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Acidogenic Mine Tailings

The Use of Biofilm Bacteria to Exclude Oxygen

Scientific Note

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Index Entries: Acid mind drainage; top-dressing; biofilms; bacteria; permeability reduction.

INTRODUCTION

Acid mind drainage (AMD) is an important contributor to the deterioration of surface and ground water quality via acidification, heavy metal pollution, and sedimentation (*1–4*). The National Stream Survey (NSS) conducted by the US Environmental Protection Agency found that approx 4590 km of streams in the mid-Atlantic and southeastern United States were acidic because of AMD and 5780 km were strongly impacted, although not acidic (*5*). AMD is the product of reactions between sulfide-containing mine tailings, air, and water, as demonstrated in the following equations (*6*):

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

$$Fe^{2+} + 1/4O_2 + H^+ = Fe^{3+} + 1/2H_2O \quad (2)$$

$$Fe^{3+} + 3H_2O = Fe(OH)_3\,(s) + 3H^+ \quad (3)$$

$$FeS_2\,(s) + 14Fe^{3+} + 8H_2O = 15\,Fe^{2+} + 2SO_4^{2-} +.\,16H^+ \quad (4)$$

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The rate of oxygen penetration into the surface pore spaces of tailings is believed to control the rate of acid generation (*7*). It is therefore critical to reduce the rate of oxygen penetration or eliminate it completely. Pyrite will remain in a reduced state if the tailings environment can be kept anaerobic (*8*).

It is well known that bacterial and plant growth in the surface layers of soil can completely consume the available oxygen, causing the remainder of the soil profile to exist in an anaerobic state. It has also been well demonstrated that bacteria are very capable of reducing the permeability of rock matrices through cell growth and exopolymer production (*9–11*). Based on these principles, mine-tailings columns were "top-dressed" in our laboratory with bacteria and nutrients to determine whether an oxygen-excluding surface layer could be established to prevent the development of AMD. This article presents our preliminary data.

MATERIALS

Mine Tailings

Uranium mill tailings, containing 5–7% pyrite, were obtained from Denison Mines (Elliot Lake, Ont.). Freshly processed tailings (unoxidized, pH = 5.5) and tailings that had been collected from outdoor "beaches" at the site (field-oxidized, pH ≈ 2.2) were used in the laboratory trials. Both types of tailings resembled sand in terms of grain size.

Tailings Columns

Laboratory tailings columns were assembled as follows, unless otherwise stated. Oxidized or unoxidized tailings were packed into separate glass columns (30-cm high, 7.5 cm in diameter) with four intervening horizontal layers of white Ottawa sand (Fig. 1). The Ottawa sand layers were included because they darken under anaerobic conditions, providing a convenient indicator. Anaerobic test strips (Anaerotest, Merck) were also taped to the inside of the glass columns.

Bacteria + Nutrient Top-Dressing

The bacterial top-dressing consisted of a 75-mL suspension of *Klebsiella* sp. (10^7 cells/mL), which had been mixed into 100 mL of tailings (Fig. 1). *Klebsiella* was chosen because of its copious exopolysaccharide production, and because it is capable of both aerobic and anaerobic growth. The nutrient top-dressing consisted of 15-mL aliquots of half-strength Brain Heart Infusion medium (½ BHI) added as stated.

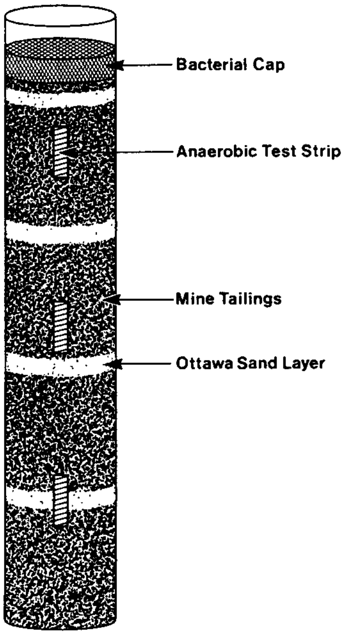

Fig. 1. Representative laboratory mine tailings column.

METHODS

Effect of Bacteria + Nutrient Top-Dressing

After top-dressing the columns with bacteria, the nutrient medium was added twice weekly. Control columns were not inoculated and received sterile distilled water (15-mL aliquots) twice weekly to maintain comparable moisture levels. After 14 d, the columns were dismantled, and soil samples were collected at four depths (6, 13, 22, and 27 cm). Standard soil procedures were used to determine pH. Samples were also plated onto ½ BHI and Brewer's agar plates for determination of aerobes and anaerobes, respectively. The ½ BHI plates were incubated for 48 h at room temperature. The Brewer's agar plates were incubated for 72 h at room temperature in an anaerobic chamber.

