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Factors Affecting Elemental Sulfur Formation in Biooxidized Samples: Preliminary Studies

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Abstract – Refractory sulfide ores and concentrates often consume large quantities of cyanide during cyanidation. Sulfides, elemental sulfur and many base metals react readily with cyanide, reducing the amount of cyanide available for leaching of the desired metal. Oxidative pretreatments such as biooxidation may remove some of these cyanide-consuming materials, resulting in a decrease in cyanide demand during cyanidation. Occasionally, however, consumption of cyanide increases following biooxidation, most likely due to the formation of elemental sulfur. Limiting the formation of elemental sulfur during biooxidation should reduce cyanide consumption. In this preliminary study, we examined parameters, which may affect elemental sulfur formation during biooxidation. In particular, we studied the effects of bacterial species, pH, temperature, and frequent solution removal. Sulfur formation was found to occur in two phases: low levels produced early in the oxidation process and high levels produced later, during rapid oxidation. Early sulfur production occurred in all flasks, and was higher at higher temperature. High sulfur always occurred in combination with rapid oxidation, regardless of the test conditions. Mechanisms are suggested for the production of both occurrences of sulfur.

Introduction

Refractory sulfide gold ores often consume large amounts of cyanide during leaching, due to the reactivity of the sulfide moiety and of base metals, if present (Marsden and House, 1992). Consumption of cyanide by ore components other than gold increases the cyanide demand and therefore the cost of recovering the gold.

Pre-oxidation of sulfide gold ores allows a higher recovery of the gold, often with lower cyanide consumption. This may be achieved by pressure oxidation, roasting, or biooxidation. In some cases, elemental sulfur is formed during oxidation of sulfide ores, causing an increase in cyanide consumption. One weight percent elemental significantly is increasing the cost of recovering the gold.

In this preliminary study, a series of shake flask experiments were performed to investigate conditions, which may affect elemental sulfur formation during biooxidation. These included bacterial species, pH, temperature, and frequent solution removal.

Materials and Methods

Mineral Samples

All shake flask studies were performed on a –100 mesh pyretic concentrate initially containing 39.2% total sulfur, o.57% elemental –sulfur, 0.46% sulfate sulfur, 38.1% Fe, and 0.31 oz/t Au.

Microorganisms

The T. ferrooxidans (Tf) culture was derived from a mixed culture of ATCC strains 14119, 19859, 23270, and 33020, which had been maintained for approximately three years in a 9k-iron sulfate fermentation. T. thiooxidans (Tt) cells were derived from a mixture of ATCC strains 19703 and 15494

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which had been maintained for about one year on 9K salts supplemented with powdered sulfur. The cell suspension was passed through an 8 um filter to remove sulfur particles, prior to inoculation. L. ferrooxidans (Lf) cells were derived from a culture of ATCC 29047 which had been maintained in 9K medium supplemented with 20 g/L $FeSO_4$ $7H_20$. The cell suspension was passed through an 8 um filter to remove iron precipitates, prior to inoculation. The T. ferrooxidans and L. ferrooxidans cultures were incubated at 35 ºC; the T. thiooxidans culture was maintained at about 25 ºC.

Cells were counted prior to inoculation using a Petroff-Hauser counting chamber. Inoculations were made at 1 X 10^7 cells/g concentrate. In cultures containing two species of bacteria, 1 X 10^7 cells/g of each type were added.

Shake Flask Experiments

Shake flask studies were performed in 2.8 L Fernbach flasks containing 300 g of concentrate, or in 1 L Erlenmeyer flasks containing 100 g of concentrate. The pulp density in each case was 20%. Concentrates were suspended 0.2X 9K, a modification of 9K salts (Silverman and Lundgren, 1959) containing 1 g/L $(NH_4)_2SO_4$, 0.03 g/L KC1, 0.017 g/L K_2HPO_4, 0.167 $MgSO_4$ $7H_2O$, with H_2SO_4 for pH adjustment.

