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METAL RECOVERY FROM LOW-GRADE ORES,
SULPHUR RECOVERY FROM GYPSUM ^{1/}

by

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The Role of Thiobacillus ferrooxidans
in Mining of Copper and Uranium

and

The Role of Bacteria in Sulfur Production

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SUMMARY

METAL RECOVERY FROM LOW-GRADE ORES,
SULPHUR RECOVERY FROM GYPSEUM ^{1/}

by

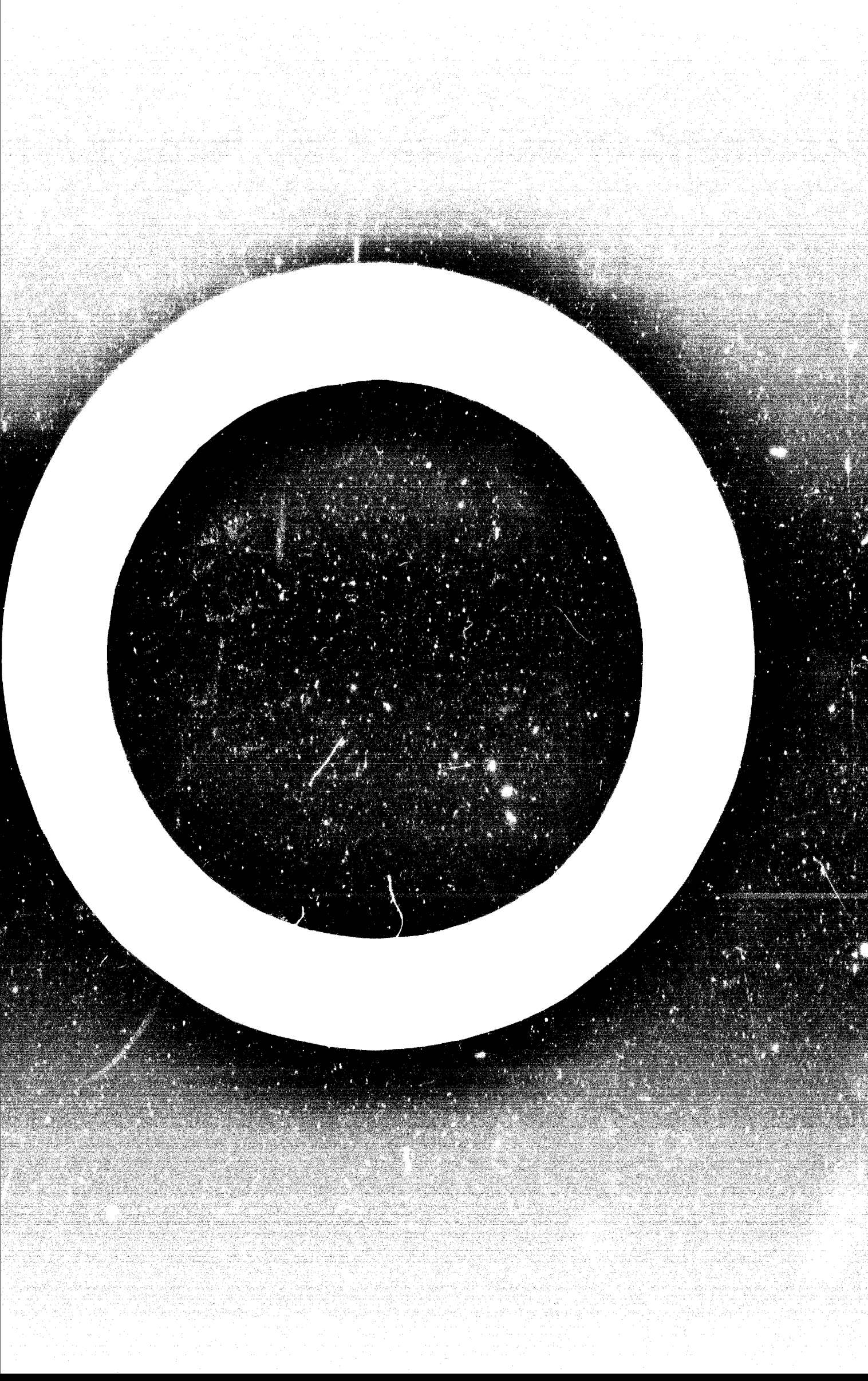
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The Role of *Thiobacillus ferrooxidans*
in Mining of Copper and Uranium

I. Copper from sulfide ores of low mineral content.

- Introduction
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- Bacterial oxidation of copper sulfide minerals
- Physiology of *Thiobacillus ferrooxidans*
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The mining and recovery of copper by leaching from low grade ores have become significant parts of the copper industry. Studies have shown that the bacterium *Thiobacillus ferrooxidans*, by rapidly oxidizing and solubilizing sulfide minerals, plays an important role in waste-ore leaching operations. Temperature and oxygen content of gases in the interior of some ore dumps are incompatible with microbial activity, but in low temperature leaching, and even in cases of high temperatures, it is suggested that bacterial oxidation and solubilization are important. Certainly, adjustment of conditions of leaching to more adequately accommodate bacterial growth has greatly increased the efficiency of waste-ore leaching. Further improvements in efficiency may be expected as more knowledge of the bacterium and its activity in leaching systems becomes available.

II. Uranium from its oxides when associated with iron pyrite.

- Chemical process for uranium extraction
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A proposed bacterial leaching process for uranium

Although bacterial leaching of uranium is of some economic importance, the process is not rapid enough for general uranium production even in those cases where steps have been taken to increase its efficiency. Recently a reverse flow, six tank continuous system has been proposed as a means of rapid extraction of uranium from its oxide ores by bacterial action. The authors state that the latter process may prove to be economically competitive to the conventional acid-oxidizer method of uranium extraction.

Major economies of bacterial leaching lie in the decreased quantity of acid required and in the lower temperature requirements. Acid leaching requires 60-80 lbs. of sulfuric acid per ton of ore, as compared to 25 lbs./ton in bacterial leaching which of course generates considerable acid from pyrite oxidation. A temperature of 70°C is essential for chemical leaching whereas bacterial leaching occurs at ambient temperatures.

The Role of Bacteria in Sulfur Production

Introduction

Bacterial formation of sulfides from sulfates

Use of domestic and industrial wastes for sulfate reduction

Other materials suitable for use in a sulfate reduction process

Description of the English pilot plant sulfur production process

Conclusion

References

Attempts to use sulfate reducing bacteria for commercial production of sulfur have been made. The most successful of these, to my knowledge, was developed in Britain through a successful pilot plant stage with a daily sulfur production of about 200 lbs. sulfur. This project was abandoned about 10 years ago, but is now being revived.