Effect of Nutrient Top-Dressing Only

A second trial was performed to examine the response of tailings columns to the addition of nutrient medium alone, as compared to top-dressing with bacteria and nutrient. The protocol above was repeated, with the following alterations:

1. Anaerotest strips were not used to indicate anaerobic conditions;
2. A nutrient top-dressing was added twice weekly to columns that had not been top-dressed with bacteria; and
3. The columns were dismantled after 24 d.

Effect of Bacteria + Nutrient Top-Dressing on Permeability

Tailings were packed into 50-mL graduated cylinders equipped with drainage spouts. Each cylinder was topped with 3 mL of *Klebsiella* suspension in 5 mL of tailings. Nutrient (2-mL aliquots) was pipeted evenly across the top of each tailings column on days 0, 8, 10, 15, and 22. The permeability of the tailings columns, defined here as the ability to allow passage of water, was periodically measured by adding 20 mL of sterile distilled water to the top of each column and timing its collection in a calibrated cylinder located beneath the base spout. The value obtained on day 0 was considered to be 100% permeability, and subsequent values were expressed as relative percentages. This trial was performed in duplicate.

RESULTS

Bacteria + Nutrient Top-Dressing

After 14 d, the Ottawa sand layers were still white, and the Anaerotest strips were still blue in the control columns, indicating that the tailings had not become anaerobic. In the top-dressed oxidized column, the Ottawa sand layer just beneath the surface had darkened, indicating the start of anaerobiosis in the column. Below this point, aerobic conditions were indicated. The impact of bacteria and nutrient top-dressing was much more dramatic on the unoxidized tailings. The full length of the column was clearly anaerobic as evidenced by the reduced Ottawa sand layers and by the colorless Anaerotest strips.

Encouraging pH increases were noted throughout the entire top-dressed unoxidized column (Fig. 2). In the top-dressed oxidized column, a dramatic pH increase was evident in the surface (upper 6 cm) zone when compared with the control (pH 2.63 vs. 8.57) (Fig. 3).

An abundant population of aerobes and anaerobes was present in the control unoxidized column (Table 1). Aerobes and anaerobes were not detected in the control oxidized tailings column, using the culture methods employed. In the bacteria and nutrient top-dressed columns, the depths at which anaerobiosis was present and at which pH increases were noted generally corresponded to those where bacterial population increases were evident.

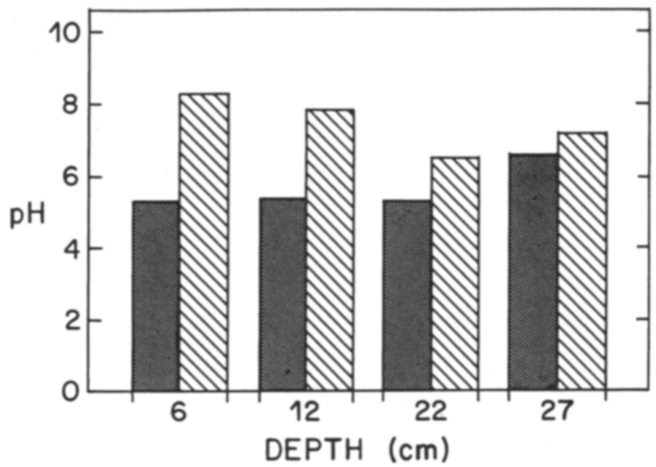

Fig. 2. pH measurements taken in the control (dark stipple) and bacteria + nutrient top-dressing (diagonal) unoxidized mine tailings columns.

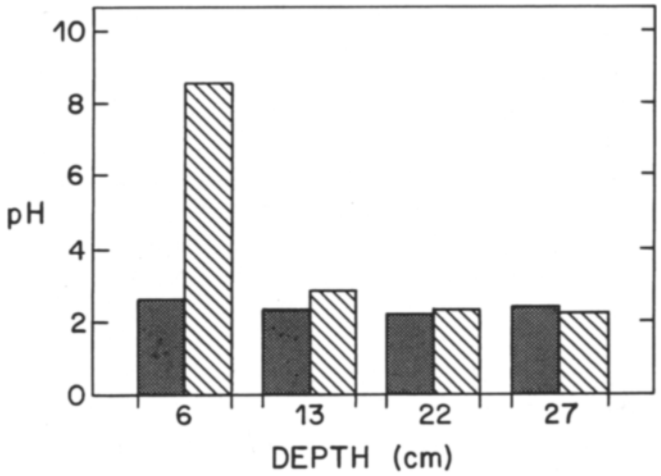

Fig. 3. pH measurements taken in the control (dark stipple) and bacteria + nutrient top-dressed (diagonal) oxidized mine tailings columns.