Flasks were set up in order to investigate effects of the following parameters:

- Bacterial inoculum: Flasks 1, 2/2A/2B, and 3
- Temperature: Flask 3 vs. 4
- PH Flask 5 vs. 6
- Frequent solution changes: Flask 7 vs. Flasks 3,5

During the stage of rapid pyrite leaching, the contents of Flask 2 were divided between two smaller flasks, 2A and 2B. Flask 2A received a partial solution change, and Flask 2B received a partial solution change and an inoculation with T. thiooxidans.

Analyses

Measurements were made of pH, redox potential, and total iron concentration. Solution pH was measured with an AccupHast probe (Fisher Scientific) and redox potential was measured with a Corning combination electrode ($AgCl_2$ in saturated KC1). Probes were double rinsed between samples to reduce cross-contamination of bacteria among flasks. Soluble iron concentration was measured by atomic absorption spectroscopy using a Perkin-Elmer model 3100. Slurry samples were taken for determination of total sulfur, elemental sulfur, sulfate, and iron. The slurry was collected during rapid mixing of the flask contents with a magnetic stirrer. This slurry was then weighed, filtered through 20 um pore-size filter paper, washed with distilled water, and dried overnight at 45 ºC. Following pulverization of the dried sample, a portion was sent to Hazen Research, Inc. (Golden, Colorado) for analysis of elemental sulfur, total sulfur, iron, and sulfate content.

Cyanide Consumption

Cyanide consumption was determined in modified bottle roll tests. 10 g samples were rolled in 50 ml conical centrifuge tubes with Ca $(OH)_2$ solution until the pH equilibrated at $\geq$ 11. The bottle contents were adjusted to 40% pulp density by solution removal, 0.5 g activated carbon was added to each sample, and NaCN was added to 5 g/L. Cyanide concentration was measured after 4 hours and 24 hours, by titration with $AgNO_3$ (Charlot and Bezier, 1957), using KI as an indicator, and adjusted back up to 5 g/L if necessary. After 26 hours, the assay was complete, at which time the total amount of cyanide consumed was calculated. Microscopic observations of the leach solutions were made in order to note the morphology of any bacteria present. This consisted of noting whether there were rods, spirals, or

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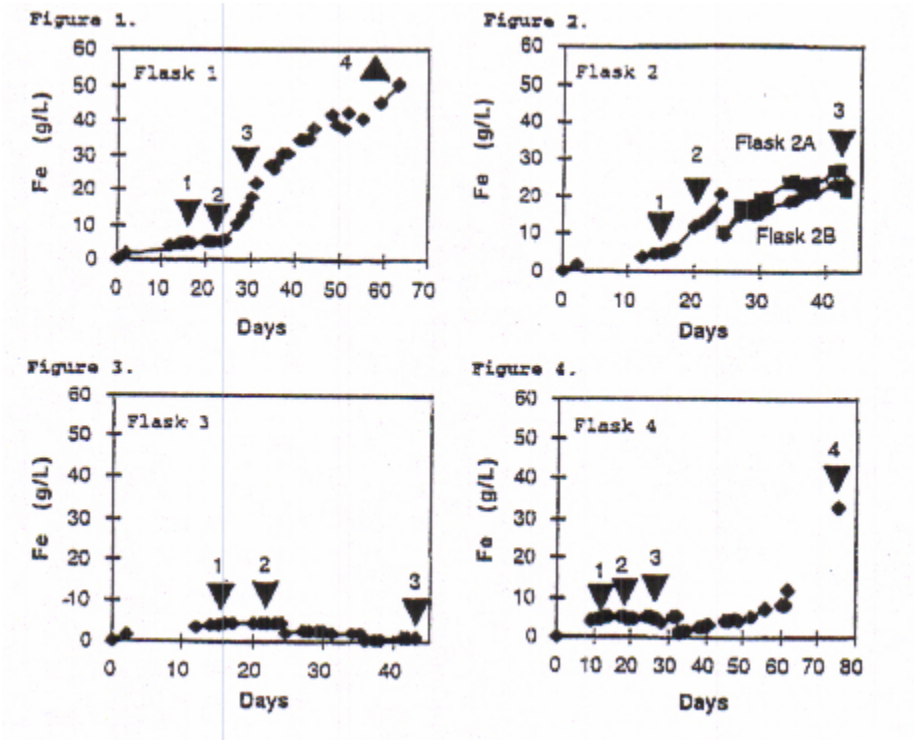
vibrios present, and the relative numbers. (Rods are indicative of Tf and Tt; vibrios and spirals indicate a population of Lf.)