An interesting and significant aspect of the British project was the use of London raw sewage as the source of the organic substance. It was hoped that the method would prove capable of removing much of the soluble organic matter from domestic sewage and at the same time produce valuable sulfur from inexpensive gypsum.

THE ROLE OF THIOBACILLUS FERROOXIDANS IN MINING OF COPPER AND URANIUM

I. Copper From Sulfide Ores of Low Mineral Content

Introduction

The cement copper yield from dump leaching operations of low grade ore in the U. S. A. in 1967 was 250,000 tons (1). This was about 15% of the total domestic production and represented an increase from leaching of about 70% since 1965 (1,2). Factors affecting the increased production from leaching were discussed by Argall (3). He suggested that an understanding of the microbiological aspects of the leaching process was in part responsible for the increased copper production.

It is the purpose of this paper to present the essential operational details of dump leaching practises and more particularly to consider the role of Thiobacillus ferrooxidans in leaching various types of copper ores. Although microbiological aspects will be emphasized, some engineering and chemical items will be discussed. Detailed treatment of the latter will be found in published reports noted in the list of references.

Historical

Alchemists of the sixteenth century knew of the replacement of soluble cupric ion by metallic iron (5). Also noted was the rapid corrosion of iron pipe by copper containing water and the deposition of metallic copper. Nadkarni and Wadsworth (6) state that the first economical application of copper precipitation or cementation in the western world was the recovery of copper from leach water at the Rio Tinto mines in Spain in the late sixteenth century. Heap leaching at Rio Tinto, which is still being practised, yielded as early as 1833, 140 tons of cement copper per year. Heap leaching is the term applied to operations where the ore being leached has a much higher copper content than that encountered in leaching of waste dump ore, and where the amount of ore per pile is usually less than 100,000 tons (7,8). Both rate of percolation and aeration are subject to careful control. Dump leaching usually involves millions of tons low grade ore (9).

In the nineteenth century, cement copper was produced in Ireland, England, Germany and Hungary (6). The productive copper areas of western U. S. A. recorded their first cement copper yield about 1890. Apparently, copper was recovered from mine water or from atmospheric water flowing over or through waste copper ore. Rights allowing processing of mine water at both Butte, Montana and Bingham Canyon, Utah, were granted by mine operators in the first decade of the twentieth century. About 2,300 tons of cement copper were produced at Butte in 1910 (6).

Forced leaching of copper ore at Tyrone, Arizona, was initiated following encouraging recovery of copper in leaching a 25 ton pile of mill tailings (7). A 20,000 ton dump of 2.71% copper ore yielded about 40% of its copper during a three year leach period. At Bisbee, Arizona, 45% recovery of copper from a 10,000 ton pile of ore was achieved during a three year period of intermittent leaching. Later a 70% recovery from 8.5 million tons of 0.74% copper ore was reported

It is interesting that metallic iron for cementation of copper has usually been scrap iron and mainly used 'tin cans'. Read (10) stated in 1913 that "a large (precipitation) plant would quickly exhaust the scrap heaps of the world"

Cement copper at present accounts for over 25% of the total copper production at Bingham Canyon, Utah whereas in 1950, it accounted for about 2% of the copper produced. The size of leaching operations that are possible by today's methods is shown by the daily production at Bingham Canyon of 200,000 tons of waste ore and the daily recovery of 400,000 lbs. of cement copper (11)

Studies on the origin of acid mine water in the eastern part of U. S. A. resulted in the discovery by Leathen (12) and by Colmer and Temple (13) of an iron-oxidizing bacterium in 1947. This microorganism was later shown to be able to oxidize free sulfur and certain metal sulfides, as well as ferrous iron, and was named Thiobacillus ferrooxidans (14, 15). Its ability to oxidize certain metallic sulfide ores explained the enhanced oxidation rates of these ores in leaching experiments (15). Numerous reports have associated Thiobacillus ferrooxidans with the natural occurrence of sulfide ores (17, 18, 19, 20, 21, 22) and recently good correlation was found between the numbers of this bacterium in certain parts of leaching operations and the known chemical reaction taking place (23). In spite of these reports, there are still questions regarding the actual role of the bacteria in leaching sulfide ores.

A typical dump leaching operation

A considerable literature exists that deals with successful copper leaching operations. Sheffer and Evans (1) have made a valuable contribution by summarizing the general leaching practices and by bringing together a comprehensive compilation of references. Although they record numerous variations in copper leaching operations, there are certain practices which are essential to any dump leaching program. These are the application of the leaching solution to a large mass of ore, collection of the solution after percolation through the ore and removal of copper from the pregnant solution. A flow sheet showing these essential steps is shown in Figure 1. Ore to be leached is moved from the mine to a site where water can be conveniently applied to it and where the effluent water can be easily collected. Another requirement of a suitable site for placement of the ore is that at least 90% of the leach solution fed to the ore is recoverable (4). The tops of the dumps are prepared so that water may be applied either by flooding through a system of dikes or by sprinkling.

The water percolates through the ore mass and is collected on the natural or artificially prepared surface of the leaching site. It then flows by gravity to a collection pond or directly into a collection system of pipes for delivery to the copper recovery plant.

The most common method for the recovery of metallic copper from the acidic cupric solution is precipitation on metallic iron. In the cementation method the pregnant solution is allowed to flow over iron metal which replaces the copper and precipitates it as cement copper according to equation 1.



Accompanying this reaction in the acidic solution is the release of hydrogen and the reduction of ferric iron to the ferrous form.



Efficient operators reduce to a minimum the loss of iron by reactions 2 and 3 by control of the contact time between iron and the acidic solution.

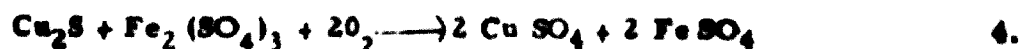
Tailings from the precipitation plant are recycled and thus serve as the major source of solution for the leaching operation. This practice is necessary in many areas because of lack of water and because it provides a suitable means for disposal of the highly acidic, mineralized effluent water.

