

# Effect of Applied Potentials on the Activity and Growth of *Thiobacillus ferrooxidans*

K. A. Natarajan

Department of Metallurgy, Indian Institute of Science, Bangalore 560 012, India

Received December 28, 1990/Accepted November 5, 1991

The effect of applied DC potentials both in the positive and negative range, on the activity and growth of *Thiobacillus ferrooxidans*, is discussed. In general, application of positive potentials up to +1000 mV in an acid bioleaching medium was found to be detrimental to bacterial activity, while the impression of negative potentials enhanced both their activity and growth through electrochemical regeneration of ferrous ions and an increase in the biomass. Ferrous-ferric ratios in a bioleaching medium could be monitored through Eh measurements.

Among the base sulfide minerals such as pyrite, chalcopyrite, and sphalerite, sphalerite could be selectively bioleached if an impressed potential of -500 mV (SCE) could be maintained in the leaching medium. Electrochemical bioleaching tests carried out under an applied potential of -500 mV with sphalerite in the presence and absence of noble minerals such as pyrite and chalcopyrite indicated enhanced zinc dissolution with negligible copper and iron in solution. Probable mechanisms and advantages of the electrochemical bioleaching process developed in the laboratory are outlined.

## INTRODUCTION

*Thiobacillus ferrooxidans* is the primary microorganism implicated in the leaching of several mineral sulfides and its commercial potential in the treatment of lean grade and refractory ores, desulfurization of coals and oils, environmental control, and in the beneficiation of difficult-to-dress minerals is ever increasing. It is a chemolithotrophic, acidophilic, mesophile deriving its energy for metabolism through the oxidation of ferrous iron and reduced sulfur compounds. Depending on the leaching conditions, direct and indirect, as well as electrochemical mechanisms, may be operative in the bio-oxidation reactions.

From a biological standpoint, two major controlling factors affecting oxidation rates are the activity as well as the biomass of the bacteria. Recent studies have indicated that the biomass of *T. ferrooxidans* can be significantly increased through electrochemical means. For example, *T. ferrooxidans* grown in an electrolytic reactor under applied current conditions were found to be more efficient as attested to by a 3.7-fold increase in protein concentration and an increase in the ferrous oxidation rate by 1.5 times.<sup>13</sup> Application of currents up to 90 mA increased cell yields of the above bacteria by sixfold.<sup>6</sup> Soviet scientists<sup>1,5,7</sup> have cultivated *T. ferrooxidans* in a ferrous-ion-containing medium with continu-

ous electroreduction of ferric ion and obtained increased biomass. The role of applied potentials in the bioleaching of some sulfides has been outlined previously.<sup>3,10</sup> However, the specific role of applied DC potentials, both in the positive and negative range on the activity of *T. ferrooxidans* in the presence and absence of mineral sulfides has not been reported earlier. In this article, the effect of positive and negative applied potentials on the activity and growth of *T. ferrooxidans* is brought out with respect to bioleaching of sulfides. Advantages of electrochemical bioleaching are illustrated with respect to selective dissolution of sphalerite from mixed sulfides.

## MATERIALS AND METHODS

A strain of *T. ferrooxidans*, ATCC 19859 was used in all the experiments. The bacterial culture was maintained in 9 K medium.<sup>11</sup> The 9 K medium used throughout the experiments was maintained at pH 2.0. The culture samples used in the tests were pure as confirmed by laboratory tests. A 4-day-old 9 K grown bacterial culture was used in electrochemical experiments to investigate the effect of applied potentials on bacterial activity and growth. Of this culture sample, 150 mL (in 9 K medium) was initially filtered through Whatman No. 41 filter paper to remove precipitates and taken in a trilimbed pyrex glass cell to study the effect of applied potentials. A G-3 frit separated the central limb from the side limbs of the cell. The central limb housed a platinum mesh electrode that constituted the working electrode. The two side-limbs housed a platinum foil auxiliary electrode and a saturated calomel electrode (SCE) as reference, respectively. The platinum electrodes as well as the cell were sterilized before each experiment and while conducting the applied potential tests, the entire potentiostat as well as cell assembly were kept in a sterile horizontal laminar flow chamber to avoid any external contamination. A BANK ELEKTRONIK Wenking Model ST 72 potentiostat was used to apply the desired potential.