Results

Effect of Bacterial Inoculum (Flasks 1, 2/2A/2B, 3)

Inoculation with Tt, either at the beginning of the experiment, in combination with Lf (Flask 1), or during the period of rapid pyrite oxidation (Flask 2B) did not appear to affect the amount of elemental sulfur produced. In fact, the amount of elemental sulfur produced by the end of the leach in Flask 2B (+ Tt) greatly exceeded that of Flask 2A (no Tt). The iron solubilization profiles and sampling times of these flasks can be found in Figures 1-3.



Figures 1-4
Fe concentration in solution (g/L) and sampling times.
Numbered arrows indicate sample number; sample data is given in Table 2.

After the initiation of rapid pyrite oxidation in Flasks 1 and 2 (inoculation with Lf +Tt , and with Lf. respectively), the amount of elemental sulfur associated with the concentrate increased considerably. Flasks 3 and 4, which showed very little iron solubilization over the first 45 days, maintained low elemental sulfur values (Table 1)

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Table 1
Elemental Sulfur Composition and cyanide Consumption

Flask No.	Sample 1 (days)		Sample 2 (days)		Sample 3 (days)		Sample 4 (days)	
	% Sº	NaCN Kg/T	% Sº	NaCN kg/T	% Sº	NaCN kg/T	% Sº	NaCN kg/T
Head (Avg.)	.63 (0)	7.57						
1	1.63 (16)	10.08	1.3 (22)	12.16	3.24 (30)	12.46	10.1 (63)	20.80
2, 2A	1.85 (16)	9.41	1.92 (22)	11.96	5.8 (43)	15.12		
2B					9.53 (43)	18.36		
3	1.33 (16)	10.74	1.02 (22)	13.38	1.51 (43	14.39		
4	2.28 (13)	12.78	1.26 19)	14.85	1.08 (27)	14.15	4.58 (75)	
5	1.47 (17)		5.16 (30)		8.82 (43)		7.49 (58)	
6	1.33 (20)		1.24 (35)					
7	1.5 (13)	11.16	9.78 (26)	33.77	5.72 (44)			

Not all samples were tested for cyanide consumption. Sample numbers correspond to numbered arrows in the figures.

Effect of Temperature (Flasks 3 and 4)

At the first sampling, Flask 4 (40 ºC) had leached about 10% more iron into solution than had Flask 3 (35ºC), in three fewer days (Table 1, Figures 3 and 4). Following this initial burst, iron leaching stopped in both flasks, and the elemental sulfur content in Flask 4 decreased. Following a medium change and a reinoculation with T. ferrooxidans, Flask 4 entered a phase of rapid pyrite oxidation with high elemental sulfur formation; no additional leaching or elemental sulfur production occurred in Flask 3, despite the same treatment.

Effect of pH (Flasks 5 and 6)

Flasks 5 and 6 were maintained at pH 1.3-1.8 and pH 2.5-3.0, respectively, in order to assess the effect of pH on elemental sulfur formation. Although Flask 5 leached more iron into solution than Flask 6, the amount of elemental sulfur produced in the two flasks during the early part of the biooxidation was similar. Flask 5 later began to leach iron into solution at a rapid rate (Figure 5), whereas Flask 6 did not. During this period of rapid oxidation, the amount of elemental sulfur produced in Flask 5 increased substantially (Table 1); the soluble iron concentration and elemental sulfur content of Flask 6 did not change.

