ASSESSMENT OF BIOLOGICAL TREATMENTS TO REMEDIATE CYANIDE HEAP-LEACHED ORE

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ABSTRACT

A new gold rush is taking place in the American Southwest, due in large part to improvements in the processes that use cyanide to extract gold from low-grade ore deposits. Along with increased gold production, however, has come an increase in cyanide contamination of surface and ground water in areas affected by gold mining. The objective of this study was to determine the potential for bioremediation of total (free and complexed) cyanide in heap-leached ore.

Two methods, including the addition of a carbon amendment and/or cyanide degrading microorganisms to cyanide heap-leached ore, were evaluated with regard to their influence on loss of total cyanide in microcosm studies. Heap-leached ore, containing approximately 400 mg cyanide/kg ore, was obtained from the Summitville Mine in southern Colorado. The presence of a cyanide degrading microbial assemblage in Summitville ore was verified by isolating microorganisms on plates supplied with free ($HCN_{(g)}$) as the sole source of nitrogen. However, stimulation of the indigenous community did not occur with respect to loss of total cyanide in ore microcosms using sucrose as a carbon amendment. Cyanide degrading inocula, consisting of *Pseudomonas fluorescens*, an Industrial (from Pintail Systems, Inc.) microbial consortium and a native Summitville species, also had no effect on the rate of loss of total cyanide compared to uninoculated controls. Loss of total cyanide in biological and control treatments and was relatively linear over time at a rate of 4.9 (±0.7) mg CN^-/kg ore/day. While bioremediation may not an be an effective treatment for remediating complexed cyanide, there exists the potential for biological degradation of free cyanide generated from the dissociation of metal complexed cyanide in heap leached ore.

Keywords: cyanide, biodegradation, bacteria, water quality, water pollution prevention, biological treatment, dissociation.

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INTRODUCTION

Industrial Cyanide Contamination

Cyanide (CN^-) is a potent inhibitor of cellular metabolism (Solomonson 1981) and concentrations of less than 1 ppm can suppress biological activity in higher organisms (Connell and Miller 1984). Accordingly, industrial operations are regulated with regard to cyanide content of the wastes they generate. Coal coking, electroplating and precious metals mining are a few of the operations that have the potential to release cyanide to the environment. In 1978, the annual industrial output of cyanide-containing waste was over 3 billion liters (Towill et al. 1978). This figure probably underestimates today's annual cyanide waste output given the recent resurgence of the gold mining industry in the United States.

Since 1980, gold mining in the US has increased by over 900% (Lawrence 1991) due in large part to improvements in the processes that use cyanide to extract gold from low-grade ore deposits. Cyanide is used in both mine mills and the heap-leach process developed by the US Bureau of Mines because it is the most effective reagent available for gold recovery. Unfortunately, cyanide contamination of soils and surface/ground water is a regrettable outcome of cyanide heap-leach gold mining (Smith et al. 1984).

Recently, water quality in several areas of the southwestern United States has been threatened with cyanide contamination from heap leach mining. A plume of contaminated ground water at the Ortiz Gold Mine site in Santa Fe County, New Mexico is a potential threat to the health of the area's citizens. Also, of the ten Superfund sites in New Mexico, the Carrizozo site in Carrizozo, New Mexico is primarily devoted to remediating cyanide pollution. Perhaps the most dire example of environmental cyanide contamination can be found at the Summitville Mine in southern Colorado. This site may be the worst ecological disaster in Colorado's history and, at the time of this study, the EPA was working daily to prevent the release of 200 million gallons of cyanide-contaminated water to the local waterways at a cost of $40,000 per day (Kosich 1993).

It is estimated that remediation of the Summitville site could eventually cost taxpayers over $100 million (Mine Monitor 1993). Clearly, the need for an expedient and cost-efficient method for dealing with cyanide contamination from mining is a high priority.

Cyanide Heap Leach Mining:

In a cyanide heap-leach process, the gold bearing ore is extracted, crushed to a nominal size and piled on an impervious pad (Hickson 1982). An alkaline cyanide solution is then sprayed on the pile. Cyanide complexes with the particulate gold and carries it from the ore in a leaching solution to a collection pond. The gold is recovered from this solution by a technique using activated carbon. The stripped cyanide solution can then be recirculated back onto the pile for further gold extraction (see Figure 1). The cyanide-leach solutions applied to heaps typically range in strength from 0.15 to 1 kg CN^-/ton (150 to 1,000 mg CN^-/L) and are maintained at a pH of 9.5 to 12. Below pH 9.3, cyanide exists mainly as gaseous HCN which is lost to the atmosphere (Figure 2). The EPA water quality guidelines of 1976 gave an ambient standard of 0.005 mg CN^-/L for fresh water aquatic life. The Public Health Services drinking water standard for human health is 0.2 mg CN^-/L (van der Leeden et al. 1990).

Bioremediation of Cyanide Heap-Leached Ore:

Many chemical processes currently used to detoxify cyanide-containing industrial wastes have serious limitations (Knowles 1976; White et al. 1988). For example, the use of sodium hypochlorite or hydrogen peroxide to oxidize cyanide can be prohibitively expensive. Also, remediation options such as gaseous chlorination of the cyanide can be potentially dangerous (Fortier and Wright 1985).

Cyanide Heap-Leach Operation

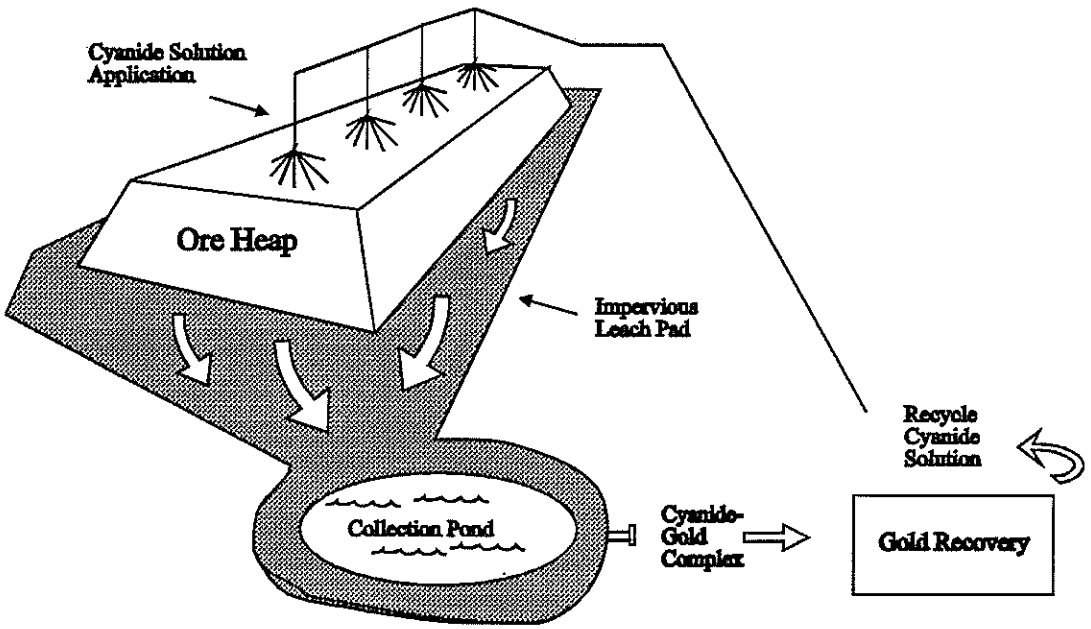


Figure 1. Generalized operation for removal of precious metals from low-grade ore using the heap-leach process (after Hickson 1982).

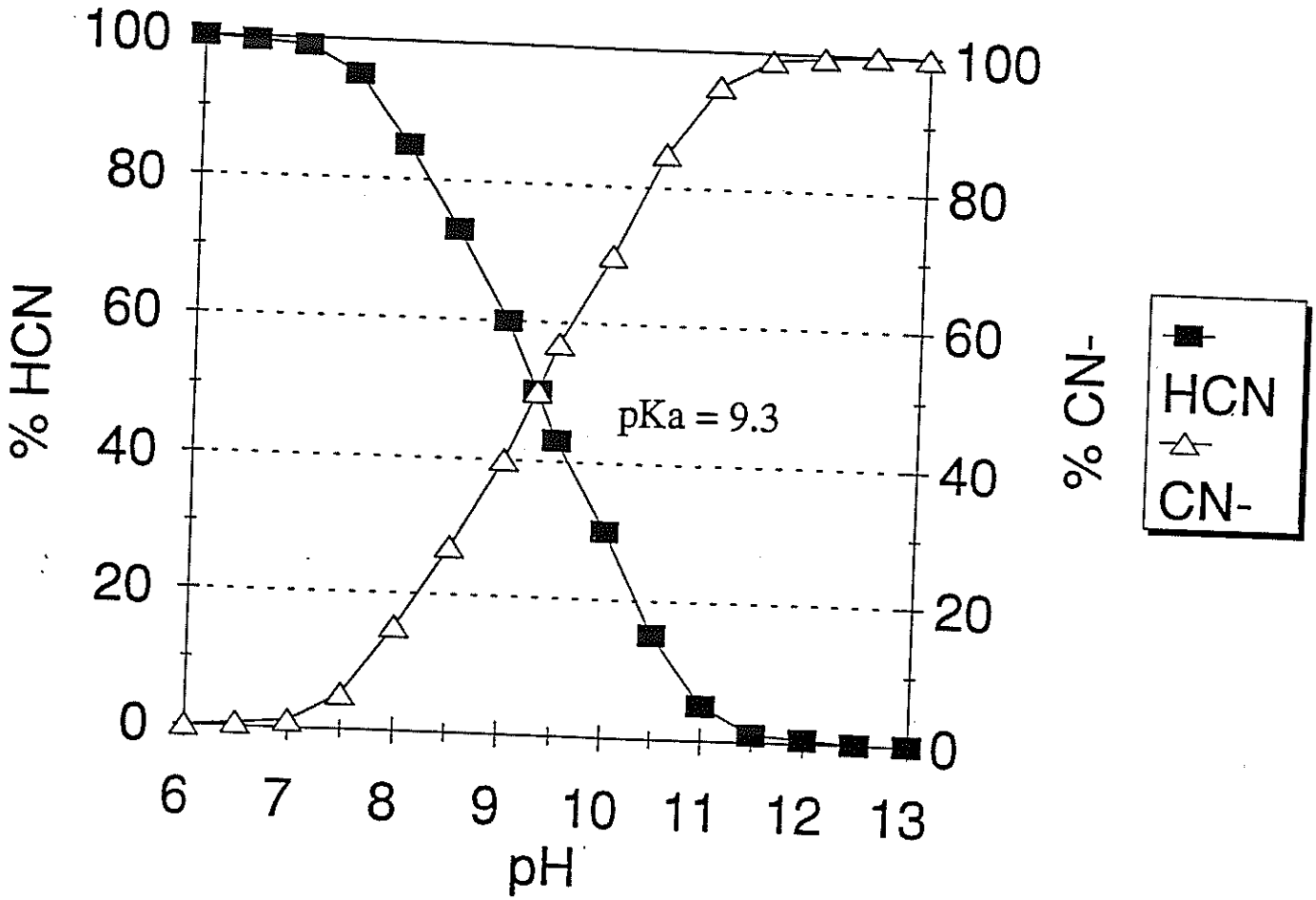


Figure 2. Cyanide dissociation equilibria as a function of pH.

Biological treatment of cyanide containing wastes may have advanteges over other remediation options. Although cyanide ingestion can be devastating to higher life forms, many microorganisms can overcome its metabolic inhibitory effects (Knowles 1976; Knowles and Bunch 1986) and utilize the cyanide molecule as a source of carbon and/or nitrogen for growth (Table 1). A generalized reaction for the aerobic (i.e. with oxygen) biological degradation of cyanide can be stated as:

$$\text{Microorganisms} + CN^- + O_2 \Rightarrow \text{Microbial Biomass} + NO_3^- + CO_2 \quad \text{(Eq. 1)}$$

The biological conversion of cyanide into additional cells and metabolites such as nitrate (NO_3^-) and carbon dioxide (CO_2) makes bioremediation an attractive alternative for cleaning up cyanide contaminated environments (Knowles 1988). Considering the plethora of research in this area, however, it is surprising that so few studies have focused on the biodegradation of complexed cyanide.

