HETEROTROPHIC BACTERIA AND GRASS COVERS ON FRESH, BASE METAL TAILINGS

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ABSTRACT

Acid mine drainage (AMD) resulting from the oxidation of base metal tailings represents a substantial environmental liability for the Mining Industry.

A reduction in the rate of acid generation is suggested through the establishment of an oxygen consuming bacterial population. Heterotrophs, which decompose organic matter provided by a vegetation cover, would outcompete oxidizing bacteria for oxygen consumption.

Experiments have been carried out to test for the presence of heterotrophs in fresh tailings. Methods have been developed which allow for germination of grass seeds on fresh tailings. Roughening of the tailings surface and an organic supplement were found to greatly assist in vegetation establishment.

The experiments have demonstrated that it is possible to vegetate fresh tailings. Heterotrophic bacteria are numerous in the root zone. Further work is now required to establish the quantities of nutrients supplied to the heterotrophs in the root zone and to produce a moisture containing and acid neutralizing layer for acid water generated in the deeper parts of the tailings.

Introduction

A reduction of the acid generation rates in tailings is expected if the movement of water and oxygen through the tailings mass can be prevented. Placing a cover over the tailings or submerging them under water are current practices of tailings disposal. An oxygen-consuming living cover over tailings is proposed, through the incorporation of oxygen-consuming bacteria into the root zone of vegetation growing on fresh tailings.

Tailings, when leaving the mill, are generally alkaline, but may generate acid very quickly. Revegetation is normally carried out after the tailings surfaces have dried sufficiently for heavy equipment to traverse. However, during this period of de-watering, acid-generation in the tailings usually proceeds, consuming the alkalinity supplied by the mill. Agricultural limestone is used to neutralize the surface prior to seeding. Acid generation proceeds underneath a typical vegetation cover, as the root region does not provide a barrier to oxygen and water. Although a vegetation cover will reduce runoff and increase evapotranspiration, water still enters the tailings mass.

A reduction in the rate at which tailings oxidize might be achieved if a population of oxygen-consuming bacteria (heterotrophs) could be maintained in the root region of a vegetation cover. Heterotrophs growing in the root region of a vegetation cover, would consume the oxygen in the water entering the tailings. Such bacteria have much higher potential growth rates than chemo-autotrophs, such as the pyrite oxidizing bacteria (*Thiobacillus* spp). Thus, if oxygen is a limiting factor in the acid generation rates, then depletion of oxygen in the pore water would reduce that activity of oxidizing bacteria.

Although the concept of an oxygen consuming cover on fresh tailings is attractive, the feasibility is dependant on

a) the presence of heterotrophs in fresh tailings, b) the ability of establishing a vegetation cover on fresh tailings with root penetration, and c) the productivity of such a vegetation cover to sustain the heterotrophic activity.

This paper summarizes the findings of field and laboratory experiments where fresh tailings were used to establish vegetation and determine the presence/absence of heterotrophs and thiobacilli in the root region.

Materials and Methods

Fresh tailings were supplied from a base metal mine producing Zn and Cu. Alkalinity and pH were determined on slurries of 1:1 (volume:volume) of tailings and distilled water using standard methods (ASTM, Method 2310). The pH of the slurries and water were determined using an Orion pH meter with a combination electrode.

Determination of heterotrophs and Thiobacilli: Enumeration of heterotrophic bacteria was achieved by plating 1 mL aliquots of tailings slurries (5 mL distilled water: 5 g tailings or dilutions thereof) on Petrifilm plates (3M Corp.). The plates were incubated at room temperature, and the number of colonies counted after 1, 2, and 4 weeks.

Thiobacilli were enumerated through serial dilution in the medium of McCready et al (1986). Ferrous sulphate was added to the medium, which when oxidized to ferric sulphate produced an orange colour in the medium. The tubes were incubated at room temperature. Presence or absence of thiobacilli were noted, based on the colour of culture medium after incubation for 40 days .

Vegetation establishment on fresh tailings: Plastic garden pots (15 cm diam.) were filled with fresh tailings. Six pots were set up with each of the following treatments: 1) Tailings vegetation + fertilizer; 2) Tailings vegetation + fertilizer + molasses; 3) Tailings only; 4) Tailings + molasses; 5) Tailings + grass seed + fertilizer; 6) Tailings + grass seed + fertilizer + molasses.

Molasses containing 50 % (w:v) sugar was added as a carbon amendment for heterotrophic bacteria, at a rate of 8 mL/pot, resulting in an equivalent of 10 g sugar/L of tailings. Slow release fertilizer (Osmocote 19:6:12) was added at 1.3 g/pot to enhance grass and bacterial growth. The grass seed (White Rose, Lawn Pleasure) was added at a rate of 0.5 g/pot.

