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Biological Detoxification of Cyanide in Northern Environments

By Microbial Technologies, Inc.

May 2000

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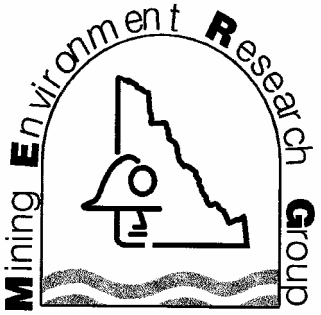

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Biological Detoxification of Cyanide in Northern Environments

A Project Report submitted to:
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and
Mining Environment Research Group

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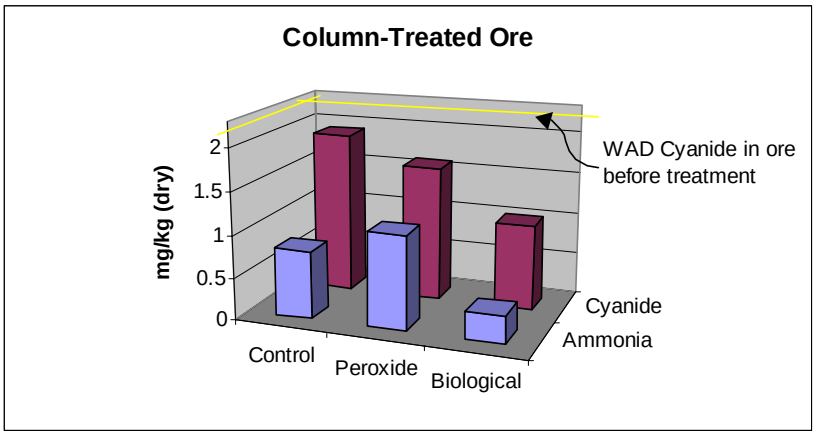
Executive Summary

Microbial Technologies investigated the technical feasibility of using cyanide-degrading bacteria to destroy cyanide associated with ore from a spent heap using bacteria native to the Yukon Territory.

A microbiological analysis of samples collected in the vicinity of the Brewery Creek Mine showed that bacteria degrading metal-cyanide complexes (nickel-cyanide, ferrous and ferric-cyanide complexes), thiocyanate are widely distributed, even in environments known not to be exposed to (anthropogenic) cyanide. Interestingly, most iron-cyanide degrading isolates also degraded thiocyanate. Ammonia oxidizing bacteria and nitrate-reducing bacteria were also common at the site.

Three columns received different treatments during a three months period: a control (water rinse) column, a peroxide treatment column, and a biological treatment column. Column effluents were monitored weekly for changes in pH, conductivity, redox potential and WAD cyanide concentrations. After the treatments, for Total and WAD cyanide and ammonia remaining on the spent ore were analyzed.

Biological destruction was more successful at removing cyanide than was peroxide treatment. WAD cyanide associated with the spent ore was reduced from an initial value of 2.10 mg/kg to 1.01 mg/kg in the biological column, compared with 1.60 mg/kg in the peroxide column. Spent ore in the control column had 1.92 mg/kg WAD cyanide at the end of the study (see figure below).



Ammonia concentrations were significantly higher in the peroxide column than in either the control or the biological columns. The biological treatment produced much lower ammonia concentrations. The biological column also had high levels of nitrate, indicating that bacteria in the column were oxidizing ammonia as well as the cyanide.

The successful removal of cyanide in the biological column provides a proof-of-concept for the biological detoxification of cyanide in spent heaps in Northern environments. The results indicate that a biological treatment can attain cyanide removal rates superior to a peroxide treatment. The bacterial oxidation of ammonia is a further, considerable benefit of a biological approach.

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Introduction

MERG (Mining Environment Research Group) and Viceroy's Brewery Creek Mine commissioned a study by Microbial Technologies, Inc. to determine the viability of biological detoxification of spent heaps in Northern (Arctic) environments. This approach has been applied at a small number of mine sites in North America, but it remains a relatively unproven technology. In particular, the detoxifying ability of microorganisms indigenous to Northern mine sites remained to be demonstrated. This approach may be advantageous for the Yukon Territory because it relies on bacteria grown on site to destroy cyanide, as compared with importing (potentially large quantities) of hydrogen peroxide.

The study was designed to answer two questions:

1. Is biological detoxification a feasible option for Brewery Creek Mine?
2. Is this process likely to be applicable to other similar operations in the Yukon Territory and other Northern locations?

A number of challenges had to be confronted to answer these questions.

There is virtually no information on the abundance and distribution of bacteria capable of degrading cyanide in the Arctic. It is known that different bacterial species degrade different metal-cyanide complexes, such as iron-cyanide or nickel-cyanide complexes[1]. Thus, it is important to determine both the abundance and the metabolic diversity of micro-organisms metabolizing metal-cyanide complexes. The first phase of the study involved collecting, enumerating, and culturing such microorganisms from the mine site.

