CHEMICAL INHIBITION OF IRON-OXIDIZING BACTERIA IN WASTE ROCK

AND SULFIDE TAILINGS AND EFFECT ON WATER QUALITY[1]

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Abstract.--The effectiveness of sodium lauryl sulfate (SLS), potassium benzoate, and potassium sorbate in controlling the population of iron-oxidizing bacteria, thereby reducing acid production, was tested on sulfide tailings and waste rock, common waste products of metal mining. The waste rock was unweathered and contained 4.07 percent total sulfur, all in the pyritic form. Two different samples of sulfide tailings were used, an extensively weathered material (5.98 percent total sulfur, 0.17 percent pyritic sulfur, and 5.81 percent sulfate sulfur) and a slightly weathered material (20.57 percent total sulfur, 19.73 percent pyritic sulfur, and 0.84 percent sulfate sulfur). Two sample sizes were used in the experiment, 7 kg and 100 kg. The 100-kg samples of each material were treated once with a 600-mg/kg dose of SLS or potassium benzoate. The 7-kg samples were treated with a high (600 mg/kg) or low (60 mg/kg) dose of SLS, potassium benzoate, or potassium sorbate. The treated samples and untreated control samples were subsequently leached once per week with filtered demineralized water (an amount equivalent to 2.5 cm of precipitation). For the 100-kg samples of the waste rock a single treatment of SLS and potassium benzoate completely inhibited iron-oxidizing bacteria repopulation for 182 and 231 days, respectively. Acidity in the leachate from the 100-kg samples treated with SLS and benzoate remained below the untreated control for 287 and 343 days, respectively. Similar results were obtained from the 7-kg samples of waste rock. In the extensively weathered sulfide tailings, none of the treatments inhibited the iron-oxidizing bacteria or reduced acidity levels in the leachate. In the slightly weathered sulfide tailings, all treatments reduced the bacterial populations, but did not significantly reduce acid production. However, in additional tests of the slightly weathered tailings, the removal of the weathered products prior to treatment with SLS or benzoate resulted in lower populations of iron-oxidizing bacteria and reduced acidity levels.

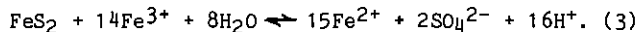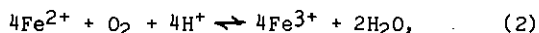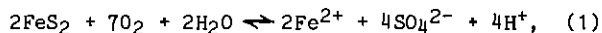[2]George R. Watzlaf is Environmental Engineer, U.S. Bureau of Mines, Pittsburgh Research Center, Pittsburgh, PA.

INTRODUCTION

The U.S. Bureau of Mines is currently researching at-source control of acid mine drainage (AMD). AMD is formed by water and oxygen reacting with the sulfide minerals that are associated with coal and metal mining wastes. A common remedy to AMD is chemical treatment of the contaminated water with alkaline materials such as lime, limestone, sodium hydroxide, or sodium carbonate. These treatments raise pH, reduce acidity, and precipitate metals. However, chemical

http://doi.org/10.21000/JASMR88010109

neutralization is expensive, may be necessary for many years, and generates large volumes of sludge.

A more direct approach is to stop or slow sulfide mineral oxidation and consequent water contamination. The chemical reactions involved in the formation of AMD can be summarized as follows:

$$2FeS_2 + 7O_2 + 2H_2O \rightleftharpoons 2Fe^{2+} + 4SO_4^{2-} + 4H^+, \quad (1)$$

$$4Fe^{2+} + O_2 + 4H^+ \rightleftharpoons 4Fe^{3+} + 2H_2O, \quad (2)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightleftharpoons 15Fe^{2+} + 2SO_4^{2-} + 16H^+. \quad (3)$$

