

Rate of Pyrite Bioleaching by *Thiobacillus ferrooxidans*: Results of an Interlaboratory Comparison

GREGORY J. OLSON†

Polymer Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899

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Ten laboratories participated in an interlaboratory comparison of determination of bioleaching rates of a pyrite reference material. A standardized procedure and a single strain of *Thiobacillus ferrooxidans* were used in this study. The mean rate of bioleaching of the pyrite reference material was 12.4 mg of Fe per liter per h, with a coefficient of variation (percent relative standard deviation) of 32% as determined by eight laboratories. These results show the precision among laboratories of the determination of rates of pyrite bioleaching when a standard test procedure and reference material are used.

The microbially accelerated oxidation of pyrite (FeS₂) has important economic and environmental consequences. For example, acidic ferric sulfate solutions resulting from pyrite oxidation assist in the commercial recovery of copper and uranium from ores and wastes (3), and microbial leaching has begun to be applied as a pretreatment step in the commercial recovery of gold from sulfidic deposits. In addition, pyrite is the dominant form of inorganic sulfur in coal, and many studies have shown the potential for microbial coal cleaning through pyrite oxidation (10, 13). Conversely, uncontrolled microbial pyrite oxidation in active and abandoned coal and metal mines leads to acid mine drainage (15), a serious water pollution problem.

Microorganisms play a key role in pyrite oxidation in these natural and commercial processes. The rate-determining step in pyrite oxidation in water is the oxidation of ferrous iron, which bacteria accelerate in acid mine water by a factor of 10⁶ or more above the abiotic rate (15). In addition, bacteria oxidize elemental sulfur, which forms on the surface of pyrite under certain oxidation conditions (8, 9).

The most widely studied pyrite-oxidizing bacterium is *Thiobacillus ferrooxidans*, which grows autotrophically on pyrite as an energy source, oxidizing iron and sulfur to produce acidic ferric sulfate solutions.

An increase in the rate of pyrite oxidation by *T. ferrooxidans* and other pyrite-oxidizing bacteria would improve process efficiencies in ore leaching or perhaps make feasible the use of bioleaching in certain metal leaching or coal-cleaning applications. The physiological factors which limit the rate of microbial pyrite oxidation are not known. Rates of pyrite oxidation by *T. ferrooxidans* are usually determined by measuring the rate of production of soluble Fe. However, rates of pyrite oxidation by *T. ferrooxidans* reported in the literature are difficult to compare, making difficult the evaluation of different bacterial strains and leaching processes. In some recent reports, rates of pyrite bioleaching by *T. ferrooxidans* range from 2.5 to 30 mg/liter (4, 5, 6, 11, 12, 18, 19). This variability in leaching rates might be due to physiological differences among strains of *T. ferrooxidans*, but it could also be due to variables associated with conducting and measuring bioleaching rates. In some cases, it is not possible to compare leaching rates because of differences in

the manner in which leaching rates are determined and reported. Another source of variability could be the pyrite substrate, as pyrite from different sources can vary widely in susceptibility to chemical oxidation (16).

Recently, standards have been developed to facilitate more-uniform testing and comparison of bioleaching rate data. Pyrite bioleaching reference material was recently prepared at the National Institute of Standards and Technology (NIST) (12), and a standard test method for conducting studies of pyrite bioleaching rates has been developed by committee E-48 (Biotechnology) of the American Society for Testing and Materials (ASTM) (2). However, the reproducibility of measuring bioleaching rates among laboratories has not been determined. An interlaboratory comparison of bioleaching rates using a single strain of *T. ferrooxidans*, the pyrite reference material, and the ASTM test method was necessary to determine the variance associated with the determination of bioleaching rates (1). This paper describes results from 10 laboratories which participated in a comparison of determination of pyrite bioleaching rates.

MATERIALS AND METHODS

Organism and culture conditions. *T. ferrooxidans* 13661 (American Type Culture Collection, Rockville, Md.) was repurified twice at NIST by being streaked on ferrous sulfate agar (7). The bacterium was maintained on 9K liquid medium (14) at 28°C on a gyratory shaker. Actively growing cultures were placed in screw-capped test tubes and distributed by mail to participants.

Pyrite. Twenty kilograms of -10-mesh pyrite (95 to 96%) from the Waldo Mine, New Mexico, obtained through the Department of Mining, Environmental, and Geological Engineering, New Mexico Institute of Mining and Technology, Socorro, was ground to -165/+250 mesh (58 to 91 μm), acid washed, rinsed, dried, sterilized, and packaged in 100-g quantities under nitrogen as described previously (12). Sample bottles were distributed by mail to participants by the Office of Standard Reference Materials, NIST.