The effluent water has a high level of ferrous iron and a reduced acid concentration, conditions considered sub-optimal for economic leaching. The ferrous iron level is reduced by storage of the spent solution in a large oxidation pond and the acid level is increased by addition of acid to the water being taken from the pond to the ore dump. Loss of water by seepage into the underlying soil and by evaporation is inevitable. Such loss may be partially replaced by natural atmospheric or surface drainage water. If natural additions are insufficient, replacement water must be added to the system. This is usually done at the oxidation pond.

Leaching of various copper minerals

Solubilization reactions that occur during leaching depend on the nature of the minerals in the ore. Most oxide, carbonate and silicate minerals of copper are soluble in dilute sulfuric acid and slow percolation of the acidic leach water through the ore effects their release into the solution. Sulfide minerals, on the other hand, are quite insoluble and must undergo oxidation prior to solubilization.

The rate of the oxidative reactions which depend on an adequate supply of oxygen may be greatly increased by catalysis and by elevated temperatures. The beneficial effect of the presence of ferric ion in leaching solutions is well recognized. It acts in acidic lixiviants to oxidize and solubilize such minerals as chalcocite (4) and bornite (25).



Equation 4 shows the overall result of chalcocite oxidation by the ferrous ion and agrees with results of laboratory studies (24).

Certain copper sulfide minerals such as chalcopyrite, which because of its wide occurrence is of great economic significance, and covellite are only very slowly soluble in acidified solutions of ferric iron (25, 26). Therefore, leaching operations involving chalcopyrite ores must depend on the use of elevated temperatures or little understood catalytic effects to be economically feasible.

It is well established that high temperatures cause the rapid oxidation of sulfide minerals including chalcopyrite (27, 28). Temperatures in piles of ore used in heap leaching reach very high levels and probably account for the mineral oxidation which occurs. Even in dump leaching, temperature levels of 60-70°C have been reported and I have observed effluent water to be 52°C. These temperatures are high enough to cause a rapid rate of chalcopyrite oxidation (29).

Most sulfide ores are more rapidly oxidized in the presence of Thiobacillus ferrooxidans than in its absence (16, 24, 25). In summary, the rate of sulfide ore oxidation in dump leaching depends on the type of mineral present, the availability of oxygen, the presence of ferric iron, the temperature in the interior of the dump and the activity of Thiobacillus ferrooxidans.

A prerequisite to any leach program is that the copper mineral be accessible to well aerated leach solution. Among important factors which affect accessibility are the nature of the gangue, size of the broken ore pieces and the ability of air to penetrate the ore dump. The ore pieces must be easily penetrable by the leaching solution. Obviously, ore gangue which is alkaline in nature may neutralize so much acid that its leaching becomes uneconomic.

Ore placement for leaching.

A number of factors must be considered in deciding whether or not a certain ore body should be used for leaching treatment (8). The nature, extent and metal content are obviously important, but equally significant for the economics of the process are the availability of a convenient site for placement of the ore, an adequate water supply, and the capability of recovering most of the percolation water. Ore is either taken from the mine to the leach area or it is prepared by breaking, caving or other means for leaching in situ. Ore being moved from the mine to a ravine or other natural drainage area may be crushed below a certain maximum size, but in most instances, ore broken by block blasting is taken directly to the leach area.

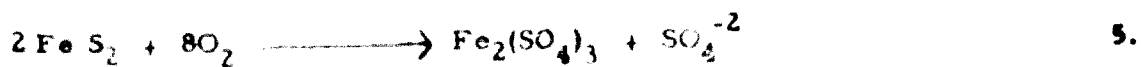
Untreated soil surfaces may be sufficiently impervious in their natural state to serve as the base of the drainage basin. In case the soil is extensively penetrated by water, it may require treatment to make it impervious. Mill sludge, hydrocarbons or plastic materials have been used to prepare the surface soil of a proposed dump

area for placement of ore (1). Treatments used must be able to withstand the destructive effect of large masses of ore being dumped on to the treated surface.

Leaching solutions

Acid solutions with or without ferric ion are considered essential for dissolving even the most soluble copper minerals. As already noted, copper precipitation results in a loss of acid and reduction of the ferric to the ferrous form. Maintenance of good leaching activity requires addition of acid to the system and reoxidation of the ferrous iron.

Sulfuric acid is produced by the oxidative activity accompanying the solution of sulfide ores by microbial or purely chemical action. Leaching of ores low in sulfides may require the addition of acid to the system to maintain the pH in the optimum range of 2.2 - 3.5. At one mine acid is produced by chemical oxidation of sulfur. In cases where iron pyrite is present it may well serve as the source of supplemental sulfuric acid. Thiobacillus ferrooxidans is able to rapidly oxidize iron pyrite with the formation of sulfuric acid and ferric iron. Figure 2A shows the rapid rate of iron pyrite oxidation in a laboratory leaching experiment using Thiobacillus ferrooxidans. Figure 2B shows a similar effect of a heavy cell suspension on iron pyrite in a short term laboratory experiment. In each case pyrite oxidation resulted in formation of acid and ferric ion. Experiments from which the data for Figure 2B were obtained showed good equivalence between oxygen utilization and ferric plus sulfate ions formed, and also showed good agreement with values predicted by use of equation 5 (Table 1).



Reoxidation of ferrous ion at the pH levels found in leaching operations is dependent on the oxidative activity of Thiobacillus ferrooxidans. This reaction requires some time and an adequate supply of oxygen. It takes place primarily in the oxidation pond, but occurs also in the shallow diked ponds formed to allow the leach water to penetrate the ore dump. Oxidation in the ponds results in covering of the surface rock with a heavy precipitate of a ferric compound that decreases the rate of water input into the dump and necessitates mechanical removal of the surface layers to restore normal input rates. Thus, it is clear that microbial activity in the oxidation pond encourages the leaching operation by maintaining the iron in the ferric state and by facilitating removal of excess iron in the oxidation pond rather than at the top of ore dumps.

Collection of leach water and copper recovery

Penetration of leach water, rate of percolation, use of intermittent or continuous flow, control of aeration, etc., are engineering problems which have been considered in several publications (1, 2, 4, 9).

Pregnant leach water from the ore dumps is collected in a system of open channels or in pipes and carried either to small collection reservoirs or directly to the precipitation plant. The usual method of copper recovery is precipitation by metallic iron, but

other methods are used in some cases (30, 31). The collected pregnant solution passes over metallic iron in tanks called launders or in cone-type (11) precipitators until the iron is completely dissolved. The cement copper is then flushed from the launders with high pressure water and collected in a large pile where water is allowed to drain from it. It is subjected to further drying and then sent to the refinery for further purification. Cement copper after drying contains 85-90% copper.