The sulfide moiety of pyrite (FeS_2) can be oxidized by oxygen (Eq. 1) resulting in ferrous iron (Fe^{2+}), sulfate (SO_4^{2-}), and acid (H^+). Ferrous iron can then be oxidized to ferric iron (Fe^{3+}), which can directly oxidize pyrite (Eq. 3). This results in additional ferrous iron, sulfate, and acid. The rate-limiting step of FeS_2 oxidation is equation 2, which proceeds slowly at low pH in the absence of iron-oxidizing bacteria such as Thiobacillus ferrooxidans (Singer and Stumm 1970). These bacteria, which are indigenous to areas where pyrite occurs, can accelerate the rate of equation 2 as much as 1×10^6 times (Singer and Stumm 1970). The catalytic activity of these bacteria may be the cause of over 80 pct of the AMD problem in the United States (Browning 1970).

Various chemicals can be used to inhibit T. ferrooxidans. Sodium lauryl sulfate (SLS) and other surfactants have been shown to reduce AMD in laboratory-scale, pilot-scale and full-scale field tests on coal refuse (cf. Stancel 1982, Kleinmann and Erickson 1982). A disadvantage of applying surfactants in solution form is the necessity to intermittently repeat the application. However, even with their short effective duration and need to reapply every three to six months, anionic surfactants can be more cost effective than treatment of the original highly acidic water with conventional alkaline materials. To remedy the need to reapply the surfactant, Kleinmann et al. (1980) developed a method using surfactant-impregnated rubber pellets that slowly release a surfactant over a longer period of time.

In an attempt to identify chemicals that have a longer effect when added as solutions, organic acids and their ability to form sparingly-soluble salts were studied (Onysko et al. 1984a). It was hypothesized that these salts would precipitate on pyrite surfaces and that these precipitates would dissolve upon decrease in pH to the biologically active form. This scheme was intended to extend the inhibitory effects of these chemicals. Salts of benzoic acid and sorbic acid were deemed appropriate for study (Onysko et al. 1984b). In laboratory and pilot-scale tests on coal refuse, benzoate and sorbate were found to be effective bacterial inhibitors; however, no increase in duration of effective inhibition was noted relative to conventional SLS solution application (ibid).

Potassium benzoate and potassium sorbate at low concentrations should have no adverse effects on the aquatic environment. These chemicals specifically inhibit acidophilic microorganisms (Eklund 1983, Eklund 1980, Cruess and Richert 1929, and Sofos and Busta 1981) and are on the Food and Drug Administration Generally Regarded as Safe (GRAS) List as suitable for inclusion in foods and beverages (U.S. Code of Federal Regulations 1983). On the other hand, small concentrations of SLS may

be deleterious to fish and other aquatic life (Margaritis and Creese 1979). Although this potential problem exists with SLS, it has never been detected in any waterway near any of the refuse sites at which the Bureau of Mines has supervised SLS application (Kleinmann and Erickson 1983).

Because various metal sulfides undergo similar chemical reactions (cf. Onysko 1985), inhibition of iron-oxidizing bacteria should also reduce acid production from these wastes. Therefore, the objectives of this study were to determine if these chemicals (surfactants and organic acids) would inhibit the iron-oxidizing bacteria, and if inhibition of these bacteria would reduce acid production.

WASTE MATERIALS

The sulfide tailings used in this experiment came from a metal mine in Quebec, Canada. The ore from this mine is passed through a concentrator and processed for recovery of copper, zinc, pyrite, gold, and silver. The ore passes through multiple stages of crushing, grinding, and flotation. The waste product from the flotation cells constitutes the sulfide tailings (70 pct less than 200 mesh). The extensively weathered tailings used in the experiment were taken from the surface of a large tailings pile. The slightly weathered tailings were taken from under the oxidized layer of the pile.

The waste rock came from a mine in British Columbia, Canada, where the sulfide ore is mined for its silver, copper, and gold content. The material used in the experiment was unprocessed because it was below ore grade. The material was unsized (15 cm x 0 cm) and represented the most acid-producing material from this mine.

