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Standard Test Method for Determining the Rate of Bioleaching of Iron From Pyrite by *Thiobacillus Ferrooxidans*[1]

This standard is issued under the fixed designation E 1357; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers procedures for determining the rate of bioleaching of iron from pyrite (FeS_2) by the bacterium *Thiobacillus ferrooxidans*.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 516 Test Methods for Sulfate Ion in Water[2]

D 1068 Test Methods for Iron in Water[2]

D 1193 Specification for Reagent Water[2]

D 4455 Test Method for Enumeration of Aquatic Bacteria by Epifluorescence Microscopy Counting Procedure[3]

3. Terminology

3.1 *Definition:*

3.1.1 *soluble iron*—the complexed and dissolved iron as determined by Vuorinen et al.[4] in their study of the species of iron released from pyrite oxidation by *T. ferrooxidans*. They found that values of complexed and dissolved iron corresponded closely with "total iron" as determined after hot sulfuric acid digestion of samples, particularly at 1 to 2 % pulp density.

4. Summary of Test Method

4.1 Cells of *T. ferrooxidans* grown on ferrous iron are added to conical flasks containing finely ground iron pyrite in an inorganic salts medium (2 % pulp density). The culture is incubated with agitation and samples are periodically withdrawn for determination of soluble iron. The rate of pyrite leaching is determined from the linear portion of a curve-plotting soluble iron produced versus time.

4.2 The average rate of soluble iron production in mg of iron/L/h is reported along with values for uninoculated controls. The standard deviation for triplicate flasks is also reported. Also to be reported is the particle size range of the pyrite and the initial and final pH values of the test solutions.

5. Significance and Use

5.1 The development and refinement of processes for bioleaching of metal ores and coal desulfurization require intercomparison of bioleaching data both to better understand metal ore bioleaching mechanisms and to develop more effective strains. For uncertain reasons, different strains of *T. ferrooxidans* exhibit different pyrite leaching rates and different sources of pyrite vary widely in susceptibility to microbial attack.

5.2 This test method has been developed to provide a standard procedure for evaluating the rate of bioleaching of iron from iron pyrite (FeS_2), a commonly used growth substrate for *T. ferrooxidans* and an important mineral that is biologically degraded in commercial bioleaching operations and in many exposed coal deposits. A high leaching rate in this test is evidence for potential degradability of the mineral in mining operations. A low rate of bioleaching suggests that the mineral is inherently not a good substrate or that it contains toxicants toward thiobacilli, and might not be readily bioleaching in a mining operation.

6. Apparatus

6.1 *An Gyratory Incubator-Shaker*, for maintaining cultures at constant temperature (28 ± 2°C) and agitation rate (200 r/min) during both inoculum preparation and the leaching test.

6.2 *An Ultraviolet-Visible Light Spectrophotometer, Colorimeter or Atomic Absorption Spectrophotometer*, for determining concentration of soluble iron.

6.3 *A Centrifuge*, for harvesting cells of *T. ferrooxidans* prior to inoculation of the pyrite suspension and for removing particles of iron from solution prior to analysis for soluble iron. A filtration apparatus may also be used for particle removal prior to analysis for soluble iron.

6.4 *Conical Flasks*, 500, 250 ml or 125 mL (non-baffled).

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that

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[1] This test method is under the jurisdiction of ASTM Committee E48 on Biotechnology and is the direct responsibility of Subcommittee E48.03 on Unit Processes and Their Control.

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] *Annual Book of ASTM Standards*, Vol 11.02.

[4] Vuorinen, A., Hiltunen, P., Hsu, J. C., and Tuovinen, O. H., "Solubilization and Speciation of Iron During Pyrite Oxidation by *Thiobacillus ferrooxidans*," *Geomicrobiology Journal*, Vol 3, 1983, pp. 95–120.

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all reagents conform to the specifications of the Committee on Analytical Reagents of the American ChemicalSociety where such specifications are available.[5] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type IV of Specification D 1193.

8. Hazards

8.1 This test method may include the use of hazardous chemicals. Avoid contact with chemicals and follow manufacturer's instructions and material safety data sheets.