The spent water issuing from the precipitation plant may be recirculated directly to the surface of the leaching area or it may flow to the oxidation pond for treatment as outlined earlier. It is obvious that adequate pumping facilities and a system of acid resistant pipes are essential parts of a leaching system. The most frequently encountered arrangement is the one in which water flows by gravity through the ore, the copper recovery area and into the oxidation pond. The pumping plant is located at the oxidation pond. The choice of arrangement is dictated by the terrain, the size and location of the ore dump, availability of sites for collection reservoirs, etc.

Microbial activity associated with copper leaching operations

Bacteria and dump leaching.

Following the report showing the presence of Thiobacillus ferrooxidans in leach water at Bingham Canyon, Utah (16), a number of workers from many parts of the world (17, 18, 28) reported similar results. This organism seems to be universally associated with areas of surface exposure of sulfide minerals and no other rigorously identified bacteria have been consistently found. There are several reports (17, 20) purporting to show the presence of Thiobacillus thiooxidans in mine leach water. I question whether or not Thiobacillus thiooxidans is present in leaching systems although it may well be found in situ in sulfide ore deposits. In my experience all enrichment cultures of sulfur-oxidizing bacteria even at high dilutions of (10^7 or 10^8) have been able to rapidly oxidize ferrous iron. The possible adaptation of Thiobacillus thiooxidans to become capable of iron oxidation over long periods of laboratory culture in the presence of high ferrous iron concentration has not been studied. If such adaptation proves to be impossible, then our results must be taken as evidence against the presence of Thiobacillus thiooxidans in leaching waters of copper mines.

An extinction dilution method was used to count the numbers of T. ferrooxidans in various parts of several mine leaching operations (23). The numbers of bacteria were observed to vary over a wide range from mine to mine and from point to point in a single mine. More recently obtained results shown in Table 2 generally confirm the earlier report. There is noted a marked decrease in the T. ferrooxidans count on passage of the leach water through tank type precipitators. A less marked decrease has been noted to result from passage through a cone-type precipitation plant, probably because of the greatly decreased contact time. Extensive growth of T. ferrooxidans occurs in the oxidation pond, and bacterial counts as high as 10^8 or 10^9 have been observed. I have been unable to observe any correlation between the numbers of bacteria in the pregnant solution and either its pH, copper or iron content. As reported (23) there was noted a definite effect of the temperature on the copper yield and the bacterial count. Further studies of this relationship have not been reported.

Thiobacillus ferrooxidans has a low growth rate (32). This must be taken into consideration in making bacterial counts and in attempts to understand leaching phenomena. Bacterial growth is made evident in the extinction dilution method by a rusty iron color and precipitate which appears at a cell density of about 10^7 cells per ml. At a generation time of 10 hours it requires about 14 days for this number of cells to be produced from a single cell. Therefore, the period of incubation of dilution series for counting should be continued beyond 15 days. The slow rate of growth of T. ferrooxidans is of significance in establishing the percolation rate schedule and deciding whether or not to use an intermittent leaching program.

A pink yeast, apparently a species of Torula, has been reported frequently in mine water samples and in laboratory enrichment cultures. This yeast, in my opinion, has no essential role in any part of the leaching process. Although it can remain viable in strongly acidic ferric iron solutions, it does not grow unless T. ferrooxidans is also present. The latter is known (33) to excrete organic compounds into the medium in which it grows and the yeast probably takes advantage of this for its growth requirements.

Bacterial oxidation of copper sulfide minerals

Significantly increased rates of oxidation during leaching of several sulfide minerals was attributed to the presence of growing cultures of T. ferrooxidans in the leach solutions (16, 17, 19).

Typical leaching data is shown in Figure 2A. These results clearly implicate bacteria as important catalytic agents in sulfide ore solubilization. The activity of the ferric ion in solubilizing and oxidizing sulfide minerals has long been known (1). Therefore, when an iron-oxidizing bacterium was shown to increase the rate of oxidation of sulfide minerals, its effect was assumed to be due to the regeneration of the ferric iron and not to a direct attack on the ore. More recently (29) results of laboratory studies using active bacterial cell suspensions suggest that T. ferrooxidans does directly attack certain sulfide minerals, oxidizing sulfides to sulfates, and iron to the ferric state and solubilizing the metals present (34).

However, it can be seen from the data of Table 3 that ferric ion in the presence of active bacteria does stimulate oxidative activity on some, but not all sulfide ores. It seems that successful dump leaching may be ascribed to a combination of microbial and purely chemical oxidation.

The latter becomes relatively more important as the temperature increases. Bryner and Jones (24) showed that in leaching pyrite under controlled temperature conditions there occurred a minimum rate of oxidation at about 50°C followed by an accelerating rate up to 70°C which was the maximum studied. Data (29) obtained from cell suspension studies over a temperature range of 35°C to 55°C showed a decreased activity at about 50°C and a non biological oxidation which became significant at 55°C . It will be recalled that in a pile of ore which is being leached, temperatures as high as 80°C have been recorded. At such temperatures it is most probable that biological activity has ceased and that the observed reactions are wholly chemical in nature. Significant bacterial activity would seem to occur only at low temperatures. On the other hand, bacteria may initiate oxidative processes which in well insulated areas in ore dumps lead to production of high temperatures. In such areas, heat induced processes may account for the leaching phenomena.

Physiology of Thiobacillus ferrooxidans

Thiobacillus ferrooxidans is an autotrophic bacterium. Its carbon requirements can be supplied by carbon dioxide and its other requirements can be obtained from simple inorganic salts which are supplied by the minerals present in most ore dumps. The possible exception to this is a source of nitrogen, usually obtained from the ammonium ion. It is likely that in blasting ore deposits with slurry explosives, sufficient nitrogenous compounds for good bacterial growth are formed and remain in the ore. Energy requirements are met by oxidizing activity using sulfide minerals and oxygen. In certain instances, where microbial activity is intense, it may be possible for some nutritional deficiencies to arise. The most likely ones would seem to be carbon dioxide, oxygen and perhaps nitrogen. Highly acidic water carries but little dissolved carbon dioxide, such a small amount that it may be unable to satisfy the carbon required for growth of large numbers of bacteria. It has been established that for every mole of O_2 utilized for bacterial growth, about 0.02 mole of carbon dioxide can be used (35). The carbon dioxide to oxygen ratio in air is less by a factor of 10 than the 0.02 value for the ratio of CO_2 to oxygen required for growth of T. ferrooxidans. Since gas samples from actively producing dumps are devoid of oxygen, it seems possible that not only oxygen but also carbon dioxide may be limiting microbial activity. The observed absence of oxygen in the interior of ore dumps is evidence of the intense oxidative activity occurring there. Some operators inject pure oxygen into dumps in an attempt to increase the rate of copper release. The results of such treatments are not available.