Cyanide-degrading bacteria may be present in the mine environment, but they may not be able to detoxify the ore from the spent heap. For example, they may be incapable of colonizing the ore or they may be inhibited by high cyanide concentrations or some toxicant in the ore (e.g., arsenic, mercury). Phase 2 of the study was a column study to determine if bacteria collected and grown in Phase 1 can detoxify spent ore collected from Brewery Creek's heap. Their ability to destroy cyanide was compared with that of hydrogen peroxide, the conventional chemical treatment of spent heaps.

Study Objective

The purpose of the study was to provide a proof-of-concept for the use of microorganisms indigenous to the Northern environment to detoxify cyanide from spent heaps. Specifically, the study sought to demonstrate that:

- Micro-organisms capable of degrading various metal-cyanide complexes and thiocyanate are present in soils from the Yukon Territory,
- These micro-organisms can become established on ore from the spent heap, and
- These micro-organisms can biodegrade various metal-cyanide complexes and thiocyanate in the solids and leachate from the spent ore.

[1] Cyanide can exist in free form or complexes with metals. However, it is doubtful that it will exist in the free form in the environment.

Phase 1 – Micro-organism Collection and Analysis

Background

An analysis of the chemistry of process water at the mine suggested that the following toxic compounds present in the spent ore may need to be degraded: nickel-cyanide complexes; ferrous and ferric-cyanide complexes; thiocyanate, ammonia and nitrate. Thus, populations of nickel- and iron-cyanide degraders, thiocyanate degraders, ammonia oxidizers and nitrate reducers were sought in the mine environment.

The above bacteria catalyze the following reactions:

Cyanide degraders:	$Me\text{-}[CN]x + 2x\ H_2O + ½x\ O_2 \rightarrow Me\text{-biofilm} + xHCO_3^- + xNH_3$	(Eq. I)
Thiocyanate degraders:	$SCN^- + 2\ H_2O + 2½\ O_2 \rightarrow SO_4^{2-} + HCO_3^- + NH_3$	(Eq. II)
Ammonia oxidizers:	$NH_4^+ + 1½\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O$	(Eq. III)
Nitrite oxidizers:	$NO_2^- + ½\ O_2 \rightarrow NO_3^-$	(Eq. IV)
Denitrifiers:	$3\ NO_3^- + 2.5\ CH_3OH \rightarrow 1.5\ N_2 + 2.5\ CO_2 + 3.5\ H_2O + 3\ OH^-$	(Eq. V)

Both cyanide and thiocyanate degradation produce ammonia (Eq. 1,II+). With the CCREM guideline for ammonia discharge set at 1-2 ppm, degradation of only 1.5 ppm CN would produce unacceptable concentrations of ammonia, assuming stoichiometric transformation of nitrogen. Hence, nitrifying bacteria will be required as part of the detoxification process. Similarly, high concentrations of ammonia can produce unacceptable concentrations of nitrate (50 ppm NH_3 is converted to 182 ppm NO_3^-, assuming stoichiometric transformations). So the presence of denitrifying bacteria may be required as part of the detoxification process.

Note that in the above reactions, all the bacteria require oxygen except for denitrifiers. Thus, these bacteria will be found in aerated environments, whereas denitrifiers will be found in environments where oxygen is excluded, e.g., water-logged sediments. In addition, the aerobic bacteria do not require a carbon source to carry out their reactions. Hence they can be found in environments that are poor in organic carbon, unlike denitrifiers. These differences affect the distribution of these microorganisms and dictate both the environment where they will be found and their numbers.

Methods

A site visit to collect environmental samples potentially containing cyanide-degrading bacteria was carried out in July, 1999. Seven samples were obtained: three samples collected from Cells 1, 2, 3 of the heap (See Figure 1); one sample of mud exposed to heap leachate (South Toe Dam), one sample from the barren pond (Barren Pond), one sample near the AVR plant (AVR), and a sample from a small pool of water below the Canadian dump (Green Pond). The presence of denitrifying bacteria was indicated by the release of gas from sediments in the South Toe Dam. The water, soil, and sediment samples were brought to our laboratory unrefrigerated and unpreserved, but were stored at 4 °C immediately upon our return.

Populations of thiocyanate degraders, nickel- and iron-cyanide degraders were determined on solid medium by the Most Probable Number (MPN) technique, based on the ability of micro-organisms to grow using these compounds as source of nitrogen. Ammonia oxidizers and denitrifying bacteria were enumerated by conventional MPN-based methods.

Bacteria from the spent ore in Cells 1, 2 and 3 were propagated in our laboratory for use in the column study of Phase 2, as explained further below.

