Acid Drainage: Microbes Present and Potential for Source Treatments

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Abstract

Acid drainage, caused by oxidation of certain sulfide minerals, representsa difficult challenge in environmental and reclamation practices. This paper describes the potential of in-situ microbial passive treatment systems for management of acid drainage and related contaminant issues.Although *Acidithiobacillus* and *Leptospirillum sp*. were common across mine site environments examined, other species were found to dominate specific geological zones and specific geochemical environments within these zones. These microbes included heterotrophic, acidophilic, sulfate reducing bacteria and Archaea in varying concentrations. Microbial population concentrations depended on zone characteristics and microenvironments withinacid producingand other mine environments. The microbial complement present is potentially capable of numerous reactions, as well as pyrite oxidation, including:

- production of organic and inorganic acids
- dissolution and/or cementation type reactions
- particle fracturing through growth in micro-cracks
- production of chelators that sequester minerals
- exchanging OH^- for H^+ ions in microcosms
- influencing the site moisture regime

All microorganisms require carbon (C), nitrogen (N), phosphorus (P), sulfur (S), and trace minerals to grow and/or to carry out life functions. Redox chemistry governs the large- and micro-scale microbial and chemical changes occurring in mine site environments. Based on the microbes present, the need for appropriate C:N:P:S ratios in most mine site environments and the energy derived from various reactions, experimental evidence indicates that acid producing source environments, and metal and inorganic impacted plumes and drainages might be remediated through site modification and in situ microbial semi-passive treatment.

Key words: microorganisms, ARD, treatment, pilot-scale, full-scale,

1.1 Introduction

Acid Drainage

Acid rock drainage or acid mine drainage, collectively called acid drainage (AD), is formed when certain sulfide minerals are exposed to oxidizing conditions. The formation of acid drainage is a function of site geology,geochemistry and hydrology. It can occur under natural conditions and where sulfides are encountered in mining, highway construction, and numerous other excavations. Acid drainage is formed by a series of geochemical and microbial reactions that occur when water comes in contact with iron and other metal sulfides, like pyrite, marcasite (FeS_2), chalcopyrite ($CuFeS_2$), covellite (CuS), and arsenopyrite (FeAsS). The resulting waters can be acidic and high in dissolved metals.

Pyrite and other metal sulfides are oxidized by oxygen and/or ferric iron. Microbial activity can accelerate this oxidation rate. A rate law derived by Williamson et al., 1994, states that the abiotic rate of pyrite oxidation increases with increasing oxygen concentration and with decreasing pH. Moreover, the rate of sulfide mineral oxidation increases further as the pH of the environment is lowered to a range conducive to bacterial mediation of ferrous iron oxidation (Nordstrom, 1982, Kleinmann et al., 1981, Singer and Stumm, 1970). Microbial pyrite oxidation rates begin to exceed chemical oxidation rates at pH of around 3.5 – 4. At lower pH values, the microbial oxidation can be several orders of magnitude greater than chemical pyrite oxidation (Kelley and Tuovinen, 1988). Both abiotic and biotic rates tend to increase with increasing temperature.

Despite the application of sound management and reclamation principles, acidic drainage can be formed and must be treated. Treatment and remedial activities might have to be continued for considerable time, specifically until the discharge meets effluent standards without treatment. Acid drainage treatment typically involves chemical neutralization of the acidity followed by precipitation of iron and other suspended solids including:

- pH neutralizing agents
- A means for mixing neutralizing agents and acidic drainage
- Procedures for ensuring iron oxidation and removal of iron and other metals
- Settling ponds, constructed wetlands, vertical flow ponds, etc., for removal of iron, manganese, and other co-precipitates

A number of factors dictate the level of treatment system sophistication required to ensure that effluent standards will be met. These factors include: the chemical characteristics of the AD, the quantity of water to be treated, climate, terrain, and sludge characteristics. Chemicals used to buffer acidity include limestone, hydrated lime, soda ash, caustic soda, and ammonia. These chemicals raise the pH to acceptable levels and decrease the solubility of dissolved metals. Chemicals and treatment equipment can be expensive and require additional costs associated with operation, maintenance, and disposal of metal-laden sludges.

A variety of passive treatment systems have been developed that do not require continuous chemical inputs and that take advantage of naturally occurring chemical and biological processes to clean contaminated mine waters. The primary current passive technologies include constructed wetlands, anoxic limestone drains, and vertical flow systems; where precipitates are settled and removed from the solution.

Microbiology

In general,under favorable physicochemical conditions, microbial populations present in these environments are potentially capable of numerous reactions, including a number of dissolution, precipitation and/or cementation type reactions. Some of the more important processes caused or influenced by microorganisms include break-up of particles, growth of microorganisms in micro-cracks or fractures, production of organic acids and chelators, pyrite oxidation, oxidization of nitrogen-containing organic materials, denitrification, and formation of nitric, carbonic and other inorganic acids.