The free cyanide ion will readily bind with metals as a strong ligand to form complexes of variable stability and toxicity (Greenberg et al. 1992). In a mine heap, a large portion of these complexed metal cyanides may consist of iron-bound cyanide (Mudder and Whitlock 1984) which can slowly dissociate in aquatic systems, potentially leading to long-term cyanide releases creating chronic contamination problems. From calculations based on redox potential, pH and total cyanide concentration, Meeussen et al. (1992) estimate that complexed iron-cyanide in most soils will eventually decompose to toxic free cyanide. Stability constants of iron-complexed cyanides and selected other metal cyanide complexes commonly found in a heap environment are listed in Table 2. These compounds are expected to constitute a significant fraction of cyanide-related wastes, but their degradation by microorganisms has not generally been investigated (see Silva-Avalos et al. 1990).

Organism (family, genus or species)	Citation
Acinetobacter species strain (RFBI)	Finnegan et al. 1991
Alcaligenes xyloxidans subs denitrificans	Ingvorsen et al. 1991
Actinomycetaceae	Ware and Painter 1955
Aspergillus niger	Rangaswami and Balasubramanian 1963
"	Iwanoff and Zwetkoff 1936
Bacillus stearothermophilus	Atkinson 1975
Bacillus megaterium	Castric and Strobel 1969
"	Castric and Conn 1971
"	Raef et al. 1977 (b)
Bacillus pumilus	Skowonski and Strobel 1969
"	Meyers et al. 1991
Chromobacterium violaceum	Brysk et al. 1969
"	Macadam and Knowles 1984
"	Rodgers 1982
Enterobacter species (strain 10-1)	Sakai et al. 1981
Escherichia coli	Dunnil and Fowden 1965
"	Lauinger and Ressler 1970
"	Cherryholmes et al. 1985
Fusarium solani	Shimizu et al. 1968
"	Shimizu and Taguchi 1969
Gloeocercospora sorghi	Fry and Myers 1981
"	Fry and Munch 1975
G. sorghi	Nazly et al. 1983
Klebsiella (3 species)	Silva-Avalos et al. 1990
Marasimius oreades	Allen and Strobel 1966
Pholiota adiposa,, P. praecox, P. aurivella	Allen and Strobel 1966
Fusarium nivale	Allen and Strobel 1966
Rizopus nigricans	Allen and Strobel 1966
Pseudomonas acidovarans	Shivaraman and Parhad 1985
"	Shivaraman et al. 1985
Pseudomonas fluorescens	Harris and Knowles 1983
"	Knowles 1988
"	Kunz et al. 1992
Pseudomonas nonliquifaciens	Putilina 1961
Pseudomonas putida (acetonitrile)	Nawaz et al. 1989
Pseudomonas aeruginosa	Cherryholmes et al. 1985
P. aeruginosa (acetonitrile)	Nawaz et al. 1991
Pseudomonas species	Mudder and Whitlock 1984
"	Harris and Knowles 1983
"	Silva-Avalos et al. 1990
"	White et al. 1988
"	Whitlock 1987
Rhizopus nigricans	Allen and Strobel 1966
Rhizopus oryzae	Padmaja and Balagopal 1985
Stemphylium loti	Fry and Millar 1972
"	Nazly and Knowles 1981
"	Nazly et al. 1983

Table 1. Microorganisms reported to degrade cyanide or cyanide-containing compounds to intermediates or innocuous end products (citation is referred to by author(s) and year).

Relative Strength of Complex	Speciation	
1. Free Cyanide	CN^-	
	$HCN_{(g)}$	(9.23, pKa)
2. Simple Cyanide Compounds		
a) readily dissociable	NaCN KCN	
b) relatively undissociable	$Zn(CN)_2$ CuCN AgCN	
3. Weak Metal-Cyanide	$Zn(CN)_4^{-2}$ $Cd(CN)_3^-$ $Cd(CN)_4^{-2}$	
4. Moderately Strong Metal-Cyanide Complexes	$Cu(CN)_2^-$ $Cu(CN)_3^-$ $Ni(CN)_4^{-2}$ $Ag(CN)^{-2}$	(24.00) (28.63) (30.12)
5. Strong Metal-Cyanide Complexes	$Fe(CN)_6^{-3}$ $Fe(CN)_6^{-4}$ $Co(CN)_6^{-4}$ $Au(CN)_2$ $Hg(CN)_4^{-2}$	(45.63) (52.62) (55.48)

Table 2. Relative stability of metal cyanide compounds and complexes in water listed in approximate order of increasing stability (from Smith and Struhsacker 1987). Values in parentheses are log formation constants (Garn and Thomsen 1984).

The little work that has been done on biological treatment of complexed cyanide has focused primarily on biodegradation of nickel cyanides (Rollinson et al. 1987; Silva-Avalos et al. 1990) and iron-cyanides (Finnegan et al. 1991; Cherryholmes et al. 1985). Pettet and Ware (1955) reported that acclimated sewage sludge was capable of destroying complexed cyanide, although the responsible microorganism(s) were not isolated. From an applied waste management perspective, much more research emphasis should be placed on biodegradation of all cyanide species (total cyanide) at a cyanide contaminated site. This is particularly the case when considering the potential for long-term release and chronic cyanide contamination problems resulting from dissociation of the metallo-cyanide complexes.

Objectives and Research Elements

The objective of this study was to determine the potential for bioremediation of total cyanide in heap-leached ore. Specifically, we used cyanide degrading microorganisms for biological treatment of heap-leached ore. Treatment of ore has advantages over remediation of an aqueous contamination plume in that it could potentially treat cyanide waste problems at their source.

For the research, we chose to focus on the heap leached ore at the Summitville Mine rather than an earlier site, the Ortiz Mine (Markwiese and White 1991, 1992: White and Markwiese 1992), for several reasons. Of primary importance was that fact that we had unlimited access to the Summitville site through the US EPA while access to the Ortiz Mine was extremely difficult pending resolution of litigation issues associated with Ortiz owners. Secondly, remediation of the Summitville site was declared a Colorado state emergency and it is currently under consideration for listing on the EPA's Superfund National Priority List. This research was performed while EPA was actively reviewing and in need of information regarding Summitville remediation options; included among these options was biological treatment.

Enhancement of the subsurface environment to maximize microbial degradation of contaminants (in-situ bioremediation) is a technology that offers the advantages of being relatively inexpensive to implement and capable of on-site contaminant attenuation with minimum impact on the soil/ground water system (Hooper 1989; Wilson and Brown 1989). On site biological treatment options can range from adding a limiting growth factor (a process referred to as biostimulation) to adding pollutant-degrading microorganisms (bioaugmentation) to contaminated material.

The first phase of work involved stimulation of the bacterial community in heap-leached ore to degrade cyanide via application of a carbon (sucrose) amendment. Microbial growth in heap-leached ore can be limited by available organic carbon relative to available nitrogen (as cyanide, CN-N and/or nitrate, NO_3-N) sources (White and Markwiese 1992). Previous research indicated that application of a sucrose amendment to dilute slurries of heap-leached ore (5 mg ore in 95 mL liquid media) was a viable means of reducing the concentration of free cyanide (Markwiese and White 1991, 1992; White and Markwiese 1992). We hypothesize that application of a carbon amendment should enhance native cyanide degradation at sites having an active microbial community capable of degrading cyanide.

The second phase of the project was designed to optimize cyanide biodegradation with the addition of specific cyanide-degrading microorganisms to mined ore previously leached with a cyanide solution. Only recently have investigators demonstrated enhanced removal of environmental pollutants by augmentation with specifically adapted microorganisms (Shirkot and Gupta 1985; Lamar et al. 1990; Brodkorb and Legge 1992; Havel and Reineke 1992; Pipke et al. 1992). The effectiveness of microbial augmentation for remediating cyanide heap-leached ore had not been investigated with a rigorous statistical design prior to this study.

METHODS

Site/Sampling Description

The Summitville Mine is located about 40 km south of Del Norte, Colorado in Rio Grande County (Figure 3). The mine is near the old historic mining town of Summitville at an altitude of 3,500 m. Formerly operated by Summitville Consolidated Mining Company Incorporated, the mine is a large tonnage open-pit, heap-leach, gold mine with approximately 10 million tons of ore processed during its operating history (July, 1986-October, 1991). Although mining ceased in 1991, leaching of the heap was only recently discontinued (Environmental Chemical Corporation 1993).

Processed ore was collected from an area that had received a rinse of ore leachate approximately one week prior to sampling. All equipment used to collect the ore samples was sterilized with ethyl alcohol (95%) prior to sampling. The material was sieved on site to <2 cm and immediately transferred to an alcohol-sterilized ice chest for transport to laboratory facilities at the University of New Mexico, Albuquerque, New Mexico.

Growth Medium

A strongly buffered minimal salts broth was used for isolation of bacteria and subsequent growth of cultures. This medium was composed of: (i) 4.25 g KH_2PO_4, 0.012 g $FeSO_4 \cdot 7H_2O$, 0.003 g $MnSO4 \cdot H_2O$, 0.003 g $ZnSO_4 \cdot 7H_2O$, 0.001 g $CoSO_4$, and 0.01 g Nitrilotriacetic acid (per liter deionized water); (ii) $MgSO_4$ (200 g / L) and (iii) $CaCl_2$ (111 g / L). The first solution was adjusted to pH 7.6 with NaOH (1 M) and all solutions were autoclaved separately for 30 min. at 121°C. After sterilization, 1 mL of the second and 0.1 mL of the third solution were added to the first. All chemicals (except for total cyanide in ore) were reagent grade.

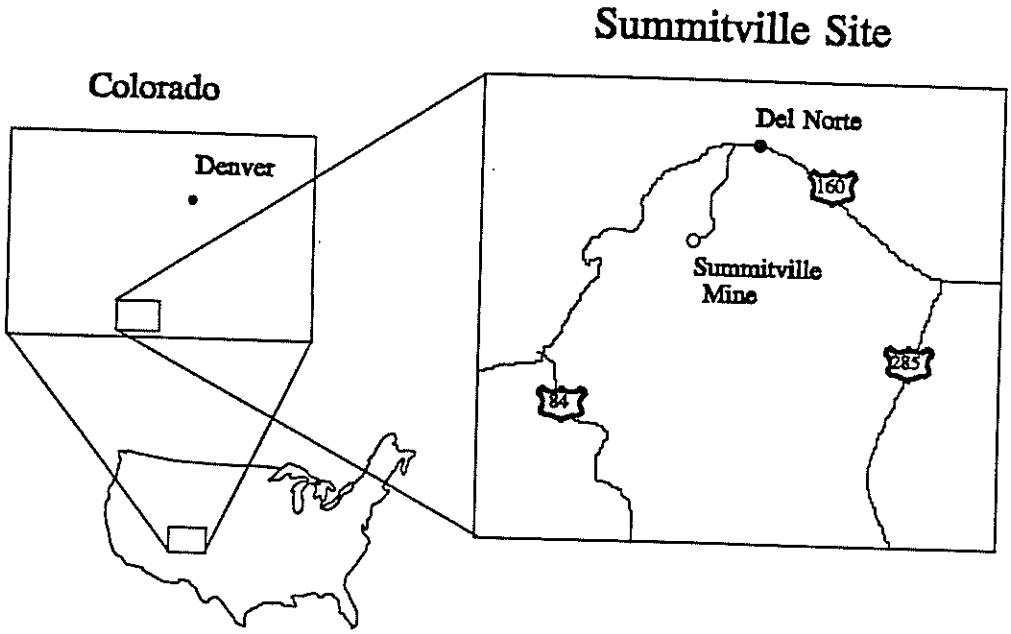


Figure 3. Location map of Summitville Mine

Carbon sources, including glucose (18.0 g/0.1 L) and sucrose (34.2 g/L), were autoclaved separately. NH_4Cl (53.5 g/L) and $Na_2S_2O_3$ (100 g/L) were also autoclaved separately. Buffered (0.443 g CAPS buffer) potassium cyanide (KCN, 0.814g/0.1 L) stock was adjusted to pH 12 with 1 N NaOH and filter sterilized (0.2 um pore-size filter). Filter sterilized potassium iron-cyanide [$K_4Fe(CN)_6$ and $K_3Fe(CN)_6$] stock solutions (0.1 M) were prepared in amber volumetric flasks to prevent photolysis of the cyanide complex.