Pots were grown under banks of fluorescent lights with a 12:12 day:night light regimen in the laboratory. They were watered daily (distilled water) to keep them moist. After 6 weeks of growth, the pots were harvested. Above ground biomass was cut off with scissors, dried at 105°C, and weighed. Bacterial counts (aerobic heterotrophs and thiobacilli) were made.

Tailings surface conditions: An experiment was set up to investigate the effect of surface treatments on seed germination and vegetation establishment on fresh tailings.
Four 50 x 50 cm plywood boxes were constructed, and filled to a depth of about 15 cm with fresh tailings. A chamber with a width of about 10 cm allowed the maintenance of a water level in the lower part of the tailings mass. Decant water from the original tailings barrel was added to the boxes. The boxes were left to settle. Surface treatments, fertilizer and seed were added 5 days later.

Each box was subdivided into the following plots: 1) tailings only; grass seed sown over half the surface; 2) surface roughened; 3) surface roughened + Hydrosource; 4) Verdyol; 5) wood chips; 6) surface roughened + wood chips. Plots were 10 x 20 cm.

Molasses was added to 1 box before it was subdivided into plots. The molasses was mixed with tailings at a rate of 4 mL/L before the tailings were added to the box. This represented half of the application rate to the pot experiment.

Surface roughening was applied by scratching the surface with a nail (3.5 mm in diameter) to a depth of 1 cm, or by adding amendments. Scratches were 2 cm apart. Organic material used in the plots were wood chips, hydrosource, and Verdyol. Wood chips were applied to give a layer approximately 1 cm thick. Hydrosource was also used to provide a moist environment for seed germination. Hydrosource is a polyacrylamide gel which absorbs many times its own volume in water. Verdyol mats, provided cover for the tailings, reducing moisture loss.

Osmocote 19:6:12 slow-release fertilizer was applied to all of the boxes at a rate of 723.7 $g.m^{-2}$. Grass seed was applied at a rate of 280 $g.m^{-2}$.

Field experiments: Field trials were carried out on fresh tailings. A plot 70 m long by 8 m wide was established in June 1990 over a moisture gradient which existed along the tailings slope (wet, moist, and dry). These Verdyol strips (4.0x 3.75m) were further divided into areas where a) a Verdyol mat cover was placed over the length of the plot, and grass seed sources added on top of the Verdyol mat; b) grass seed sources and amendments were distributed directly on the tailings surface without a Verdyol mad; and c) grass seed sources and amendments were distributed directly on the tailings surface, then a Verdyol mat was placed over the seeded areas.

The tailings were seeded with a combination of common lawn seed supply containing 60% annual rye grass, 30% Timothy grass, and 10% Creeping Fescue and a mixture with 60% Kentucky Bluegrass. The two seed blends were mixed in a 4:1 ratio and the mix was applied at a rate of 27 g/m2 over each of the designated grass plots.

Fertilizer was added to the plots which contained controlled-release, sulphur-coated urea. A dose of 147/g m2 was applied to each of the designated grass plots.

At the end of the second growing season, after regrowth during the winter, three blocks of tailings (35 x 35 cm) were dug to spade depth in each plot containing grass growth. The shoot material was cut off with scissors for dry weight determination. Tailings blocks were cut into two halves. One half was used for separation of tailings from litter and root material.

The material was placed in a sieve and the tailings washed through with a high pressure hose. The litter, shoots, and roots were dried at 105 °C for dry weight determination. Tailings cores were cut from the remaining half blocks. These cores were used for microbiological and chemical studies.

Results

In Table 1, the numbers of heterotrophic bacteria in various tailings samples in the milling circuit and exposed in the tailings pond are shown. It is evident from the results that in the mill, heterotroph numbers are low or below detection limits. However in a sample from the tailings beach, where flotation reagents frequently accumulate, a substantial populations of heterotrophs were found. As the tailings acidify, the populations diminish, as exemplified by the sample taken from the old tailings ponds and a ditch with tailings seepages.

Sample	Heteroprophs (bacteria/mL)	pH
Sagmill before addition	23	6.7
Sagmill after addition	550	7.0
Ballmill discharge	0	7.7

Sample	Heteroprophs (bacteria/mL)	pH
Thickener underflow	0	11.5
Thickener overflow	0	12.5
Barrel tailings-surface	<1	n.d.
Barrel tailings-sub-surface	6	n.d.
Barrel water	3	11.5
Tailings beach	1404000	8.1
Tailings seepage ditch 1	40500	2.1
Tailings seepage ditch 2	10800	4.8
Old Pond	9	2.2

Table 1: *Bacterial counts in tailings*

Vegetation establishment in pots: The results of the bacterial colonisation in the seeded pots is given in Table 2. Counts for total aerobic heterotrophs and thiobacilli were made at harvest after 6 weeks.