After the application of the desired DC potentials for different periods of time, 10-mL portions of the exposed samples were inoculated into 90 mL of a fresh 9 K medium, and the ferrous iron oxidation was moni-

tored. Corresponding uninoculated blanks were maintained for comparison purposes.

Iron-free cell suspensions were used in bioleaching experiments under applied potential in the presence of the mineral samples. For this purpose, 1 L of a 4-day-old bacterial culture was first passed through Whatman No. 41 filter paper in order to remove the precipitates. The filtrate was then centrifuged in Sorvall-RC 5B refrigerated centrifuge at 15,000 rpm in SS-34 rotor for 15 min. The pellet was suspended in a solution of sulfuric acid at pH 2.0 and allowed to stand for 2 h in a refrigerator to permit settling of any precipitate. The supernatant containing cells was again centrifuged as mentioned earlier. Washing with sulfuric acid was repeated until the cells were free from iron (as confirmed by a color test). The cell pellet was finally suspended in 100 mL of sulfuric acid (pH 2.0) and stored at 4°C.

In the inoculum, cell concentrations were maintained at  $10^8$  cells/mL.

Estimated protein content in the inoculum amounted to 0.024 mg/mL on an average, and this value was used as reference when increase in protein content after application of potentials was determined.

Highly mineralized samples of pyrite (97% pure), chalcopyrite (65% pure), and sphalerite (98% pure) were used in bioleaching tests at 5% pulp density, the particle size of all the mineral samples corresponding to 53–75  $\mu\text{m}$ . In tests involving binary mixtures of the above sulfides, the respective minerals were mixed in a 1:1 ratio.

Electrochemical bioleaching tests under an applied potential using different sulfide minerals were carried out using a pyrex glass cell of cylindrical cross section with a capacity of about 200 mL. Thermostated water was circulated through an outer glass jacket in the cell to maintain a constant temperature of 30°C. Five grams of the mineral sample was taken in 100 mL of 0.9 K Fe medium. A platinum mesh electrode submerged into the mineral slurry served as the working electrode. A platinum foil auxiliary electrode was introduced into the cell close to the working electrode, but separated through a fritted tube compartment. A saturated calomel electrode connected through a Luggin capillary served as the reference electrode. The mineral slurry was agitated using a magnetic stirrer. Desired potentials were applied through a BANK ELEKTRONIK Wenking Model ST 72 potentiostat. A 10% iron-free inoculum was used in all the tests, while the corresponding uninoculated blanks contained a 5% alcoholic thymol solution.

Copper, zinc, and total iron in leach solutions were analyzed using an atomic absorption spectrophotometer, while ferrous and ferric ion concentrations were monitored using visible spectrophotometry.<sup>12</sup> Eh and pH of the medium were also frequently monitored. Bacterial cell numbers were estimated by direct microscopic count using a Petroff-Hausser counter. Cell

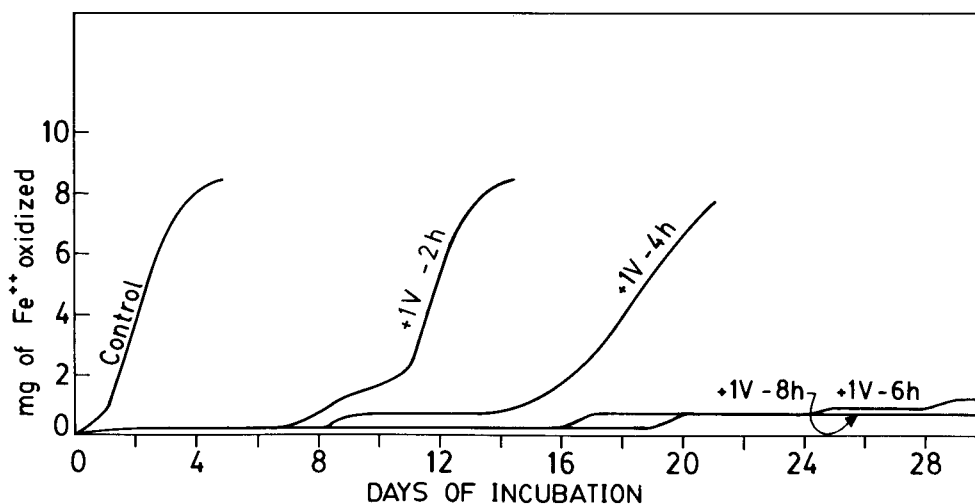
biomass in solutions was also estimated indirectly through determination of total protein.<sup>8</sup>