Thiobacillus ferrooxidans is remarkably resistant to high concentrations of heavy metals. Viable cells have been reported in solutions containing over 2,000 ppm cupric ion, 10,000 ppm aluminum ion, 5,000 ferric ion and smaller amounts of several other metallic ions such as manganese, zinc, nickel, uranium and cobalt.

Presence of rare metals in leaching solutions

Studies in our laboratory, field observations and discussions indicate that certain elements which are not periodically removed from leach water accumulate until a certain, apparently equilibrium concentration is reached. Large quantities of aluminium and smaller quantities of nickel, cobalt and manganese have been found in all leach water samples examined. Periodic removal of these ions by an inexpensive resin adsorption method may prove to be an economic operation. Other metals of greater economic value also occur in leach water systems (36, 37). Although the concentration of these ions may not exceed a few parts per million, the amount in a fifty million gallon leaching operation would be significant.

Conclusion

The mining and recovery of copper by leaching from low grade ores have become significant parts of the copper industry. Studies have shown that the bacterium Thiobacillus ferrooxidans, by rapidly oxidizing and solubilizing sulfide minerals,

plays a major role in waste-ore leaching operations. Temperature and oxygen content of gases in the interior of some ore dumps are incompatible with microbial activity, but in low temperature leaching, and even in cases of high temperatures, it is suggested that bacterial oxidation and solubilization are important. Certainly, adjustment of conditions of leaching to more adequately accommodate bacterial growth has greatly increased the efficiency of waste-ore leaching. Further improvements in efficiency may be expected as more knowledge of the bacterium and its activity in leaching systems becomes available.

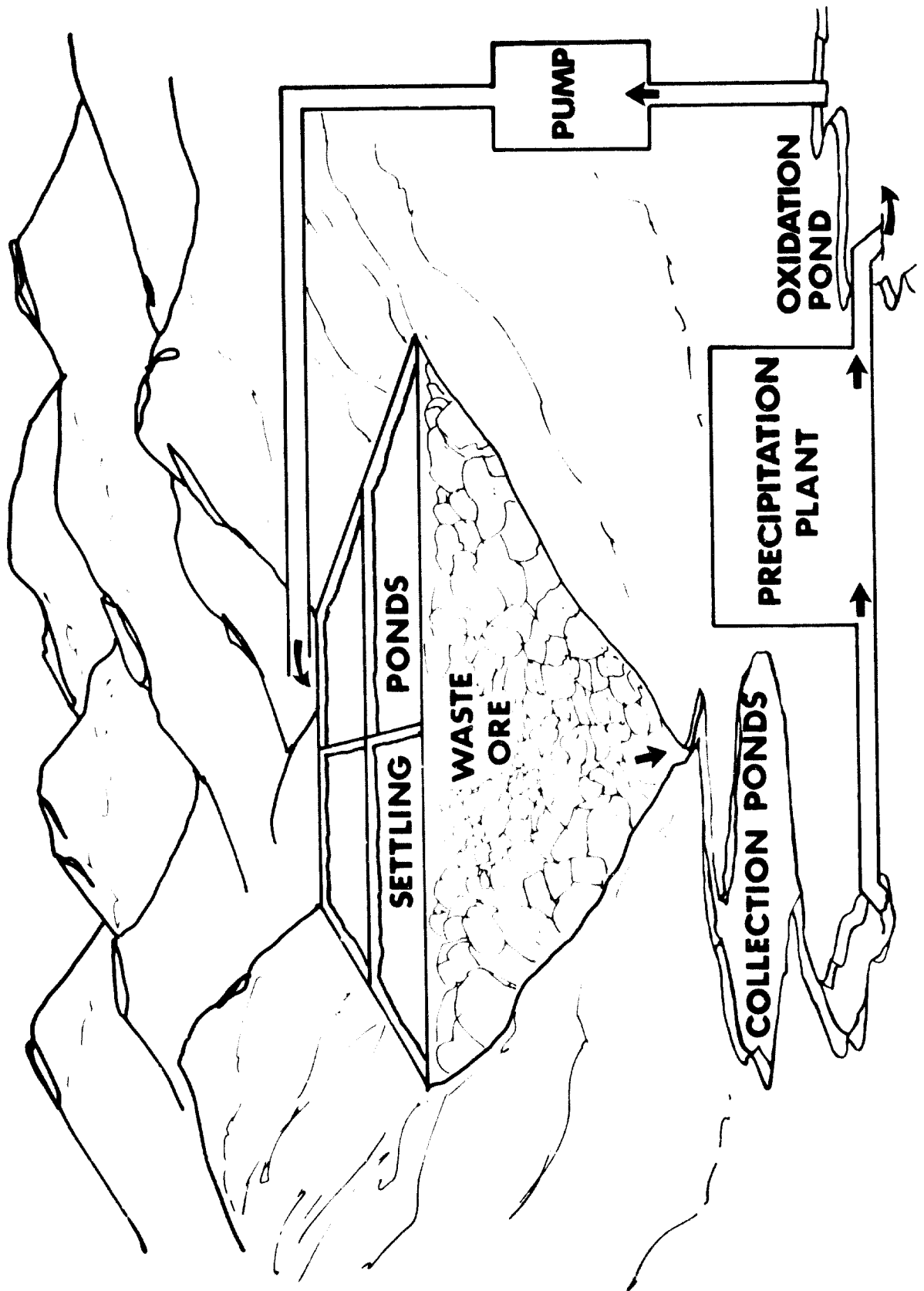
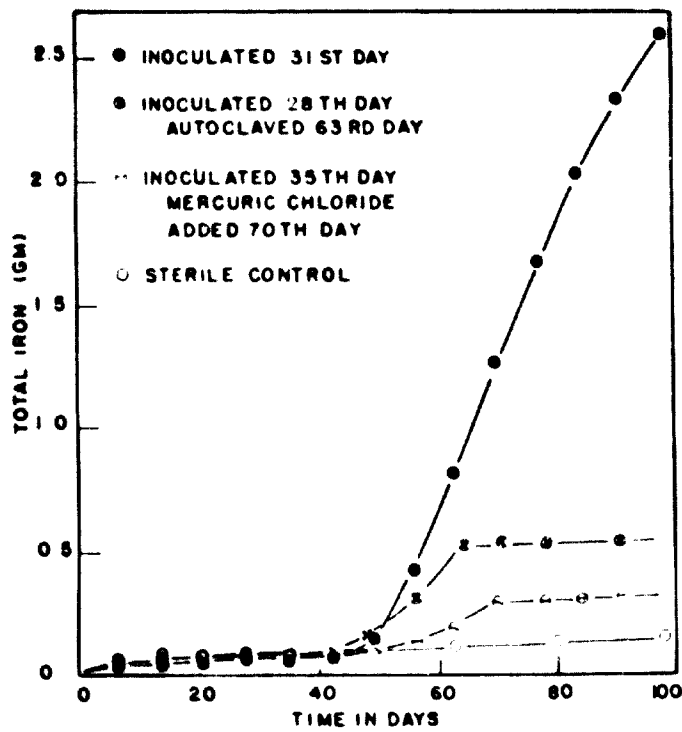
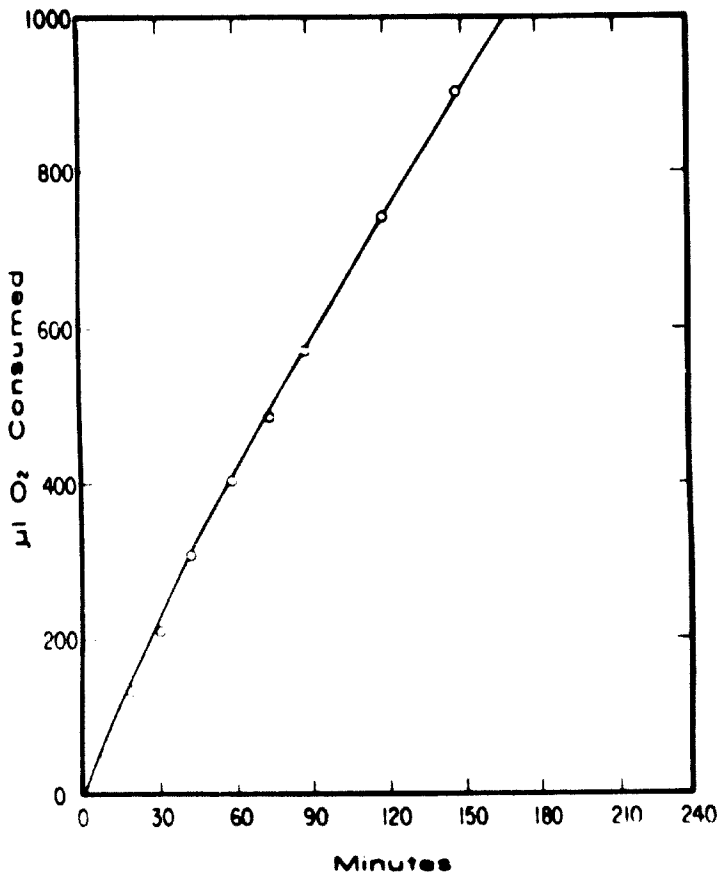