Microbes influence the moisture, pH, and redox regimes in microcosms. As microorganisms and products of microbial metabolism participate in and accelerate many geochemical reactions within the environment, they use various minerals present as electron donors or terminal electron acceptors. Microbes can accelerate environmental, AD, and rock pile chemistry changes, and respond to rock pile geochemistry. Various relationships between geochemical parameters and microbial populations are presented below.

Acid is typically formed by the oxidation of pyrite (FeS_2) and it contains reduced iron and sulfur species. When solid-phase pyrite in rock is exposed to oxygenated water, the following chemical reaction occurs(Johnson et al., 1993):

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+$$

As a result of this oxidation, the concentration of reduced iron and acid in drainage waters increase significantly. The simultaneous presence of reduced iron and oxygen promotes the growth of acidophilic, autotrophic iron- and sulfur-oxidizing bacteria. These include *Acidithiobacillus species, Acidiphilium sp.,* *Leptospirillum sp.*(the colorless sulfur-oxidizing bacteria)and others such as *Sulfolobusacidocaldarius*(Baker etal. 2006)(Evangelou and Zhang, 1995).

It is widely believed that *T. ferrooxidans*and other iron oxidizers act as a catalyst for acid production by constantly oxidizing reduced iron (Fe^{2+}) to ferric iron (Fe^{3+}), which then acts as a chemical oxidant of pyrite, thereby liberating more reduced iron, sulfate, and large amounts of acidity as shown in the following two reactions (Harrison, 1984).

$$4Fe^{2+} + O_2 + 4H+ \rightarrow 4Fe^{3+} + 2H_2O$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4{}^{2-} + 16H^+$$

Oxidation of Fe^{2+} serves as the rate-limiting step in biologically mediated acid production (Singer and Stumm, 1970). Because bioavailable organic carbon tends to be relatively scarce in most mine rock piles, especially those with acid drainage, *T. ferrooxidans*and other iron oxidizers often compete quite well with other bacterial species for oxygen and nutrients.

Various groups of bacteria are able to rapidly attack a variety of minerals under the right environmental conditions. A "succession" or change in species population domination takes place as the pH of the rock pile environment is lowered by the production of sulfate. Similar types of microbial succession can be observed in relationship to site and/or rock pile temperatures, moisture, geochemistry, and other environmental parameters.

All microbial species require nutrient components; carbon, nitrogen, phosphate, sulfur, and a number of other elements and vitamins, in certain ratios. Since they are required in specific ratios for microbial growth, energy, and contaminant transformation, any nutrient component can be limiting. A limiting nutrient component will affect the utilization of the other nutrient components and the transformation, sequestration, or mobilization of the minerals present. Nutrients present can also determine the microbial types and population composition within an environment or rock pile. Shortage of carbon, nitrogen, and/or phosphate is often associated with many rock pile environments, particularly hard rock mines. Consequently, there is often not a well-defined sequence of reduction or redox reactions affecting the inorganic species in the system, which presents unique opportunities for potential source treatment.

MATERIALS AND METHODS

Microbiology

General and specific standard operational procedures (SOP) were developed for collection, microbial analysis and bench-and full-scale applications. In general,the samples were collected based on visual and geophysical variations observed ingeochemical and geophysical sample boundaries; extensive drilling and sampling programs characterized the full-scale applications.

SOP's were adjusted as on-site conditions and laboratory culturing, characterization and testing of diverse microbial populations required. Microbes were examined via classical and nucleic acid methods (not presented) in association with University of Utah contract laboratory services. Enrichment and isolations were completed to characterize and test the different microbial and nutrient supplement preparations. Samples were collected from a number of different mine site and rock pile environments for correlation analysis, including:

- General site and pit locations
- Roads and road cuts
- Pits, and trenches

- Surface and rock pile samples with depth
- Wells, and streams

In general, methods of microbial data collection also provided the capability for quality control between samples, sample replicates, and samples analyzed over time. Microbes obtained from samples were used as inoculum in a variety of different media, which were incubated under aerobic and microaerophillic, and limited anaerobic conditions. Metabolic and biochemical characterizations were carried out, including assessment of growth rates, and their range of metabolic capability.

Sampling and Sample Storage
Defining the site microbiology entails collecting, isolating, and characterizing microbes from samples collected at specific sites, with specific lithologies and geochemistry. In order to evaluate the microbial influence on the rock pile stability, microbiological samples from site rock pile trenches, drill cuttings, hot spots, associated soils and rock outcrops, crust materials, and rock pile vent areas were collected.