Samples of each material were taken at the beginning of the experiment. Total sulfur was measured using the combustion furnace method (American Society for Testing and Materials 1983). To differentiate the sulfur forms, two acid extractions (hydrochloric acid and nitric acid) were performed. The residues from these extractions were analyzed in a combustion furnace, and the percentages of total, pyritic, sulfate, and organic sulfur were calculated (table 1).

EXPERIMENTAL PROCEDURES

The supply of waste materials limited the experiment to three 100-kg samples of each material: one untreated control, one treated with SLS, and one treated with potassium benzoate. In addition to these 100-kg samples, eight 7-kg samples of each material were treated as follows: two untreated controls, a high and low dose of SLS, a high and low dose of potassium benzoate, and a high and low dose of potassium sorbate. The experimental design is summarized in table 2.

For the 100-kg samples, nine 200-L plastic barrels were set up on wooden platforms. Two holes were drilled in the bottom of each barrel, and a plastic bucket was placed underneath each hole to collect leachate. For the 7-kg samples, twenty-four 20-L plastic buckets were used. Each bucket had two holes drilled in the bottom and was

Table 1.--Sulfur content of waste materials.

Material	Sulfur form, pct by weight			
	Total	Pyritic	Sulfate	Organic
Slightly weathered sulfide tailings	20.6	19.7	0.84	-
Extensively weathered sulfide tailings	5.98	0.17	5.81	-
Waste rock	4.07	4.07	-	-

Table 2.--Treatment of samples for each waste material.

Sample weight, kg	Chemical treatment	Treatment dosage, mg/kg	Volume of water used in weekly leachings, L
100	Untreated	-	8.0
100	SLS	600	8.0
100	Benzoate	600	8.0
7	Untreated	-	1.2
7	"	-	1.2
7	SLS	600	1.2
7	"	60	1.2
7	Benzoate	600	1.2
7	"	60	1.2
7	Sorbate	600	1.2
7	"	60	1.2

positioned over another bucket to enable leachate collection.

Each of the mine wastes was used to fill three barrels and eight buckets with 100 kg and 7 kg of material, respectively. The waste materials were added (without compaction) to their respective containers, and within one week, the experiment began. An enclosed hanger-type building in which the air temperature ranged from approximately 15 to 25° C was used for the experiment.

Each barrel (100-kg samples) designated to receive chemical treatment was treated with 8 L of a 7,500-mg/L solution of SLS or benzoate. These treatments were equivalent to 600 mg of treatment chemical per kilogram of waste material, however, any treatment solution that did not adsorb to the waste material was allowed to drain freely from each barrel. The barrels designated as controls received 8 L of deionized tap water.

Two of the eight buckets (7-kg samples) of each material were designated as untreated controls. The remaining six buckets of each material received a high or low dose of SLS, potassium benzoate, or potassium sorbate. The high treatment dose was added to each bucket using 1.2 L of the appropriate 3,500-mg/L solution. The low treatment dose was applied using 1.2 L of a 350-mg/L solution. The high and low treatment doses were equivalent to 600 and 60 mg, respectively, of treatment chemical per kilogram of material. The buckets designated as controls received 1.2 L of deionized tap water.

The weekly leaching program began one week after treatment. Tap water was passed through a 0.20-μm capsule filter and a two-bed ion-exchange-resin system before storage in a 500-gal plastic reservoir. Periodic sampling of this reservoir showed the water to be acceptably low in dissolved solids, and the population of iron-oxidizing bacteria was below detectable limits (<2 cells/100 mL). A plastic sprinkling can was used to distribute water evenly on each waste material. The 100-kg and 7-kg samples were leached with 8.0 and 1.2 L of water, respectively. These volumes are equivalent to 2.5 cm of precipitation on the waste material per week.

