Artesian Mineral Development & Consolidated Sludge, Inc.

Iron Mountain Mines, Inc. *insitu* remediation summary

History of copper cementation and bioleaching

Cementation of copper began with the discovery of copper and the beginning of copper mining at Iron Mountain around 1896. By 1908 the State Geologist reported that the operation was so extensive that a building was being constructed over and around it.

In 1919 copper prices crashed and the mine closed, in 1920 fish kills were reported.

In 1921 copper cementation resumed and was thereafter operated continuously until the EPA implemented their High Density Sludge treatment.

After WWII Iron Mountain mines produced sulfur and iron for fertilizers until 1963.

Iron Mountain has 20,000,000 tonnes proven and 5,000,000 tonnes probable reserves.

The naturally occurring archaea living in the Richmond mine are reported to be capable of producing the most acidic natural mine waters on the planet, pH-3.6.

Iron Mountain Mines, Inc. bioleaching naturally produces about 8 tons of metals per day.

One of the earliest records of the practice of leaching is from the island of Cyprus. Galen, a naturalist and physician reported in AD 166 the operation of in situ leaching of copper. Surface water was allowed to percolate through the permeable rock, and was collected in amphorae. In the process of percolation through the rock, copper minerals dissolved so that the concentration of copper sulphate in solution was high. The solution was allowed to evaporate until copper sulphate crystallized. Pliny (23-79 AD) reported that a similar practice for the extraction of copper in the form of copper sulphate was widely practiced in Spain.

Prior to invention of electrolysis, the only practical method for the recovery of copper from copper sulphate was by cementation, a process that derives its name from the Spanish word cementacion, meaning precipitation. It is thought that the cementation of copper was known in Pliny's time, but no written record of its commercial application seems to have survived. The cementation of copper was also known to the Chinese, as documented by the Chinese king Lui-An (177-122 BC). However, the Chinese implemented the commercial production of copper from copper sulphate using a cementation process in the tenth century. The Chiangshan cementation plant started operation in 1096 with an annual production of 190 t per annum of copper. In the Middle Ages, the alchemist Paracelsus (AD 1493-1541) described the cementation of copper as an example of the transmutation of Mars (iron) into Venus (copper).

Heap leaching of copper sulphide on an industrial scale was carried out at the Rio Tinto mine in Spain since about 1752. The ore was crushed and laid on a gently sloping impervious pad. The layers of ore were alternated with beds of wood. Once the heap was

constructed, the wood was ignited, resulting in the roasting of copper and iron sulphide. Water was then distributed over the top of the heap. As the water percolated through the heap, the copper and iron dissolved, forming copper and iron sulphate. In 1888, this method of extraction was prohibited by law, because of serious environmental damage caused by the clouds of sulphur dioxide formed. The process of heap leaching, without the roasting step, continued with success until the 1970's at Rio Tinto. The reason for its success was unknown, but it was thought to be due to 'some obscure quality either of the Rio Tinto ore or the Spanish climate'. Today, it is known that the microorganism Thiobacillus ferrooxidans plays the critical role in the success of Rio Tinto.

Several reports in the early part of last century associated soil microorganisms with weathering of sulphide minerals and of coals containing sulphides. Indeed, it was estimated that in 1940 the production of sulphuric acid due to the weathering of subbitumuous coals resulted in the discharge of several million tons of sulphuric acid into the Ohio River. This level of pollution was alarming, and universities and several US government institutions, such as the US Bureau of Mines, began to search for the causes of this sulphuric acid. These investigations found that the cause of the sulphuric acid was the oxidation of pyrite contained in subbitumuous coal, and that this oxidation occurred at rates in excess of those suggested by inorganic chemistry. In addition, the occurrence of sulphur oxidising bacteria was noted. A couple of years later, in 1950, a new species was identified and named Thiobacillus ferrooxidans. This organism is able to oxidise elemental sulphur and ferrous ions at much higher rates than can be achieved by inorganic chemistry. Indeed, it is this catalysis of the oxidation of ferrous ions that makes Thiobacillus ferrooxidans and other iron and sulphur oxidising microorganisms such important catalysts in the extraction of metals in bioleaching processes.