The highest numbers of heterotrophs were found in the pots amended with molasses. The lower numbers were found in the unamended pots. This contrasts with the findings for the fresh tailings prior to planting (Table 1), indicating that unamended tailings have the ability to support populations of heterotrophs. There were no thiobacilli found in any of the pot treatments after 6 weeks.

In the presence of molasses, few seeds germinated due to the growth of pathogenic fungi on the tailings surface. Root penetration was poor in all treatments, no more than 1 cm into the fresh tailings. In these conditions, seeds did not germinate well and vegetation establishment was poor. The conditions for seed germination and establishment had to be improved.

Treatment	Heterotrophs (x10 ^5/g tailings)	Thiobacillus (pres +/abs -)
Tailings Only		
T2	143	-
T3	130	-
Tailings/Molasses		
T2	6760	-

Treatment	Heterotrophs (x10 ^5/g tailings)	Thiobacillus (pres +/abs -)
T4	2690	n.d.
Tailings/Seeded Grass		
T5	216	-
T6	115	n.d.
Tailings/Molasses/Grass		
T2	231	-
		(n.d. = not determined)

Table 2: *Pot Experiment - Bacterial Numbers*

Tailings surface treatments for seed germination: In the tailings boxes, were sections of the surfaces were roughed, covered with woodchips , Verdyol and/ or Hydrosources, germination was first observed after 4 days. Measurements were taken on days 6,7,8, and 13 after sowing. Results shown in Table 3 are mean values of the different surface treatments after 13 days. Values from the box which contained tailings mixed with molasses represent single values and not means.

Treatment	No Molasses Seedlings/Box	Molasses Seedlings/Box
Control	51	0
Roughened	194	12
Rough/Hydrosource	125	0
Verdyol	114	37
Wood Chips	134	30
Rough/Wood Chips	132	23

Table 3: *Tailings Box Experiment - Grass Germination*

All surface treatments (roughening, Hydrosource, Verdyol, wood chips) gave better germination rates than untreated controls. Surface roughening gave the highest germination rates. Hydrosource on a roughened surface gave lower germination rates than roughening alone. Germination rates on Verdyol were initially similar to controls (6 days from sowing), but subsequently, rates increased. Treatments with wood chips had higher germination rates than those with surface roughening alone. Very few seeds germinated on molasses-treated tailings, confirming the results of obtained previously in the pots. With these result it was possible to proceed to field trials.

Field experiment: Standing biomass and percent cover were measured on plots 3 months after set up (data not

shown). The Verdyol mats with seed below them or seeded on top of them greatly increased grass vegetation covers, reaching 100% cover in some plots. Grass germinated and established in cracks in the bare tailings, while seeds were washed from flat surfaces. However, where grass did establish, and fertilizer was applied, tall grass clumps developed.

After overwintering, and growing through one more summer season, the plots with 100% healthy grass cover were remeasured. Cores were collected for determination of shoot and root productivity as well as examination of microbial activity, in particular, for the presence or absence of oxidising bacteria in relation to the vegetation.

Shoot and root biomass dry weight data are summarised in Table 4. A well defined root mat, about 1 cm thick, was present under all five plots. Individual roots were found throughout the depth of the cores (i.e. to 20 cm at least). The highest shoot weight was found in plot 1A2, which was located on a Verdyol mat within an areas defined as "wet". The next highest shoot production was in plot IC2, which was classified as "dry". The lowest shoot production came from plot 3B4 which was "moist". This suggests that this apparent moisture gradient did not affect vegetation establishment.

Plot	Moisture	Shoot wt (g/sq.m)	Root/Litter (g/sq.m)	Total biomass (t/ha)
1A2	wet	164.8	682.4	8.47
3B4	moist	25.5	353.3	3.79
1C2	dry	136.8	863.9	10.01
1C4	dry	62.7	352.5	4.15
3C4	dry	99.6	310.1	4.1

Table 4: *Tailings Vegetation Biomass after Second Year*

Verdyol placement, whether on top of, or beneath seeds did not seem to make much difference in grass cover after one growing season. Of the 5 plots with a grass cover from the entire experiment, plots 1A2, 1C2, 1C4 were sown with seeds over Verdyol whereas plots 3B4 3C4 were sown with seeds beneath the Verdyol. In plots without Verdyol the grass cover was absent or poor.