Hydrogen Cyanide Plates

Plates containing minimal salts broth solidified with 15 g Bacto-agar / L were used with sucrose (20 mM) or glucose (20 mM) as carbon sources. These plates contained no nitrogenous substrate. Plates were inverted and filter disks (1 cm^2) were placed on the lid. Free cyanide solution was prepared by adding 0.8 mL of KCN stock to 4.2 mL buffered (pH 6) dilution water and the 0.2 mL of the resultant solution (20 mM CN^-) was immediately applied to the filter paper. This supplied cyanide in gaseous phase ($HCN_{(g)}$) as the sole nitrogen source (Figure 4). The filter papers were replaced approximately every two days and freshly prepared free cyanide solution was added to the replacement filters. Glucose plates were used to verify cyanide degrading ability only, while sucrose plates were used for both verification of cyanide degrading ability and enumeration of cyanide degrading microorganisms.

Free and Total Cyanide Measurements

Total cyanide in samples of ore slurry were acid-refluxed/distilled in concentrated sulfuric acid for 1 hour and 15 min. (Greenburg et al. 1992; 4500-CN^-C.). Reflux in acid converts complexed cyanide samples to HCN gas which is absorbed in a NaOH solution (Figure 5). Subsequent analysis is for free cyanide. Cyanide determination by acid reflux/distillation

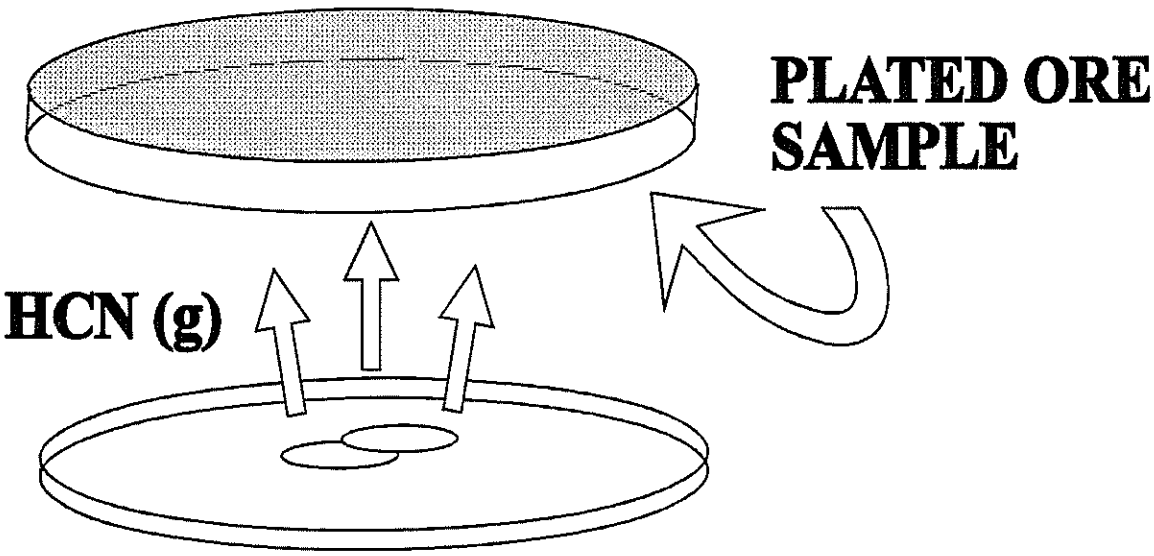


Figure 4. Selection for cyanide-degrading microorganisms in heap-leached ore. Nitrogen for growth supplied as hydrogen cyanide ($HCN_{(g)}$) by applying 0.2 mL of KCN stock (20 mM, pH 6.0) to sterile filter paper on lid of petri plate.

eliminates or reduces virtually all known cyanide interferences and provides the best estimate of total cyanide. This method can measure the cyanide content of most cyanide-complexed metals (excluding cobalt), (Greenberg et al. 1992). A subset of samples (n=3) was also analyzed for weak acid dissociable (WAD) cyanide (Greenberg et al. 1992; 4500-CN^-I.).

Free cyanide (in distillate from ore slurry and in broth culture samples) was measured colorimetrically with chloramine-T (Greenberg et al. 1992; 4500-CN^-E.). This method was adapted for automated analysis on a Technicon Autoanalyzer II which allowed for rapid and highly accurate processing of a large number of samples. Samples were collected in 15-mL polystyrene sterile test tubes and refrigerated until analyzed. At the time of collection, samples were diluted to working range for the Autoanalyzer (0.1 - 1.0 mg CN^- / L) in 20 mM NaOH.

Total Cyanide Calculations:

Total cyanide was calculated as follows:

$$(\mathbf{A} \times \mathbf{B} \times \mathbf{C}) / \mathbf{D} = \text{mg } CN^- / \text{Kg ore} \quad \text{(Eq. 2)}$$

where, **A** = sample cyanide concentration (mg / L), **B** = sample dilution volume (L), **C** = 1000 (g / kg ore) and **D** = moisture corrected sample weight (g)

Screening/Isolation of Cyanide Degrading Microorganisms:

Cyanide heap-leached ore samples were diluted 100-fold with sterilized deionized water and 0.1 mL portions were spread onto HCN plates and incubated in the dark at room temperature. Individual microbial colonies appearing after 4-18 days exposure were streaked onto fresh plates. Cyanide degrading ability was verified by transferring isolates at least five times to fresh plates as indicated above. Growth was compared with control plates lacking nitrogen (after Harris and Knowles 1983). Ore samples were examined for cyanide degraders using HCN plates.

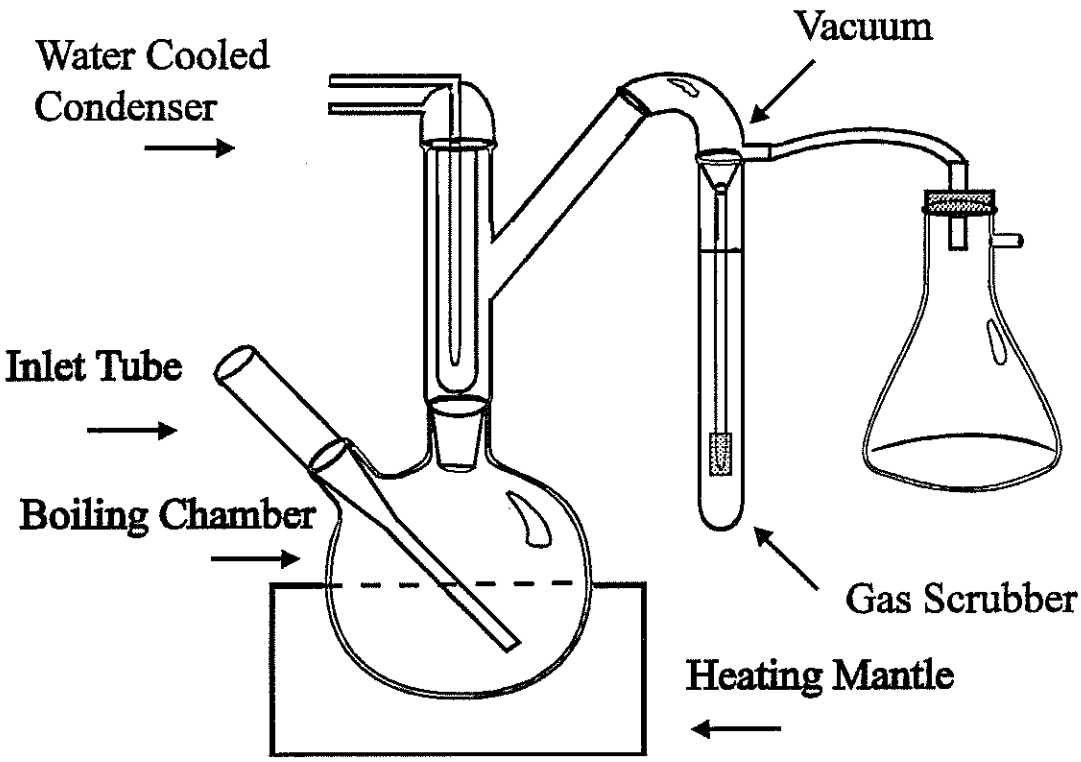


Figure 5. Cyanide distillation apparatus (after Greenburg et al. 1992; 4500-CN-C.)

An isolate from the Summitville ore which showed particularly vigorous growth on HCN plates was identified by its ability to use a characteristic set of carbon and energy sources with Biolog GN Microplates®. Preparation for the carbon-substrate-characterization tests involved culturing on solid Trypticase soy broth (DIFCO, Detroit, MI) prior to Biolog® analysis.

In addition to screening the native microbial community, two other bacterial groups were examined with respect to cyanide-degrading ability using both free and total cyanide. A well characterized cyanide degrader (Harris and Knowles 1983; Knowles 1988; Kunz et al. 1992), identified by the National Collection of Industrial and Marine Bacteria as *Pseudomonas fluorescens* NCIMB 11764, was obtained for use in experiments from culture collection in Torrey, Scotland. Also, a consortium of microorganisms utilized for commercial cyanide bioremediation (hereafter referred to as the Pintail consortium) was obtained from Pintail Systems Inc., Aurora, Colorado. Recent research (Kang and Kim 1993) has indicated that selectively enhanced consortia may be more effective in treating cyanide pollution than use of pure cyanide degraders alone.

Culturing of Cyanide Degraders in Broth on Free Cyanide

Free cyanide utilization in a liquid medium was evaluated in broth culture. All bacterial groups were tested in duplicate flasks. Single colonies from HCN plates were cultured on complete broth media (20 mM sucrose or glucose, 10 mM NH_4Cl) brought to 100 mls total volume in 250 mL Erlenmeyer flasks. Flasks were capped with sterilized sponges to facilitate gas transfer. All cultures flasks were stirred at 500 rpm at 25°C on a 9-station stir plate (Fisher Scientific model 2008).

Samples from a subset of the cultures in complete minimal media (5 mL) were used as bacterial inocula for cyanide decomposition broth experiments. Cells were grown to early stationary phase, harvested by centrifugation (8,000 rpm for 8 min. at 25°C) and resuspended in minimal salts broth (pH 7.6). Growth on minimal media (including NH_4-N) in broth was also

examined with respect to nitrogen (as NH_4Cl) limiting (10 mM vs. 1 mM) conditions for the Pintail consortium. This consortium was grown on sucrose with a single pulse of cyanide as the sole source of nitrogen or in combination with NH_4Cl and/or thiosulfate. In all other tests, cultures were supplied with repeated pulses of the cyanide solution (0.25 mM) to compensate for bacterial utilization and volatilization (as $HCN_{(g)}$). Growth was assessed by absorbance measurements with a Beckman DU ®-64 spectrophotometer (540 nm). In addition to growth (absorbance) measures, cyanide utilization was also determined for *P. fluorescens* on sucrose by comparing free cyanide concentration over time for inoculated cultures and sterile controls.

Culturing of Cyanide Degraders in Broth on Complexed Iron-Cyanide.

Attempts to grow both the Pintail consortium and *P. fluorescens* on complexed iron cyanide (using either $K_4Fe(CN)_6$ or $K_3Fe(CN)_6$) at two concentrations (5 mM and 0.5 mM) were carried out using batch cultures. Respective colonies from HCN plates were transferred to Trypticase Soy Broth (TSB). Cells (5 mL) grown in TSB were harvested at mid-exponential growth phase and used in iron-cyanide decomposition experimental flasks. Total volume for iron-cyanide growth tests was 50 mL $flask^{-1}$. Media for experimental flasks included either sucrose (20 mM) or glucose (20 mM) and minimal salts broth. Bacteria were cultured as in free cyanide broth experiments with the exception that amber flasks were used for protecting the iron cyanide complex from photochemical dissociation. All test flasks were run with an uninoculated control.