The site microbial analysis and subsequent speculation on microbial roles is based on a total of 148 paired samples that included 15 duplicate samples, and sample sets across two geological units. Site materials were aseptically collected into sterile propylene screw-capped, plug-sealed tubes. Microbiological and geochemistry analysis were not conducted from the same sample, but from the same sample zone.Samples were collected from freshly exposed surfaces using gloved hands and an alcohol cleaned stainless steel trowel or spoon, filling the tubes so as to eliminate as much air space as possible and to preserve the sample moisture content. Filled tubes were stored in a cooled ice chest and transferred to a refrigerator at ~4^0 C as soon as possible after sampling. Once in the laboratory, one sample from each set was frozen at -80^0 C; the other paired sample remained at 4^0 C for various analyses. Frozen samples were used for checks to ascertain any decrease in viability, for random reproducibility checks of samples stored at 4^0 C, and as a repository of samples for additional analysis.

Media Development and Testing
Media was developed and optimized specifically for culture of site rock pile microbes falling into general microbial categories. This was completed by visually, physically, and chemically screening composite samples for microbial growth and diversity from different mine site environments. This nutrient/media screening was used to reduce the number of different culture media combinations used for study of the culturable microbes at the mine site. Briefly, media was developedthat yielded optimal microbial diversity and numbers for all samples and microbes examined. Screening included replicate samples and the following procedures:

- Metabolic profile analyses on environmental, gram positive, and gram negative BIOLOG™ plates and select formulated media combinations
- Samples at pH's of 3.5 to 7.0
- Testing in media and saline solutions with various trace elements and screening in various standard, classical, and specially formulated media
    - Comparing site results on specific media with cultivation of stock control samples from American Type Culture Collection,laboratory stock cultures, and various control samples including *Acidithiobacillus ferrooxidans, A. thiooxidans*, *Leptospirillum sp.,* a general acidophilic microbial mix, several sulfate reducing bacteria, anda general heterotrophic microbial mix
- Replicate plating on solid media and comparative triplicate MPN analysis in liquid samples of all media types
- Acid generating materials were examined in duplicateafter blending and splitting,in humidity cell-type bench-scale experiments, for treatment potential, microbes present, microbial processes,

and pH change during experiments. Columns were leached with simulated rainwater until they initiated acid production. At this point they were treated with a pad that reduced oxygen penetration into the column and introduced both nutrients and microbes into the column materials.

- Pilot and a full-scale in situ application and testing was characterized by 33 monitoring wells and extensive microbial and chemical analysis for a pilot-scale rock pile estimated at about 2,493,000tons and full-scale, 25 million ton rock pile application

RESULTS AND DISCUSSION

Microbial Culturing and Characterization

It is now accepted that the vast majority of microbes have not yet been isolated, identified or characterized as to how these organisms survive, grow, or their range of functions in natural habitats. When one also considers that a bacterium is often only a part of a larger, more complex community or ecosystem with possible co-dependence on other members, it is understandable why traditional culturing techniques fail to accurately reflect the large microbial diversity in an environmental sample.

A microbial study based only on the culturable microbes can be constrained with respect to viewing the microbial diversity, but is very useful when developing treatment approaches. The media and analysis of bacteria in the rock pile system was designed around bacterial metabolism rather than specific bacteria; however, specific types of bacteria were also identified. Analysis is looking at bacteria that perform specific biochemical functions or transformations rather than just focusing on specific bacteria of a particular genus and species. Bacteria can be thought of as small chemical factories; under the right conditions, many different genus and species of bacteria can perform similar geochemical transformations. This is stated because the methods of analysis determine the types and relationships of the information obtained.

Microbial enumeration and characterizations have defined the site microbiology in both general and specific characteristics. Variations of stock media were tested over a broad pH range and numerous minor adjustments in component levels to determine the optimal pH and component concentration. This was assisted by evaluation of 10 visually different site wide sample combinations to determine how effective the different media would be in maximizing the successful culture of site microbes.

Five main metabolic groupings, from a total of 56 nutrient media screened were used to adjust culture media content in an attempt to cultivate as many different microbes from site samples as possible, Figure 1. This approach was useful in reducing the number of different culture media combinations required for this effort and was confirmed through culture of known environmental sample controls. Control samples included *Acidithiobacillus ferrooxidans,A. thiooxidans*, *A. denitrificans*, *Leptospirillum* sp., a general acidophilic microbial mix, several known sulfate reducing bacteria, and various laboratory repository rock pile cultures, including heterotrophs.