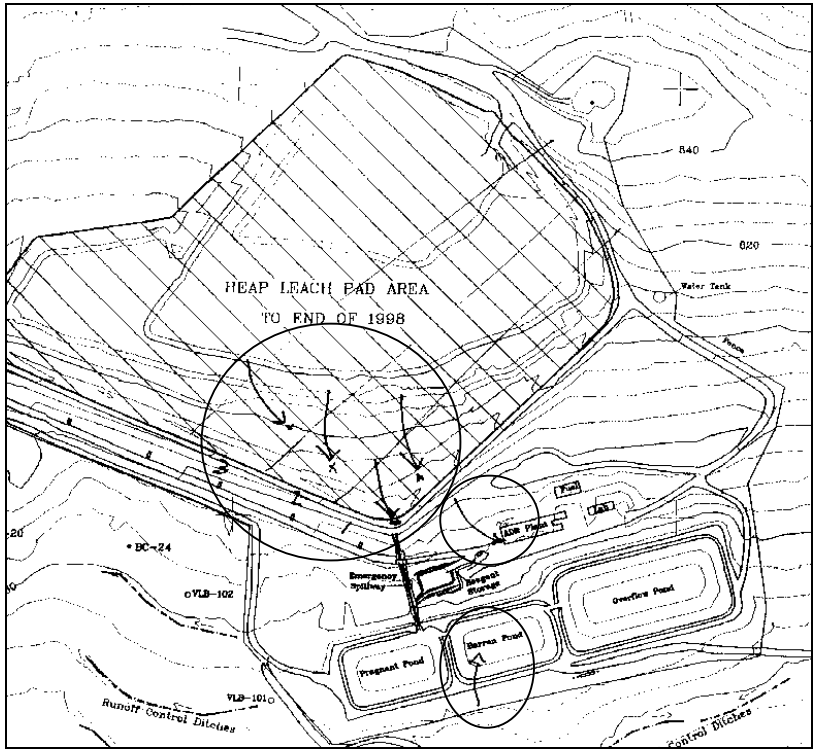

Figure 1. Sample sites near leach pad.

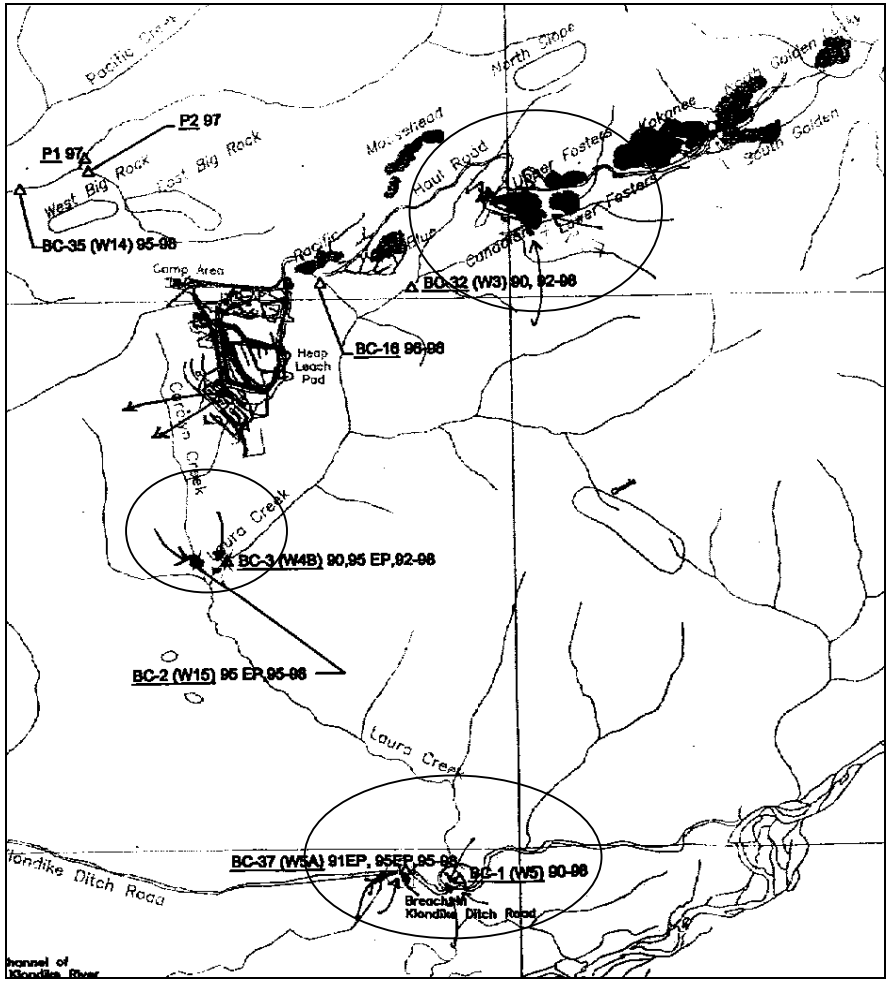

Figure 2. Sample sites at Brewery Creek Mine.

Results

All the samples analyzed contained bacteria capable of degrading metal-cyanide complexes, thiocyanate, and their nitrogenous breakdown products. Cyanide and thiocyanate degraders were relatively abundant, whereas nitrifying bacteria were less abundant. Results of the enumerations are presented in Table 1. The cell numbers in the Barren Pond sample were not directly comparable to that of other samples because it is expressed in MPN(cells)/mL, whereas the others are expressed in MPN(cells)/dry gram. Nevertheless, a few general conclusions could be easily drawn from a comparison of cell numbers in all the samples.

Table 1. Enumeration of Cyanide Degrading Bacteria and associated nitrogenous compounds.