Bioremediation of Total Cyanide in Heap-Leached Ore:

Batch incubations were used to evaluate the effects of application of either a sucrose amendment or various inocula of cyanide-degrading microorganisms on biodegradation of free and complexed cyanides in cyanide heap-leached ore. The experimental design consisted of the following treatments: 1) gamma irradiated; 2) minimal salts control; 3) sucrose amended;

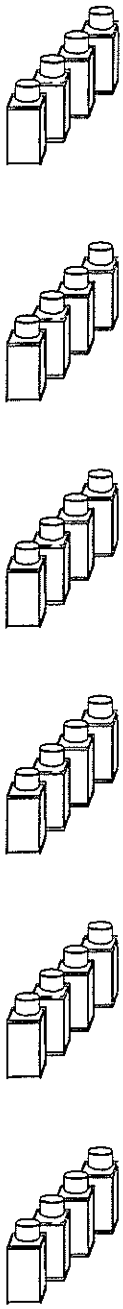
4) *P. fluorescens* inoculum; 5) Summitville inoculum and 6) Pintail inoculum (Figure 6). Each treatment consisted of four replicate flasks. All flasks were sterilized by autoclaving.

Individual flasks received 30 g of ore and 30 mL of minimal salts broth in standard 160 mL dilution flasks. Ore for the gamma-irradiated group was exposed to cobalt source irradiation at 1.5 kilorads/hr for at least 72 hrs at the Department of Nuclear Engineering, University of New Mexico and was used as an abiotic control. The minimal salts control treatment was used to examine the effects of the carrier solution's influence on total cyanide dynamics.

Sucrose (20 mM) was added to the minimal salts broth for the sucrose-amended treatment. In addition to minimal media broth and sucrose, treatments 4, 5 and 6 used bacteria (taken from respective HCN plates) grown to early stationary phase in complete (20 mM sucrose, 10 mM NH_4Cl) broth as inoculants. Large quantities of cells were produced this way. Cells were enumerated using a hemocytometer, harvested by centrifugation (10,000 rpm for 10 min. at 25°C), resuspended in minimal salts broth and added to flasks for a final inoculum density of 10^8 cells/g slurry. Incubations were conducted for 52 days in the dark and under aerobic conditions at 25°C. Flasks were capped with sterilized sponges to facilitate gas exchange.

Intense sampling was conducted at the experiment's initiation and at approximately two week intervals thereafter. Immediately prior to sampling, flasks were shaken and a pair of sub samples (1 mL ea.) of the ore slurry were taken using a Rainin pipetman (p-1000) fitted with a sterile, wide-hole plastic tip (i.e. with the end cut off). Of the pair, one sample for total cyanide measurement was emptied into a sterile 15-mL polystyrene dilution tube, weighed and immediately frozen. Analysis of fresh and frozen ore samples showed that freezing had no effect on total cyanide concentration. The remaining sample was combined with samples from the other flasks (within each treatment), weighed, oven dried (100°C for 24 hrs) and reweighed for determination of percent moisture.

Rates of total cyanide loss in experimental treatments were compared to gamma-irradiated and non-sterile ore samples receiving minimal salts only. Comparisons involved



1. Gamma Irradiated

2. Minimal Salts Media

3. Sucrose Amended

4. *Pseudomonas fluorescens* + Sucrose

5. Summitville Isolate + Sucrose

6. Pintail Consortium + Sucrose

Figure 6. Experimental design for total cyanide biodegradation study.

generating regression lines of total cyanide over time (52 days, 6 sampling intervals) for individual flasks (n = 4) in each treatment (n = 6). The average rate of total cyanide loss for each treatment (i.e. mean of slopes for individual flasks/treatment) was analyzed with a one-way analysis of variance procedure followed by Tukey's and Scheffe's pairwise multiple comparisons test (SAS Inst. Inc., 1985).

RESULTS AND DISCUSSION

Screening/Isolation of Cyanide Degrading Microorganisms

Screening for cyanide degrading microorganisms is an inexpensive and relatively straightforward process. This procedure can provide information on the native microflora's ability to degrade cyanide in addition to providing data on abundance of cyanide degraders within heap-leached ore.

Two ore samples from the Summitville site were plated for isolation of cyanide degrading microorganisms on HCN plates. Circumstantial evidence from this revealed that numbers of cyanide degraders at the Summitville site in Colorado were quite low with less than 100 cyanide degrading microorganisms/g ore material. However, both ore samples tested were positive for growth of bacteria on cyanide. These results lend further support to the concept that microorganisms are capable of colonizing cyanide heap-leached ore. A study at the Ortiz Gold Mine, a cyanide heap-leach operation located in Cerrillos, New Mexico revealed numbers of cyanide degrading microorganisms ranging from 10^3-10^5 bacteria/g ore in the spent ore pile at the Ortiz Gold Mine (Markwiese and White 1991). These microorganisms may have been introduced to the Ortiz pile (as air-borne microorganisms or on dust particles) during the leaching process, or during storage of the spent ore (White and Markwiese 1992).

At least four colony morphologies comprised the cyanide-degrading community within the Summitville ore. Colonies were isolated by streaking on plates with HCN (g) as the sole source of nitrogen. Of the four isolates, one microorganism showed particularly vigorous growth on cyanide and was selected for further experiments. This organism is referred to as the Summitville isolate. Gram staining showed that this isolate is a gram-negative rod. Results from a Biolog® analysis tentatively identified it as *Pseudomonas fluorescens* type II with a similarity coefficient of 0.725.

It may not be surprising that the Summitville isolate was identified as a Pseudomonad (*P. fluorescens* type II) considering the diverse enzyme systems exhibited by this group. Pseudomonads are particularly well suited for environmental remediation work because they can utilize a diverse array of organic substrates. In addition, many species of *Pseudomonas* do not require specific vitamins, growth factors or amino acids and grow on several carbon sources (Chapelle 1993). Relative to other microorganisms, *Pseudomonas* species often are able to withstand a much wider range of environmental conditions, making them ideal candidates for bioremediation of cyanide wastes in a variety of settings.

In addition to the Summitville isolate, *Pseudomonas fluorescens* NCIMB 11764 and the Pintail consortium showed positive growth on HCN plates (Table 3). Positive growth was indicated by colony formation on HCN plates after successive (5) transfers. It should be noted that not all members of the Pintail Consortium showed equal ability to utilize cyanide as a source of nitrogen on the HCN plates. Visual inspection indicated that particular colony morphologies were selected for differentially upon repeated subculturing. Subculturing probably selected for those organisms able to outcompete other species for available nitrogen only available as CN-N.

Culturing of Cyanide Degraders in Broth on Free Cyanide

The bacteria's ability to use cyanide in broth culture may be particularly important when assessing the potential to bioremediate aqueous waste streams contaminated with cyanide. Broth culture more closely mimics conditions of an aqueous sample and bioremediation plans typically involve inoculation of contaminated material using a liquid carrier solution. Therefore, evaluation of batch culture growth for each bacterial group was carried out prior to inoculation experiments.

GROWTH MEDIA						
Culture	**HCN Plates $HCN_{(g)}$**		**Broth CN^-**		**Broth [$K_3Fe(CN)_6$ or $K_4Fe(CN)_6$]**	
	glucose	sucrose	glucose	sucrose	glucose	sucrose
Summitville Isolate	+	+	-	-	-	-
Pintail consortium	+	+	+	-	-	-
P. fluorescens	+	+	+	+	-	-

Table 3. Qualitative assessment of cyanide degrading ability for Summitville Isolate, Pintail consortium and *Pseudomonas fluorescens.* Cyanide supplied as sole source of nitrogen in plates ($HCN_{(g)}$) and broth culture (CN^- or $K_3Fe(CN)_6$ or $K_4Fe(CN)_6$) with glucose or sucrose serving as carbon sources. Symbols: (+) growth; and (-) no growth.

Summitville Isolate

Growth was scored as positive in broth culture if the microorganisms showed an increase in optical density. Attempts to culture the Summitville isolate with free cyanide as the sole source of nitrogen were unsuccessful in broth (using both glucose and sucrose) experiments (Table 3). However, this isolate could grow on broth media with a nitrogen source provided (10 mM NH_4Cl) and demonstrated cyanide utilization from plating studies. Relative to other inoculants (Pintail consortium and *P. fluorescens*) used in this study, the Summitville isolate appears less likely to grow under more strict culture conditions where cyanide serves as the sole source of nitrogen.

Pintail Consortium

Two concentrations of NH_4Cl (10 mM and 1 mM) were used to examine the effects of growth in complete minimal media on subsequent cyanide degradation tests in broth for the Pintail consortium (Figure 7). The 10 mM NH_4Cl treatment resulted in an order of magnitude greater number of cells than the 1 mM treatment (data not shown). These results are logical as the growth-limiting substrate (nitrogen) was 10 times more concentrated in the former treatment. Inoculum density was normalized between treatments by adding ten-fold more of the more dilute (1 mM NH_4Cl) culture to experimental flasks. Results of culture tests (Figures 8 and 9) indicate that pre-inoculation conditions do not dramatically alter subsequent growth in cyanide degradation tests.

Attempts to culture inocula from either the 10 mM or 1 mM NH_4Cl treatments on cyanide as the sole source of nitrogen were unsuccessful with sucrose as the carbon source (Figure 8 Table 3). However, when this experiment was repeated with added nitrogen as NH_4Cl (10 mM) growth occurred (data not shown). Growth for the Pintail consortium was positive when cyanide (0.25 mM), NH_4Cl (10 mM), $Na_2S_2O_3$ (57 mM)

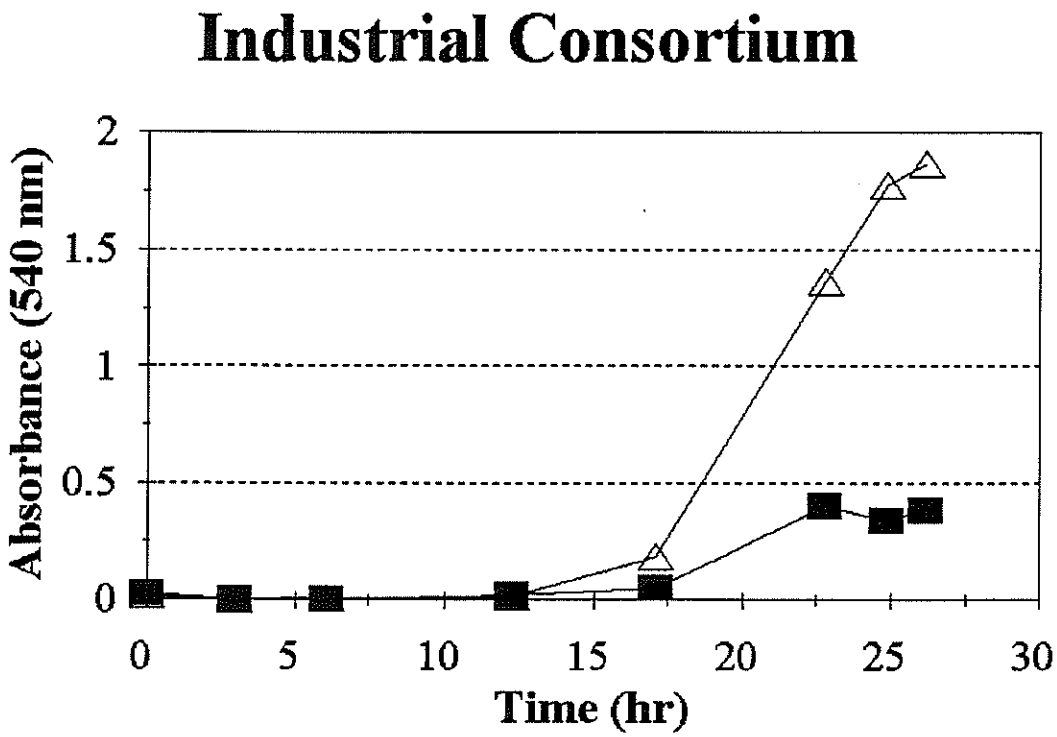


Figure 7. Growth of Pintail consortium in sucrose (20 mM) minimal medium in batch culture at two NH_4Cl levels. Symbol: Δ, 10 mM NH_4Cl; and ■, 1 mM NH_4Cl.