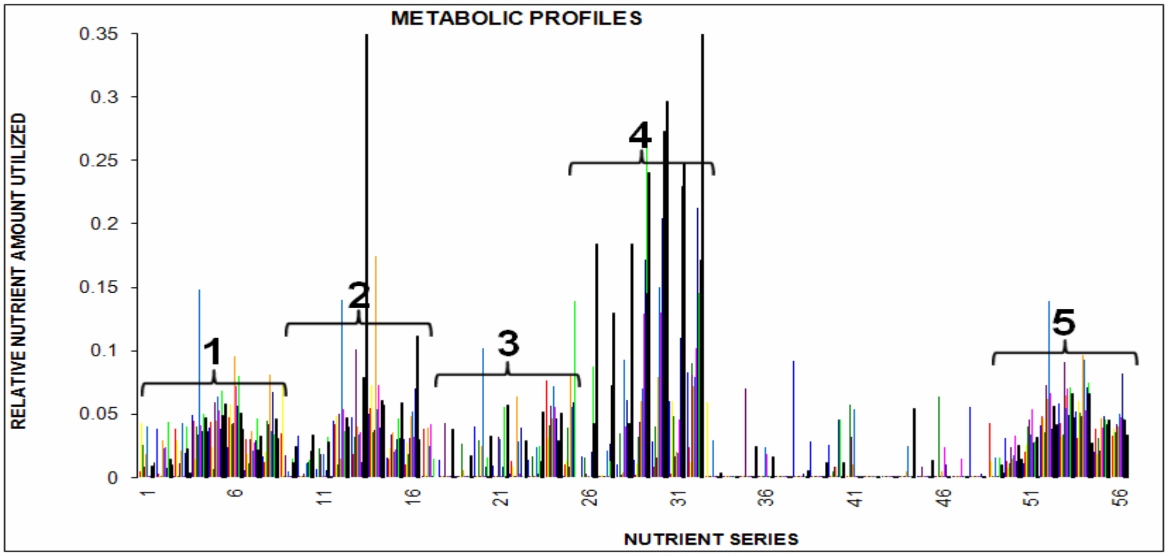

Figure 1. Metabolic profiles of 10 site sample combinations collected across zones shown in Figure 3, indicating the five metabolic profiles, of the 56 nutrient combinations tested and amount utilized by various microbial preparations (modified from Adams 2012).

In the microbial population analysis, based on metabolism profiles, it should be noted that statistical analysis of the populations indicates that the acidophilic microbes may be a subset of the heterotrophic microbes that grow well at lower pH,around 3.5. However, this was contradicted with observances that acidophilic microbes are definitely the predominant microbes in some samples and heterotrophs in others rather than both being high in the same samples.

Geochemistry

Iron oxyhydroxides generated by pyrite oxidation, as well as other reaction products, can aid in the cementation of soil and sand materials. Formation of these types of aggregates would tend to increase rock pile stability. Ferricretes represent iron transported by reducing acidic waters and precipitated as hematite and goethite under more oxidizing, less acidic conditions (Eh above 0.4mV and pH above 6). . These conditions represent major shifts in redox chemistry and possibly in the site microbiology. Weathering and microbial actions can influences slope stability both positively and negatively.

Sulfur bio-oxidation takes place from a pH of 8.5 to 1.9; however, the species involved will vary.Moreover, it is somewhat dependent on rock pile geochemistry, moisture, etc., oxidizing sulfur to sulfate. Although *A. ferrooxidans* is often considered to be the most important member of a rock pile population in terms of acid generation, mixed bacterial cultures can be more efficient at mineral decomposition than is *A. ferrooxidans* alone. Additionally, *A. ferrooxidans* seldom, if ever, grows as a pure culture. Environments at a pH above3.0 contain a consortium of heterotrophic bacteria and a variety of other acidophilic, chemolithotrophic, and sometimes sulfate reducing bacteria.

The microbial complement present at a siteis potentially capable of numerous dissolution and cementationtype reactions as well as pyrite oxidation when changes in rock pile conditions favor such reactions. Bacteriacapable of denitrification were present at low to moderate levels in the site samples examined and their relative concentration varied with sample pH. Based onsite observations and analysis, the site examined had both past and present acid generating areas within the rock piles.

For each of the major redox elements there is a specific range of pE for the initiation of the reduction of the element. Microorganisms use oxidized forms of Fe, S, Mn, and some metal oxyanions as terminal electron acceptors. Nitrate respiration below pE 8 produces products that include NO_2^-, N_2, N_2O, NH_4^+. In combined samples examined for microbial population profiles, it was shown that denitrifiers were present. Denitrification is a fairly common trait in many microbial genera and with the past presence of blasting compounds; the presence of denitrifiers was expected. It was not surprising to find that while microorganisms capable of denitrification were found in all pH environments examined they were most common and active in circumneutral environments.