After the waste materials were watered, they freely drained into the sample-collection buckets for 24 hours. Samples from each leachate were taken and the remaining volume was measured and discarded. Water samples of the leachate from each bucket and barrel were analyzed for pH, acidity, ferrous iron, total iron, calcium, magnesium, aluminum, sodium, manganese, and sulfate. Ferrous iron was measured by potassium dichromate titration using the sodium salt of diphenylaminesulfonic acid as an indicator. Other metal concentrations were determined by inductively coupled argon plasma (ICAP) spectroscopy. After passing the sample through a cation-exchange resin, sulfate was analyzed by barium chloride titration using thorin as an indicator. Acidity analysis consisted of a fixed endpoint titration to pH 8.3 using a 1N sodium hydroxide solution and converted to mg/L as $CaCO_3$.

Analyses for iron-oxidizing bacteria were performed for the 100-kg samples (barrels) only. The multiple-tube, most probable number (MPN) method (American Public Health Association 1985) using the media described by Cobley and Haddock (1975) was used. This method uses a series of 10-fold sample dilutions that are added to a low-pH, ferrous-iron-enriched media. Bacterial densities are estimated by observing a color change in the tubes, which indicates oxidation of ferrous iron by iron-oxidizing bacteria.

The percent of iron in the ferrous (as opposed to ferric) state in the raw samples was found to be a good indicator for the density of iron-oxidizing bacteria. Higher ferrous iron percentages indicated that the bacterial density was low. Conversely, lower ferrous iron percentages indicated higher numbers of bacteria. Therefore, chemical analysis of the leachate (for ferrous and total iron) enabled a rough estimate of the number of iron-oxidizing bacteria in the 7-kg samples without actual MPN analysis. Figure 1 shows these correlations for the 100-kg samples of waste rock and slightly weathered sulfide tailings that were treated with SLS.

RESULTS

The following sections cover the results of the three treatment chemicals on the three mine waste materials. Although direct measurements of the bacterial populations were made, the effectiveness of the chemicals was based on leachate water quality. Graphs of acidity concentration and cumulative acid load over time are presented because acidity directly relates to water treatment costs and acidity was correlated with other water quality parameters of interest (table 3). All acidity measurements are expressed in $CaCO_3$ equivalence.

Table 3.--Spearman's correlation coefficients* of acidity with other water quality parameters.

Sulfate	0.97
Iron	0.94
Aluminum	0.94
Manganese	0.89
pH	-0.78

*All significant at $p < 0.001$

Waste Rock

In the 100-kg samples, the iron-oxidizing bacteria were reduced below detectable limits by SLS and benzoate for 182 and 231 days, respectively (fig. 2). Both SLS and benzoate significantly reduced acidity levels in the leachate (fig. 3). After 250 days, the cumulative acid load generated by each treated sample was 15 percent of the cumulative acid load generated by the control (fig. 4). Forty days after the bacterial inhibitors began to lose effectiveness, the iron-oxidizing bacteria repopulated to levels greater than those found in the control. The concentration of acidity from each treated sample also increased above the control level. These increases in acidity occurred about 100 days after the increases in iron-oxidizing bacteria.