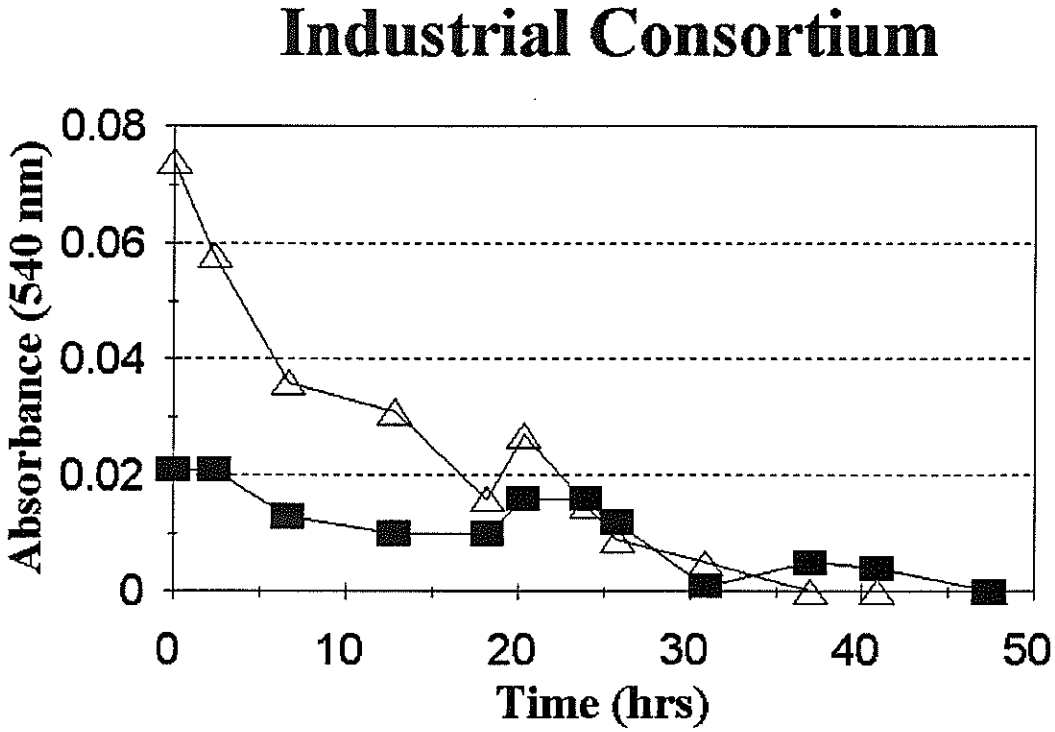


Figure 8. Industrial (Pintail) Consortium in sucrose (20 mM) minimal medium in batch culture with 0.25 mM CN^- as the sole source of nitrogen. Inocula taken from the 1 mM and 10 mM NH_4Cl broth treatments. Symbol: Δ, 1 mM; and ■, 10 mM NH_4Cl treatments.

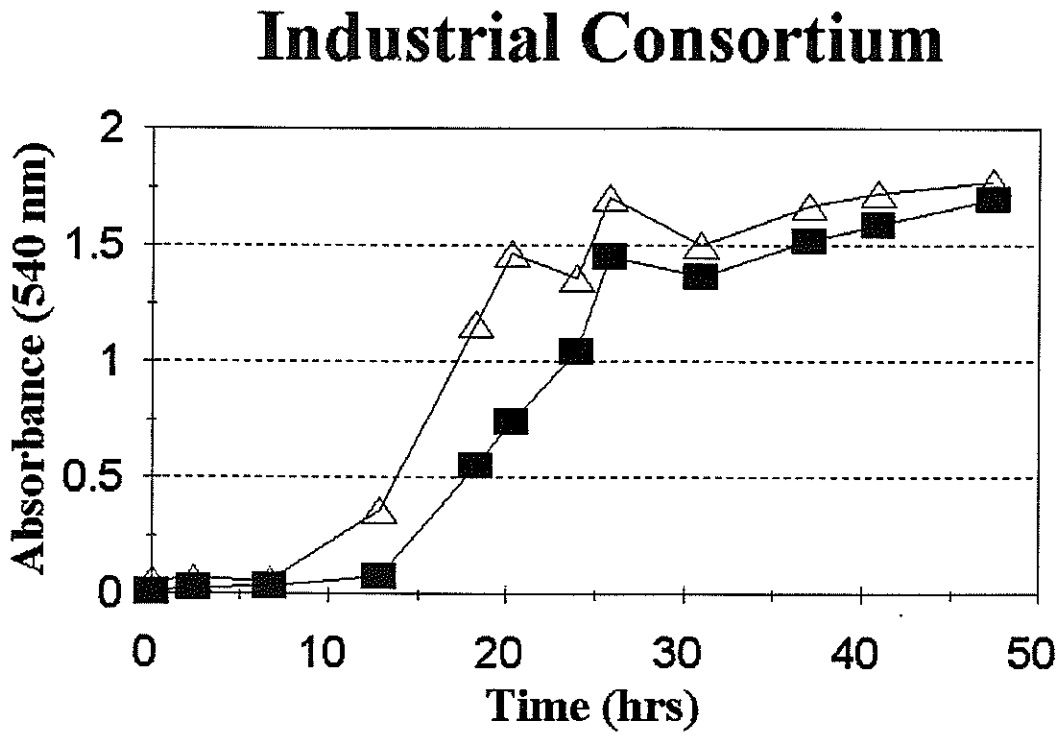


Figure 9. Growth of Pintail consortium in sucrose (20 mM) minimal medium in batch culture with 0.25 mM CN^-, 10 mM NH_4Cl, and 57 mM $Na_2S_2O_3$. Inocula taken from the 1 mM and 10 mM NH_4Cl broth treatments. Symbol: Δ, 1 mM; and ■, 10 mM NH_4Cl treatments.

and sucrose (20 mM) were used in the broth medium (Figure 9). Growth was also positive on glucose with cyanide (CN^-) as the sole source of nitrogen.

It is likely that at least one member of the consortium was able to reduce cyanide toxicity by catalyzing the following reaction:

$$CN^- + S_2O_3^{-2} \Rightarrow CNS^- + SO_3^{-2} \qquad \text{(Eq. 3)}$$

An enzyme common to many bacterial genera, rhodanase (thiosulfate:cyanide sulfur transferase, EC 2.8.1.1), is responsible for the enzymatic cleavage of the S-S bond of thiosulfate to form thiocyanate and sulfite (Volini and Alexander 1981). Thiocyanate (CNS^-) is much less toxic to aquatic life than free cyanide (Doudoroff 1976).

In contrast to the Summitville Isolate, the Pintail consortium showed positive growth on cyanide in broth culture when glucose was used as the carbon source (Figure 10, Table 3). In addition, there was a greater acclimation phase to cyanide additions observed for the Pintail consortium compared to that evidenced by *P. fluorescens* with glucose as a carbon source (Figures 10 and 11). This lag prior to the onset of rapid cyanide degradation and bacterial growth may represent a community shift within the consortium to cyanide degraders which subsequently metabolized the available glucose. The Pintail consortium used for broth tests (with glucose as a carbon source) had already been transferred 5 times on HCN plates with presumed selection at each transfer. Broth conditions probably represent another selective environment as evidenced by growth following a prolonged lag phase (distinct growth increase after 125 hrs of incubation, Figure 10).

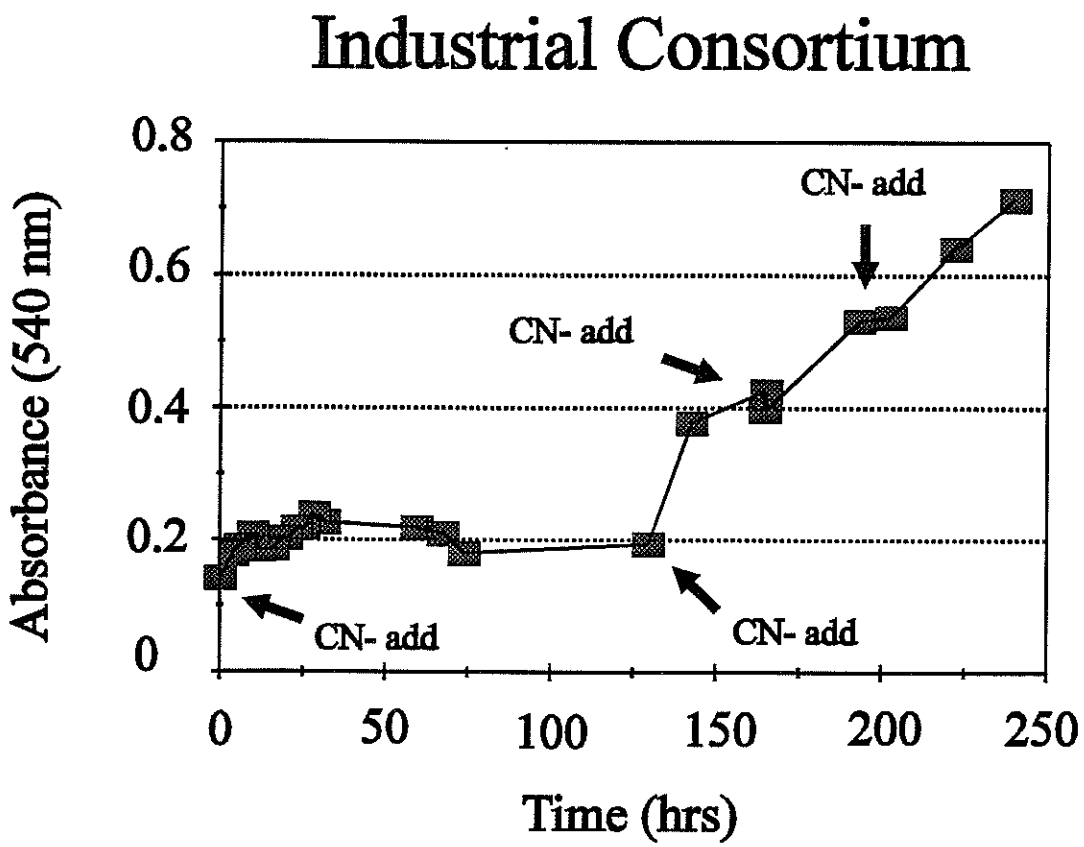


Figure 10. Growth of Pintail consortium in a glucose (20 mM) minimal medium in batch culture pulsed at the times indicated with 0.25 mM cyanide as the sole nitrogen source. Note the growth response to cyanide additions at approximately 125 hrs. Symbol: ■, growth.

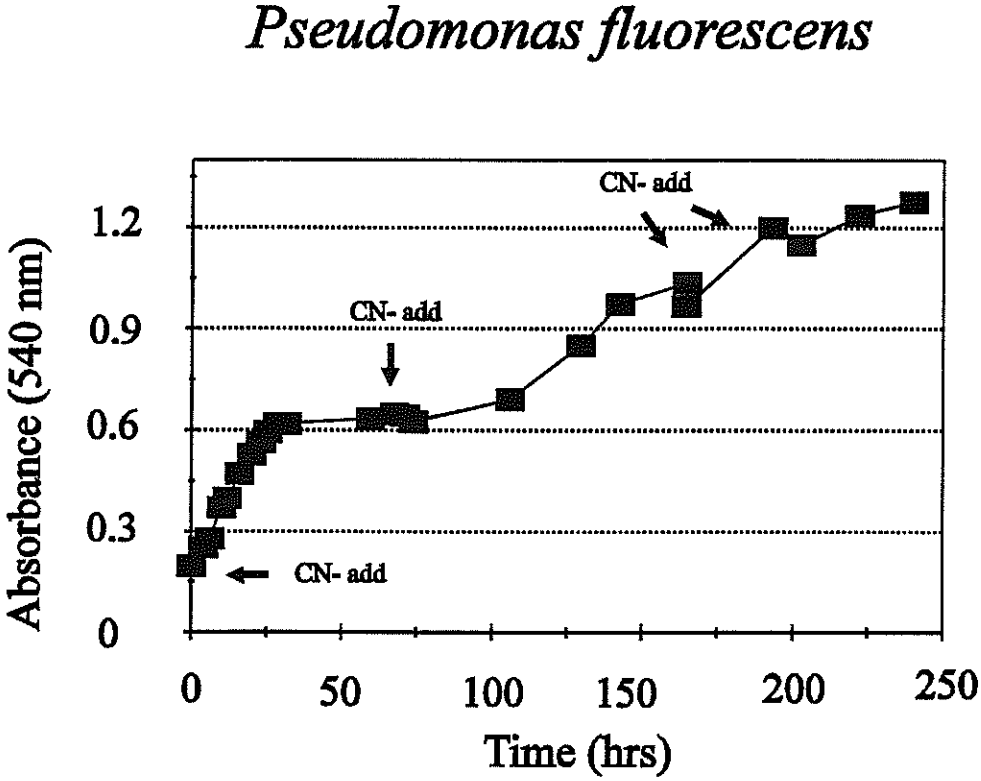


Figure 11. Growth of *P. fluorescens* in a glucose (20 mM) minimal medium in batch culture pulsed at the times indicated with 0.25 mM cyanide as the sole nitrogen source. Note the growth response to cyanide additions. Symbol: ■, growth.