Figure 1. Flow sheet of a typical leaching process for low grade copper ores.



A.



B.

Figure 2. Oxidation of iron pyrite by Thiobacillus ferrooxidans.

A. Leaching experiment.

B. Heavy suspension of intact cells in manometric apparatus with 200 mg pyrite.

Table 1

Oxidation of iron pyrite by a cell suspension
of Thiobacillus ferrooxidans

	Oxygen consumed	Products formed	
		ferric ion	sulfate ion
	μ mole	μ mole	μ mole
control	1	0	0
cells	63	18	39

Table 2 **Thiobacillus ferrooxidans in copper mine leaching waters**

Mine No.	Sample Taken	Precipitation Plant		Oxidation Pond
		Input ml ⁻¹	Effluent ml ⁻¹	ml ⁻¹
1	1969a	100	10,000	100,000
	1969b	10,000	1,000	1,000,000
2	1968	1,000	-	800,000
	1969	10	100	50,000
3	1966	1,000	10	-
	1969	50,000	1,000	5,000
4	1969	1,000,000	5,000	1,000,000
5	1968	100,000	100,000	10,000

Table 3

The effect of ferric ion on the rate of oxidation of pyrite and chalcopyrite by suspensions of intact cells of Thiobacillus ferrooxidans

Ferric ion added μ mole/ml	Sulfide mineral oxidized		
	pyrite μl O ₂ /hr	chalcopyrite μl O ₂ /hr	
0	initial*	375	200
	final**	375	100
12.5	initial*	380	600
	final**	380	200

* initial 30 minutes

** final 60 minutes of 4 hr experiment

Warburg flask contained 200 mg mineral, 0.5 mg Cellular nitrogen, 2 ml Total volume, pH = 2.8, T = 35°C
Aerobic conditions

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II. Uranium From Its Oxides When Associated With Iron Pyrites

Chemical process for uranium extraction

Uranium is obtained from its oxide ores by leaching the finely ground (-400 mesh) material with acidic oxidizing solutions. The lixiviant is a dilute solution of sulfuric acid (pH 2.0 or below) some soluble iron and an additional oxidizing agent. Several specific oxidizing agents have been used, but most frequently employed is sodium chlorate. The oxidizing agent maintains the iron in the oxidized ferric state, which then effectively converts the insoluble tetravalent uranium oxide to the hexavalent state as shown in equation 1.



Hexavalent uranium forms an acid soluble sulfate complex which is separated from the gangue by filtration and is then removed from the solution by ion-exchange reactions (1, 2, 3).

The ore is removed from the mine, crushed and ground to the required particle size and placed in large tanks to be leached.

Thiobacillus ferrooxidans in uranium mine water

The extensive uranium oxide ore deposits of Ontario, Canada, consist of brannerite, uraninite, etc. in a porous conglomerate mixed with substantial amounts of iron pyrite. The mines are subject to considerable water and shortly after mining operations began it was noted that the water was becoming acidic. The increasingly high acid content of the water finally began to cause extensive corrosion of the water removal system of pipes and pumps. It was also noted that the acidic water contained ferric ion. Chemical oxidation of the pyrites in the ore was assumed to be the cause of the acid and ferric iron in the effluent mine water. Further consideration and observation led to the idea that the pyrite oxidation occurring in the mines was biological. Thiobacillus ferrooxidans has been found (4) in large numbers in mine waters of the Lake Elliot, Ontario area, and undoubtedly is responsible for the observed iron pyrite oxidation. As the acidity of the effluent mine water increased, there was noted an increased uranium content which finally reached levels satisfactory for economic recovery.