Effect of Nutrient Top-Dressing Only

Anaerotest strips were not used in this trial, because we were concerned about air channelling down the sides of the columns if the packing was not adequate. The Ottawa sand layers have the advantage that they represent a horizontal slice through the tailings, and thus, if areas near the column sides are aerobic because of channelling, anaerobic areas in the middle can still be detected. Unfortunately, the Ottawa sand did not visibly reduce in this trial. Subsequent tests with Ottawa sand placed in

Table 1
Viable Cell Counts of the Aerobic and Anaerobic Bacterial Population in Unoxidized and Oxidized Mine Tailings Columns[a]

		Colony-forming Units/g			
		Unoxidized Tailings		Oxidized Tailings	
		Control	Bacteria + Nutrient	Control	Bacteria + Nutrient
Aerobes	a	1.61×10^4	$>3.00 \times 10^7$	0	$>3.00 \times 10^7$
	b	1.67×10^5	2.26×10^7	0	0
	c	3.40×10^5	7.10×10^6	0	0
	d	1.93×10^6	1.09×10^7	0	0
Anaerobes	a	2.01×10^5	1.77×10^7	0	9.10×10^5
	b	2.01×10^5	3.10×10^4	0	0
	c	6.90×10^5	1.00×10^4	0	0
	d	7.50×10^3	5.20×10^3	0	0

[a] Samples were taken at four depths (a = 6, b = 13, c = 22, and d = 27 cm) from the control and bacteria + nutrient top-dressed columns.

an anaerobic hood indicate that it will not blacken when dry. Assuming that anaerobiosis was achieved, this suggests that the tailings material used in this trial no longer contained sufficient moisture to allow visible reduction of the Ottawa sand, even with periodic nutrient additions.

In addition, the pH in the unoxidized control cores approached that of the oxidized control cores, indicating that the unoxidized material had oxidized during storage. The results of the unoxidized material will therefore not be presented.

After 24 d (Fig. 4), the pH in the upper 6 cm of both the nutrient only and the bacteria + nutrient treated oxidized tailings had increased to approx the same level (pH 6.38–6.95), representing a significant increase over that of the control (pH 2.06). As in the previous oxidized tailings trial, increases in pH were not detected at the other depths. Bacteria were not detected in the control oxidized tailings column, using the culture methods employed (Table 2). However, in both the nutrient only and the bacteria + nutrient treatments, aerobes became well established in the upper 13 cm of the tailings columns after 24 d. Anaerobes were detected in the top 13 cm of the nutrient only column, but were only found in the top 6 cm of the bacteria + nutrient column.

Effect of Bacteria + Nutrient Top-Dressing on Permeability

Although the unoxidized material had begun to oxidize during storage, the permeability trial was performed from a grain-size point of view, based upon the assumption that the field-oxidized tailings may have undergone grain-size alterations as a result of weathering. The relative decreases in

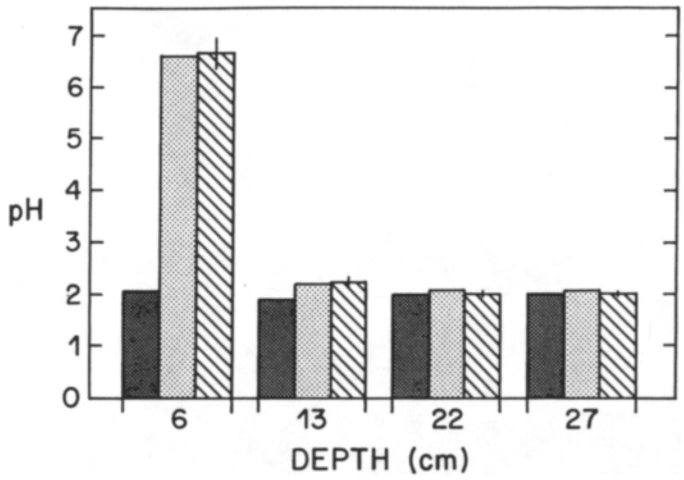

Fig. 4. pH measurements taken in oxidized mine tailings columns that were not treated (dark stipple), or were top-dressed with nutrient (light stipple) or bacteria+nutrient (diagonal). The bacteria+nutrient top-dressing was done in duplicate ($\bar{X}$ with range).