The isolation of sulphur-oxidising microorganisms from hot springs by Brierley and by Brock in the late 1960's provided the opportunity of operating reactors at much higher temperatures. Woese proposed in the late 1970's that these and similar microorganisms belonged to an entirely new kingdom of life, called the archae. Woese's proposal that these thermophilic microorganisms are as different from bacteria as eucaryotes are from bacteria was verified by detailed genetic studies in 1996. Iron- and sulphur-oxidising archae are presently the microorganisms of choice in the development of tank leaching processes for the extraction of base metal sulphides.

Microorganisms currently used in commercial bioleaching operations (both stirred tank and bioassisted heap leaching) are ubiquitous in nature. Wherever a suitable ore is exposed to the surface and water is present, the microorganisms will be found occurring naturally. Microorganisms used in commercial operations are exactly the same as those found in nature, the only difference is that in some cases they have been selected for rapid growth on the ore or concentrate concerned. The growth of biomining microorganisms is inhibited or prevented in the presence of organic matter. The microorganisms also only function in specific temperature ranges, require iron or reduced sulphur as an energy source and grow optimally at pH < 2.5. These extreme conditions or requirements prevent the growth of the microorganisms on plants, insects or animals, including humans. They are therefore non-pathogenic and there have been no reports of illness due to these microbes.

Over the last three decades or so, the technology of heap leaching (particularly for the extraction of copper, gold and silver) has advanced by leaps and bounds. Heap leaching was originally developed for the treatment of oxidized copper, gold and uranium ores. More recently, heap leaching has become increasingly applicable for the extraction of copper from sulfide ores, particularly those in which copper occurs in chalcocite. In addition, the technology is being extended to leaching of nickel and zinc.

Bioheap leaching of copper from sulfide ores has also made significant advances. Brierley & Brierley (2000) summarized the results for ten commercial heap leaching operations treating chalcocite copper ores. Eight of theses are in Chile and two in Australia. Bruynesteyn (2000) and Young (1999) reported progress in developing technology for bio-leaching chalcopyrite.

The in-situ leach concept that is the focus of this white paper lends itself to the application of the same leaching technology used for heap leaching and bioheap leaching, but applied to selected deposits that cannot be economically mined by conventional techniques. In addition the new in-situ leach technology promises to create a means of optimizing the leach process even beyond that possible in conventional run-of-mine heap leaching.

APPLICATIONS OF IN-SITU LEACHING

There are references to primitive forms of in-situ leaching of copper in Roman times, and maybe even long before that in China. In-situ leaching occurs prolifically in nature. When uncontrolled, it is called acid rock drainage.

In tropical environments, it is known that gold is sometimes leached from surface deposits in nature and is then redeposited below at an Eh or pH interface. Likewise copper is leached during the oxidation of porphyry deposits near the surface, transported in descending fluids and reprecipitated as a chalcocite blanket at the Eh boundary. Such processes, called "supergene enrichment" by geologists, are a natural form of in-situ leaching. Acid rock drainage is a form of natural in-situ leaching "gone wild". Preventing this natural form of in-situ leaching remains a formidable environmental challenge.

These natural forms of in-situ leaching take place over a time span of millions of years. Commercially interesting applications of in-situ leaching must provide a means of greatly accelerating the process and improving the recovery of metals.

Sulfur

The Frasch process produces sulfur through the injection of hot water into underground sulfur deposits as practiced in West Texas and offshore Louisiana. This was probably the first major commercial application of solution mining and has been a major source of sulfur for well over a century. Superheated water is forced down into underground deposits, melting the sulfur. Compressed air is introduced, and the less dense, liquid sulfur emulsion flows up through an outlet pipe to settling vats. Following cooling, very pure sulfur is produced in this way. This application falls more under solution mining and is less likely to benefit from the new concept described in this report.

Copper

Thus far, only two commercial currently operating ISL copper recovery operations have been identified in North America, namely the rubble leaching operation at ASARCO's Silver Bell copper mine located NW of Tucson, AZ and in-situ leaching of mined out stopes at BHP's San Manuel copper mine, also in Arizona. Both of these successful in-situ leach demonstrations, however, involved previously mined sites as opposed to virgin ore deposits. The National Academy of Sciences (2002), Bartlett (1992, 1998), Coyne and Hiskey (1989), Schlitt and Hiskey (1981) and Schlitt and Shock (1979) considered these to be successful in-situ leach demonstrations and concluded that in-situ leaching of copper requires a means of creating sufficient permeability for lixiviants to contact ore minerals.

