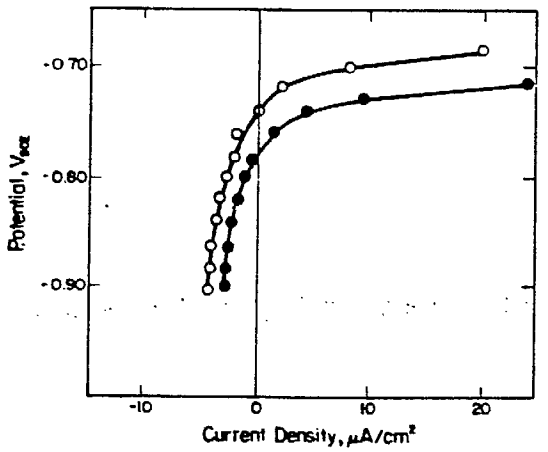

Fig. 15. Potentiostatic polarization curves for AISI 1020 steel in artificial sea water contaminated by sulphate-reducing bacteria (total sulphides 10^{-3} M, pH 7·8, redox potential −510 mV) (○) and in sea water with the addition of 10^{-3} M Na_2S (pH 8·0) (●) (Salvarezza & Videla 1980). With permission 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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