Designation: E 1357 – 90 (Reapproved 1996)ε1

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.

ε[1] NOTE—Section 3 was updated and Section 12 was added editorially in October 1996.

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 The culture is incubated with agitation and samples are periodically withdrawn for determination of soluble iron.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).

[1] This test method is under the jurisdiction of ASTM Committee E-48 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.