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Steven J. Onysko, Robert L. P. Kleinmann and Patricia M. Erickson

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NOTES

Ferrous Iron Oxidation by *Thiobacillus ferrooxidans*: Inhibition with Benzoic Acid, Sorbic Acid, and Sodium Lauryl Sulfate

STEVEN J. ONYSKO,† ROBERT L. P. KLEINMANN,* AND PATRICIA M. ERICKSON
Pittsburgh Research Center, U.S. Bureau of Mines, Pittsburgh, Pennsylvania 15236

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Benzoic acid, sorbic acid, and sodium lauryl sulfate at low concentrations (5 to 10 mg/liter) each effectively inhibited bacterial oxidation of ferrous iron in batch cultures of *Thiobacillus ferrooxidans*. The rate of chemical oxidation of ferrous iron in low-pH, sterile batch reactors was not substantially affected at the tested concentrations (5 to 50 mg/liter) of any of the compounds.

Acid mine drainage is formed by the weathering or oxidation or pyritic materials exposed during the mining of coal deposits and is a major water pollution problem in the Appalachian region of the United States (15). The rate of pyritic material oxidation can be greatly accelerated by certain acidophilic bacteria such as *Thiobacillus ferrooxidans*. These bacteria promote indirect oxidation of pyrite through the catalysis of the oxidation of ferrous iron to ferric iron (5, 14, 16–18, 20), which is an effective oxidant of pyrite (18). These bacteria also may catalyze direct oxidation of pyrite by oxygen (1, 3, 9). A number of organic compounds, under laboratory conditions, can apparently inhibit both the oxidation of ferrous iron to ferric iron by *T. ferrooxidans* (12, 21–24) and the weathering of pyritic material by mixed cultures of acid mine drainage microorganisms (8, 11, 12). Sodium lauryl sulfate (SLS), an anionic surfactant, has proved effective in this regard (6–8, 11, 12).

Our laboratory at the Bureau of Mines has demonstrated in full-scale experiments (10, 13) that SLS can effectively control acid mine drainage formation under field conditions also. Single applications of 0.25% SLS solutions at ca. 5,000 liters/ha (i.e., one 210-liter drum of 30% SLS [diluted ca. 1:100] per 4 ha) can sufficiently sorb to coal and refuse materials to effect dramatic reductions in acidity, sulfate, and dissolved iron concentrations in discharge waters for 3 to 6 months. The technology is now in use at 40 to 50 surface mining operations in the United States that have acid drainage problems. Pilot-scale tests by our laboratory (12) also indicate that single applications of SLS embedded in rubber pellets that slowly dissolve can be added to refuse material to effect a more prolonged release of SLS and bactericidal action.

Current research efforts at the Bureau of Mines relate to the problem of acid drainage from abandoned underground mines—the single largest source of this pollution in Appalachia (15)—and other sites not suitable for either of the demonstrated SLS treatment techniques. The objectives have been to identify environmentally acceptable compounds that can (i) be added to active acid-producing sites to neutralize existing acidity, (ii) precipitate as insoluble compounds from these waters as a means of storage of reserve compound after neutralization is accomplished, and (iii) redissolve and exhibit bactericidal action upon reappearance of the acid conditions conducive to recolonization by *T. ferrooxidans* (12).

Experiments in this laboratory indicate that addition of 0.1% solutions of sodium benzoate or potassium sorbate to mine waters can result in the formation of organic precipitates during neutralization of the waters. Sodium benzoate has been previously studied (7) as a fungicidal additive for preservation of SLS solutions used in experiments on the control of bacterially catalyzed acid formation in refuse material, although no bactericidal action was ascribed directly to the benzoate. If the precipitated compounds consist of ferric salts of sorbate or benzoate, as we suspect, the species liberated from the precipitates during redissolution in acidified waters may include sorbic and benzoic acids. Determination of the inhibitory effect of sorbic and benzoic acids on ferrous iron oxidation by *T. ferrooxidans* is the subject of this report. The efficacy of SLS inhibition is also reevaluated.

In the study, 250-ml Erlenmeyer flasks were autoclaved and subsequently filled with 100 ml of filter-sterilized (0.22-μm-pore cellulose acetate membranes; Millipore Corp., Bedford, Mass.) culture medium. Controls contained the stock ferrous iron and mineral medium of Cobley and Haddock (C & H medium) (4). This medium has an initial pH of 1.6 and a ferrous iron concentration of 10,000 mg/liter. Each of 12 supplemented media consisted of 5, 10, 25, or 50 mg of SLS, benzoic acid, or sorbic acid per liter in stock C & H medium. No formation of precipitates in the stock medium was observed as a result of the addition of these concentrations of supplements. In the experiment, two parallel series of 15 culture flasks consisted of three stock C & H medium controls and one flask of each of the 12 media that were supplemented with SLS, benzoic acid, or sorbic acid. Each flask in the first series was inoculated with 0.1 ml of a subculture of *T. ferrooxidans* derived from strain ATCC 13661 that had been incubated for 7 days in 1 liter of the stock C & H medium. Each flask in the second series was inoculated with 0.1 ml of sterilized water. Culture flasks were shaken at 200 rpm on gyratory shakers mounted inside a darkened incubator operated at 30°C.