At present most mines in the Lake Elliot area obtain all their uranium from mine effluents (5). Such effluents (6) contain in excess of one million Thiobacillus ferrooxidans per ml, and even after removal of the uranium by ion exchange reactions, the bacterial count may exceed one hundred thousand per ml. The barren solution is recycled into the mine where it slowly moves downward to the pumping station. In passing through areas of ore in old stopes or through broken ore in situ the lixiviant oxidizes pyrite and dissolves uranium oxide ore. The pregnant solution in the mine usually contains some ferrous ion which is oxidized as the solution is moved to the surface or as it is treated at the surface.

Thiobacillus ferrooxidans has a remarkable capacity to rapidly adapt to high concentrations of heavy metal ions. Bryner et al. in 1954 (7) reported that cultures maintained in the absence of copper for some time were unable to grow in a medium containing 500 ppm copper ion. Cultures that had been freshly obtained from the copper mines, or which had been exposed to increasing copper ion concentration through a series of transfers, showed rapid and immediate growth at 500 ppm copper. Similar results were reported by Trussell et al. (8) which show that although "untrained" cultures of *T. ferrooxidans* have a low uranium tolerance, they rapidly adapt to concentrations of 500 ppm U_3O_8 .

A proposed bacterial leaching process

Although bacterial leaching of ore in situ in the mine is of economic importance, the process is not rapid enough for major uranium production. Studies have been made therefore to develop bacterial leaching procedures on a large scale. Such a process developed to the pilot plant stage has been proposed (9). The method uses a reverse flow of the finely ground ore and lixiviant in a six tank continuous system.

Major economies of the bacterial process are stated to reside in the decreased quantity of acid required and in optimum recoveries at lower temperatures. Chemical acid leaching requires 60-80 lb of sulfuric acid per ton of ore at about 70°C, whereas in bacterial leaching only 25 lb of acid is needed and the bacteria are active at ambient temperatures. The authors state that the microbial process is competitive with the conventional acid-oxidizer method of uranium extraction.

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THE ROLE OF BACTERIA IN SULFUR PRODUCTION

Sulfur and its conversion product sulfuric acid are major items of the chemical market (1). Sulfur production in the U. S. A. in 1968 was 9.7 million tons (1). This was produced by the Frasch mining process and by recovery from petroleum and natural gas.

The world demand for sulfur fluctuates considerably (2). This results in alternating periods of excess and of tight supply. In times of scarcity there have been attempts to exploit other sulfur sources. Perhaps the greatest reservoir of sulfur is in the form of sulfate that occurs most abundantly as gypsum. Massive deposits of this mineral occur in all parts of the world. The development of economically competitive methods for the mass production of sulfur from gypsum would enable almost every area of the world to be self sufficient with regards to this most important chemical product.

Methods for the recovery of sulfur by reducing sulfates to sulfides have long been considered. Some of these have been developed to the pilot plant stage and at least one to industrial production level (3). The details of the latter process are not available but it probably is a purely chemical process using natural gas as the reducing agent for reduction of the sulfate ion.

Biological reduction of sulfates to the sulfide level makes possible the biosynthesis of the sulfur containing amino acids in all green plants and many bacteria and fungi.

Another function of biological sulfate reduction became apparent following the description of the sulfate-reducing bacteria by Beijerinck in 1895 (4). These bacteria utilize the sulfate ion as the primary electron acceptor and produce as the reduced product hydrogen sulfide. Discovery of dissimilatory sulfate reduction by bacteria led to new concepts regarding the origin of sulfur deposits and the microbial origin of many major sulfur deposits is now widely accepted (5). The occurrence of hydrogen sulfide in certain lakes (6) and in other areas of anaerobiosis results from microbial activity. Naturally occurring hydrogen sulfide eventually is converted to free elementary sulfur (S^0) by chemical and/or biological action.

Conditions essential for microbial sulfate reduction are now known and a number of attempts have been made to develop economically feasible methods for sulfur production using bacteria. One such idea using natural gas as the reducing agent was suggested in 1967 (7). Details of this process are not available.

Characteristics of the sulfate-reducing bacteria and a general review of the proposals that have been made for their use in sulfur production will be presented in this paper.

Characteristics of sulfate-reducing bacteria

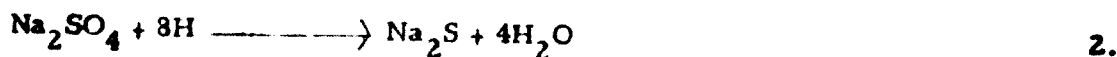
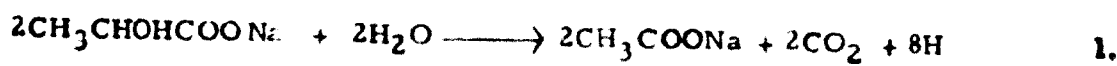
The sulfate-reducing bacteria have been adequately described and their physiology discussed in a monograph by Postgate (8) and in an extensive work by Ivanov (5). In the metabolism of these bacteria the sulfate ion is reduced to hydrogen sulfide.

The organisms are strictly anaerobic and they utilize the sulfate ion as the hydrogen and electron acceptor for the oxidation of organic substances or of hydrogen (8). Beijerinck (4) first described this phenomenon and showed the responsible bacterium to be a small curved rod. Later Van Delden (9) was able to isolate pure cultures of the sulfate-reducers. He confirmed Beijerinck's earlier observations. In 1936 (10) the genus name Desulfovibrio was proposed. An earlier observation that some Desulfovibrio cultures possessed remarkable heat resistance was correctly assumed to indicate the presence of spores (11). The anomaly of a spore-forming vibrio was explained when Campbell (11) succeeded in isolating a typical peritrichously flagellated spore-forming bacterium from cultures of Desulfovibrio. The spore-former was originally named Clostridium nigrificans but this has been changed to Desulfotomaculum nigrificans and two other well characterized sulfate-reducing bacteria have been placed in the genus Desulfotomaculum.