Solid phase manganese reductions can occur in the presence of NO_3^-. However, solid phase iron reduction does not occur in the presence of NO_3^- or O_2 and the Mn:Fe ratio is an indication of whether a denitrifying environment is present. Changes in pE(pE≡ -log[e^-]) induce both chemical reduction sequences as well as sequences in microbial ecology. Relative microbial correlation with site minerals is shown in Figure 2.

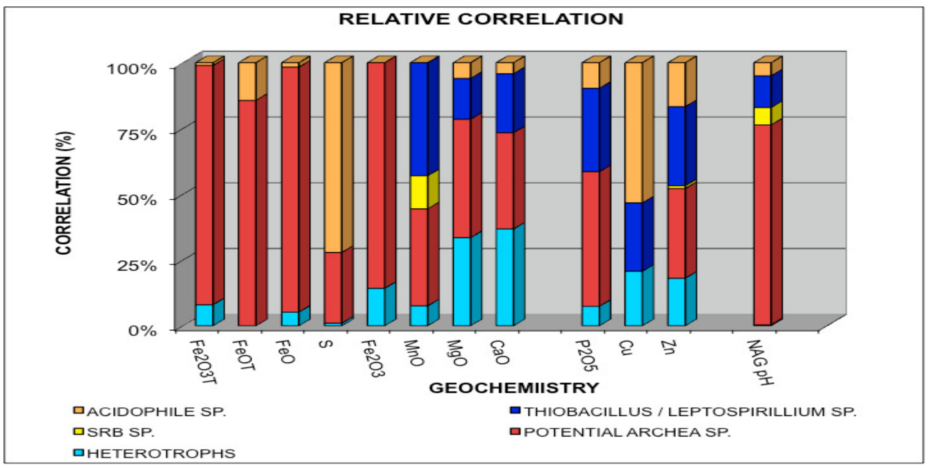

Figure 2. Microbial metabolic type found associated with several mineral environments.

Acidophilic and heterotrophic populations were shown to be associated with different microbial population profiles and therefore could indicate geologic zones where a succession of microbial populations is taking place. In these areas, higher concentrations of *Acidithiobacillus/ Leptospirillum sp.*were associated with the higher concentrations of acidophilic microbes than with higher concentrations of heterotrophic microbes. All other microbial metabolic types, *Acidithiobacillus/Leptospirillum sp.*, *Archaea*, and SRB were all shown to be distinctly different metabolic types and significantly different at the 99% or higher confidence level.

General Site Microbial Analysis

In the examined sites, it was common to find combinations of different microbial types (with different metabolic characteristics), present even at lower pH ranges. Both the rock pile trench samples and the general site microbial profiles were similar and have similar population profile ratios; *Acidithiobacillus/Leptospirillum sp.*> heterotrophs ≥ Acidophilic microbes ≥ Archaea > SRB populations.

Out of a total of 133 different samples examined, 81 samples had dominant populations. In about 36% of the dominant population samples, *Acidithiobacillus/Leptospirillum sp.* were the predominant species, representing 50% or more of the total microbial number present. In about 32% of the samples, either heterotrophs or acidophilic microbes were dominant. Acidophiles are organisms that can withstand and even thrive in acidic environments (pH range 1-5) and include types of Bacteriaand Archaea. It has only been recently that the importance of general acidophiles, other than*Acidithiobacillus/Leptospirillum* *sp.,*and their role in acid mine drainage, as well as the presence of SRB species in highly acidic environments, has been recognized; Figure 3.

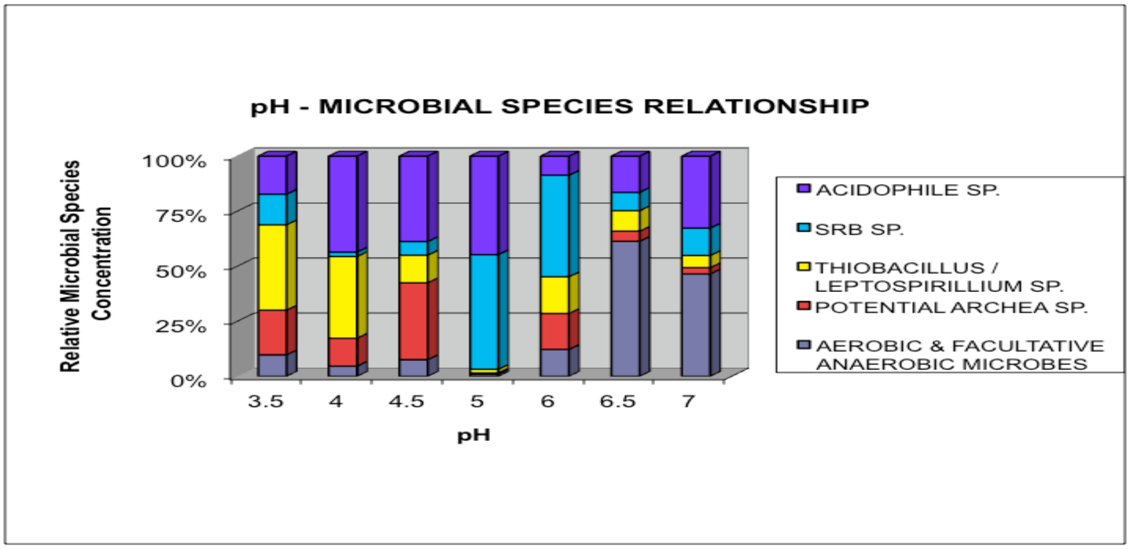

Figure 3. Relative microbial distribution across the mine sitepH environment.

