Microorganisms in Leaching Sulfide Minerals

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Leaching the products of oxidation of copper sulfide minerals and the recovery of the copper has been a profitable operation in the mining industry for many years. It has been shown by Bryner *et al.* (1954) and Bryner and Anderson (1957), that iron pyrites, chalcocite, chalcopyrite, and molybdenite are oxidized biologically in exposed ore bodies to sulfates, surfuric acid, and other soluble products. It has also been shown by Colmer and Hinkle (1947); Leathen *et al.* (1953 a, b), that sulfur and iron sulfides occurring in coal beds in the Pennsylvania and West Virginia area are oxidized biologically.

The purpose of this paper is to report the results of laboratory studies on the isolation, characterization, and nutrient requirements of two chemosynthetic autotropic bacteria found in the leaching streams from exposed ore bodies in Bingham Canyon, Utah. Also, to report similarities of these bacteria to those found in leaching streams from Cananea, Sonora, Mexico (Jameson, 1957).

Materials and Methods

The basic apparatus used in these studies was an air lift percolator previously described (Bryner and Anderson, 1957). These percolators consisted essentially of an air lift which enabled nutrient solution and microorganisms to pass freely through a suspension of the substrate in Ottawa sand. The air lift served the additional purpose of maintaining a high concentration of oxygen and carbon dioxide in the nutrient solution.

The percolator was charged with 5 g of mineral suspended in 100 g of Ottawa sand. This charge was placed in the main tube on top of a porcelain filter disc and glass wool. After the charge was placed in the tube, 100 ml of nutrient solution was added to the percolator. All entrances to the percolator were either sealed off or fitted with cotton plugs. This apparatus could be sterilized and maintained indefinitely under aseptic conditions.

The microorganisms used in this investigation were obtained from the effluent streams from waste dumps in Bingham Canyon, Utah, and Cananea, Sonora, Mexico. The sources of the various cultures which were used are summarized in table 1.

Several oxidizable substrates were used in the isolation procedure and physiological studies on the isolated bacteria. Analyses of these substrates are shown in table 2.

The compositions of the nutrient solutions which were used are shown in table 3. Nutrient I was used initially to simulate naturally occurring conditions. Nutrient II was the optimum nutrient found. Alternate sources of nitrogen are given.

TABLE 1

Source of bacterial cultures

Culture No.	Source
1	Bingham Canyon stream solution
2	Culture 1 after enrichment on iron pyrite
3	The organism isolated on sulfur from culture 1
4	The organism isolated on ferrous iron from culture 1
5	Cananea stream solution (Veta)
6	Cananea stream solution (Ronquillo)

TABLE 2

Analysis of substrates used

Substrate	Per Cent				
	Mo	S	Cu	Fe	Insol.
Molybdenite concentrate	57.58	28.42	0.1	0	3.9
Chalcopyrite II	Trace	33.45	32.15	18.55	15.85
Pyrite III	0.04	44.45	6.57	46.0	0.08
Pyrite V	Trace	42.5	5.36	34.25	17.89
Sulfur	Reagent grade				
Ferrous sulfate	Reagent grade				
Copper sulfide (CuS)	Reagent grade				

TABLE 3

Various nutrients used in the studies of the physiological properties of the isolated bacterium

Nutrient Component	Nutrient No.	
	I	II
	Conc in g/L	
$(NH_2)_2CO$	—	0.05
$(NH_4)_2SO_4$	1.0	(1.0)
K_2HPO_4	0.30	0.10
KCl	0.05	—
$Al_2(SO_4)\cdot 18H_2O$	8.0	—
$MgSO_4\cdot 7H_2O$	3.0	—
$MnSO_4\cdot H_2O$	0.05	—
$Ca(NO_3)_2\cdot 4H_2O$	0.10	—
Na_2SO_4	0.05	—
Distilled H_2O	1000 ml	1000 ml
pH	2.65	—

The pH was adjusted to 2.65 with sulfuric acid.