The presence or absence of oxidising bacteria (thiobacilli) and heterotrophs was determined in root/tailings cores taken at a depth of 3 cm and 15 cm below the vegetation plot. The results are summarised in Table 5. Thiobacilli were detected in samples showing clear signs of oxidation (orange colouration) and absent from samples with no orange zones. Thiobacilli were present in all samples except for plot 1A2 at 15 cm, and plot 1C3 at 15 cm.

Sample (depth)	Heterotrophs (bacteria/mL)	Thiobacillus (+/-)	pH	Acidity (mg/L/g)	Alkalinity (mg/L/g)
Control 3 cm	174	+	7.3	310	669
Control 15 cm	1455	+	7.1	298	681
1A2 Wet 3 cm	1380000	+	6.7	263	373

Sample (depth)	Heterotrophs (bacteria/mL)	Thiobacillus (+/-)	pH	Acidity (mg/L/g)	Alkalinity (mg/L/g)
1A2 Wet 15 cm	1270000	-	7.0	224	287
3B4 Moist 3 cm	324000	+	6.3	449	357
3B4 Moist 15 cm	35700	+	6.9	210	294
1C3 Dry 3 cm	1940000	+	6.6	345	356
1C3 Dry 15 cm	62100	-	7.2	160	404

Table 5: *Bacterial Counts in Tailings with Vegetation*

Heterotroph numbers from the same cores give some indication total microbial activity. The largest populations of bacteria were found in a dry plot at 15 cm depth. The next highest numbers were in wet plots (1A2). Heterotroph numbers were generally higher in root zone (3 cm) than deeper in the profile (15 cm), and also higher in vegetated areas than in controls.

Discussion

In order to realize the concept of introducing oxygen consuming bacteria into the root zone of a vegetation cover on fresh tailings, several components had to be established first in laboratory and field experiments. The presence of heterotrophs in the tailings had to be determined and the methods required to establish a vegetation cover with root penetration on relatively fresh tailings had to be developed.

Laboratory and field experiments were initiated to identify conditions necessary for the development of vegetation on fresh mine tailings. Laboratory studies in pots and larger "tailings boxes" led to initial field trials in 1990.

The pot experiment demonstrated that root penetration in settled tailings was a problem. Molasses, while it increased bacterial populations, was highly inhibitory to germination of the seeds. Heterotrophic bacterial populations were similar both with and without vegetation, over the 6 week experiment. Thiobacilli were not detected in any of the pots.

Grass seedlings germinated well in surface-roughened (Verdyol, sawdust of direct surface roughening) tailings. Therefore, a roughening treatment (Verdyol) was appropriate for the field trials.

The field trials demonstrated conclusively that a grass cover can be established on fresh tailings and form a continuous organic layer on the tailings surface. This vegetation cover overwintered, and biomass at the end of the second year exceeded that of year one. Total standing crop biomasses reached 10 t/ha, after the second growing season. Considering that the growing season is only about 80-90 days, this standing crop compares favourably with agricultural crops (Black 1982).

Vegetation layers in fresh tailings provide a source of carbon and nutrients for heterotrophic bacteria. Heterotrophic bacteria, including *Pseudomonas*, *Escherichia* and *Bacillus* spp. commonly inhabit soils (Paul and Clark 1989). Growth rates of these bacteria under optimum (laboratory) conditions range from *Pseudomonas putida* (0.75 hr), *Escherichia coli* (0.35 h), and *Bacillus subtilis* (0.43 h; Stanier et al. 1970). Growth rates of *Thiobacillus*, however, are considerably slower (0.12 h; Barron and Lueking 1990). Thus, under the same ideal conditions, heterotrophic bacteria will probably outgrow *Thiobacillus*.

Assuming that respiration rates are proportional to growth rates, heterotrophs will very effectively outcompete the thiobacilli for oxygen. Further, if oxygen transport through the tailings is a limiting factor for the tailings oxidation rate, then the presence of heterotrophs in large numbers will significantly reduce the tailings oxidation rate.

The presence of heterotophs in the root zone of the grass from the field plot after the 2nd year has been confirmed. Relatively few bacteria were found in the milling circuit, suggesting that bacterial growth in tailings areas was the result of providing nutrients. Vegetated areas had more heterotrophs than non vegetated controls. Thiobacilli were found in almost all samples, both vegetated and non-vegetated.

In conclusion, these experiments have demonstrated that it is possible to vegetate fresh tailings. Heterotrophic bacteria are numerous in the root zone. Further work is now required to establish the quantities of nutrients supplied to the heterotrophs in the root zone and to produce a moisture containing and acid neutralizing layer for acid water generated in the deeper parts of the tailings.

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