In the 7-kg samples, the high dose (600 mg/kg) of SLS, benzoate and sorbate kept acidity concentrations below 50 pct of the lowest of the two untreated controls for 343, 147, and 147 days respectively (fig. 5a). The low dose (60 mg/kg) of SLS and benzoate reduced acidity concentrations below 50 pct of the lowest control for 63 and 77 days, respectively. The low sorbate dose did not reduce acidity levels (fig. 5b).
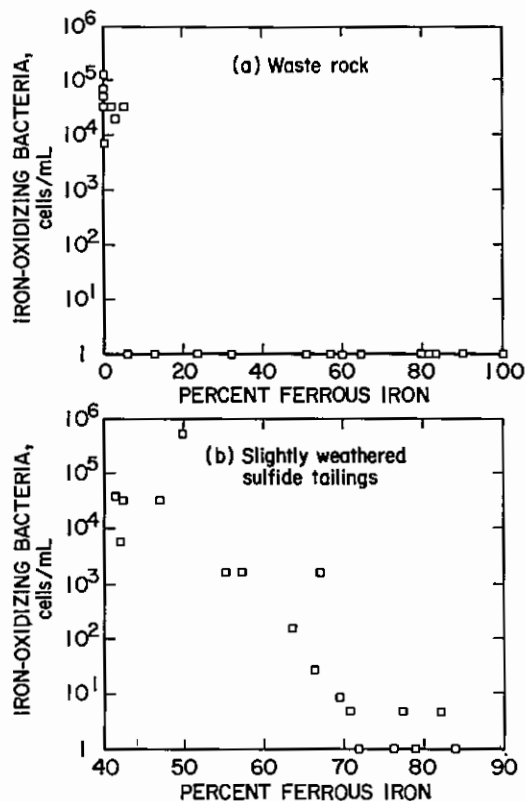

Figure 1.--Relationship between the population of iron-oxidizing bacteria and percent ferrous iron in 100-kg, SLS-treated samples of (a) waste rock and (b) slightly weathered sulfide tailings.
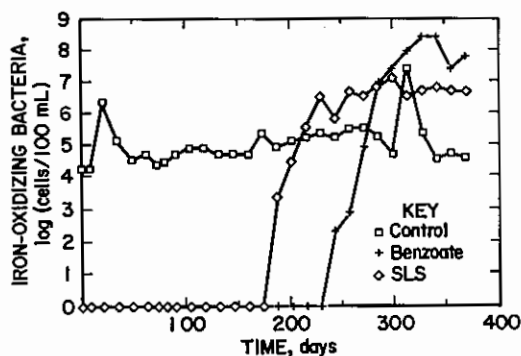

Figure 2.--Population of iron-oxidizing bacteria vs. time for 100-kg samples of waste rock.
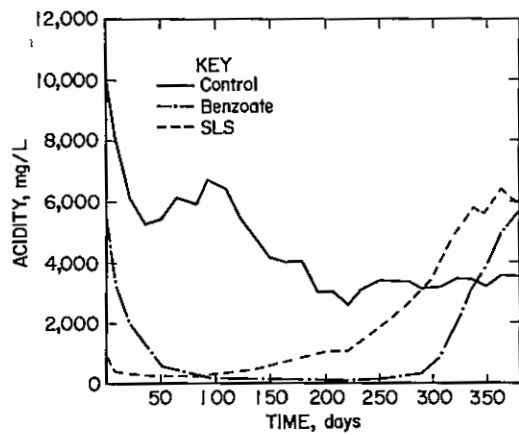
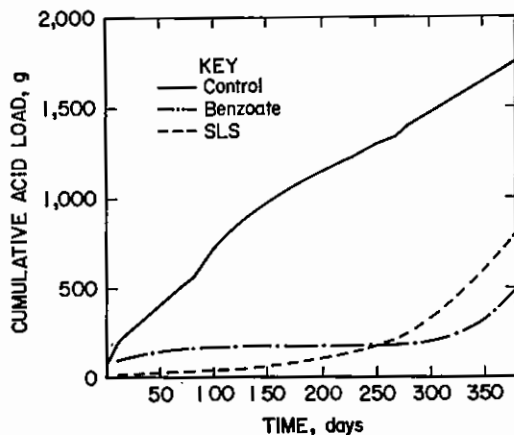Figure 3.--Acidity concentration vs. time for 100-kg samples of waste rock.