From the results obtained, it is thought that the acidophilic and heterotrophic bacterial species may play an important role in mineral leaching, dissolutions and transformations that provide opportunities for re-precipitation and crust formation. These acidophilic heterotrophic microbes probably play important roles on both sides of the mineral leaching and re-precipitation event zones within a rock pile environment.Archaea microbes were dominant in about 9% of the samples and were noticeably present in populations where *Acidithiobacillus/Leptospirillum sp.* dominated, but not the other way around.Sulfate reducers were dominant in about 4% of the samples.

Specific environments within the rock pile were more favorable for particular microbial types. This may be explained through the observation of the different geologic zones and the zones of increasing oxidation or reduction. In general, the proportions of microbial types appear to vary depending on the site geology, geochemistry, and the specific site conditions. Samples were analyzed in most geologic zones that ranged from a pH of approximately 3.5 to 8.1. While many samples had*Acidithiobacillus/ Leptospirillum sp.,* theywere not the dominant microbes in all samples. A number of samples contained dominant populations of heterotrophs, acidophiles, sulfur reducing and other sulfur-oxidizing bacteria. These various microbial metabolic types were present across a broad site geochemistry and pH range, which opens the potential opportunity for modification of subsurface environments and source treatments.

Modification of Sub-surface Environments

Bench-scale Testing (Acid Generating Materials)

Bench-scale experiments were completed in humidity-cell type columns to monitor microbial populations and pH during experiments. Columns were leached with simulated rainwater until they initiated acid production. At this point they were treated with a pad that reduced oxygen penetration into the column and introduced both nutrients and microbes into the column materials. Within a week, both sets of column materials started reversing the acid generation within the column. With the provision of a balanced nutrient supply that provided organic carbon, ammonia, and phosphate that favored the growth of acidophiles/heterotrophs, these microbes started increasing and at the same time the number of *Acidithiobacillus* and *Leptospirillum* type species started decreasing, Figure 4.

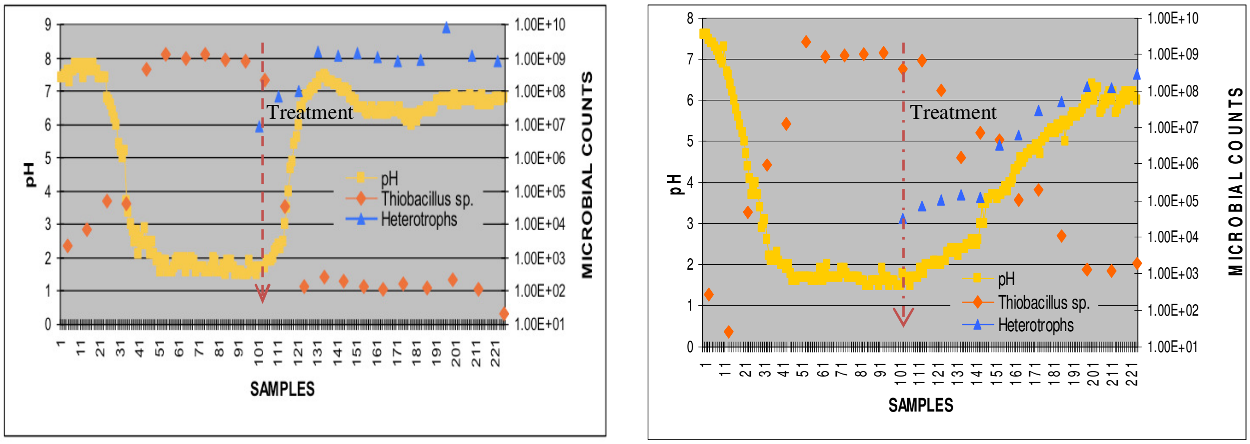

Figure 4. Bench-scale tests demonstrate in situ approach for reversal of acid generation in two similar mine site materials.