## RESULTS AND DISCUSSION

### Effect of Applied Potentials on Bacterial Activity and Growth

The effect of applied DC potentials on the growth and activity of *T. ferrooxidans* was studied in the range of +1000 mV to -1000 mV (SCE). Eh, pH,  $\text{Fe}^{++}$ , and  $\text{Fe}^{+++}$  levels in solution as a function of time were monitored. Under an impressed potential of +1000 mV for a period of up to 4 h, no significant changes in Eh, pH, and ferric ion concentrations were observed. These experiments were carried out in the 9 K medium where all the ferrous sulfate was oxidized to the ferric state by initial bacterial growth. Bacterial culture in the 9 K-grown medium was used as such. The ferric ion concentration in the medium remained constant at about 4.4 g/L throughout, and there was no ferrous iron traceable. The redox potential too remained at about 600 mV (SCE) with a little change in pH. This could be expected because there was no ferrous iron produced under the influence of the applied potential. Prolonging the duration of the above experiment up to 8 h yielded similar values for these parameters. At the end of different periods of exposure, 10-mL portions of the bacterial suspension were removed from the electrolytic cell and inoculated into 90 mL of a fresh 9 K medium at pH 2.0, and the ferrous iron oxidation by the bacteria was monitored. Typical results are illustrated in Figure 1. As could be readily seen, the bacterial activity was deleteriously affected with increasing periods of imposed potential. Compared to an unexposed control, there was significant increase in the lag periods for ferrous oxidation by the bacteria after exposing to +1000 mV. For periods beyond about 4 h, under the applied potential, the ferrous oxidation activity of bacteria was observed to be completely crippled. Such an inhibitory effect was also found to be irreversible in that even prolonged subculturing of the exposed bacteria in fresh 9 K media did not bring back their iron oxidation ability.

It is known that nascent oxygen will be generated under electrolytic conditions in an acid medium, and the bacterial deactivation observed earlier could be attributed to this electrochemical effect. Also, under electrolytic conditions, oxygen is liberated under pressure. cursory tests have indicated that growth of *T. ferrooxidans* was impeded when pure oxygen was bubbled into an inoculated 9 K medium instead of air. Atomic oxygen as well as intermediates such as  $\text{H}_2\text{O}_2$  generated in an acid medium under electrolytic conditions can interfere with bacterial metabolism and reproduction.<sup>2</sup> Also, all the iron in the medium was essentially present in the ferric state under the influence of the positive (oxidizing) potential, and, hence, no ready energy source for



**Figure 1.** Effect of period of application of +1000 mV on ferrous iron oxidation by *Thiobacillus ferrooxidans*.

the bacteria was available. To prove the earlier conclusion, further tests under similar applied potential conditions were carried out in the presence of oxygen scavengers and ferrous iron in the bacterial medium. Glutathione (GSH) and sodium sulfite were used as oxygen scavengers, while chemical ferrous sulphate and pyrite mineral served as ferrous ion-yielding substrates. The presence of even a small quantity of glutathione (1  $\mu$ M) could minimize and ultimately prevent inhibition of bacterial activity caused by positive applied potentials. Similarly, in the presence of up to 10 g/L of sodium sulfite, there was no deleterious effect caused by the applied positive potential of +1000 mV. The earlier observations indicate that by the simultaneous removal of electrolytically generated oxygen, the deleterious effect of positive applied potentials could be controlled. Likewise, if sufficient ferrous iron concentrations in the medium could be maintained during the application of the positive potentials, no deleterious effect could be observed. Results of tests carried out in the presence of 9 g/L of ferrous iron during the application of +1000 mV up to 4 h duration confirmed the earlier observation. The results indicate that if the electrolytically generated oxygen is effectively consumed (removed), its deleterious effect on bacterial growth and activity could be minimized. Also, ensuring the presence of sufficient ferrous iron in the medium during application of potential would not only provide the energy source for the bacteria, but it would also lead to oxygen removal through chemical ferrous iron oxidation.