9. Procedure

9.1 The inoculum consists of an active culture of *T. ferrooxidans* grown on ferrous iron as the energy source in a medium[6] containing (in g/L of water): $(NH_4)_2SO_4$, 3.0; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.1; $Ca(NO_3)_2 \cdot 4H_2O$, 0.01; $FeSO_4 \cdot 7H_2O$, 44.22 and 10N H_2SO_4, 1.0 mL. The ferrous sulfate is dissolved separately in 300 mL water and the other salts are dissolved in 700 mL water. The two solutions are autoclaved (121°C, 15 min), combined when cool, and 100 mL portions added to sterile, loosely capped 250-mL conical flasks and inoculated with *T. ferrooxidans*. The temperature is maintained at 28°C with shaking at 200 r/min on a gyratory shaker. Cells are harvested when the culture has reached the late logarithmic phase of growth as monitored by cell number (direct counts by Petroff-Hauser counting chamber or epifluorescence microscopy (see Test Method D 4455)) or by determination of residual ferrous iron in solution (for example, using Test Method D 1068, Test Method A for ferrous iron or by permanganate titration[7]). Cells are harvested by centrifugation, washed twice in 0.01M H_2SO_4, and resuspended to a concentration of 10^9 to 10^{10} cells/mL. This cell suspension is then diluted into 25 mL (for 125-mL flasks) 50 mL (for 250-mL flasks) or 100 mL (for 500-mL flasks) of the above medium at one-tenth strength (diluted with 0.1N H_2SO_4), minus ferrous sulfate but containing pyrite at 2.0 % pulp density (2 g/100 mL). Make sure that starting cell concentrations are 1 to 5×10^7 cells/mL. The flasks containing the liquid medium and pyrite are sterilized at 110°C and cooled prior to inoculation. Make sure that the pH of the solution after autoclaving is near 2.0. The flasks are weighed so that losses of water due to evaporation can be replaced.

NOTE 1—Where samples of pyrite contain appreciable acid buffering capacity (for example, associated carbonates), the pH in the testing solution may rise to levels unsuitable for optimal growth of *T. ferrooxidans*. Although the elevated pH indicates that the sample of pyrite may not be a good substrate for *T. ferrooxidans*, the investigator may wish to determine the inherent bioleachability of the pyrite free from associated acid-neutralizing minerals. In this case, the pyrite may be washed first in 5*M* HCl, followed by several rinses in water. The initial pH of the test solutions can affect the amount of total soluble iron produced by the action of *T. ferrooxidans* on pyrite, despite the fact that the final pH may drop to low levels.[4]

NOTE 2—200 mL of 9K medium normally yields sufficient numbers of cells in a final washed suspension to inoculate triplicate pyrite leaching flasks. Iron precipitates harvested with the cells can be separated by allowing the washed cell suspension to stand in a test tube for 2 to 3 h, then collecting the supernatant by pipet (most of the iron precipitates settle out).

9.2 Flasks are incubated at 28°C with shaking at 200 r/min on a gyratory shaker and sample aliquots are removed every 1 to 2 days for determination of total soluble iron. Flasks are weighed prior to each sampling and the amount of water lost by evaporation is replaced by addition of sterile water. Also, the amount of sample removed is replaced with sterile 0.01M H_2SO_4. Samples are centrifuged or filtered (0.45 µm or less) and soluble iron is determined by atomic absorption analysis or by colorimetric procedures (for example, Test Method D 1068). Samples are removed periodically until the rate of soluble iron production slows markedly.

NOTE 3—Make sure that sample size is small as possible (1.0 mL or less) to avoid excessive dilution of the culture. This is especially critical where 125-mL flask sizes are used.

9.3 Sulfate is determined also (for example, Test Methods D 516) initially and at the end of the test. Determine the percentage of pyritic iron and sulfate converted to soluble iron and sulfate.

10. Report

10.1 The rate of iron solubilization is determined by plotting the concentration of iron in solution with time. The rate is obtained by determining the slope of the linear part of the leaching curve and is expressed as mg of iron L/h.

10.2 Also reported is the duration of the test (days), the initial and final pH of the solutions and the percentage of the pyritic iron and sulfate converted to soluble iron and sulfate.

11. Precision and Bias

11.1 This section will be added on completion of interlaboratory testing of a pyrite research material and culture of *T. ferrooxidans*.

12. Keywords

12.1 bioleaching; iron; ore leaching; pyrite; soluble ion; *Thiobacillus Ferrooxidans*

[5] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Analar Standards for Laboratory U.K. Chemicals," BDH Ltd., Poole, Dorset, and the "United States Pharmacopeia."

[6] Silverman, M. P., and Lundgren, D. G., "Studies on the Chemoautotrophic Iron Bacterium *Ferrobacillus ferrooxidans* I: An Improved Medium and a Harvesting Procedure for Securing High Cell Yields," *Journal of Bacteriology*, Vol 77, 1959, pp. 642–647.

[7] Skoog, D. A., and West, D. M., "Fundamentals of Analytical Chemistry," 3rd ed., Holt, Rinehart and Winston, New York, 1979.

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