Effect of Solution Changes (Flasks 3, 4, 5, 6 and 7)

Following the initial phase of oxidation, the solutions in Flasks 3 and 4 were replaced with fresh medium. When no change occurred in either flask, both were reinoculated with Tf. Twenty days later, the solutions of Flasks 3 and 4 were changed again. There was no change in the leach rate in Flask 3, but the leach rate of Flask 4 began to increase. Elemental sulfur analyses made after the medium change indicated that additional sulfur was produced in Flask 4, but not in Flask 3. An increase in leach rate and elemental sulfur formation also occurred following a solution change in Flask 5, but not in Flask 6 (Figure 5).

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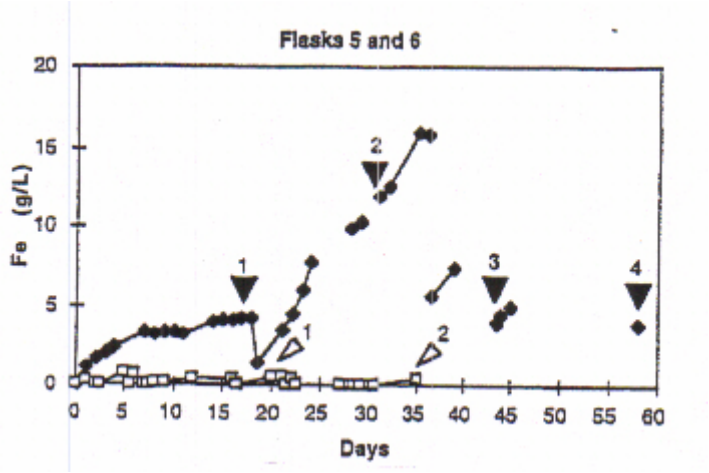

Figure 5
Fe concentration in solution and sampling times for Flasks 5 (filled symbols) and 6 (open symbols). Numbered arrows indicate sample number; sample data is given in Table 2.

Flask 7 was set up in order to determine whether frequent medium changes early in the leaching process would reduce the amount of elemental sulfur produced during the first few days. The amount of elemental sulfur produced initially was similar to that in the other flasks. After rapid pyrite oxidation commenced, however, the percentage of elemental sulfur in the sample increased dramatically (Fig. 6).

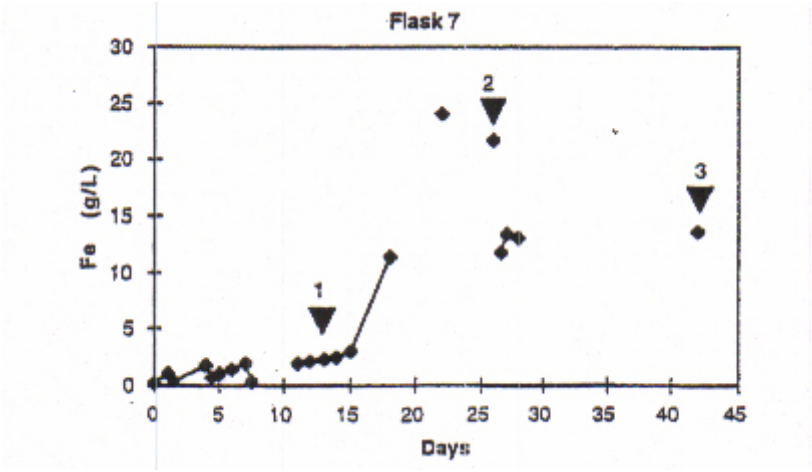

Figure 6
Fe concentration in solution and sampling times for Flask 7. Numbered arrows indicate sample number; sample data is given in Table 2.

Relationship of Elemental Sulfur to Cyanide Consumption

In all cases, the cyanide consumption of the first sample exceeded that of the unbiooxidized (head) sample. In general, for a given flask biooxidation, cyanide consumption increased with increasing elemental sulfur concentration (Table 1).

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Discussion

In order to investigate the effect of different bacterial species on elemental sulfur formation during pyrite biooxidation, flasks were inoculated with known numbers of one or more of the following species (Table 2): Thiobacillus ferrooxidans, which exidizes ferrous iron, sulfur, and some soluble sulfur compounds; Leptospirillum ferrooxidans, which oxidizes ferrous iron only; and Thiobacillus thiooxidans, which oxidizes sulfur and some soluble sulfur compounds (Harrison, 1984; Norris, 1990).