On each sampling day, the 15 flasks in each series were weighed, the mass of water that had evaporated since the previous sampling day was replenished with sterile water, the flasks were reshaken, and 1-ml samples were aseptically

* Corresponding author.
† Participated in this research while a Ph.D. Candidate in the Mineral Engineering Department, University of California, Berkeley, CA 94720.

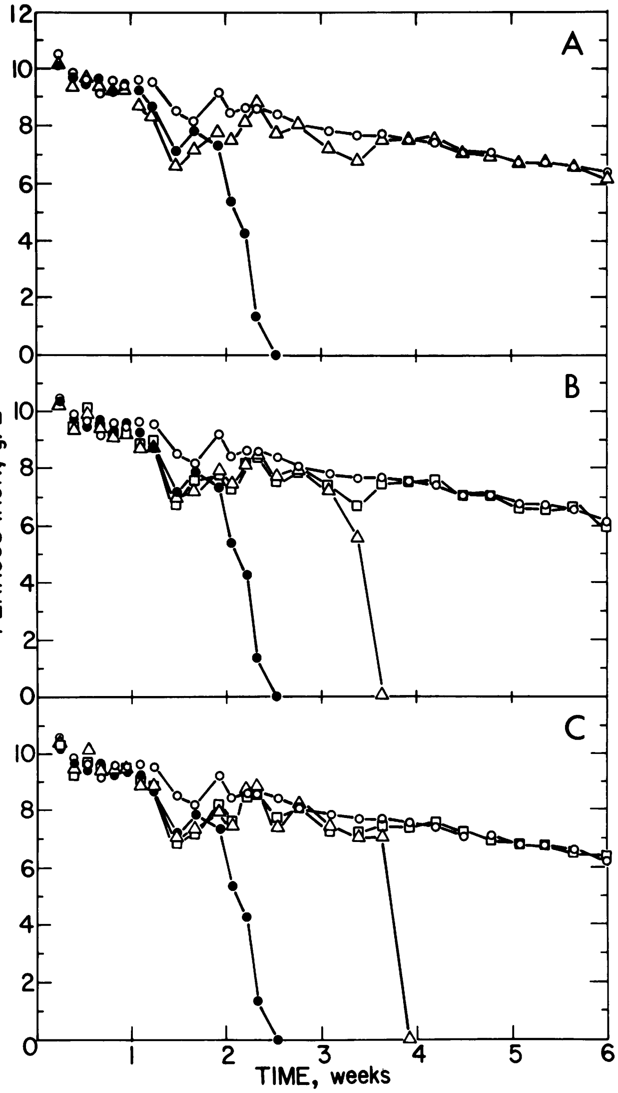

FIG. 1. Ferrous iron disappearance from *T. ferrooxidans*-inoculated media supplemented with 5 (△) or 10 (□) mg of (A) SLS, (B) sorbic acid, or (C) benzoic acid per liter. (The average data for the three sterile, unsupplemented [○] and the three *T. ferrooxidans*-inoculated, unsupplemented [●] replicates are indicated with each inhibitor data set.

removed for electrometric determination of pH (Orion model 407A pH meter) and colorimetric determination of ferrous iron concentration (Coleman-Hitachi model 124 double-beam spectrophotometer) by the phenanthroline method of Tamura et al. (19). The decrease in ferrous iron concentration with respect to time was assumed to be indicative of the rate of ferrous iron oxidation by chemical means (sterile flasks) or by combined biological and chemical means (*T. ferrooxidans*-inoculated flasks).

During the experiment, the pH of the sterile and inoculated reactors gradually increased and stabilized at pH 2.0 ± 0.1, a range where bisulfate and ferric ions in partially or fully spent C & H medium can buffer system pH from changes associated with ferrous iron oxidation at low pH. Under these conditions, it is difficult to correlate pH changes with ferric iron production. However, ferrous iron disappearance data from the *T. ferrooxidans*-inoculated flasks (Fig. 1) indicated that ≥10 mg of sorbic or benzoic acid per liter effectively inhibited biological ferrous iron oxidation. SLS demonstrated effective inhibition of ferrous iron oxidation by *T. ferrooxidans* at each of the tested SLS concentrations (i.e., ≥5 mg/liter). The correlation between the average data from the sterile, unsupplemented flasks and the data from the sterile flasks supplemented with 5, 10, 25, or 50 mg of benzoic acid (r^2 = 0.96, 0.94, 0.93, or 0.92, respectively), sorbic acid (r^2 = 0.97, 0.95, 0.94, or 0.94, respectively), or SLS (r^2 = 0.91, 0.92, 0.91, or 0.94, respectively) per liter indicated that the rate of chemical oxidation of ferrous iron was not substantially affected at the tested concentrations of inhibitors. More rigorous kinetic experiments would be necessary to substantiate any general conclusions, however.

The study indicated that benzoic and sorbic acids merit further investigation for use in the inhibition of ferrous iron oxidation by *T. ferrooxidans*. In this regard, the Bureau of Mines has initiated field experiments in the Pittsburgh Research Center experimental underground coal mine to evaluate the performance of sodium benzoate and potassium sorbate as inhibitors of acid mine drainage formation. It remains for future research to determine the exact nature of the inhibitory effect of the studied chemicals on the oxidation of ferrous iron by *T. ferrooxidans*. Additionally, the effect of benzoic and sorbic acids on the catalysis of direct oxygen oxidation of pyrite by *T. ferrooxidans* should be addressed in that effective control of acid production in drainage may require that both the indirect and direct mechanisms of formation be controlled.

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