Earlier reports that some sulfate reducers are thermophilic must now be interpreted in terms of impure cultures consisting of a mixture of Desulfotomaculum nigrificans and Desulfovibrio desulfuricans. Postgate (12) states that there are probably no thermophilic Desulfovibrio strains, although some relatively more heat resistant strains have been developed by training procedures. None of the latter have temperature optima above 50°C.

The non-sporogenous sulfate-reducers are recognized as belonging to the genus Desulfovibrio. A new species was described by Le Gall (13). This is a much larger member of the genus and has been named Desulfovibrio gigas whereas the most frequently isolated types have been identified as Desulfovibrio desulfuricans.

Sulfate-reducing bacteria oxidize a variety of organic substances (12) and thereby obtain electrons, most of which are used to reduce the sulfate ion to hydrogen sulfides. The half reactions for the oxidation and reduction steps are shown in equations 1 and 2 using sodium lactate as the energy source.



Although most physiological studies of the sulfate reducers have used Desulfovibrio strains, it is believed that the sporogenous types have similar metabolic activities.

The association of sulfate-reducing bacteria and hydrogen sulfide with crude oil led to studies on the ability of these bacteria to utilize hydrocarbons as the electron source for sulfate reduction. Crude oil contains a number of substances in addition to saturated hydrocarbons, and in view of several unsuccessful attempts to demonstrate growth under strictly anaerobic conditions on paraffin hydrocarbon and sulfate (15) media, it seems most unlikely that such substances are attacked.

Most strains have hydrogenase activity and are able to effect the reduction of sulfate with hydrogen gas. Carbon dioxide fixation by certain strains was reported (14) and although some radioactive carbon dioxide can be shown to be used for cell

substance formation, the amount so fixed is no greater than that absorbed by the typical heterotrophic bacterium. At present, sulfate-reducing bacteria are not considered to be autotrophic.

There is a general agreement that growth and hydrogen sulfide formation are increased by the addition of yeast extract or other sources of bacterial growth factors and amino acids. The cause of the enhancement effect may be direct, that is metabolic use of the additive materials, or it may be a result of the additions making certain nutritional ingredients such as iron more readily available to the cell. At any rate growth response is encouraged by media rich in organic nutrients which in natural conditions also contribute to establishment of suitable redox values. The latter must be no greater than -0.200 V (5) for growth of the sulfate reducers. Failure to provide these low Rh conditions may be the cause of difficulties reported in the culture and maintenance of Desulfovibrio strains.

Growth rates of Desulfovibrio are usually linear with time. Postgate (8) suggests that this response is due, at least in part, to the removal of the necessary ferric ion from the culture medium by the hydrogen sulfide which is formed. The lowered level of ferric ion concentration reduces the rate of cell growth from the exponential to linear. Reduction of growth rate affects the rate of hydrogen sulfide formation and becomes an important consideration in establishment of an industrial process.

Sulfur production from sulfate takes place in two steps: the reduction of the sulfate ion to form hydrogen sulfide and oxidation of the latter to free sulfur. The reductive step requires the expenditure of considerable energy the cost of which makes chemical reduction economically non-competitive at the current price of about 2c/lb for sulfur.

The conditions required for microbial sulfate reduction may be easily established and maintained with but little cost beyond capital investment for plant construction and operation. Sulfate in the form of gypsum is readily available. The oxidizable material which supplies energy for biological sulfate reduction may be any of a large number of substances as has been demonstrated in many laboratory studies (8). Most of these, of course, could not be used in a large scale industrial process because of their cost. Several low cost industrial materials including hydrogen, sewage and sewage sludge, yeast plant waste, sulfite liquor waste, crude oil, organic composts and cow dung have been used. Waste from potato processing plants, whey from butter manufacturing, molasses and other waste organic matter could conceivably be used. For many of these substances the mineralization of the waste material under controlled conditions, thus facilitating waste disposal, may make an otherwise uneconomic sulfur process sufficiently attractive for subsidy by government or industrial agencies.

Large scale studies using most of the possible organic matter sources for sulfur production have not been reported. Subba Rao (15) has described so called "sulfur farming", Schulman (15) and others (15) have given detailed reports on the use of yeast plant waste liquors and Butlin (15, 16) has described the London County Council pilot plant study using sewage sludge. Details beyond those given by Postgate (15) for the former two methods are not available to the writer. The British method will be presented in some detail, although it would be unprofitable to attempt to summarize the extensive reports that are available concerning this project (16, 17).

The London County Council sulfur production project

Addition of small amounts of sulfates to the sludge digestion tanks of sewage works causes a greatly increased production of hydrogen sulfide. Thus, it seemed possible to develop a sewage digestion procedure which would produce economic amounts of sulfur from the hydrogen sulfide formed. Laboratory studies were encouraging and pilot plant studies were undertaken using a 50 gal. digestion tank containing sewage sludge with 8% gypsum added.

Several problems became apparent in the early work. The inhibitory effect of hydrogen sulfide prevented formation of methane and in high concentrations completely inhibited further sulfide formation. After considerable study it was decided that an economic process could not simultaneously produce methane and hydrogen sulfide. Further work was therefore directed towards methods for maximal sulfide production at the expense of methane formation. High yield of sulfide was achieved by sweeping hydrogen sulfide from the digestion fluid with a mixture of 70% methane and 30% carbon dioxide. A special extraction procedure was adopted using an external stripping column maintained at 40°C which was found to be the optimum temperature for sulfide removal.

The residual sludge contained sulfide to the extent of about 50 mg per cent even after the most efficient removal of hydrogen sulfide gas. This amount, although small, caused the residual sludge to have an offensive odor and to be highly corrosive. Treatment of the sulfide sludge with activated sludge and continuous aeration yielded a product free of sulfide, which settled readily and yielded a low water sludge residue.

Sulfur formation from hydrogen sulfide in nature may be biological, a chemical oxidation or a more complex series of reactions involving carbon dioxide, and yielding a carbonate salt plus sulfur. Industrial sulfur production processes have apparently employed a direct chemical oxidation of dissolved hydrogen sulfide (8) with subsequent purification by filtration.

A process using a 25,000 gal. fermenter was designed which was to yield 1.5 to 2 lbs. of sulfur per 100 lb. of 4-5% raw sewage sludge. As far as I can determine, such a plant was never built, the sulfur project having been abandoned prior to initiation of construction.

Descriptions of other processes for fermentative sulfur production are either inaccessible or so lacking in detail that it would seem inadvisable to attempt to discuss them. The list of references should be of some value in starting a serious study of this interesting and perhaps economically important process.

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