Table 2
Initial Conditions of Shale Flasks

Flask	Inoculum	Temp.	Initial Conditions
1	Lf + Tt	35 ºC	0.2X 9K, pH 0.5-1.5
2	Lf	"	0.2X 9K, pH 0.5-1.5
2A	Lf	"	Continuation of Flask 2
2B	Lf + Tf	"	Addition of Tt
3	Tf	"	0.2X 9K, pH 1.3-1.8
4	Tf	40 ºC	0.2X 9K, pH 1.3-1.8
5	Tf	35 ºC	0.2X 9K, pH 1.3-1.8
6	Tf	"	0.2X 9K, pH 2.5-3.0
7	Tf	"	0.2 X 9K, pH 1.3-1.8, frequent medium changes

All flasks exhibiting rapid pyrite leaching contained L. ferrooxidans, regardless of their initial inoculum. These bacteria could have been present on the pyrite concentrate, or they may have entered the flasks on the pH or redox probes, in spite of careful rinsing of the probes between measurements. These bacteria probably altered the characteristics of the bioleach, since they utilize a different range of substrate, reproduce at different rates, and oxidize iron at different rates than T. ferrooxidans. (Hallmann et al., 1992; Norris, Barr, and Hinson, 1987). Flask 5, for example, which had the same initial conditions as Flask 3 (Table 1), rapidly oxidized pyrite, whereas Flask 3 did not. L. ferrooxidans was found in Flask 5 but not in Flask 3. As a result of this contamination, the bioleaching processes were most likely the result of a mixed culture, comprising L. ferrooxidans in addition to the type of cells initially inoculated.

Stirred-tank and column biooxidation have been reported to evolve to contain a large mixed population of L. ferrooxidans and T. thiooxidans, with T. ferrooxidans as a major population (Espejo et al., 1995; Garcia and Jerez, 1995; Rawlings, 1995). With this in mind, one flask (Flask 1) was inoculated with both L. ferrooxidans and T. thiooxidans. Inoculation with Thiobacillus thiooxidans was expected to decrease the amount of elemental sulfur formed during the bioleaching of pyrite, as has been speculated elsewhere (Helle and Onken, 1987), and to increase the rate of pyrite oxidation by providing additional acid from the oxidation of sulfur (Curutchet, Tedesco, and Donati, 1996).

As can be seen from the results (Table 1, the presence to T. thiooxidans did not keep elemental sulfur in check. It is possible that the T. thiooxidans population was overwhelmed by the amount of sulfur produced during active leaching, as a reaction product of ferric leaching. Or, the T. thiooxidans may have been inhibited by the high ferric concentration that coincided with rapid leaching (Curutchet, Tedisco, and Donati, 1996). It is also possible that soluble sulfur intermediates, such as tetrathionate, thiosulfate, and sulfite, which may be produced during ferric leaching (see below), are the preferred substrates of T. thiooxidans, rather than elemental sulfur.

Flask 4, at 40 ºC, produced more iron and elemental sulfur during the initial leach period that did Flask 3, at 35 ºC (Table 1, Figures 3 and 4). This was expected, due to the higher rate of chemical reactions at higher temperature. The higher leach rate after the medium change in Flask 4 may have been due to the production of enough ferrous iron for L. ferrooxidans activity to begin, leading to ferric leaching.

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Initially, the biooxidation performed at low pH (Flask 5) and at high pH (Flask 6), produced similar amounts of elemental sulfur (Table 1. Although Flask 5 later began rapid leaching, with concomitant elemental sulfur formation, the soluble iron and elemental sulfur content of Flask 6 remained low.

A pH of 2.5 should not inhibit pyrite oxidation by T. ferrooxidans (Rodiguez-Leiva and Tributsch, 1988), but it might select against L. ferrooxidans, which has a lower pH optimum (Hallman et al., 1992). If L. ferrooxidans plays an important role during rapid pyrite leaching, then pyrite biooxidation and sulfur formation would be expected to be inhibited at higher pH. Chemical oxidation of pyrite should continue at pH 2.5-3.0 (Nordstrom, 1992), but the iron might precipitate at the higher pH (Lawrence, 1990), removing ferric iron from solution, making it unavailable for pyrite oxidation.