Pseudomonas fluorescens

P. fluorescens was able to use both of the carbon substrates in broth culture (sucrose and glucose) with cyanide as its sole source of nitrogen (Figures 11 and 12). This reflects the diverse enzymatic capabilities of this genus (Chapelle 1993). Definitive evidence of bacterial cyanide utilization was provided by both growth of the organism (GROWTH) and faster rates of cyanide loss in inoculated flasks (EXP [CN-]) versus sterile controls (CTL [CN-]) (Figure 12). Tests in our laboratory with *P. fluorescens* have repeatedly demonstrated its ability to degrade free cyanide after being cultured on a variety of carbon and nitrogen substrates and after prolonged storage at -70°C. Such attributes may be particularly useful for transport and culture of the organism at remote sites for eventual application to cyanide contaminated material. Considering the capacity of *P. fluorescens* to use cyanide with sucrose and glucose in both broth and on plates, it is somewhat surprising that the Summitville isolate did not share these abilities as it was also identified as a *P. fluorescens* (Type II).

Culturing of Cyanide Degraders in Broth on Complexed Iron-Cyanide

Attempts to culture bacterial groups on complexed iron cyanide (using either $K_4Fe(CN)_6$ or $K_3Fe(CN)_6$) were unsuccessful (Table 3). All cultures (n=3) tested could grow in the presence of either iron cyanide compound (at either 5 mM or 0.5 mM) with sucrose or glucose and dilute Trypticase Soy Broth (i.e. amount carried over with the inoculant). However, iron cyanide as the sole source of nitrogen could not support growth (data not shown). Free cyanide was not used as a potential nitrogen source in these experiments.

There have been two reports of bacterial iron-cyanide degradation in the literature. These studies are, however, equivocal. Cherryholmes et al. (1983) found that, in the

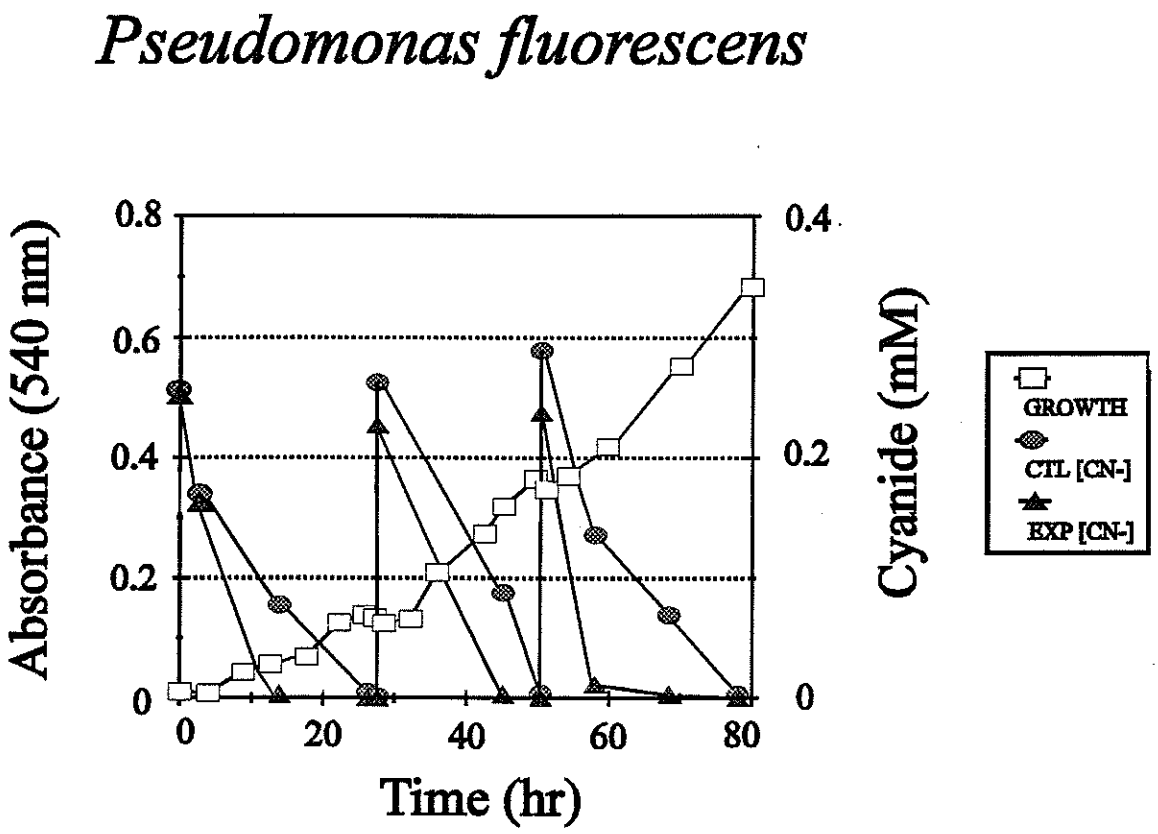


Figure 12. Growth of *P. fluorescens* in a sucrose (20 mM) minimal medium in batch culture pulsed at the times indicated with 0.25 mM cyanide as the sole nitrogen source.

presence of microorganisms, free cyanide (CN^-) is formed in solutions containing hexacyanoferrate ($K_3Fe(CN)_6$). Meeussen et al. (1992) have criticized this study pointing out that the release of free cyanide is not necessarily directly induced by microbial activity.

Cherryholmes et al. (1985) report a pH drop in inoculated treatments (pH 7.9 to 5.7 after 25 days) and a slight pH increase (pH 7.5 to 7.8) in uninoculated treatments over the experimental period indicating the effects of biotic activity. The shift towards acidic conditions in the inoculated treatment could have increased the rate of chemical dissociation of the complexed hexacyanoferrate. Doudoroff (1956, 1976) has shown that the rate of dissociation of metallocyanide complexes increases with decreased pH. Consequently, the microbial role in Cherryholmes et al. (1983) may not have been direct biotic utilization of the metal complexed cyanide but an indirect effect of lowering pH and consequently increasing the rate of dissociation of metal complexed cyanide to free cyanide. It was not reported whether microorganisms were able to utilize the free cyanide generated in these experiments.

Finnegan et al. (1991) investigated the microbial assimilation of a range of cyano-metal complexes (including complexed iron-cyanide) by an *Acinetobacter* species. This organism was reported to grow on ferri/ferro complexed cyanide although there is no mention of redox or light conditions in the experimental design. Meeussen et al. (1992) have shown that exposure of complexed iron-cyanide to even diffuse daylight results in complete dissociation of the complex within days. Consequently, the observed growth of the *Acinetobacter* species in the Finnegan et al. (1991) experiments could be the result of incorporation of free cyanide released by abiotic mechanisms and not utilization of the complex by bacteria.

Biodegradation of Total Cyanide in Heap-Leached Ore:

The results of the experiments showed that glucose was an excellent carbon source for free cyanide biodegradation experiments. Bacterial growth on HCN plates with glucose as the carbon source was positive for all groups tested (Table 3). Glucose was also able to support growth for the industrial consortium in broth (Figure 8 vs. Figure 10) when cyanide was the sole source of nitrogen (Table 3). However, sucrose was used as the carbon source in the following total cyanide biodegradation experiments for several reasons: 1) sucrose is considerably less expensive than glucose and, as this study was targeted at remediation of an actual site, the most economically feasible carbon source was used; 2) all bacterial groups used in this study could use sucrose as the carbon source with cyanide as the sole source of nitrogen on HCN plates; 3) growth of *P. fluorescens* was comparable for both carbon sources at 80 hr (Figures 11 and 12) in broth culture and 4) glucose binds with cyanide (Pigman and Horton 1972) which could add a potential abiotic component to cyanide removal (Raef et al. 1977a) in the experimental system.

Tests on the composition of complexed cyanide species in Summitville ore showed that a large portion of the total cyanide is in a strongly complexed form. Weak acid dissociable (WAD) cyanide analyses on Summitville ore represented about 55% of the cyanide from total cyanide distillations (data not shown). Consequently, the proportion of strongly complexed cyanide in Summitville heap leached ore was approximately 45%.

Rates of total cyanide loss in experimental treatments were compared to gamma irradiated and non-sterile ore samples receiving minimal salts only. Rates are presented as the average of individual flasks for the sucrose amendment (Figure 13), *P. fluorescens* (Figure 14), Summitville Isolate (Figure 15) and Industrial consortium (Figure 16) treatments. Results of the F-test on the rate of total cyanide loss indicate some treatment effect at the alpha = 0.05 level ($F_{5,18} = 3.06$, $P = 0.036$). However, when pairwise multiple comparisons using both Tukey's and Scheffe's test (SAS Inst. Inc., 1985)

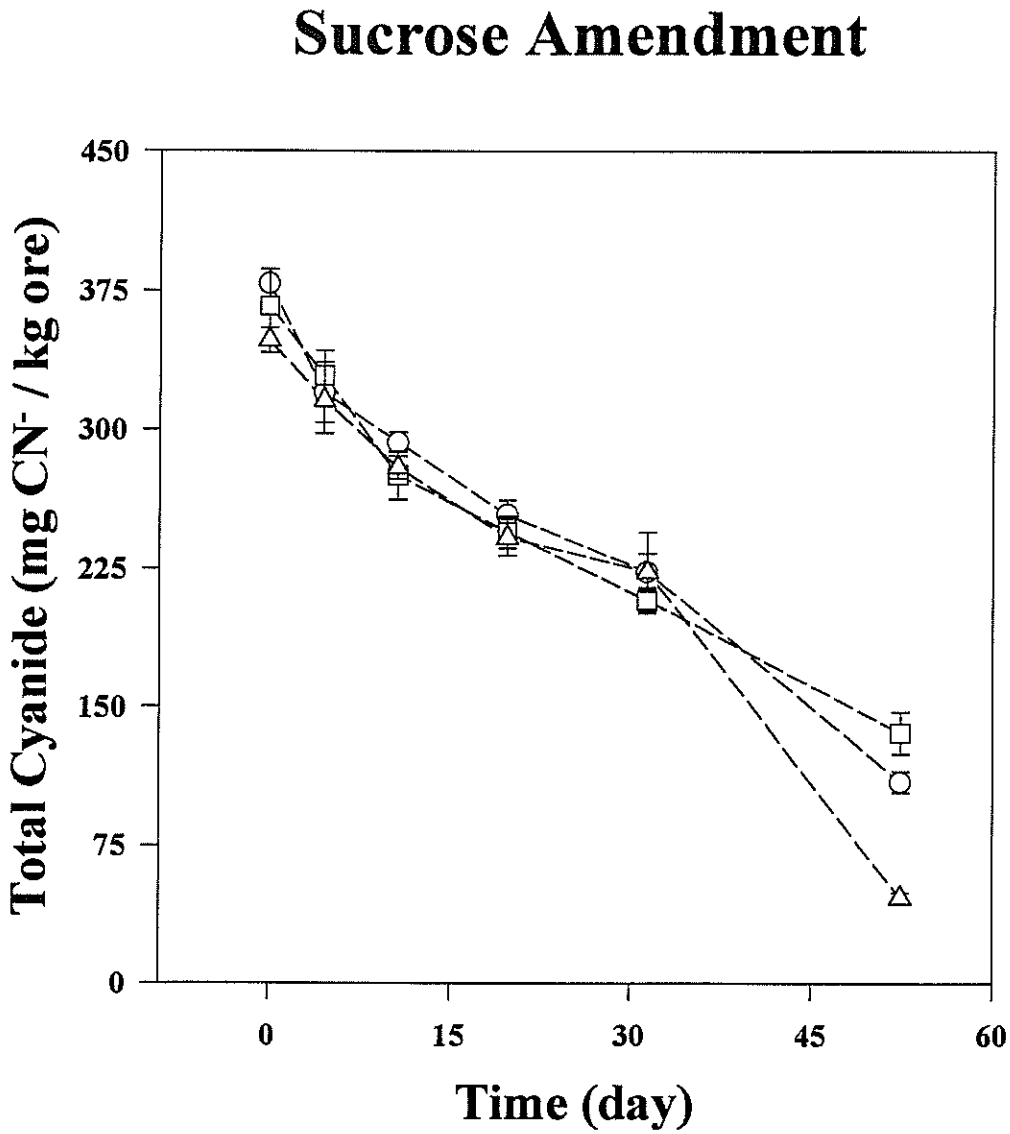


Figure 13. The concentration of total cyanide in heap-leached ore from the Summitville site is shown over a 60 day period. Error bars are ± 1 SE of the mean (n=4). Symbols: □, Sucrose Amended; ○, Minimal Salts Media; and Δ, Gamma Irradiated.