Two methods of isolation were used, isolation on a solid medium and the enrichment-dilution technique.

RESULTS

Isolation studies. It was previously shown (Bryner *et al.*, 1954) that bacteria found in the leaching streams in Bingham Canyon, Utah, were capable of the oxidation of pyritic materials. Among these were pyrite, various copper sulfide minerals, and molybdenite.

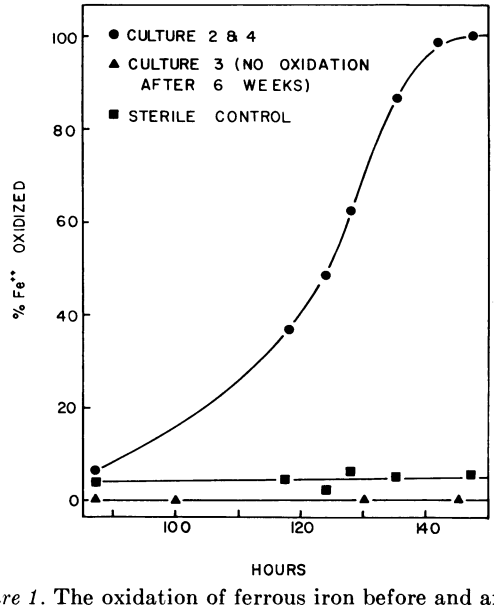

Figure 1. The oxidation of ferrous iron before and after the isolation of the oxidizing organism.

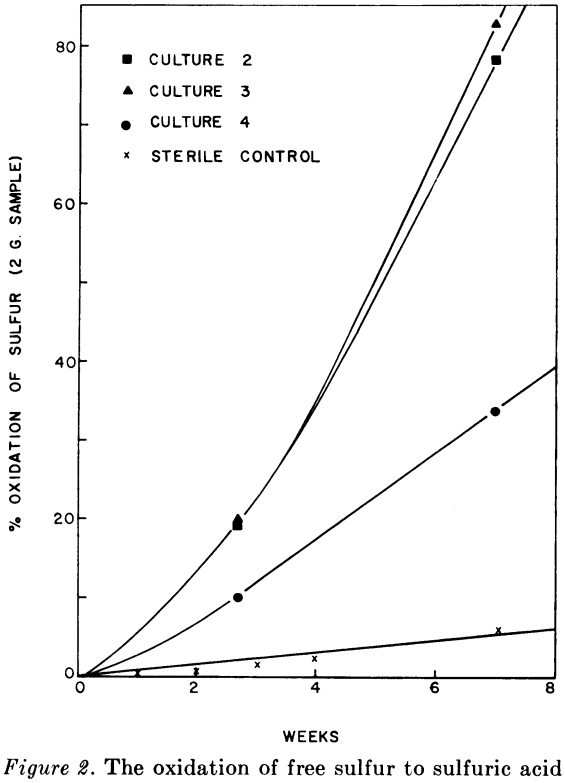

Figure 2. The oxidation of free sulfur to sulfuric acid

To isolate the bacterial species found in the original stream solution, a single oxidizable substrate was used. Chalcopyrite and pyrite, containing both iron and sulfur, were oxidized to soluble sulfates. An enriched culture (culture 2) was developed on pyrite from culture 1. Since these enriched cultures oxidized both ferrous iron and sulfur from the pyrite, they were run individually as follows: Two g of sulfur, 100 g of Ottawa sand, and 100 ml of nutrient solution were added to a percolator and sterilized in the autoclave. This was inoculated with one drop of culture 2. Ferrous iron in a concentration of 2000 ppm was used at a pH of 2.5 in a percolator without the Ottawa sand, the percolator and contents were sterilized, and one drop of culture 2 was introduced aseptically.

The results of this study are given in figures 1 and 2. It may be seen that both ferrous iron and sulfur support bacterial growth. These two materials were used as oxidizable substrates in the isolation.