In another test, a sterilized sample of  $-75 + 53 \mu\text{m}$  pyrite mineral at 5% pulp density was added to the medium during application of positive potentials. A considerable portion of the bacteria was found to be associated with the pyrite mineral under the conditions, which, when reinoculated into a fresh 9 K medium, oxidized all the ferrous iron just as an unexposed bacterial culture. Scanning electron microscopic examination of the exposed pyrite mineral samples re-

vealed the presence of a large number of bacterial cells attached to the mineral substrate. Also, the mineral attached bacteria were separated through centrifugation, and subsequent protein estimation after alkaline digestion of the mineral sample indicated the presence of significant bacterial association with the pyrite surface.

All the earlier experimental observations indicate that the deleterious effect of positive applied potentials on bacterial activity could be due to the absence of ferrous iron as well as the generation of nascent oxygen under electrolytic conditions.

The effect of negative applied potentials on the activity of *T. ferrooxidans* was then similarly studied. Potentials of  $-100$ ,  $-200$ ,  $-500$ , and  $-1000$  mV were applied for this purpose, and the various parameters such as Eh, pH, ferrous and ferric concentrations were continuously monitored in the medium. No ferrous iron was initially present in the medium. (Only ferric iron was present.) Typical results corresponding to applied potentials of  $-500$  and  $-1000$  mV are presented in Tables I and II. Unlike the case with +1000 mV, the application of negative potentials resulted in the electrochemical reduction of ferric iron to the ferrous form with a corresponding decrease in solution Eh. Ferric ion was reduced at a relatively higher rate at  $-1000$  mV than at  $-500$  mV. Further tests using chemical ferric sulphate indicated that after about 90 min of application of the negative potentials, almost 92% of ferric iron was reduced under  $-1000$  mV potential compared to a value of about 65% under  $-500$  mV. Thus, more time would be

**Table I.**  $E_{\text{SCE}}$ , pH,  $\text{Fe}^{++}$  and  $\text{Fe}^{+++}$  changes in *T. ferrooxidans* culture when exposed to  $-500$  mV.

	0	15 min	30 min	2 h	4 h
$E_{\text{SCE}}$ (mV)	600	500	460	400	360
pH	2.0	2.0	1.9	1.9	1.9
$\text{Fe}^{++}$ (mg/mL)	—	0.7	1.4	2.8	3.8
$\text{Fe}^{+++}$ (mg/mL)	4.4	3.7	3.0	1.6	0.6

**Table II.**  $E_{SCE}$ , pH,  $Fe^{++}$ , and  $Fe^{+++}$  changes in *T. ferrooxidans* culture when exposed to  $-1000$  mV.

	0	15 min	30 min	2 h	4 h
$E_{SCE}$ (mV)	630	480	460	380	230
pH	2.0	1.8	1.7	1.6	1.4
$Fe^{++}$ (mg/mL)	--	1.4	2.1	4.5	4.8
$Fe^{+++}$ (mg/mL)	5.0	3.6	2.9	0.5	0.2

required to reduce all the ferric iron under lower negative potentials. Further, the rate at which ferric iron was reduced at a fixed negative potential was also found to be a function of the initial ferric ion concentration in the culture medium. For example, at an applied potential of  $-800$  mV, while almost 90%–95% of ferric ion was reduced at the end of about 2 h, for an initial ferric iron concentration in the range of 4.5–8.5 g/L, only about 80% was so reduced if the initial ferric concentration was about 1–2 g/L. It is pertinent to mention in this context that the ferric ion levels generally encountered in 9 K-grown bacterial cultures are of the order of 4–5 g/L. Under such negative potentials, bacterial activity was not found to be inhibited as in the case under positive potentials. Ready availability of electrolytically generated ferrous ions in the medium ensured a continuous supply of the energy source for the bacteria.

However, in the absence of any iron species in the medium, the effect of applied potentials on the activity of *T. ferrooxidans* has to be viewed differently. To test this, iron-free cell suspensions of the bacteria were suspended in iron-free 9 K medium ( $10^8$  cells/mL), and DC potentials of  $-500$  and  $-1000$  mV were applied for different periods of time. It was observed that, although the cell numbers as well as the protein content remained more or less the same in the unexposed control, there was a drastic decrease in protein content with time in the inoculated medium when exposed to the earlier described DC potentials in the absence of ferrous iron. Virtually, no viable cells in the media exposed to  $-500$  mV could be seen under the microscope after 4 days. The results indicate that an induced electrical field could affect bacterial metabolism as well. The role of ferrous iron in offsetting the deleterious effect of applied potentials also becomes clear.