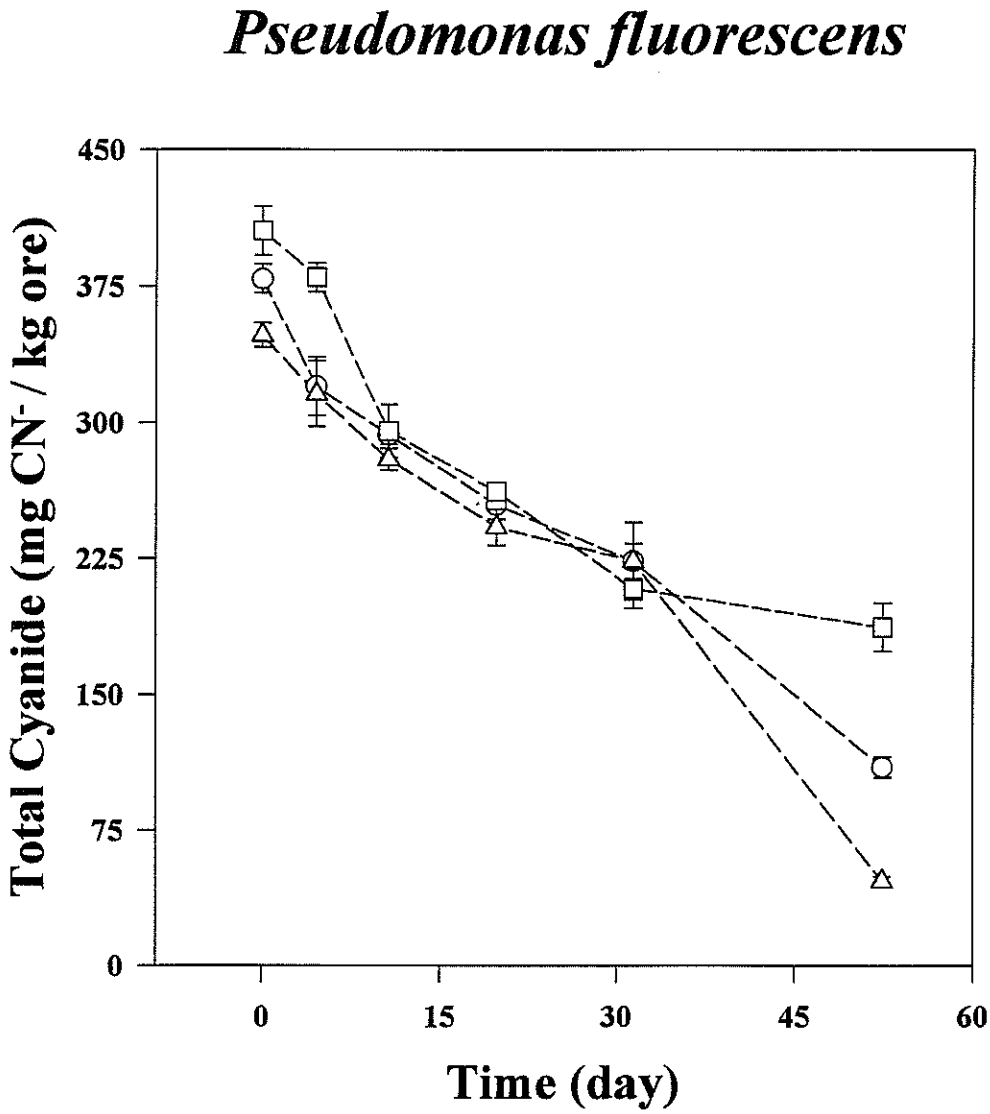


Figure 14. The concentration of total cyanide in heap-leached ore from the Summitville site is shown over a 60 day period. Error bars are ±1 SE of the mean (n=4). Symbols: □, *P. fluorescens*; ○, Minimal Salts Media; and Δ, Gamma Irradiated.

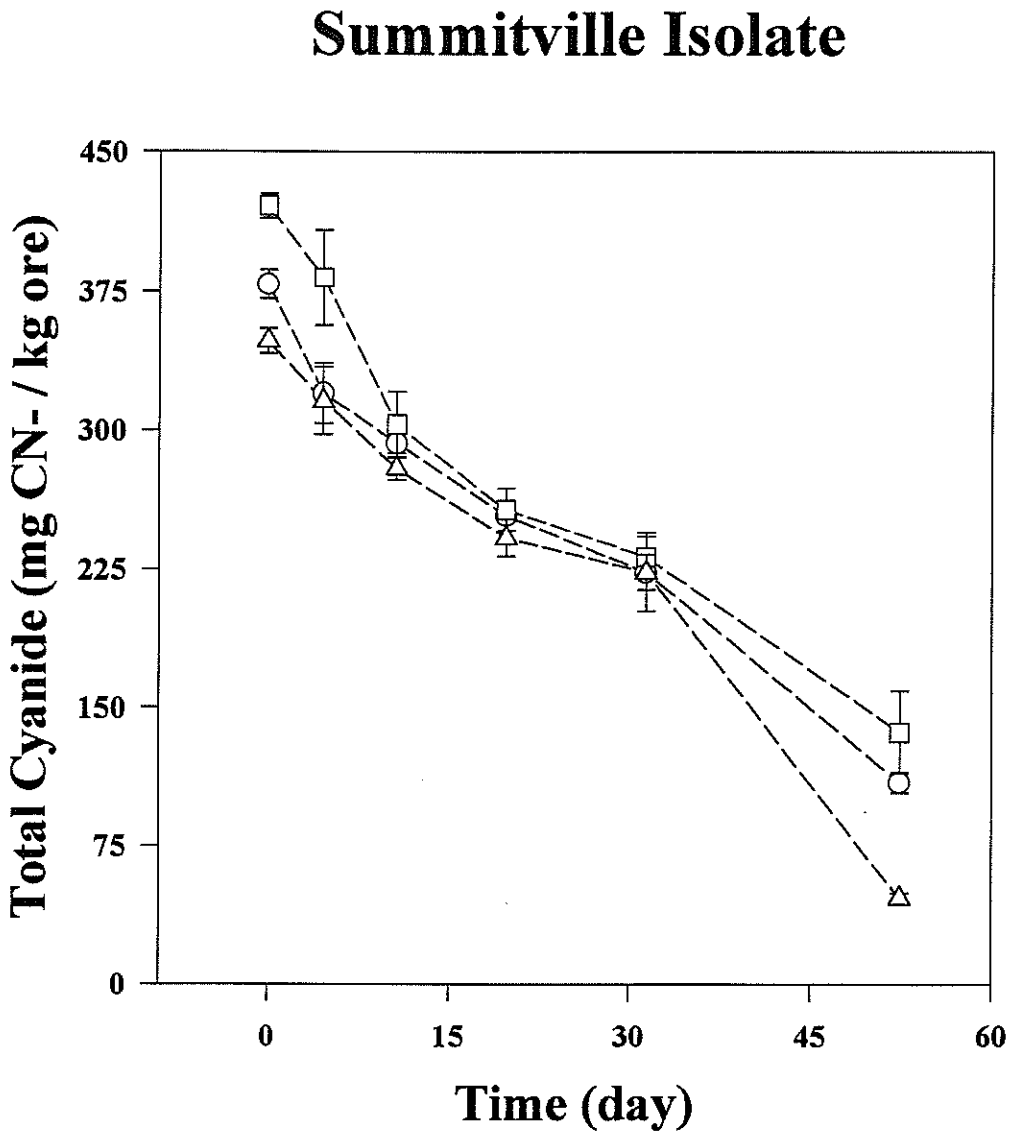


Figure 15. The concentration of total cyanide in heap-leached ore from the Summitville site is shown over a 60 day period. Error bars are ± 1 SE of the mean (n=4). Symbols: □, Summitville Isolate; ○, Minimal Salts Media; and △, Gamma Irradiated.

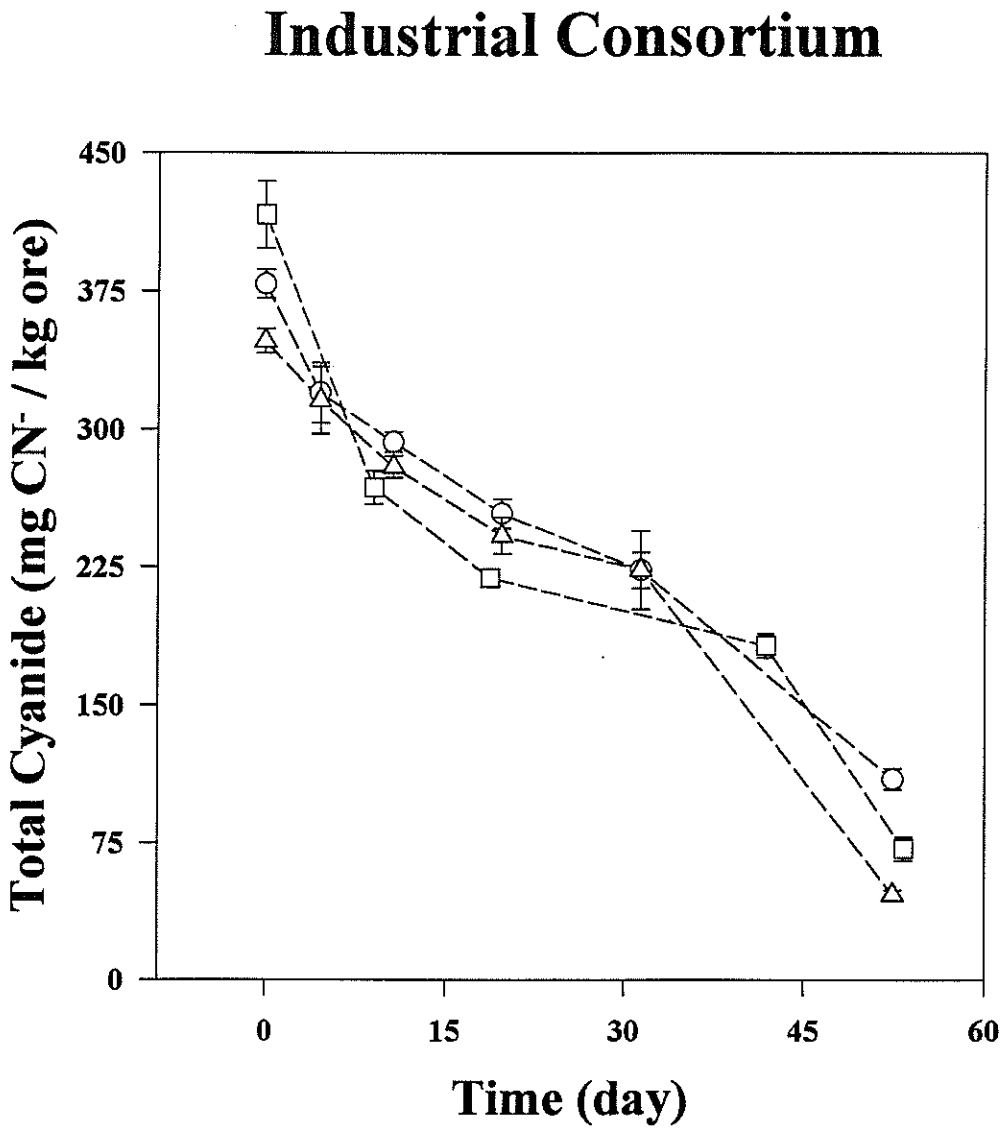


Figure 16. The concentration of total cyanide in heap-leached ore from the Summitville site is shown over a 60 day period. Error bars are ± 1 SE of the mean (n=4). Symbols: □, Industrial (Pintail) Consortium; ○, Minimal Salts Media; and Δ, Gamma Irradiated.

were performed, this effect was not strong enough to show significant differences in total cyanide loss between any two treatments.