In the cases in which it is possible, isolation on a solid medium is much preferred over the enrichment-dilution technique because single, isolated colonies can be grown from one organism. These single colonies can be easily seen and are recognized as colonies descending from a single cell.

After several attempts to grow cultures on agar-agar, it was evident that it was not suitable for the isolation of this type of bacteria. It had previously been shown that the bacterium in question was a chemosynthetic autotroph (Bryner *et al.*, 1954). Since organic materials tend to have a deleterious effect on this type of bacteria, a completely inorganic solid medium was used. Thus, a silica gel containing the desired nutrients and substrates was used in this phase of the investigation. The silica gel was prepared according to the method described by Kingsbury and Barghoorn (1954).

The photographs in figure 3 show the colonies as they appeared on the Petri plates growing on ferrous iron. A photomicrograph of the organisms is also shown (1200 ×). Unsatisfactory results were obtained using a sulfur suspension in silica gel.

The small colonies were grown on ferrous iron from a single or a very few cells. To insure against contaminant organisms, a small representative colony was used as the inoculum for another silica gel plate. After several transfers in which the characteristics of the colonies remained the same, the bacterium responsible for the substrate oxidation was assumed to be isolated.

The most generally used technique for enrichment-dilution isolation consists of diluting an enriched culture aseptically to such an extent that only a very few organisms remain. Theoretically, the enriched culture is diluted until only the organism present in the greatest amount remains; thus, by dilution, the slower growing organisms were eliminated.

A modified enrichment-dilution technique, particularly suited for use with the slow growing organisms studied in this investigation, was employed. This method consisted of an enriching step similar to the usual procedure. In this step, a high concentration of organisms was obtained. A single drop of solution was then transferred aseptically to another sterile percolator. When growth was first noticed, a single drop of the re-