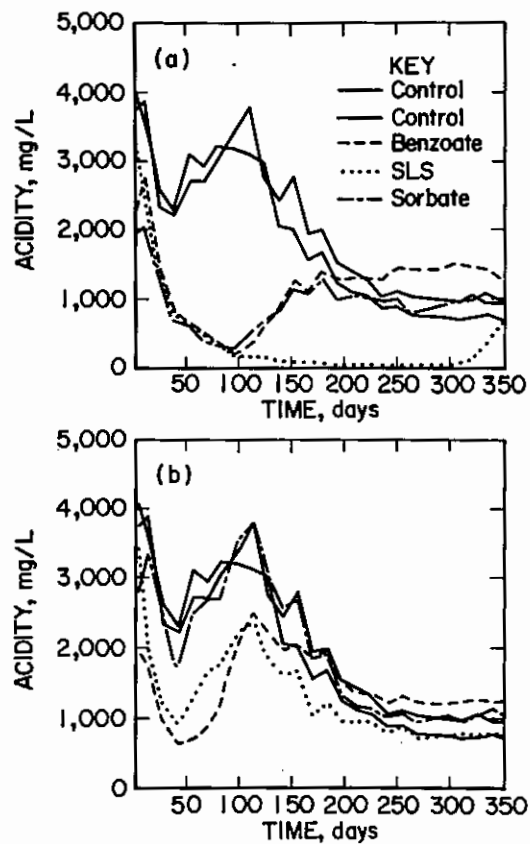Figure 4.--Cumulative acid load vs. time for 100-kg samples of waste rock.

Figure 5.--Acidity concentration vs. time for (a) high treatment dose (600 mg/L) and (b) low treatment dose (60 mg/kg) on 7-kg samples of waste rock.
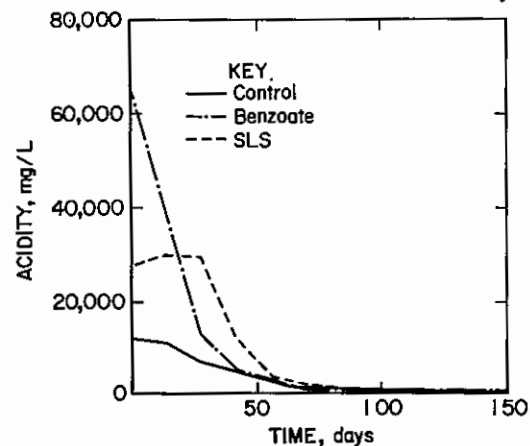

Figure 6.--Acidity concentration vs. time for 100-kg samples of extensively weathered sulfide tailings.

Extensively Weathered Sulfide Tailings

In the 100-kg samples, greater concentrations of acidity were leached from the SLS- and benzoate-treated samples than from the control in the first 40 to 56 days (fig. 6). Bacterial populations were not inhibited, and ranged from 3×10^3 to 2×10^5 cells/100 mL.

Similarly, results from the 7-kg samples showed that all of the treatments increased acidity over control levels in the first 28 to 56 days of the experiment with no subsequent difference in acidity levels between treated samples and controls for the remainder of the experiment. Ferrous iron data suggested that no significant bacteria inhibition occurred.

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In the 100-kg samples, neither SLS nor potassium benzoate reduced acidity levels relative to the control in the leachate from the slightly weathered sulfide tailings. However, both treatments kept the bacterial population significantly below that of the control for approximately 130 days (fig. 7). In the 7-kg samples, none of the treatments had any significant effect on leachate water quality. However, based on the high percentages of total iron remaining in the ferrous form, the iron-oxidizing bacteria were inhibited for over 100 days.

. An additional test was performed to determine why the inhibition of bacteria in the slightly weathered sulfide tailings did not result in lower acidity levels. In this test, the tailings were washed with a dilute hydrochloric acid solution to remove any oxidized products before the treatments were added. The experiment used six 7-kg samples, three of which were not treated with bacterial inhibitor to act as controls. Two samples were treated once with a 600 mg/kg dose of SLS and one sample was treated once with a 600 mg/kg dose of benzoate. Procedures used for leaching and water quality analyses were the same as outlined in the experimental procedure section.