Total cyanide in heap-leached ore decreased at a relatively linear rate of approximately 4.9 (± 0.7) mg CN^-/kg ore/day (Figures 13-16). There was no indication of bacterial control on this loss rate. . The loss of complexed cyanide occurred at the same rate in abiotic and biotic samples. Total cyanide loss were not significantly influenced by the addition of a carbon amendment, single cyanide degrading isolates or a consortium of cyanide degraders. Comparison of experimental treatments with the sterile (gamma irradiated) control indicate that abiotic processes of complex dissociation control rates of total cyanide loss in the experimental system.

Free cyanide released by dissociation from the metal-complexed cyanide may have been utilized by the cyanide degrading community or volatilized to the atmosphere. The fate of released free cyanide in experimental flasks was not determined. Growth experiments with iron-cyanide as the sole nitrogen source in batch culture support the conclusion of no direct utilization of metal-complexed cyanide by the microorganisms tested.

These results indicate that complexed cyanide in heap-leached ore will dissociate and liberate free cyanide under saturated conditions. Four major sinks exist for loss of free cyanide from this type of system (Figure 17). Of particular concern is infiltration of dissociated free cyanide into local aquatic ecosystems (arrow 1) due to the high toxicity of cyanide in this form. Although gaseous cyanide ($HCN_{(g)}$) is also toxic, dilution in the atmosphere minimizes the risk associated with volatilization (arrow 2). Bacteria can naturally incorporate cyanide-carbon and -nitrogen into cell tissue (arrow 3), respire the carbon as carbon dioxide (CO_2, arrow 4) or convert the nitrogen into ammonia (NH_3, arrow 5).

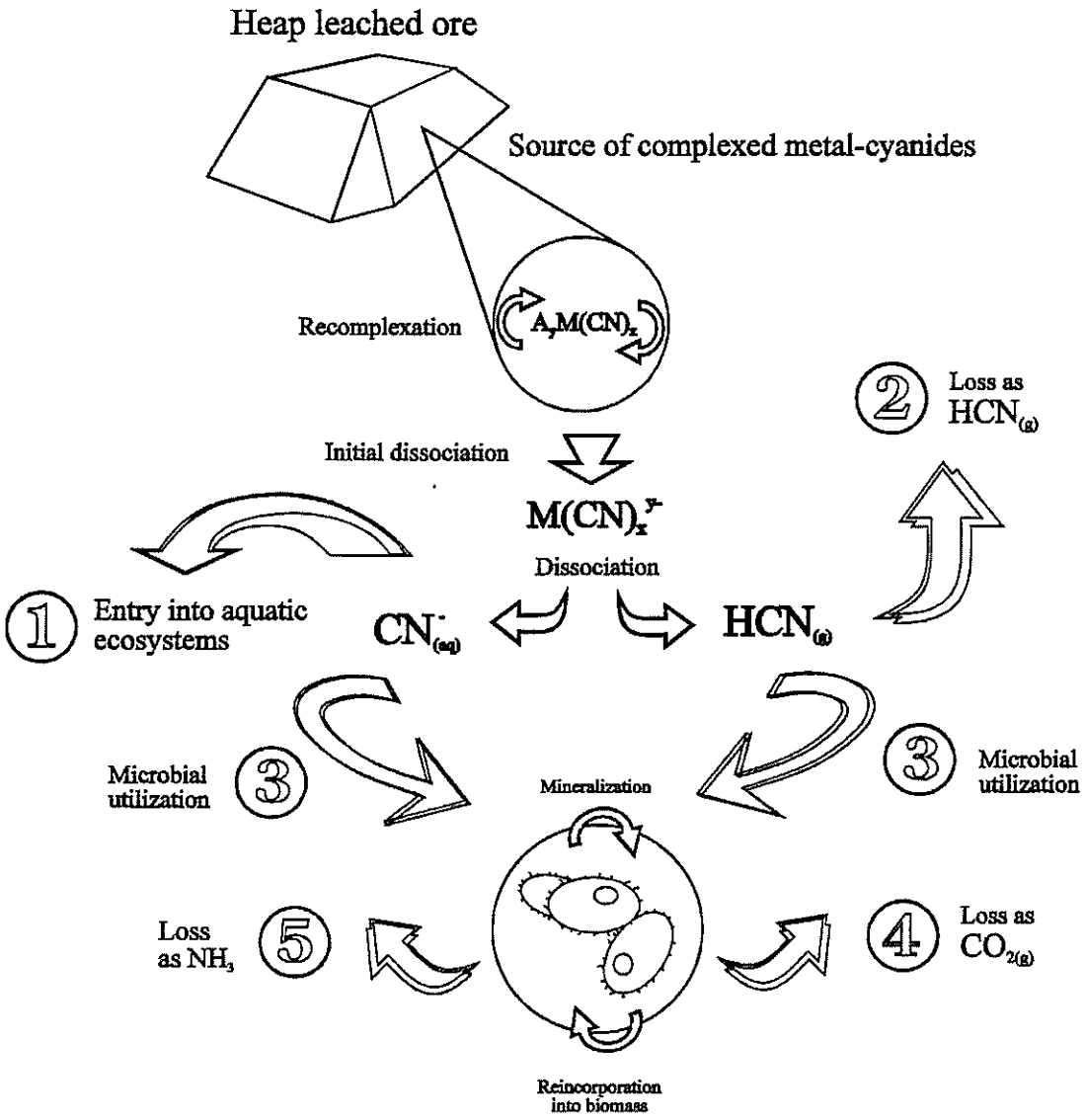


Figure 17. Potential fates of free cyanide generated from dissociation of complexed metal cyanide in heap leached ore. In the formula $A_yM(CN)_X$, A represents an alkali element present y times, M the heavy metal (ferrous or ferric iron, copper, silver and others), and x the number of CN groups. See text for explanation of numbered arrows.

Bacteria in cyanide-contaminated ore may provide an important filter to scrub free cyanide from leachate prior to entry into local waterways. The fact that Summitville microorganisms can use free cyanide may be an important consideration for bioremediation of aqueous waste streams or leachate from contaminated ore, where cyanide may exist largely in the uncomplexed form, prior to entry into the surrounding surface water or groundwater ecosystems.

PRINCIPAL FINDINGS

1. The Summitville ore has an indigenous population of microorganisms capable of degrading and utilizing free cyanide as a sole source of nitrogen. These organisms were successfully cultured and isolated in the laboratory on plates with cyanide supplied as $HCN_{(g)}$. A microorganism from the Summitville site, dubbed the Summitville isolate, was cultured in complete (20 mM sucrose or glucose, 10 mM NH_4Cl) broth as well. This organism has been tentatively identified as *Pseudomonas fluorescens* Type II.
2. *P. fluorescens* NCIMB 11764 and an industrial microbial consortium from Pintail Systems Inc. were able to use cyanide as a sole nitrogen source on HCN plates and under certain broth conditions, although they were not able to use complexed iron-cyanide (either $K_3Fe(CN)_6$ or $K_4Fe(CN)_6$) as a sole source of nitrogen in the broth culture conditions employed in this study.
3. The addition of a carbon (sucrose) amendment had no significant effect on loss of total cyanide in ore microcosms.
4. The addition of various cyanide-degrading bacterial inocula, including a native isolate, a well characterized cyanide degrader and a microbial consortium, did not result in significantly enhanced rates of total cyanide removal compared controls in ore microcosms.
5. Abiotic processes controlled the rates of total cyanide loss in cyanide heap-leached ore. This loss was relatively linear over time at an approximate rate of 4.9 ($\pm$ 0.7) mg CN^-/kg ore/day for an ore with an initial contamination level of approximately 400 mg total CN^-/kg ore.

CONCLUSIONS

1. Free cyanide in the gaseous form ($HCN_{(g)}$) is readily utilized by cyanide degrading microorganisms from known cultures and isolates from heap-leached ore piles.
2. Screening indigenous microbial communities associated with cyanide heap-leached ore for the ability to use cyanide is a relatively simple process. Isolation of bacteria on HCN plates provides qualitative and quantitative information about the cyanide degrading community in contaminated ore in approximately 4-18 days.
3. Certain bacteria can use free cyanide (CN^-) in an aqueous medium. This has important implications for bioremediation of waste streams contaminated with free cyanide.
4. The microorganisms used in this study did not demonstrate the ability to degrade complexed iron-cyanide under the experimental conditions employed. The lack of growth on iron-complexed cyanide in broth and the negligible biotic effect on total cyanide loss rates in cyanide contaminated ore support this conclusion.
5. Abiotic processes that control the rate of dissociation of the metal cyanide complexes are the rate-limiting step in the loss of total cyanide from contaminated ore from Colorado's Summitville Mine.
6. Total cyanide was not persistent in cyanide heap-leached ore under the conditions employed in this experiment. A loss rate of approximately 4.9 ($\pm$ 0.7) mg CN-/kg ore/day was measured. The addition of a carrier fluid, minimal salts broth, to contaminated ore in batch incubations provided an approximation of current remediation efforts (rinsing the ore pile with water) at the Summitville site. Loss of total cyanide in heap-leached ore can be expected to occur at a comparable rate if physical-chemical conditions of the site are similar to those employed in this experiment.

SUMMARY AND RECOMMENDATIONS

Two techniques were evaluated for potential in situ biological treatment of total cyanide. These techniques included the addition of a carbon amendment and bacterial inoculum to heap-leached ore. Previous research with cyanide heap-leached ore showed that the addition of a carbon amendment enhanced loss of free cyanide. The effectiveness of microbial augmentation for remediating cyanide heap-leached ore had not been investigated with a statistically rigorous experimental design prior to this study.

All bacterial groups (inocula) in augmentation experiments were thoroughly examined for free cyanide degrading ability on several media prior to studies using actual ore material. Native bacteria were selectively enriched for cyanide degradation potential and successfully isolated from heap leached ore. Isolation involved a plating procedure in which cyanide was supplied as the sole source of nitrogen. One isolate was tested for its influence on the degradation of total cyanide after reintroduction into cyanide heap-leached ore. In addition, two other cyanide degrading cultures, *Pseudomonas fluorescens* NCIMB 11764 and an Industrial microbial consortium from Pintail Systems Inc., were applied to cyanide heap-leached ore.

Rates of total cyanide loss in experimental treatments were compared to gamma irradiated and non-sterile ore samples receiving minimal salts only. Actual comparisons involved generating a regression of total cyanide over time (52 days, 6 sampling intervals) for individual flasks ($n = 4$) in each treatment ($n = 6$). A comparison of the average rate of total cyanide loss in all treatments (i.e. mean of slopes in each treatment) was computed with an analysis of variance procedure and pairwise comparison of means. Results of this study indicate that the addition of either sucrose and/or bacteria does not significantly influence rate of total cyanide loss from heap-leached ore when a large portion of the cyanide is in the form of metal-complexed cyanide.

Abiotic processes influencing the dissociation of metal-cyanide complexes control rates of total cyanide loss from heap-leached ore. Under the saturated conditions employed in this study, total cyanide was not persistent and was lost from the system. The cyanide loss rate in ore from the Summitville site was approximately 4.9 (± 0.7) mg CN^- /kg ore/day. The major pathway for loss of total cyanide was dissociation of the complexed metal cyanides with subsequent volatilization or microbial utilization of free cyanide. Dissociation of the complexed cyanide at the site will result in production of free cyanide which can be leached into local waterways, incorporated into bacterial biomass, respired as CO_2, or lost as $HCN_{(g)}$. Cyanide in its free form is much more toxic than cyanide complexed with other metals.

The research presented here, in addition to earlier studies (Markwiese and White 1991, 1992; White and Markwiese 1992), indicate that cyanide degrading microorganisms are common in heap-leached ore. Bacteria in heap-leached ore may provide an important filter to scrub free cyanide out of ore leachate prior to entry into local waterways. Methods outlined herein allow screening and evaluation of the native microbial population's ability to degrade free cyanide. This knowledge may facilitate attempts to bioremediate aqueous waste streams or leachate from contaminated ore, where cyanide exists mainly in the free uncomplexed form, prior to entry into the surrounding surface water or groundwater ecosystems.

These results provide an important first approximation of the efficacy of biological treatment of cyanide heap-leached mine ore. This type of information will be critical to remediation specialists and regulatory personnel when evaluating clean-up options for cyanide contaminated sites.

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