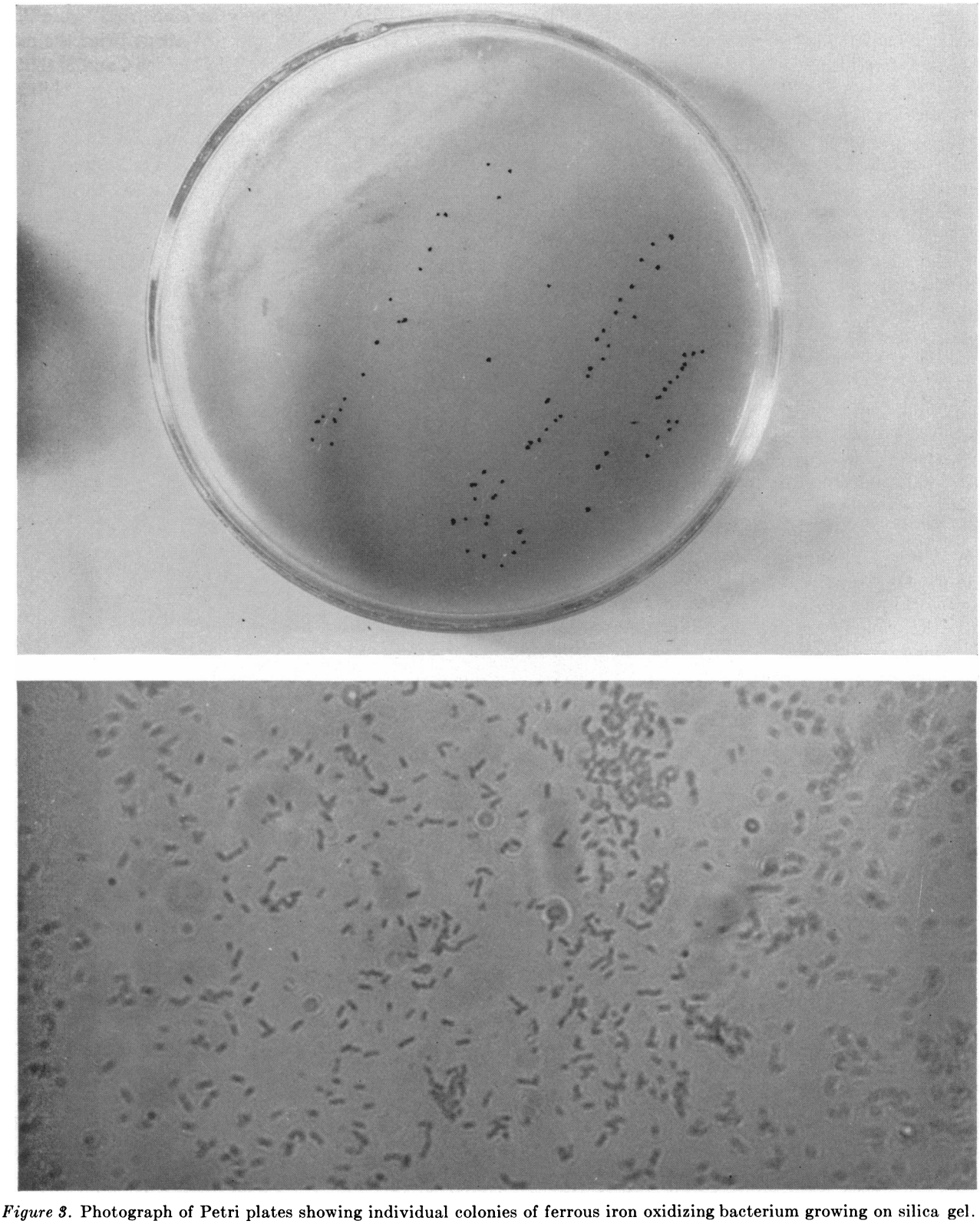

Figure 3. Photograph of Petri plates showing individual colonies of ferrous iron oxidizing bacterium growing on silica gel. The lower part is a photomicrograph of the stained organism. (1200 ×)

sulting solution was aseptically transferred to a third percolator. This was continued through several steps. Each step drastically reduced the concentration of slow growing and foreign organisms. At some point the foreign organisms reach a very low concentration. At this concentration, the chances for a single droplet to contain one bacterial species became very good. Several further transfers insured that a pure culture had been obtained.

Both ferrous iron and sulfur were used as oxidizable substrates in the enrichment-dilution isolation. After several transfers, it was assumed that the isolation of the organism had been accomplished. This was substantiated by observing that stained preparations showed uniform morphological characteristics. It was also found that the culture isolated by this means on sulfur failed to oxidize ferrous iron as shown in figure 1, culture 3.

Both methods of isolation on ferrous iron gave similar results. All tests showed that the culture was exactly the same. About the same rates of oxidation on ferrous iron, pyrite, and sulfur indicated that they had the same physiological properties.

Properties of the isolated organisms. The organism isolated on ferrous iron was found to be a gram negative rod. The conventional Gram stain was used. However, excess acid had to be removed by washing and centrifuging. The organism varied slightly in size and shape depending on the substrate. It was motile and about 0.5 to 0.8 μ by 1.0 to 1.3 μ. On ferrous iron it was almost round while on pyrite it had a definite rod shape. A photomicrograph of the organism as it grows on ferrous iron is shown in figure 3.

On silica gel with ferrous iron as the substrate (figure 3), the colonies gave the following characteristics: Color, orange to red (iron precipitate); size, 1 to 2 mm in diameter; luster, dull; margin, circular; surface, smooth, hard crust; elevation, flat. On silica gel with pyrite and chalcopyrite as the substrate, the following characteristics were exhibited: color, yellow-orange; size, 1 mm (maximum); luster, dull; margin, circular; surface, smooth; elevation, flat.

Culture 3 was found to consist of a bacterium which was morphologically similar to culture 4. The colonies of culture 3 on silica gel were very hard to distinguish. The proper characterization of each isolated bacterium requires a knowledge of its ability of oxidize various substrates. As shown in figure 1, culture 4 oxidized ferrous iron but culture 3 does not.