Bacterial generation time is also affected under electrolytic growth conditions. Results of a few cursory tests indicated that the doubling period for the *T. ferrooxidans* strains used in this study was about 16 h under normal growth conditions in 9 K medium, while it decreased to about 10 h under an applied potential of  $-500$  mV in the same medium. There was also clear evidence to indicate a significant increase in the biomass if the cell cultures grown in 9 K medium were exposed to negative potentials, such an increase being promoted under aeration. Experiments were performed using a 4-day-old culture of *T. ferrooxidans* under an applied potential of  $-500$  mV for periods extending

upto 8 days in the presence and absence of aeration. Sterile air at a rate of 80 mL/min was introduced into the cell for purposes of aeration. There was a 10-fold increase in the biomass under aeration as established by increase in protein content and cell number. On the other hand, in the absence of aeration, only about a fourfold increase in biomass was observed under similar experimental conditions.

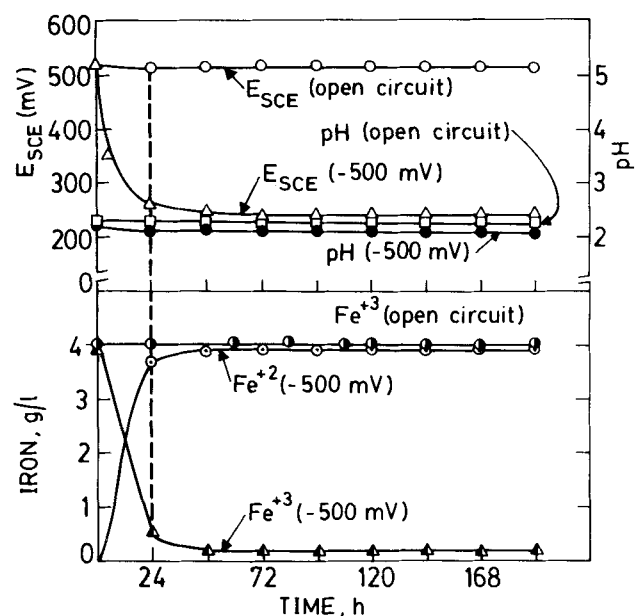
Measurements of Eh in a bioleaching system could serve as an indicator of bacterial growth and activity. Results presented in Figure 2 illustrate this point. The measured Eh values attained a lower steady state corresponding to complete conversion of ferric to ferrous iron.

### Electrobleaching of Sulfide Minerals

In any acid bioleaching system, ferric ions are continuously generated because of bacterial activity. If a negative DC potential could be maintained in a bioleaching reactor, a continuous and efficient reconversion of the biochemically produced ferric iron to the ferrous state could be achieved. Such an imposition of a DC potential would be advantageous at least in two respects:

1. Ensuring a continuous in situ supply of the ferrous iron energy source for the bacteria without the necessity for replenishing the ferrous iron externally.
2. Effectively minimizing and often eliminating an excessive build up of ferric iron (which is not conducive for bacterial activity). Precipitation of iron in the form of hydroxides and jarosites can also be controlled under the conditions described earlier.

Both of these effects achieved under electrolytic conditions would improve bioleaching rates, besides their



**Figure 2.** Variation of redox potential and pH with time indicating reduction of ferric to ferrous iron under an applied potential of  $-500$  mV.

contribution toward increasing the biomass, as mentioned already.

In an electrochemical bioleaching system, ferrous iron oxidation by the bacteria takes place simultaneously with the electrochemical reduction of ferric iron. Two opposing reactions, i.e., bacterial oxidation of ferrous iron and electrolytic reduction of biologically generated ferric iron thus take place, and their rates need to be so optimized as to increase bioleaching rates.

Applied potentials would also aid the dissolution of jarosites and iron-oxyhydroxides formed in an acid medium.

Let us now examine the role of applied potentials in the bioleaching of sphalerite. DC potentials in the range of  $-1000$  mV to  $+1000$  mV (SCE) were applied in an electrolytic cell containing the sphalerite mineral suspension for a period of 1 h and the resulting current and zinc dissolution monitored in the presence of the bacteria. It could be seen from Figure 3 that the dissolution of sphalerite would be promoted if an applied potential of about  $-500$  mV could be maintained in a bioleaching system. Detailed studies were carried out using sphalerite mineral samples in a 0.9 K Fe medium in the presence and absence of bacteria to bring out clearly the role of negative applied potential in enhancing zinc dissolution. Results are illustrated in Figure 4. The following observations are noteworthy.