Table 2
Viable Cell Counts of the Aerobic and Anaerobic Bacterial Population in Oxidized Tailings Columns Top-Dressed with Nutrient Only or with Bacteria+Nutrient[a]

		Colony-forming Units/g Oxidized Tailings			
				Bacteria+Nutrient	
		Control	Nutrient only	Rep. 1	Rep. 2
Aerobes	a	0	$>3.00\times10^7$	7.10×10^7	1.18×10^8
	b	0	3.10×10^4	1.35×10^4	1.43×10^5
	c	0	0	0	0
	d	0	0	0	0
Aerobes	a	0	2.01×10^6	2.77×10^6	3.15×10^6
	b	0	1.72×10^3	0	0
	c	0	0	0	0
	d	0	0	0	0

[a]Depths are as in Table 1.

tailings permeability as a result of bacteria and nutrient top-dressing are given in Fig. 5. All of the columns reached <5% of their initial permeability within 29 d, with the exception of one of the "unoxidized" columns, which decreased to only 23.5%. The surface of this latter column had cracked and lifted up during the course of the trial. This is a very important finding, because it indicates that moisture levels must be maintained in order to preserve the integrity of the biofilm-occluded pore spaces.

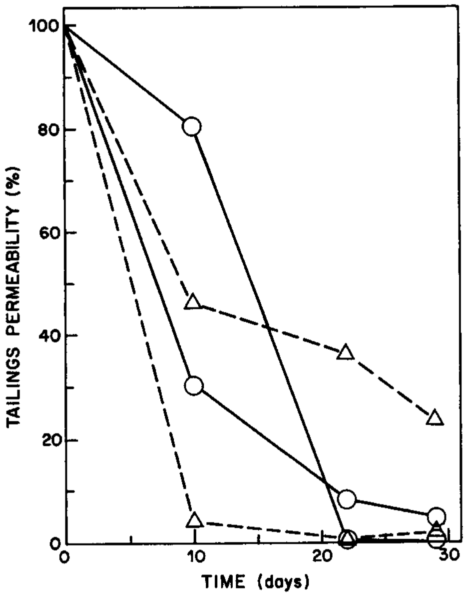

Fig. 5. Percent decrease in mine tailings permeability after top-dressing with bacteria+nutrient. Two unoxidized cores (triangles) and two field-oxidized cores (circles) were monitored.

CONCLUSIONS

The data obtained in these preliminary experiments clearly demonstrate that a bacteria and nutrient top-dressing is capable of generating anaerobiosis and of increasing pH. These changes appear to correspond to the depths at which increases in the bacterial population are noted. This suggests that the bacterial population had utilized the available oxygen, and had promoted anaerobic conditions and reduced acid production by plugging the available pore spaces through cell growth and exopolymer production. This interpretation is strongly supported by the reduction in permeability noted after top-dressing with bacteria and nutrient.

As indicated by the cell counts and pH data, the bacteria and nutrient top-dressing prevented the development of acidogenic conditions throughout the 30-cm unoxidized Denison tailings column. This strategy was less successful in the oxidized Denison tailings, although an abundant bacterial population and large increases in pH were generated in the upper 6 cm in two experiments.

It is of interest that the pH and bacterial population increase noted in oxidized tailings top-dressed with nutrient only was very similar to tailings top-dressed with both bacteria and nutrient. It may therefore be possible, in a field situation, to treat tailings with nutrient alone to enhance the indigenous population. However, before this strategy can be entertained, further testing is obviously required to determine if the rate of permeability reduction is equivalent for both treatments. A bacteria and nutrient top-dressing may possibly provide a greater initial rate of permeability reduction than a nutrient top-dressing alone, which must first stimulate the indigenous population.

Our data suggest that it may be expedient to top-dress unoxidized tailings before oxidation can occur. The oxidized tailings are obviously a very harsh environment as evidenced by their low pH and low bacterial populations. However, a viable bacterial capping layer was produced in the surface 6 cm of the oxidized tailings, which may prevent further oxidation and acid generation. Further trials will be performed at the Canada Centre for Mineral and Energy Technology (CANMET) in Ottawa.

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