It was found that the bacterium isolated on ferrous iron (culture 4) could also oxidize iron pyrites while the bacterium isolated on sulfur (culture 3) could not. Curves comparing growth on pyrite with growth before isolations are shown in figure 4. Initially, culture 4 oxidized pyrite very slowly, but later the oxidation rate increased. A second transfer showed an activity which approaches that of culture 2.

As shown in figure 2, both cultures 3 and 4 were found to oxidize sulfur to sulfuric acid. However, isolation of the sulfur oxidizer (culture 3) by enrichment-dilution technique requires that it be faster growing than all other organisms present in the impure culture. This is shown to be true. As may be seen from figure 2, cultures 2 and 3 oxidize sulfur at about twice the rate of culture 4. It may be seen from tables 4 and 5, that culture 3 fails to oxidize either chalcopyrite or molybdenite while culture 4 is able to oxidize both minerals.

The progress of the biological reactions were followed by analysis of the oxidation products. It was observed that the solutions in the active percolators became turbid and frothed somewhat. This was an indication of increased cell formation. To check this, 100 ml samples of culture 4, growing in the percolators on sulfur, were centrifuged, dried (105 C), and analyzed for nitrogen content. The oxidation of 560 mg of ele-

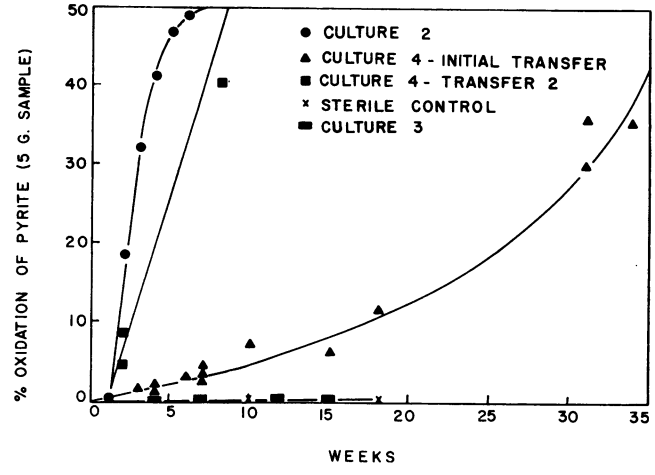

Figure 4. A comparison of oxidation of pyrite before and after isolation.

TABLE 4

A comparison of the amount of chalcopyrite solubilized by cultures 2, 3, and 4 after 13 days

Culture No.	Solubilized Copper	Solubilized Iron
	mg.	*mg.*
2	7.9	454
3	0.9	12
4	7.9	491
Sterile control	0.7	8

TABLE 5

A comparison of the molybdenite oxidized by cultures 2, 3, and 4 after 13 days

Culture No.	Oxidation of MoS_2
	%
2	0.61
3	0.09
4	0.53
Sterile control	0.06

mental sulfur to sulfuric acid by culture 4 produced 8.9 mg of dried cells with an amino nitrogen content of 14.3 per cent.

The effect of various substances on the biological oxidation of pyrite. Chemosynthetic autotrophic bacteria have the capability of living in a completely inorganic environment, utilizing carbon dioxide from the atmosphere as a source of carbon. No organic materials are needed, indeed some organic materials may actually impede or completely stop growth (Frederick *et al.*, 1957). The purpose of this phase of the investigation was to determine the effect of various organic and inorganic materials on the oxidation of pyrite by culture 2.