Both SLS and benzoate significantly reduced acidity below control levels (fig. 8). At the end of 150 days, the cumulative acid load generated by, each treated sample was 18 percent of the acid loads produced by the control samples (fig. 9). Analysis for iron-oxidizing bacteria showed that populations in the SLS- and benzoate-treated samples remained below 2 cells/100 mL for 112 and 98 days, respectively. The population of iron-oxidizing bacteria in the control samples ranged from 2.3 x 10^4 to 1.3 x 10^5 cells/100 mL.

DISCUSSION

The leaching procedure used in this experiment represents an acceleration of actual field rainfall conditions. Although 2.5 cm/week of precipitation does not seem excessive, the procedure does not allow for runoff or evaporation that might occur in the field. The duration of bacterial inhibition for the treatment chemicals may have been diminished by the short period of time that the water was added. This may have increased the amount of chemical that was washed off; however, the treatment was not lost to runoff and may have been deposited at a lower position in the sample. Because of these variables, caution must be exercised during extrapolation of the results of laboratory leaching tests to intended field use.

Results from the benzoate- and SLS-treatments of the waste rock indicate that both chemicals may be applicable for field use. SLS has reportedly been applied to waste rock by a mining company in the field, but no reductions in contaminant levels were observed. The blocky nature of the waste rock permits air to penetrate deeply into the pile. Consequently, in order to inhibit bacteria and reduce contaminant levels, a large portion of the pile must receive the chemical inhibitor and not just the uppermost layer.
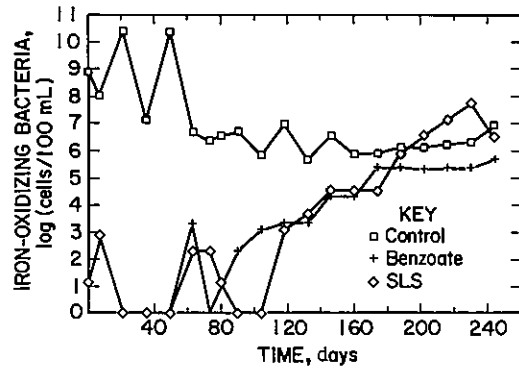

Figure 7.--Population of iron-oxidizing bacteria vs. time for 100-kg samples of slightly weathered sulfide tailings.
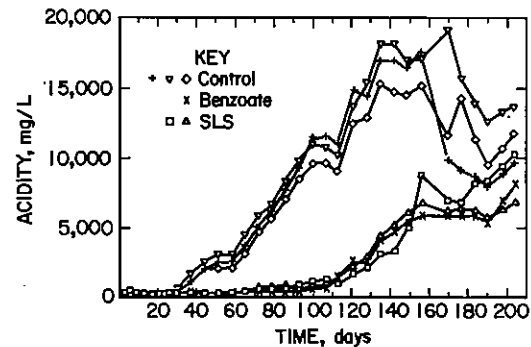

Figure 8.--Acidity concentration vs. time for 7-kg samples of hydrochloric-acid-rinsed sulfide tailings.
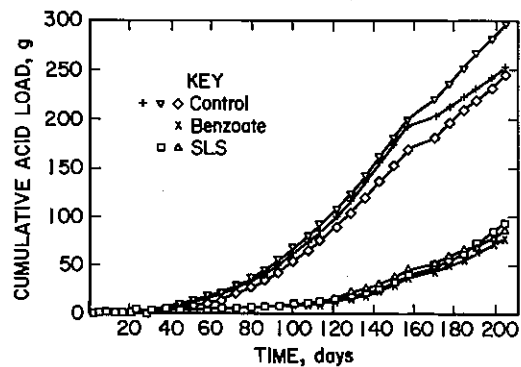

Figure 9.--Cumulative acid load vs. time for 7-kg samples of hydrochloric-acid-rinsed sulfide tailings.