Sample	SCN	$Ni(CN)_4$	$Fe(II)[CN]_6$	$Fe(III)[CN]_6$	NH_3	NO_3-r
Barren Pond[1]	2.8×10^4	3.5×10^4	3.5×10^4	7.0×10^3	-	-
South Toe Dam[2]	4.6×10^6	2.4×10^5	2.4×10^5	5.4×10^6	5.4×10^4	9.6×10^4
Green Pond	6.1×10^6	1.6×10^6	1.6×10^6	4.2×10^6	1.1×10^4	1.1×10^6
AVR	3.8×10^6	1.0×10^6	7.3×10^6	7.3×10^6	2.4×10^3	-
Cell 1	1.1×10^6	5.2×10^6	9.1×10^6	5.2×10^6	-	-
Cell 2	2.7×10^5	2.0×10^5	5.2×10^6	8.8×10^6	-	-
Cell 3	1.1×10^6	8.0×10^5	2.6×10^6	6.7×10^6	-	-

SCN: Thiocyanate degraders; $Ni(CN)_4$: Nickel-cyanide degraders; $Fe(II)[CN]_6$: Ferrous-cyanide degraders; $Fe(III)[CN]_6$: Ferric-cyanide degraders; NH_3: Ammonia oxidizers (Nitrifiers); NO_3-r: Nitrate-reducers (denitrifiers).
[1]Cell numbers expressed as MPN(cells)/mL. [2]Cell numbers expressed as MPN(cells)/dry gram.

Thiocyanate, nickel- and iron-cyanide degraders were present. They appeared to be least abundant in the Barren Pond, but equally abundant in all the other samples. Observing the colonies grown on solid media revealed the following:

1. Distinct microbial populations were seen for the Barren Pond, South Toe, Green Pond, and AVR samples. In contrast, microbial populations from the heap samples (Cells 1, 2 3) were comparable among one another, but distinct from the others. This is presumed to reflect the different environments represented by these samples.

2. A consistent finding among all the samples was that the cells degrading thiocyanate also degrade ferrous- and ferric-cyanide complexes. However, the populations of thiocyanate/iron-cyanide degraders from each of the areas were distinct. The populations of nickel-cyanide degraders were distinct from the populations of thiocyanate/iron-cyanide degraders.

3. There was a noticeable diversity of contaminant-degraders in any given sample. Thus, Cell 1 had at least 3 distinct species of thiocyanate/iron-cyanide degraders, and 2-3 distinct species of nickel-cyanide degraders. Generally, thiocyanate/iron-cyanide degraders were comprised of at least 3-5 species in any given sample, whereas nickel-cyanide degraders were less diverse, typically comprising 1-3 species.

Nitrifying bacteria are not normally abundant in soils and sediments[2]. The populations of nitrifying bacteria in the South Toe Dam, Green Pond, and AVR samples were comparatively low, consistent with the norm. Other samples were not tested for these bacteria because the known sensitivity of nitrifying bacteria to toxicants suggests that they would be much less abundant in these samples.

[2] Belser, L.W. 1979. Population ecology of nitrifying bacteria. Ann. Rev. Microbiol. 33: 309-333.

The highest numbers of nitrifying bacteria were sample in South Toe Dam sample, suggesting that it receives high ammonia concentrations. The fact that this sample is also exposed to high concentrations of cyanide suggests that cyanide is not toxic to these bacteria.

Denitrifying bacteria are more abundant in the environment than nitrifying bacteria. They are typically found at the sediment-water interface of aquatic ecosystems receiving high nitrate concentrations. This was reflected in our analyses. They were present in the mud sample at the South Toe Dam, and in the Green Pond sample (in greater numbers). The lower abundance of denitrifiers in the South Toe Dam sample reflects the fact that it had less organic carbon than the Green Pond sample.

Samples collected from different environments had distinct bacterial populations. This suggests that cyanide degraders growing on the spent ore in Cells 1, 2, and 3 are adapted to the ambient conditions (temperature, pH, conductivity, cyanide concentrations, etc) in this material, and are probably best suited for its detoxification. This material should be used as a source of bacteria for future testwork. Similarly, the nitrifying bacteria collected from the South Toe Dam sample are expected to tolerate relatively high concentrations of cyanide, and should also be used for detoxification.

More generally, the detection of thiocyanate, nickel- and iron-cyanide degraders in every sample tested, including the Green Pond sample (supposedly not previously exposed to cyanide) suggests that these micro-organisms are widespread in the environment.

Phase 2 – Column Study

Test Design

Three columns measuring four feet high by four inch diameter were constructed and loaded with spent ore obtained from the mine. Each column was filled with approximately 13.5 kg of ore. The material rested on perforated plastic plates at the bottom, and was covered at the top with one inch of cleaned aquarium gravel to aid in the distribution of water into the column. Prior to loading the columns, the ore was agglomerated with a synthetic polymer[3] and was thoroughly mixed. Approximately 1 kg washed aquarium gravel was mixed with the ore to maintain porosity within the column. The columns were mounted in a walk-in incubator, which is maintained at approximately 28 °C.

The columns ran for 64 days from January 13 to March 16, and assessed three treatments, as follows:

Column 1: Water rinse. Only water was added during the test. Water-leachable cyanide was rinsed off, but there should otherwise be no significant detoxification of the cyanide from this column. This column was used as a Control, for comparison with the other two treatments.