All flasks produced elemental sulfur early in the oxidation reaction. At this point in the biooxidation, the redox potential was low, the pH was unchanged, and the amount of iron that had leached during this stage tended to plateau (Figures 1-5). A possible explanation for this effect can be found in the following equation describing the electrochemical dissolution of pyrite:

$$FeS_2 \rightarrow Fe^{2+} + 2S^o + 2e^- \qquad [1]$$

(Lowson, 1982; Nordstrom, 1982). During this reaction, which occurs at pH $\leq$ 3, no acid is produced, all the iron produced, all the iron produced is of the ferrous form, and elemental sulfur is formed. These parameters correspond with the conditions observed in the flasks.

The leach solution was replaced in some flasks, in order to remove suspended or soluble sulfur compounds, which might degrade to elemental sulfur and coat the pyrite surface. Replacement of the leach solution during the first few days did not reduce elemental sulfur formation. This implies that the elemental sulfur that is forming during this time remains closely associated with the pyrite, possibly even as part of the crystal lattice (Nordstrom, 1982). We recognized that replacing the solution might also remove soluble inhibitors to pyrite leaching but this possibility was not investigated further.

All flasks, which were undergoing rapid pyrite oxidation, produced large amounts of elemental sulfur. The conditions present during this time (high redox potential, high iron concentration, presence of L. ferrooxidans, low pH) suggested that indirect, ferric leaching was occurring. The high redox potential indicated that essentially all of the iron in solution was in the ferric form, and therefore available for ferric leaching. L. ferrooxidans could perpetuate ferric leaching by converting ferrous iron to ferric, thus maintaining high redox conditions and a high ferric concentration. The low pH provides a suitable environment for growth of L. ferrooxidans and for stability of ferric ions (Hallman et al., 1992; Nordstrom, 1982).

Ferric leaching of pyrite could result in high concentration of elemental sulfur, according to the following reactions:

$$FeS_2 + 6Fe(H_2O)\,6^{3+} \rightarrow Fe^{2+} + S_2O3^{2-} + 6Fe(H_2O)5^{2+} + 3H_2O + 6H^+ \qquad [2]$$

and at low pH,

$$S_2O_3^{2-} + 2H^+ \rightarrow S^o + H_2SO_3 \qquad [3]$$

(Moses et al., 1987; Peters, 1976; Sand et al., 1995). Higher polythionates, which form via other reactions, also yield elemental sulfur during decomposition (Hazeu et al., 1988; Steudel, 1989).

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Conclusions

Based on the results of this preliminary study, we can make the following conclusions:

- Elemental sulfur is formed early in the pyrite oxidation process, probably as a result of electrochemical dissolution of the pyrite. Higher temperatures increase the production of this sulfur. None of the other parameters tested had an effect on this sulfur formation
- Rapid oxidation conditions (high ferric, high redox potential, low pH) result in high elemental sulfur formation, even in the presence of T. thiooxidans.
- Biooxidation can take place even in the presence of high amounts of elemental sulfur.
- Cyanide consumption increases with increasing elemental sulfur concentration.

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References

Charlot, G & D. Bezier, 1957 – Quantitative Inorganic Analysis.London: Wiley

Curutchet, G., P. Tedesco & E. Donati, 1996 – Combined degradation of covellite by Thiobacillus thiooxidans and Thiobacillus ferrooxidans. Biotech. Lett. 18 (12) : 1471-1476.

Espejo, R.T., J. Pizarro, E. Jedlicki, O. Orellana & J. Romero, 1995 – Bacterial population in the bioleaching of copper as revealed by analysis of DNA obtained from leached ores and leaching solutions. Biohydrometallurgical Processing, November 19-22, 1995, Santiago, Chile, 1-8.