While the samples behaved slightly differently, using the same treatments, both achieved the desired results; a redox change within thecolumns creating an environment that allowed heterotrophs to out compete the acid generating microbes. Nutrient additions were balanced to help neutralize the acid in the microenvironments that would allow the acidophilic/heterotrophic microbes to establish. Experimental pH endpoints of approximately6 and 7 were obtained in these experiments.

Pilot-scale Denitrification (Circumneutral Environment)

A lined spent ore padwas constructed in 2002 and 2003. It holds ~2,493,000 tons of spent ore with a moisture content of about 6.98% and a total nitrate/nitrite load of 24.73 tons. Nitrates were from ANFO in blasting compounds and the degradation of cyanide used in the ore leaching process. Portions of the pad materials weresequentially inoculated with a heterotrophic microbial population consisting of a high concentration of several denitrifying microbes and nutrients (Adams etal.1996, ITRC, 2002,Adams and Waterland, 2008).

The liner underneath the test pad held ~2.5 million tons of material/water and the associated sump used for water recirculation and inoculation held about 200,000 gallons. In 2003 and 2004, approximately 125,000 gallons of water from the sump were treated to remove nitrate and applied to the pad. From July 2005 through November 2005, approximately 6.2 million gallons of treatment solution was applied to the western third of the pad. In July 2006 through October 2006 an additional 6.8 million gallons of treatment solution was applied to the middle third of the pad, Figure 5.

In situ denitrification of the spent ore was accomplished by applying a treatment solution of denitrifying bacteria and nutrients to the top of the depository through several application methods. Treatment solution was applied by recirculation through the depository using sprays, drip lines and depressions - whichever worked best with the inoculum and weather conditions. A detailed sampling program of the spent ore was conducted in December 2008 to determine in situ denitrification effectiveness and to estimate the remaining nitrate load.

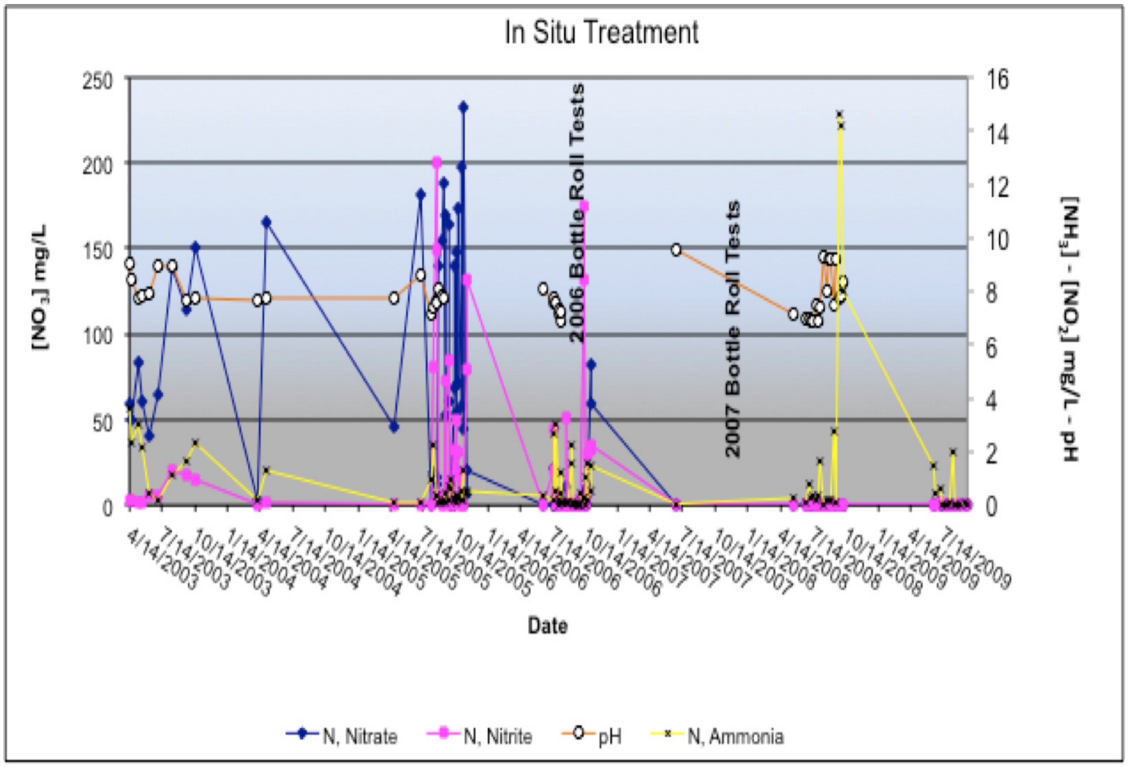

Figure 5. Pilot-scale denitrification testing monitoring NO_2, NO_3 and $NH_3$to establish parameters for future full-scale applications (modified from Adams and Waterland, 2008).