Column 2: Peroxide treatment. A 1% peroxide (28.6ml of 35% H_2O_2 in 971.4 ml H_2O) solution was circulated through this column for the duration of the test.

Column 3: Biological treatment. Cultures of cyanide and thiocyanide degrading bacteria were added to this column. A source of carbon (methanol and acetate) and phosphorus (as orthophosphoric acid) were added to sustain the bacteria. The amounts of C and P added varied throughout the test (see Table 2) to accommodate the projected growth needs of the culture.

Table 2. Additions to Biological Column solution

Week	Orthophosphoric Acid (as P)	Methanol/Acetone blend (as C)
1	0.5 mg/L	0.1 g/L
2	5.0 mg/L	1.0 g/L
3	2.0 mg/L	0.3 g/L
4	0.5 mg/L	0.1 g/L

The solutions were circulated through these columns at a rate of approximately 1 L/day from Jan.13 to March 16 (day 1 to day 46). The columns were given a two-week rest, then flushed with two column volumes of water. The rest period allows solute dissolved in micropores time to equilibrate with the bulk porewater, so if significant quantities of cyanide were still associated with the ore, concentrations would be high in the flush effluent.

Monitoring

Weekly tests of column effluent were performed to determine the WAD-CN concentration, pH, conductivity and redox potential. The rationale for measuring these parameters is as follows:

[3] Nalco 9704 (polyacrylamide) and Nalco 9760 (acrylamide/acrylate polymer), applied at a rate of ½ lb per ton.

- WAD-CN concentrations indicated the degree of treatment achieved in each column
- pH indicated the change in acidity during the treatment
- Conductivity reflected the amount of leachable salts lost from the solids during the treatment
- Oxidation reduction potential (ORP) was monitored to detect breakthrough of peroxide in the peroxide column. This measurement was also used as a surrogate for dissolved oxygen.

Populations of nickel-cyanide and iron-cyanide degraders in column effluents were measured on February 14 to compare their numbers and assess their activity in the different columns. Total CN, ammonia, nitrate and dissolved metals concentrations were also measured during the study.

Nitrate concentrations in the effluent of the different columns were determined by spectrophotometric analysis using a Hach NitraVer Nitrate reagent kit. They were measured at the end of the regular test cycle and in the flush water.

Ammonium concentrations were measured in-house using a colorimetric test kit. The precision of this test kit is comparatively low, and final determinations were obtained by submitting samples to a commercial analytical laboratory (ASL Analytical Services Laboratory).

Ore samples were taken from each column, at the end of the treatment period (Day 64). Two hundred grams of ore was mixed with 500 ml of 0.2N NaOH and leached for five days. The solution was decanted and sent for analysis to determine concentrations of ammonia associated with the ores from the different columns. Another 200g sample was shipped to a commercial lab to determine Total CN and WAD-CN concentrations in the ores after column treatments.

Results and Discussion

Effluent Data

Measurements of pH, WAD cyanide, conductivity and oxidation reduction potential for the three column discharges are presented below. The vertical blue line marks the end of the six-week test period.

In general, the columns appeared to take two to three weeks to stabilize. During this time, there was a decrease in WAD-CN concentrations and in conductivity, and an increase in redox potential in all columns.

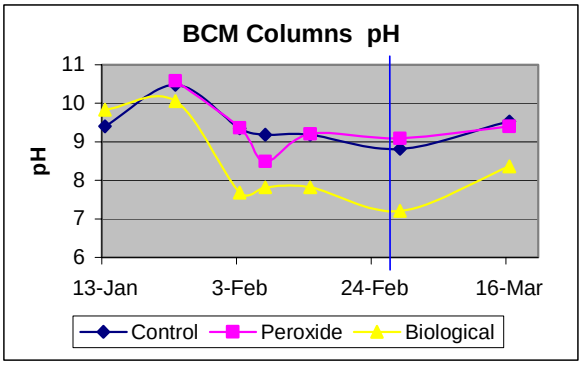

Figure 3. Column Effluent pH

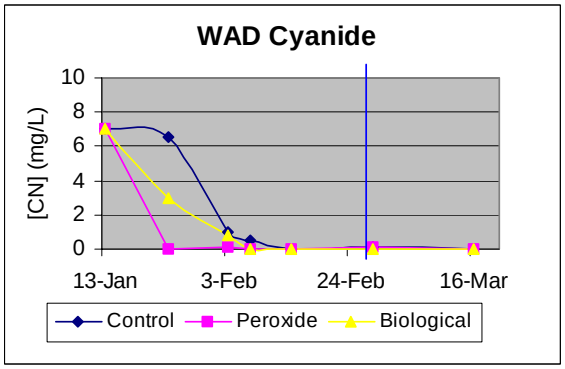

Figure 4. Column Effluent WAD Cyanide

An initial short-lived increase in pH in column effluents was followed by a period where pH remained consistent. The control and peroxide columns maintained a pH around 9, while the biological column averaged 7.6 once it stabilized. This lower pH can be attributed to the addition of phosphoric acid used to provide phosphate for the bacteria. The effluent from the final flush of water had a slightly higher pH in all columns.