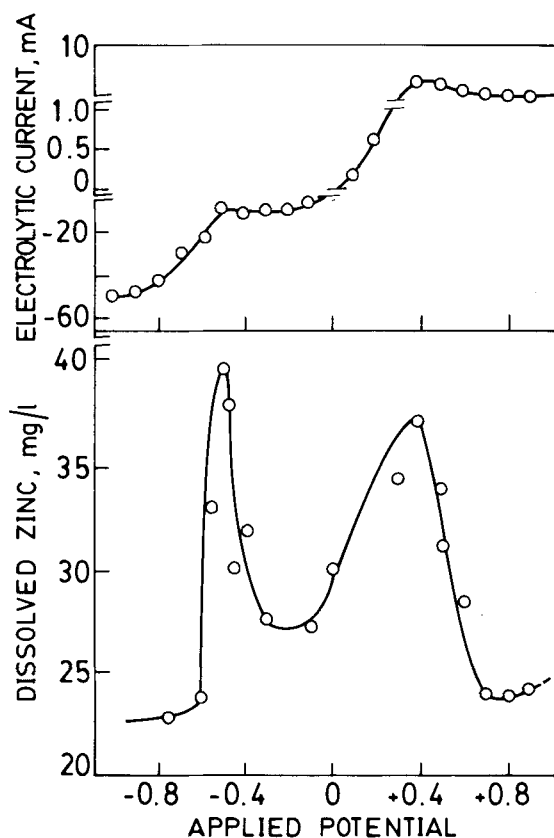


Figure 3. Variation of electrolytic current and zinc dissolution as a function of applied potentials (duration 1 h).

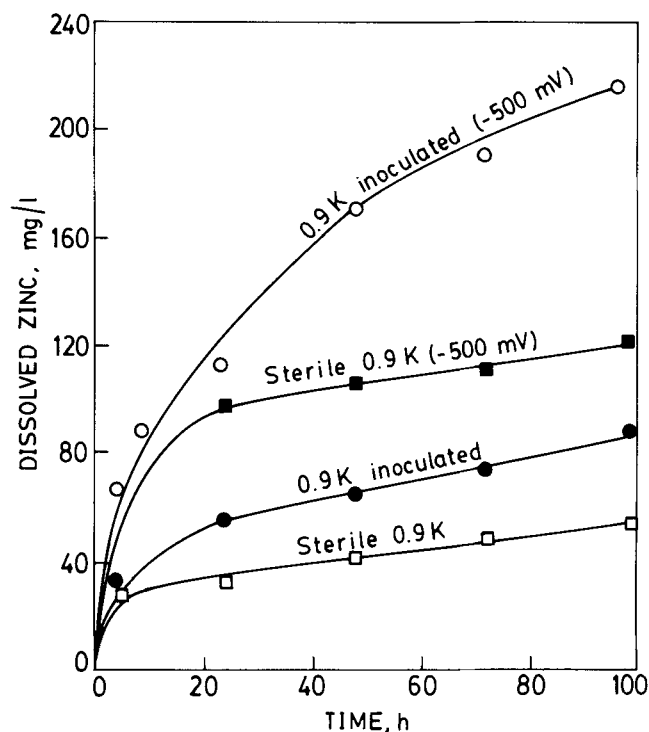
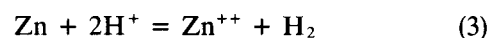
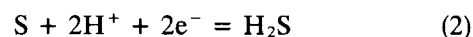
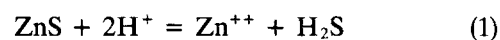


Figure 4. Dissolution of zinc from sphalerite as a function of time under an applied potential of  $-500$  mV in the presence and absence of *T. ferrooxidans*.

Zinc dissolution from sphalerite is enhanced under an applied potential of  $-500$  mV, even in the absence of *T. ferrooxidans*. Compared to chemical dissolution, electrochemical leaching of sphalerite is more efficient in an acid medium. This could be termed reductive dissolution under a negative potential that promotes the following reactions.



After about 100-h of leaching in the absence of bacteria, about 120 ppm of zinc were dissolved under the applied potential as compared to a value of only about 50 ppm caused by simple chemical leaching.