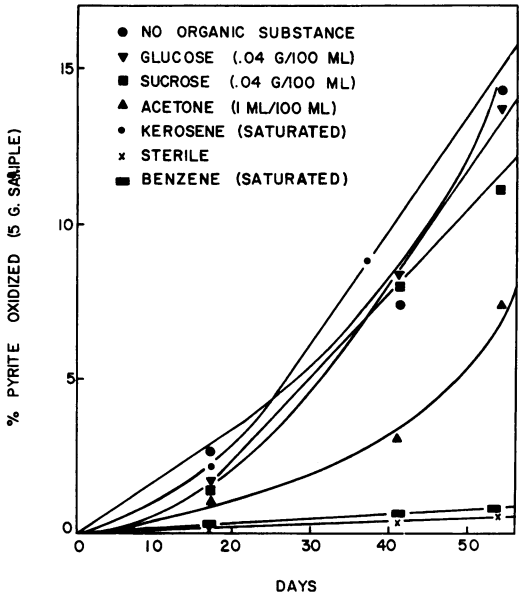

Figure 5. The oxidation of pyrite in the presence of various organic compounds.

TABLE 6

The effect of addition of thiourea and sodium thiocyanate to Nutrient II

Time Days	Cumulative Per Cent Oxidation of Pyrite			
	Sterile control	Nutrient II	Nutrient II plus 0.1 g per L thiourea	Nutrient II plus 0.1 g per L thiocyanate
18	—	—	—	0.40
25	—	—	—	0.65
32	—	—	—	0.90
34	0.2	24.0	0.6	—
90	0.4	68.0	0.8	—

TABLE 7

The effect of various substances as nitrogen sources in the biological oxidation of pyrite

Time Days	Per Cent Oxidation of Pyrite					
	Sterile control	Nutrient II $(NH_4)_2SO_4$	Urea 0.10 g per L	β-Alanine 0.10 per L	L-Cystine 0.06 g per L	Sodium nitrate 0.10 g per L
34	0.2	27.9	14.1	0.1	8.6	0.29
90	0.4	74.0	53.2	2.7	35.3	0.60

Figure 5 shows the effect of several representative organic compounds on the oxidation of pyrite. Benzene definitely inhibits pyrite oxidation, acetone has some inhibitory action, whereas kerosene, glucose, and sucrose exhibit little if any effect.

Table 6 shows the effect of thiourea and sodium thiocyanate on the biological oxidation of pyrite by

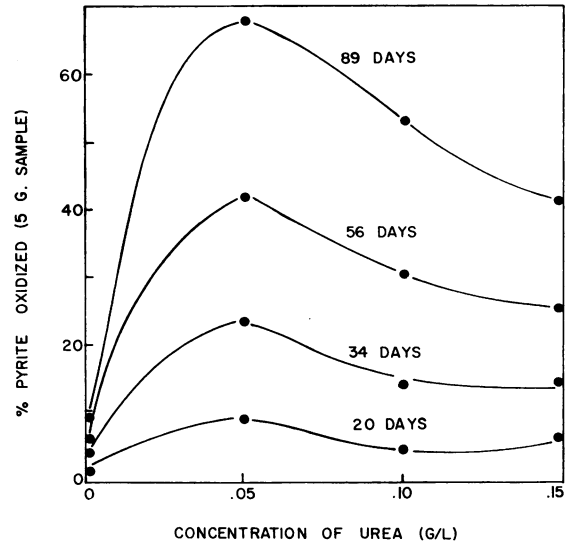

Figure 6. Urea as a nitrogen source in the biological oxidation of pyrite.

TABLE 8

Pyrite oxidized by organisms from various geographical locations

Time Days	Cumulative Soluble Iron (mg)			
	Sterile	Culture 2	Culture 6	Culture 5
12	20	187	152	193
26	25	404	313	482
51	30	985	1098	1127

TABLE 9

Chalcopyrite oxidized by organisms from various geographical locations after 13 days

Culture No.	Solubilized Iron	Solubilized Copper
	mg.	*mg.*
2	539	7.9
5	428	11.5
6	494	8.5
Sterile control	8	0.7

TABLE 10

Molybdenite oxidized by organisms from various geographical locations after 13 days

Culture No.	Oxidation of MoS_2
	%
2	0.70
5	1.00
6	0.61
Sterile control	0.06

culture 2. From these results it is apparent that both compounds greatly inhibit pyrite oxidation.