WAD cyanide decreased to zero or near-zero concentrations in effluents from all columns. This occurred most quickly in the column treated with peroxide, more gradually in the biological column, and slowest in the control column. These values are WAD cyanide in the effluent only, and do not necessarily reflect the cyanide in the spent ore.

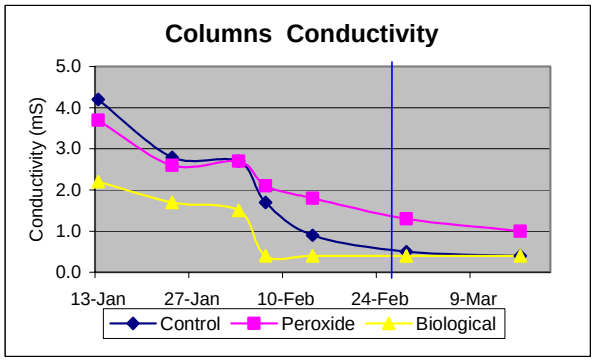

Figure 5. Effluent Conductivity

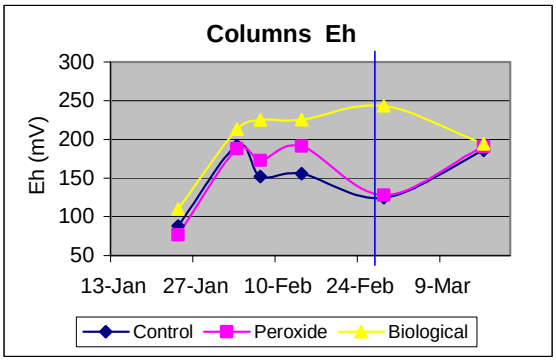

Figure 6. Effluent ORP Potential

Conductivity decreased steadily in all three columns. Presumably this was a result of salts washing out from the solids throughout the test. There was no increase in conductivity with the final flush of water, indicating that available salts had been rinsed during the test period. Surprisingly, the conductivity in the effluent from the peroxide column remained higher than that from the other columns.

Oxidation Reduction Potential (ORP) was highest in the biological column. The fact that effluent from the column with peroxide was not significantly higher than the control column indicates that most of the peroxide was consumed within the column. Peroxide may be consumed by cyanide or by other compounds (e.g., Fe) within the spent ore. ORP for all columns was similar after the final flush – suggesting that biological activity had decreased in the biological column.

Enumeration of Cyanide-degrading Bacteria in Column Effluent

Populations of nickel-cyanide and iron-cyanide degraders in column effluents were measured on February 14 (Day 31) and are presented below in Table 3. The column undergoing biological treatment had 10-100 times more cyanide degraders than the control column, with cell numbers exceeding 10^4 MPN/mL for both nickel- and iron-cyanide degraders. Surprisingly, there was a significant number of cyanide degraders in the effluent of the control column. In contrast, the number of iron-cyanide degraders was substantially depressed in the peroxide-treated column, suggesting that these bacteria, but not nickel-cyanide degraders, are sensitive to hydrogen peroxide.

Table 3. Numbers of cyanide degrading bacteria in column effluents.

COLUMN	NICKEL-CYANIDE DEGRADERS	IRON-CYANIDE DEGRADERS
Control	1.2×10^2	5.9×10^3
Peroxide treatment	2.0×10^2	43
Biological treatment	1.4×10^4	3.3×10^4

Cell numbers are expressed in MPN/mL, equivalent to cells/mL.

Dissolved Metals in Column Effluent

Dissolved metals were measured in the effluent just prior to the flush on February 28 (Day 46). These data are summarized in Table 4 and are tabulated in the appendix. Only those metals with significantly elevated concentrations are included here.

Table 4. Dissolved metals in column effluent.

	Control	Peroxide	Biological
Al	0.9	0.5	< 0.2
Ca	108	352	29.2
Mg	< 0.1	< 0.1	0.4
Na	16	39	51
Sr	0.235	0.564	0.076
Zn	< 0.005	< 0.005	0.038

Magnesium and sodium concentrations were higher in the biological column as a result of their addition with nutrient for the bacteria. Lower concentrations of Ca and Sr in the biological column may be the result of precipitation with phosphorus, which was added as a nutrient. Lower Al concentration in the biological column is likely due to the slightly lower pH in the column.
A higher zinc concentration in the biological column (0.038 mg/L) than in the control column (<0.005 mg/L) is likely a result of the biological breakdown of ZnCN. However, the absence of dissolved zinc (also <0.005 mg/L) in the peroxide column does not necessarily mean that ZnCN was not broken down in that column. It is possible that zinc released in the peroxide column was also oxidized by the peroxide and hence was unavailable in a dissolved form.