Bioleaching under the applied potential described earlier is more beneficial in that still higher leaching rates could be achieved by the combined action of bacteria and applied potential. Electrochemical reaction products such as  $\text{H}_2\text{S}$  and  $\text{S}^0$  would be further oxidized by the bacteria promoting leaching. For example, after about 100 h of bioleaching in a 0.9 K Fe medium, almost two times more zinc were solubilized under applied potential conditions than that observed in the absence of impressed potential. Thus, the presence of the bacteria substantially increases zinc dissolution rates. As brought out earlier, the applied negative potential promotes bacterial activity through a continuous electrochemical re-

generation of ferrous iron in the system as well as by increasing the biomass.

Sphalerite is electrochemically very active unlike pyrite and chalcopyrite, which are nobler minerals as evident from the rest potential values measured in a bioleaching medium.

Mineral	Rest potential (SCE) measured in a bioleaching medium (mV)
Pyrite	450
Chalcopyrite	310
Sphalerite	-10

Such an active behavior of sphalerite could be taken advantage of in achieving its selective dissolution from mixed sulfides. The role of galvanic interactions in the selective bioleaching of zinc from mixtures containing pyrite, chalcopyrite, and sphalerite has already been discussed elsewhere.<sup>3,4,9</sup>

Electrochemical leaching behavior of the three base metal sulfides, i.e., pyrite, chalcopyrite, and sphalerite with respect to their dissolution tendencies under different applied potentials for a period of 1 h in a bioleaching medium (0.9 K) is illustrated in Figure 5. In the negative range of applied potentials, only sphalerite exhibited a dissolution maximum at about -500 mV unlike pyrite and chalcopyrite, both of which possess dissolution maxima only at positive applied potentials of about +400 mV and +600 mV, respectively. Also, iron dissolution from chalcopyrite reaches a maximum only at positive potentials of the order of about 600-700 mV. The earlier observations indicate that higher selective dissolution of sphalerite could be attained from a mixture of

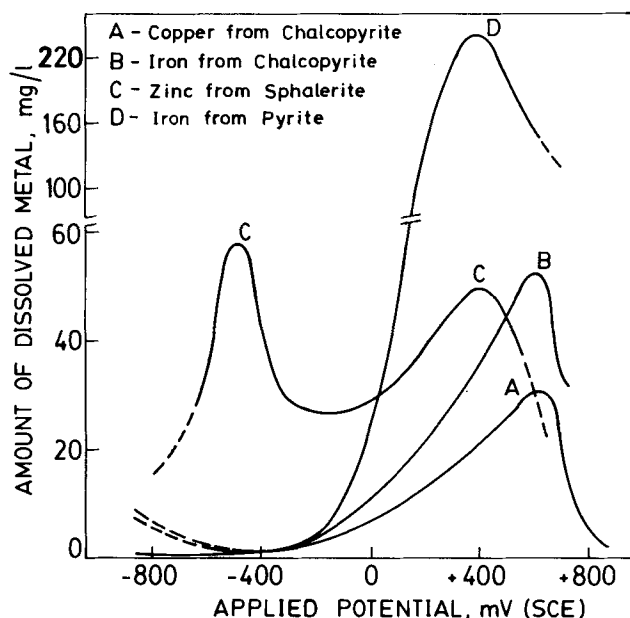


Figure 5. Electrochemical dissolution of pyrite, chalcopyrite, and sphalerite in a bioleaching medium under different applied potentials.

the three sulfides if an applied potential of about -500 mV could be maintained in a bioleaching reactor.

Under the influence of such a negative potential, dissolution of both pyrite and chalcopyrite would be minimized, while the bioleaching of sphalerite alone would be promoted. In the context of what has already been discussed, it could be reiterated that such negative applied potential would not only promote in situ electrolytic reduction of ferric ions, but also selective zinc dissolution.

Electrobioleaching of active-noble mineral mixtures would also involve the galvanic component besides the applied potential contribution in conferring selectivity of dissolution to the most active mineral in the combination. With the result, selective dissolution of the most active mineral would be further enhanced because three factors are now acting simultaneously, i.e., biological, applied potential, and galvanic effects. Typical results presented in Table III illustrate these facts quantitatively. The presence of pyrite and/or chalcopyrite along with sphalerite significantly enhances zinc dissolution with negligible dissolution of copper and iron.