The effect of various compounds as nitrogen sources for the bacterial oxidation was investigated. The substances were substituted into nutrient II in place of the nitrogen containing compound. The results, shown in table 7, indicate that urea and L-cystine may supply the nitrogen requirements whereas β-alanine and sodium nitrate do not.

Since urea was found to be nearly the equal of ammonium sulfate as a nitrogen source, the optimum was determined for use in an alternate nutrient solution. This is shown in figure 6. The optimum urea concentration was found to be 0.05 g per L. Thus, on the basis of contained nitrogen, urea has about 10 times the effectiveness of ammonium sulfate.

Sulfide oxidation by organisms from Mexico. It has been known for several years that biological action was responsible for the oxidation of various sulfides in the waste dump at Bingham Canyon. Weed (1956) has described operations that are being carried out successfully at Cananea, Sonora, Mexico, which are similar to those at Bingham Canyon.

Preliminary runs under aseptic conditions showed that the oxidation of pyrite by stream solutions from both the Cananea waste dumps (Ronquillo, culture 6) and the underground operation (Veta, culture 5) were biological. A series of sterile percolators were set up to compare the rate of sulfide oxidation by the organisms from Bingham Canyon with that by the organisms from Mexico. The initial inoculum in each case was from an enriched culture on pyrite. These results are shown in tables 8, 9, and 10. The results show that all the cultures act similarly on pyrite, chalcopyrite, and molybdenite.

Discussion

In the leaching streams from exposed ore bodies in Bingham Canyon, large amounts of soluble iron and copper are found. It was previously shown that this oxidation was biological. The primary result from this series of investigations was the isolation of the chemosynthetic bacterium responsible for this oxidation.

The isolated bacteria may be compared with similar organisms described in the literature. They correspond most closely to the genus *Thiobacillus*. Only two organisms from this group tolerate the same high acid concentration (pH 2.0 to 3.5) exhibited by the bacteria isolated in this investigation. They are *T. thiooxidans* and *T. ferrooxidans*. Differences were observed in the physiological properties exhibited by the various organisms. *T. thiooxidans* oxidizes sulfur but not pyrite or ferrous iron (Leathen *et al.*, 1953 a, b). *T. ferrooxidans* oxidizes pyrite and ferrous iron in acid solution but not free sulfur (Temple and Colmer 1951; Temple and Delchamps, 1953). One bacterium isolated in this study oxidized ferrous iron, sulfur, and pyrite as well as other sulfide minerals. Thus, this organism exhibited somewhat different characteristics than either *T. thiooxidans* or *T. ferrooxidans*, although nearly the same as *T. ferrooxidans*.

A second organism was isolated on sulfur and exhibited characteristics very similar to those of *T. thiooxidans*. An investigation was made to determine the optimum nutrient material for oxidation of pyrite by culture 4.

A process similar to that in the waste dumps at Bingham Canyon has been observed at Cananea, Sonora, Mexico. The stream solution from this source exhibited the same general characteristics as those from Binham Canyon. Oxidation of pyrite, chalcopyrite, and molybdenite was observed in nearly the same amounts. Thus, it may be concluded that the biological oxidation of sulfide minerals is not unique to any one area but occurs wherever conditions are favorable.

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Summary

The isolation and characterization of two chemosynthetic autotrophic bacteria from the leaching streams in Bingham Canyon, Utah, has been accomplished. One of these organisms is able to oxidize free sulfur, ferrous iron, iron pyrites, molybdenite, and several copper sulfide minerals. This organism is very similar to *Thiobacillus ferrooxidans*. The other organism oxidizes free sulfur but does not oxidize the sulfide minerals. This one is similar to *Thiobacillus thiooxidans*.

The nutrient requirement of these organisms has been investigated.

A comparison was made with the bacteria isolated from Bingham Canyon and those found in the leaching streams from a similar operation at Cananea, Sonora, Mexico.

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