Gold was measured in column effluent after the column flush. Concentrations of dissolved gold were low and relatively similar in all three columns:

Table 5. Gold Concentrations in Column Effluents

COLUMN	GOLD (ppb)
Control	4.24
Peroxide treatment	2.88

Biological treatment	3.60

Residual Porewater Cyanide

The amount of cyanide retained in porewater after treatment was determined by allowing a two-week rest period, during which no solution was circulated through the columns. Total and WAD cyanide were measured in the effluent of flush water after the two-week rest. The results indicated that there were not significant quantities of cyanide released with the flush water. The data indicate that Total cyanide consisted largely of WAD-CN in the control and peroxide columns, but not in the biological column, where Total-CN exceeded greatly WAD-CN. The latter result is suspect because all other data suggest that the majority of the cyanide in column effluents is WAD-CN. It is not uncommon for 0values near detection limits to be suspect.

Table 6. Column Effluent CN concentrations after Flush

	CONTROL	PEROXIDE	BIOLOGICAL
Initial WAD-CN	4.16	4.37	5.6
Flush WAD-CN	0.12	0.07	0.04
Flush Total CN	0.13	0.08	0.24

Ammonia in Column Effluent

Ammonia concentrations in column effluents were measured four times between days 25 and 64 by a colorimetric method. Results from this method were corroborated by duplicate analyses from a commercial analytical laboratory on day 46 and day 64 samples. Data from these analyses are presented in Figure 7 and Figure 8.

Both analytical methods showed significantly higher concentrations of ammonia in the peroxide column (as much as 40 mg/L early on, decreasing to 4.9 mg/L by the end) than in the other two treatments. Concentrations in effluent from the control column were around 50% to 67% of the peroxide-treated column, while ammonia concentrations in effluent from the biological column were much lower.

The decrease in ammonia from a very high initial value in the peroxide column most likely corresponds to a decrease in the amount of WAD cyanide available for breakdown by peroxide. The fact that ammonia concentrations were higher than in the control column indicates, as expected, that more cyanide was oxidized in the peroxide column than in the control column, and that ammonia persisted through the two week dry-out period before the flush. The consistently low ammonia concentrations in the biological column indicated either that cyanide was not degraded as extensively as in the peroxide column, or that nitrifying bacteria quickly oxidized it to nitrate. Since cyanide concentrations were later shown to have been reduced in the ore in the biological column (see “Final CN and Ammonia Concentrations in Ore” below), it follows that the low ammonia in the column effluent reflect the fact that it was biologically oxidized to nitrate.

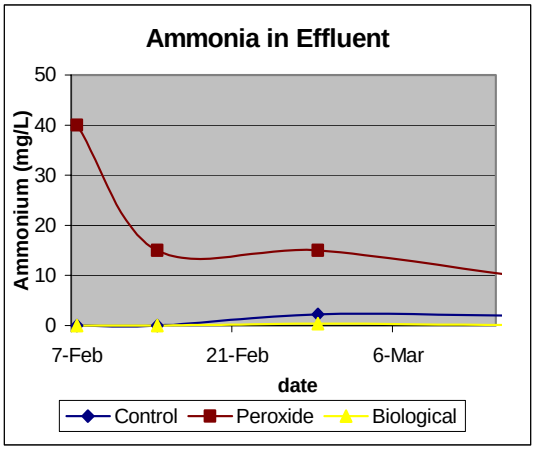

Figure 7. Ammonia concentrations in effluent (Colorimetric method)

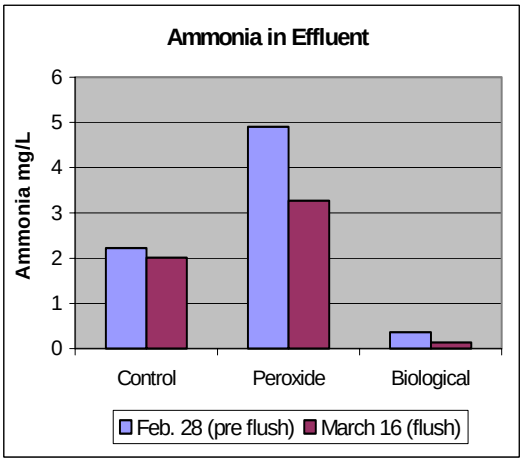

Figure 8. Ammonia concentrations in effluent (Commercial Laboratory)

Nitrate in Column Effluent

Nitrate concentrations were high in the biological column, and undetectable in the other two. These data are presented below in Table 7.

Table 7. Column Effluent Nitrate data

	Day 46 (Pre-Flush)	Day 64 (Flush)
Control	0 mg/L	0 mg/L
Peroxide	0 mg/L	0 mg/L
Biological	36mg/L	28 mg/L

Higher nitrate concentrations in the biological column correspond with the lower ammonia concentrations, and indicate that nitrification has occurred. This information, coupled with the low ammonia levels in the biological column, were an indication that cyanide was being destroyed in the biological column. That is, cyanide was first degraded, producing ammonia, and the latter was nitrified to nitrate.

Final CN and Ammonia Concentrations in Treated Ore

The most indicative test of treatment effectiveness is not in measurements of effluent concentrations, but of final cyanide concentrations in the ore itself. Accordingly, cyanide and ammonia concentrations associated with the ores were measured at the end of the study.