Results from the extensively weathered sulfide tailings showed that all of the treatments increased acidity levels in 100- and 7-kg samples. The material was considerably oxidized with 5.81 pct sulfate sulfur and only 0.17 pct pyritic sulfur; therefore, most of the contamination in the leachate was from the flushing of previously oxidized material. Apparently, the treatment chemicals caused an increased flushing of contaminants. SLS could have increased contaminant flushing by its surfactant properties. Benzoate and sorbate readily form complexes with iron which could result in dissolution of weathered products, thereby increasing contaminant flushing.

The results from the slightly weathered sulfide tailings showed that SLS, benzoate, and sorbate effectively inhibited iron-oxidizing bacteria but did not reduce contaminant levels. However, in a subsequent test in which the tailings were washed with dilute HCl to remove any oxidized products prior to treatment, SLS and benzoate inhibited the bacteria and reduced acidity levels. These results indicate that these chemicals may be applicable to field use if the tailings can be treated before they begin to oxidize.

In all three materials, differences between the results from the 100-kg samples and the 7-kg samples were noted. In the 100-kg samples of extensively weathered sulfide tailings, SLS and benzoate initially increased acidity to 28,000 and 67,000 mg/L, but the leachate from the control sample only contained 12,000 mg/L; however, in the 7-kg samples initial acidity levels were only 18,000 mg/L for the treatments compared to 10,000 and 15,000 mg/L for the two controls. Likewise, in the 100-kg samples of the slightly weathered sulfide tailings, much larger increases in acidity levels for the treated samples were observed in the larger scale test than in the 7-kg samples. Also, the magnitudes of acidity concentration were much larger from the 100-kg samples (up to 150,000 mg/L) than from the 7-kg samples (below 25,000 mg/L). In the waste rock, using the 600 mg/kg dose of treatments, the 100-kg samples showed benzoate to be slightly more effective than SLS, but in the 7-kg samples, SLS was much more effective than benzoate. Again, magnitudes of contaminant concentration differed between the two sample sizes. Although basic trends were fairly consistent between the 7- and 100-kg sample results, large discrepancies in contaminant concentration were observed even under these controlled conditions. Therefore, trying to use this laboratory data to extrapolate to anticipate field results (viz. correct chemical treatment dose, duration of effectiveness, reduction of acidity and metals levels, and corresponding reduction in water treatment costs) cannot be accurately made. However, these methods can be used to screen potentially effective chemicals and provide some guidance for larger scale field studies.

CONCLUSIONS

The amount of weathering a mine waste has undergone prior to treatment with SLS, potassium benzoate, or potassium sorbate determines the effectiveness of these chemicals to inhibit iron-oxidizing bacteria. Application of these chemicals

on weathered mine wastes can increase the liberation of previously oxidized contaminants. The presence of even a small amount of weathered products on mine wastes can render these treatments ineffective. However, if the material is unweathered (all sulfur in the pyritic form), these treatments can inhibit iron-oxidizing bacteria and significantly reduce acidity, sulfate, and metal concentrations in the leachate.

In our tests using sulfide tailings, SLS, potassium benzoate, and potassium sorbate did not reduce contaminant concentrations in the leachate from the any of the weathered tailings. However, following removal of the weathered products from the slightly weathered sulfide tailings, SLS and benzoate effectively reduced contaminant concentrations in the leachate. Respectively, SLS and benzoate reduced the density of iron-oxidizing bacteria below detectable limits (2 cells/mL) for 100 and 86 days, and reduced the cumulative acid load to 18 percent of the control acid load after 150 days.

Results of the tests using 100-kg samples of waste rock showed that SLS and potassium benzoate inhibited the iron-oxidizing bacteria below 2 cells/100 mL for 182 and 231 days, respectively. In these samples, SLS and benzoate reduced the cumulative acid load to 15 percent of the acid load from the control after 250 days.

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