Bioleaching procedure. A standard test procedure developed by the ASTM (2) was followed. Briefly, 1.0 g of pyrite was added to 50 ml of 1/10-strength 9K salts (minus iron) in 250-ml conical flasks. To minimize pyrite oxidation from autoclaving, flasks were sterilized at 110°C (10 min). After being cooled, flasks were inoculated with an actively growing culture of *T. ferrooxidans*. The inoculum had been grown

† Present address: Pittsburgh Energy Technology Center, P.O. Box 10940, Pittsburgh, PA 15236.

TABLE 1. Results of interlaboratory comparison^a

Laboratory	No. of flasks	Rate (mg/liter/h)	Within-laboratory COV ^b	No. of days ^c	pH		Final measurement		
					Start	End	Fe (mg/liter)	Day	% Leached ^d
1	3	8.8	19	1-8	2.0	1.6	3,128	30 ^e	33
2	No report								
3	3	7.8	14	1-6	1.8	1.3	1,800	12 ^e	19
4	No report								
5	3	17.0	6	2-7	2.8	1.5	4,252	15 ^e	45
6	6	10.0	4	9-17	2.5	1.5	3,073	29 ^e	40
7	5	17.8	11	12-21	2.6	1.5	3,220	26	35
8	3	15.6	9	11-19	2.0	1.8	5,400	25	42
9	3	13.2	26	4-15	2.3	1.5	3,470	15	37
10	9	9.1	4	2-12	3.4	1.3	2,553	12 ^e	27

^a Participants in this study (not listed in order shown above): Gregory J. Olson, Polymer Division, National Institute of Standards and Technology, Gaithersburg, Md.; Henry L. Ehrlich and A. R. Ellett, Biology Department, Rensselaer Polytechnic Institute, Troy, N.Y.; P. Greg McMillan, Tonkin Springs Gold Mining Co., Elko, Nev.; Elizabeth Baglin and Deborah L. Yopps, U.S. Bureau of Mines, Reno, Nev.; James A. Brierley, Newmont Metallurgical Services, Salt Lake City, Utah; Arpad E. Torma and Franz Schinner, State Mining and Mineral Resources Research Institute, New Mexico Institute of Mining and Technology, Socorro; Franco Baldi and Milva Pepi, Department of Environmental Biology, University of Siena, Siena, Italy; Giovanni Rossi, Pirpaolo Trois, and Giovanni Loi, Department of Mining and Mineral Engineering, University of Cagliari, Cagliari, Italy; Robert M. Kelly and Sachiko Hirose, Department of Chemical Engineering, Johns Hopkins University, Baltimore, Md.; Allan M. Johnson and Donald R. Leuking, Department of Mining Engineering, Michigan Technological University, Houghton.

^b COV, Coefficient of variation.

^c Approximate interval after inoculation used in calculation of bioleaching rate.

^d Percent iron solubilized from pyrite.

^e Rate of iron bioleaching had not leveled off at time of final measurement.

in 9K medium, harvested by centrifugation, washed in 0.01 M sulfuric acid, and suspended in a small volume of 0.01 M sulfuric acid. Cells were added to the pyrite to give an initial density of 1.0×10^7 to 5.0×10^7 cells per ml as determined by direct cell counts using phase-contrast microscopy with a Petroff-Hauser bacteria counter or by epifluorescence microscopy employing membrane filters. The cultures were incubated at $28 \pm 2^\circ\text{C}$ on a gyratory shaker at 200 rpm. Liquid samples were removed periodically, filtered or centrifuged, and measured for total soluble iron by atomic absorption spectrophotometry or by a colorimetric ortho-phenanthroline method (12). The rate of bioleaching was determined from the slope of a curve plotting soluble iron versus time from a minimum of three inoculated flasks and was reported as milligrams of Fe per liter per hour. The rate was not corrected for the small amount of iron removed during sampling. The slope was determined during the linear portion of the leaching curve (17), which represents active leaching at a constant rate. Among the participants, this time interval occurred as soon as 1 to 6 days and as late as 12 to 21 days after inoculation.

RESULTS

The results of the bioleaching rate comparison from 8 of the 10 laboratories are shown in Table 1. Participating scientists and the institutions at which the tests were performed are listed in a footnote to Table 1. None of the participants knew of the results from other laboratories in advance of the reporting deadline. However, after the results of the comparison had been shared with all 10 laboratories, reports were received from laboratories 2 and 4. These results were not included in the calculation of the mean bioleaching rate since they were received after the results from the rest of the participants had been made known.