At the end of the column treatments, **cyanide concentrations were lowest in the biological column, slightly higher in the peroxide column, and highest in the control column**, as shown in Table 8 and Figure 9. The data are somewhat confounded by the fact that WAD-CN concentrations were higher than Total CN for both the peroxide and the biological column samples. This apparent error was attributed to the heterogeneity of the ore. If nothing else, it indicates that most of the cyanide in the ore was WAD cyanide. As such, the cyanide concentrations presented in Figure 9 are averages of WAD and Total CN values[4].

[4] If Total CN ≈ WAD-CN, averaging Total and WAD-CN concentrations reduces sampling and analytical error.

Despite these potential sources of error, it is clear that ore detoxification was highest with the biological treatment, followed by peroxide treatment. Averaging Total and WAD-CN values, we calculate that these two treatments reduced ore-associated WAD-CN by approximately 55% and 40%, respectively. The water rinse reduced ore-associated WAD-CN by approximately 10% during the treatment. Removal could be greater for both peroxide and biological columns in an optimized system.

Table 8. Cyanide and Ammonia concentrations in spent ore after column treatments.

COLUMN	TOTAL CN (MG/KG)	WAD CN (MG/KG)	AMMONIA (MG/KG)
Before Treatment		2.06	
Control	2.07	1.76	0.8
Peroxide treatment	1.19	2	1.1
Biological treatment	0.79	1.23	0.325

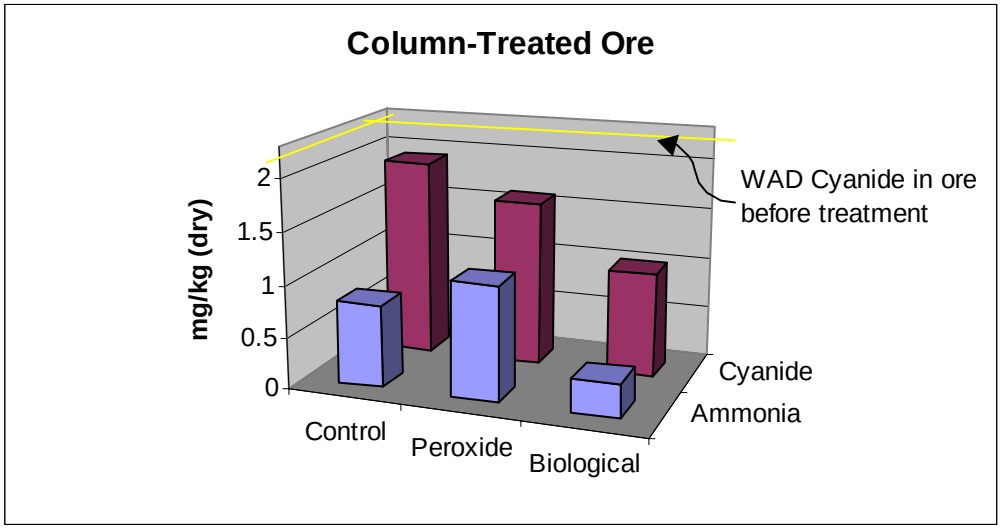

Figure 9. Cyanide and Ammonia concentrations in spent ore after column treatment.

Ammonia concentrations were highest in ore from the peroxide column, and lowest in the biological column (Table 8, and Figure 9). Assuming that nitrogen is conserved during the transformation of cyanide to ammonia, we calculate that the ore in the water rinse and peroxide columns retained approximately the same amount of nitrogen (as CN or NH_3), whereas there was a net loss of nitrogen in the biological treatment. This nitrogen was likely lost in the column effluent as nitrate (See Table 7, above).

Conclusions and Recommendations

This study provides the first proof-of-concept for the biological detoxification of cyanide in Northern locations. By documenting and employing cyanide-destroying micro-organisms native to Brewery Creek Mine (located in the Yukon Territory), we have shown that the desired bacteria are acclimatized to the Northern environment, and are capable of removing cyanide from the ore.

Phase 1 of the study established that cyanide-degrading bacteria are naturally abundant and relatively widespread at the Brewery Creek mine site. The presence of these bacteria on ore samples from the heap suggests that they produced some of the ammonia in the pregnant solution during mine operation.

Phase 2 confirmed that these micro-organisms can be established on ore from the spent heap, and that they can biodegrade metal-cyanide complexes associated with the ore. Additionally, nitrifying bacteria were also present and able to oxidize ammonia to nitrate. The biological breakdown of ammonia is one of several benefits of a biological treatment. Other advantages over a chemical treatment include the low setup and operational costs of such systems. The decrease in final Total Cyanide concentration on the ore itself indicates that the bacteria adhere to the ore, rather than being in porewater and only degrading cyanide in solution. Consequently, one would expect improved performance over time.

Although successful, the system tested was not optimized. Now that the concept has been validated, further work is required to answer some questions.

Specifically,

- What is the effect of temperature on these cyanide-degrading bacteria,
- What levels of carbon and nitrogen yield optimum results,
- What analytical methods can improve the accuracy of cyanide analyses on solids?

The next phase of work should involve a scale-up, and could be performed this summer. The results of this study are encouraging, and further work could have far-reaching applicability to Northern mine sites with the need for cyanide detoxification.