## CONCLUSIONS

The following major conclusions could be arrived at based on this study:

1. Applied DC potentials in the range of +1000 mV to -1000 mV (SCE) have an effect on the activity of *T. ferrooxidans*, depending on the magnitude and duration of impression of the potential. Generally, positive potentials tend to inhibit bacterial activity possibly because of electrolytic generation of nascent oxygen in the absence of ferrous iron availability. Such a deleterious effect could be minimized if a continuous supply of ferrous iron could be maintained in the electrolytic cell or by the addition of oxygen scavengers such as glutathione and sodium sulfite.
2. Application of negative DC potentials in the range of -500 mV to -1000 mV to a bacterial culture containing ferric iron effectively converts ferric iron to the ferrous state, promoting bacterial activity and growth.
3. Application of negative DC potentials helps not only in the regeneration of ferrous iron, but also decreases bacterial generation time with an increase in cell biomass.

Table III. Electrochemical bioleaching of sphalerite at -500 mV in the presence and absence of pyrite and chalcopyrite (96 h, ratio of mixing 1:1).

Mineral	Amount dissolved (ppm)		
	Zn	Cu	Fe
Sphalerite alone	218	—	—
Sphalerite + pyrite	1100	—	19
Sphalerite + chalcopyrite	1000	25	18

4. Electrochemical bioleaching tests carried out with sphalerite in the presence and absence of pyrite and/or chalcopyrite indicated that at an applied potential of  $-500$  mV (SCE), sphalerite could be selectively bioleached. Bioleaching under an applied potential is more advantageous because it promotes leaching rates and ensures selectivity of dissolution.

The author acknowledges with thanks the financial grant received from the Department of Science and Technology, government of India under the Indo-US STI program. Thanks are also due to Dr. G. P. BrahmaPrakash and Ms. K. N. Sudha for laboratory assistance and technical input.

## References

1. Grishin, S. I., Skakun, T. O., Adamov, E. V., Polkin, S. I., Kovrov, B. G., Denisov, G. V., Kovalenko, T. F. 1985. Intensification of bacterial oxidation of iron and sulfide minerals by a *T. ferrooxidans* culture at a high cell concentration. p. 259–265 In: G. I. Karavaiko and S. N. Groudev (ed.), Proc. Int. Sem., modern aspects of microbiological hydrometallurgy. Projects GKNT, Moscow.
2. Heckly, R. J. 1976. Free radicals in dry biological systems. p. 135–156 In: W. A. Pryor (ed.), Free radicals in biology, vol. II. Academic Press, London.
3. Jyothi, N., Sudha, K. N., BrahmaPrakash, G. P., Natarajan, K. A., Ramananda Rao, G. 1989. Electrochemical aspects of bioleaching of mixed sulfides. p. 9–16 In: B. J. Scheiner, F. M. Doyle, and S. K. Kawatra (ed.), Biotechnology in minerals and metal processing, SME (AIME). Littleton, CO.
4. Jyothi, N., Sudha, K. N., Natarajan, K. A. 1989. Int. J. Miner. Process. **27**: 189–203.
5. Karavaiko, G. I. 1985. Microbiological processes for the leaching of metals from ores. U.N.E.P., Moscow.
6. Kinsel, N. A., Umbreit, W. W. 1964. J. Bacteriol. **87**: 1243–1244.
7. Kovrov, B. G., Denisov, G. V., Sedelnikov, S. M. 1984. The culturing of ferrooxidising bacteria on electric energy. Nauka, Novosibirsk, U.S.S.R.
8. Lowry, O. H., Rosenborough, N. J., Farr, A. L., Randall, R. J. 1951. J. Biol. Chem. **193**: 265–275.
9. Natarajan, K. A. 1988. Miner. Metall. Process **5**: 61–65.
10. Natarajan, K. A. 1990. Electrochemical aspects of bioleaching of base metal sulfides. p. 79–106. In: H. L. Ehrlich, C. L. Brierley, and J. A. Brierley (ed.), Microbial mineral recovery. McGraw Hill, New York.
11. Silverman, M. P., Lundgren, D. G. 1959. J. Bacteriol. **77**: 642–647.
12. Vogel, A. I. 1985. Textbook of quantitative inorganic analysis. p. 741–743. English Language Book Society, Longman, Essex, England.
13. Yunker, S. B., Radovich, J. M. 1986. Biotechnol. Bioeng. **28**: 1867–1875.