The rate of bioleaching of pyrite as determined by the appearance of soluble iron in the medium ranged from 7.8 to 17.8 mg of Fe/liter/h, with a mean of 12.4 mg/liter/h and a coefficient of variation of 32%. The within-laboratory coefficient of variation for replicate flasks ranged from 4 to 26%.

The mean abiotic rate of leaching (sterile controls) was 0.36 mg of Fe/liter/h, as determined by four laboratories. Thus, *T. ferrooxidans* cultures produced soluble iron from this pyrite material at 34 times the abiotic rate.

Leaching of iron reached a steady linear rate within the first 4 days after inoculation in five of the eight laboratories. However, an extended lag phase occurred in three laboratories before significant iron solubilization began. Nonetheless, the rates of bioleaching in these three laboratories were comparable to those obtained by the other participants. The initial pH values ranged from 1.8 to 3.4, and final pH values ranged from 1.3 to 1.8. A few of the laboratories reported starting pH values significantly higher than that specified in the ASTM procedure. However, there was no clear correlation between initial pH and leaching rate.

Although not a primary goal of this comparison, the final concentration of iron in solution was determined and is shown in Table 1 to range from 1,800 to 5,400 mg/liter, corresponding to 19 to 45% of the iron added in the form of pyrite. This variance is not surprising, given the differences in the times at which experiments were concluded, which ranged from 12 to 30 days after inoculation. In many cases, pyrite leaching was still proceeding at the termination of the experiments.

One of the two laboratories whose results were not included reported two successive failures to achieve growth of *T. ferrooxidans* on pyrite after waiting 180 and 288 h after inoculation. However, on a third attempt, this laboratory obtained a mean leaching rate (in three flasks) of 13.6 mg of Fe/liter/h, with a relative standard deviation of 26%. Lag phases in triplicate flasks ranged from 1 to 3 weeks. The other laboratory was unable to grow the organism on pyrite after a single 13-day attempt.

DISCUSSION

The results of this study show that the rate of bioleaching of a pyrite sample using a single strain of *T. ferrooxidans* and a specific procedure was determined with a coefficient of variation of 32%, with the bioleaching rate varying over

approximately a twofold range. These results are considered reasonably good, given the within-laboratory coefficient of variations as high as 26% and the potential cumulative measurement errors and procedural variances associated with rate measurements, even with standardized tests.

A previous study (12) helped form the basis for the ASTM bioleaching procedure (2). That study showed that inoculum size does not affect bioleaching rates above an initial cell density of 10^6 cells per ml. This was determined by measuring bioleaching rates of pyrite (1.0 g of 100/200-mesh pyrite in 50 ml of 1/10-strength 9K salts) by *T. ferrooxidans* at six different initial cell concentrations (ranging from 5×10^5 to 2.4×10^8 cells per ml). Only at the lowest cell concentration tested (5×10^5 /ml) were bioleaching rates appreciably slower (about 50% less). In addition, the previous study showed that determinations of bioleaching rate in an individual laboratory are reasonably repeatable in replicate samples in temporally separated experiments and that the use of baffled flasks to increase sample aeration does not increase bioleaching rates with this pyrite and strain of organism. In addition, a relatively low starting pH (2.0) coupled with the use of 1/10-strength salts medium (which does not decrease the bioleaching rate) helped to minimize the precipitation of jarosite, $x\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6$ (where $x = \text{Na}^+, \text{K}^+, \text{NH}_4^+, \text{or } \text{H}_3\text{O}^+$), which would lead to underestimation of amounts of leached iron. It was also determined that atomic absorption spectrophotometry and colorimetric measurements of soluble iron gave comparable results.

Some participants reported departures from the ASTM procedure used in the intercomparison. Laboratory 5 sterilized pyrite at 121°C for 10 min. A few laboratories reported initial pH values higher than the specified value of near 2.0. However, there was no clear correlation between these departures from procedure and the leaching rate.

The difficulties of laboratories 2 and 4 in obtaining growth on pyrite has not been resolved, nor has the cause of the extended lag phase which occurred in laboratories 6, 7, and 8. Nonetheless, this interlaboratory comparison has illustrated the precision which can be expected when standard materials and procedures are used in studies on pyrite bioleaching rates. This study also suggests that other mineral standards (i.e., copper sulfide ores and concentrates) or coal pyrite standards could be developed to assist in the comparison of bioleaching rates of these substrates.

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