Garcia, A. & C.A. Jerez, 1995 – Changes of the solid-adhered populations of Thiobacillus ferrooxidans, Leptospirillum ferrooxidans and Thiobacillus Thiooxidans in leaching ores as determined by immunological analysis. Bioohydrometallurgical Processing, November 19-22, 1995, Santiago, Chile, 19-30

Hallman, R., A. Friedrich, H.-P. Koops, A. Pommerening-Roger, K. Rohde,C.Zanneck & W. Sand, 1992 – Physiological characteristics of Thiobacillus ferrooxidans and Leptospirillum ferrooxidans and physicochemical factors influence microbial metal leaching. Geomicrobiology Journal 10: 193-206.

Harrison, A.P., Jr, 1984- The Acidophilic Thiobacilli and other Acidophilic Bacteria that share their Habitat. Ann. Rev. Microbial. 38:265-292

Hazeu, W., W.H. Batenburg-van derVagte, P. Boc, R.K. van der Pas & J.G. Kuenen, 1988- The production and utilization of intermediary elemental sulfur during the oxidation of reduced sulfur compounds by Thiobacillus ferrooxidans. Arch. Microbial. 150:574-579

Helle, U. & U. Onken, 1987- Continuous bacterial leaching of a pyritic flotation concentrate by mixed cultures. BioHydroMetallurgy, Warwick United Kingdom, 61-76

Lawrence, R.W., 1990 – Biotreatment of Gold Ores. In Microbial Mineral Recovery, edited by H.L. Ehrlich and C.L. Brierley. New York: McGraw-Hill

Lowson, R.T., 1982- Aqueous oxidation of pyrite by molecular oxygen. Chemical Reviews 82 (5): 461-497

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Marsden, J. & I. House, 1992- The chemistry of gold extraction. New York, London: Ellis Horwood.

Moses, C.O., D.K. Nordstrom, J.S. Herman & A.L Mills, 1987- Aqueous pyrite oxidation by dissolved oxygen and by ferric iron. Geochimica et Cosmochimica Acta 51: 1561-1571.

Nordstrom, D.K., 1982- Aqueous pyrite oxidation and the consequent formation of secondary iron minerals. In Acid Sulfate Weathering, edited by E.A. Hossaer. Madison, WI: Soil Science Society of America.

Norris, P.R., 1990- Acidophilic Bacteria and Their Activity in Mineral Sulfide Oxidation. In Microbial Mineral Recovery, edited by H. L. Ehrilich and C. L. Brierley. New York: McGraw-Hill.

Norris, P.R., D.W.Barr & D. Hinson, 1987- Iron and mineral oxidation by acidophilic bacteria: affinities for iron and attachment to pyrite. BioHydroMetallurgy, Warwick United Kingdom, 43-60.

Peters, E., 1976- Direct leaching of sulfides: Chemistry and applications. Metall. Trans. B 7B: 505-517

Rawlings, D.E., 1995- Restriction enzyme analysis of 16S rDNA genes for rapid identification of Thiobacillus ferrooxidans, Thiobacillus thioxidans and Leptospirillum ferrooxidans strains in leaching environments. Biohydrometallurgical Processing, November 19-22, 1995, Santiago, Chile, 9-18.

Rodriguez-Leiva, M. & H. Tributsch, 1988- Morphology of bacterial leaching patterns by Thiobacillus ferrooxidans on synthetic pyrite. Arch. Microbial. 149: 401-405

Sand, W., T. Gerke, R. Hallman & A. Schippers, 1995- Sulfur chemistry, biofilm, and the (in) direct attck mechanism – a critical evaluation of bacterial leaching. Appl. Microbiol Biotechnol 43:961-966

Silverman, M.P. & D.G. Lundgren, 1959- Studies on the chemoautotrophic iron bacterium Ferrobacillus ferrooxidans I. An improved medium and a harvesting procedure for securing high cell yields. J. Bacteriol. 77:642-647

Steudel, R., 1989- On the nature of the “elemental sulfur” S (0) produced by sulfur-oxidizing bacteria – a model for S(0) globules. In Autotrophic bacteria, edited by H.G. Schleger and B. Bowien: Brock/Springer.