Fifteen vertical borings, five angle borings and thirteen 20-foot deep trenches were used forcomposite sample collection every 20 feet. The effectiveness of the in situ treatment of the spent ore was evaluated by bottle roll tests and sampling analysis. As a result, in 2007, an additional 65 million gallons of treatment solution was applied to the eastern, final third of the pad materials. In situ treatment was discontinued in September 2008.Based on the sampling analysis, in situ denitrification of the spent ore was highly effective, reducing the original estimated nitrate and nitrite load by approximately 97% and treatment was considered complete. Lessons learned from this and other pilot-scale treatments were applied at full-scale to adjust denitrification and metals stabilization treatments.

Metal Stabilization (Circumneutral Environment)

Application of in situ treatment technologies have been applied to a site impacted with historically placed rock pile and spent ore materials (McCarty et al. 1969,Dahab, 1993,ITRC, 2002). Similar treatment and monitoring approaches, as described in the previous section, were used in situ, to remove nitrate and stabilize metals, primarily arsenic. Nitrate removal times were shortened significantly and the following results were obtained for arsenic stabilization in down gradient waters, Figure 6.

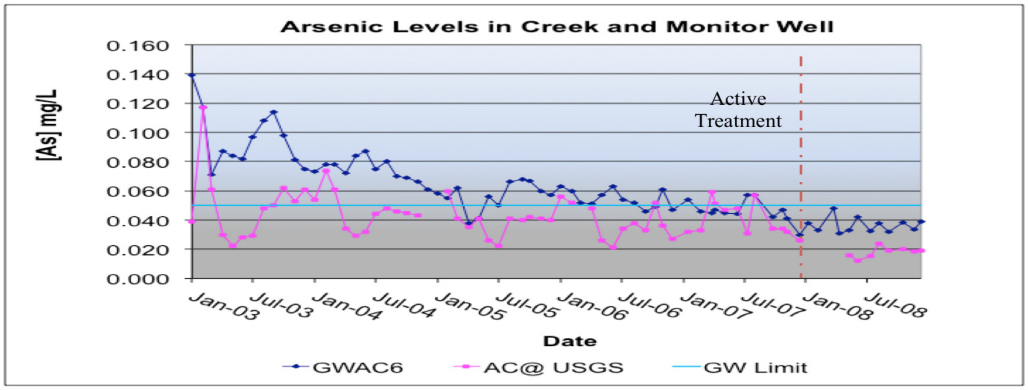

Figure 6. Arsenic stabilization in drainage area impacted from past mining activity. Site waters also contained nitrate that was removed from the system (not shown)(modified from Adams and Waterland, 2008).

Over a five-year period, site materials were treated to achieve nitrate reduction in source materials. Nitrate levels in down gradient waters were reduced from about 45 mg/L to below 10 mg/L. The microbial inoculum for this site was different than in the above pilot-scale test in that approximately 1/3 of the active inoculum was designed for metal stabilization(Adams etal.1996, O'Dayetal. 2004). Active treatment for arsenic stabilization was continued through the end of 2007 when both monitoring well and creek arsenic levels were below the groundwater limit. The site is still being monitored and receives a periodic treatment for both denitrification and metal stabilization. Periodic treatment is based on any trends that indicate an increase in arsenic load; the site has been in compliance since mid 2007(Adams and Waterland, 2008).

CONCLUSIONS

- In general, the proportions of microbial types appear to vary depending on the site geology, geochemistry, and the specific site conditions
- While many samples in potential acid generating sites have *Acidithiobacillus sp., Acidithiobacillus sp*. was not dominant in all samples
    - *Acidithiobacillus/Leptospirillum sp.* were the dominant microbes present; 50% or more of the microbial number present, in about 36% of the samples
    - Heterotrophs or acidophilic microbes were dominant in about 32% of the samples
    - Possible *Archaea* type microbes are dominant in about 9% of the samples
    - Sulfate reducers are dominant in about 4% of the samples
    - General site microbial profiles have shown quite similar microbesoccurring in other rock piles
- In rock piles, microbial populations can often be at low levels due to lack of moisture, nutrients, or other appropriate environmental conditions
- From the results obtained in pilot- and full-scale studies, it is thought that the acidophilic, acidophilic/heterotrophic, and sulfate reducing bacterial species may play an important role in both mineral leaching, dissolutions and transformations that provide opportunities for in situ treatment/remediation within selected rock pile environments
- The spectrum of microbes present likely plays an important role in the succession of different microbial types within the rock pile environments, again providing opportunity for in situ treatment
- Different microbial populations and site specific in situ treatment adjustments are important in achieving the desired in situ treatment

- Successful full-scale in-situ treatment is obtainable in a number of different rock pile and spent ore environments, but requires appropriate characterization and monitoring

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