At its Silver Bell operations, ASARCO fractured a body of low-grade oxide ore adjoining a mined-out open pit, creating permeability for leaching with sulfuric acid. Pregnant leach solution collects in a pond at the bottom of the pit from where it is pumped to the solvent extraction (SX) plant for copper extraction. Bob Washnock (2004, personal communication), former VP metallurgical operations at ASARCO Silver Bell, estimates that the in-place leaching of rubblized rock at Silver Bell achieved a recovery of perhaps 20% to 25% of the contained copper. The relatively low recovery is probably the result mainly of inadequate contact between leach solution and the ore minerals and poor oxygenation. Recovery of copper from material in this type of environment could potentially be improved by the new ISL concept described here.

Bob Washnock is now developing the Lisbon Valley copper heap leach project in Utah, belonging to Constellation Copper. Lisbon Valley mineralization is sandstone-hosted. Ore is located in an upper aquifer that will be dewatered around the open pit mine with a strip ratio around 2.2 tonnes waste to 1 tonne ore. The upper aquifer is separated from an underlying aquifer by 152 meters (500 ft) of shale that would be expected to protect water quality below. It is possible that there are satellite ore zones that can be evaluated for amenability to in-situ leaching in the future. However, this possibility is not a current focus as the flat lying copper ore deposits are spread out and may not be continuous. Copper occurs in oxide minerals as well as the secondary sulfide chalcocite.

Gold deposits, e.g. gold porphyries appear to offer the best potential for application of the new ISL concept.

Magma Copper in the 1990's carried also out a significant demonstration ISL copper leaching and recovery operation at Florence, AZ employing sulfuric acid. Florence is a porphyry copper deposit believed to be located at around 518 to 610 meters (1,700 ft to 2,000 ft) depth. Other observers estimated that the tests demonstrated, it is reputed, good permeability and connectivity between wells. Some US$ 20 to 30 million was spent on this leach test. Following the acquisition of Magma by BHP, however, the leach test was cut short followed by extensive reclamation pumping and water treatment to restore the aquifer. This is believed to have been successful. In 2002 the project was listed for sale along with other BHP assets and it was hoped to see it reactivated on a commercial scale. At least one mining company is believed to have been seriously interested when copper prices escalated to over US$1.25/lb in 2004. However, the property was sold to a real estate developer, and can now never be developed for ISL. It is unfortunate that after all the effort and investment that went into Florence, the results of the reputedly successful "hard rock" in-situ project have not been made available to those interested in advancing the science.

ASARCO, in partnership with the US Bureau of Mines, undertook an in-situ leaching test at Casa Grande, AZ. The ore deposit, like Florence, was a copper porphyry with similar mineralogy, only at a depth of around 914 meters (3,000 ft). The well-documented Casa Grande ISL project, however, yielded very little copper. Observers believe this was because porosity of the porphyry was poor, possibly on account of its depth. The Casa Grande ISL project is well documented in publicly available USBM reports published during the 1990's and probably also in papers published at conferences.

Magma Copper Company, (subsequently acquired by BHP) recovered copper from solutions applied to mined out stopes at San Manuel, Arizona. The oxide cap at the surface was to a large extent removed by open pit mining. It was underlain by a zone of mixed oxide and sulfide mineralization with chalcocite mineralization, grading to primary sulfide ore at depth. Some degree of ground movement occurred and there is reported to have been some subsidence. Towards the end, underlying sulfides were mined at depth. Later, leach solutions were injected into the transition zone, and residual ore was rubblized into the open stopes. About 50% of the ISL copper produced at San Manuel was recovered from solutions collected from the stopes below, the remainder from solution recovery wells. Due to ground movement, the earlier drill hole assays were no longer valid after 45 years of mining. Consequently, copper recovery was very hard to estimate with any accuracy. Therefore, the 50-60% copper recovery figure mentioned by The National Academy of Sciences Committee (2002) is likely to be unreliable. The stopes were located within an aquifer, and solutions flowed inwards to replace solutions pumped to the surface. Rock permeability outside the stopes was higher than that of the rubblized and open areas than within the stopes. Monitor wells placed around the perimeter indicated no sign of leakage. Industry observers are comfortable that the ISL project at San Manuel was environmentally sound.

Ranchers Exploration in 1972 set off 1.8 million kg (4 million pounds) of ammonium nitrate/fuel oil (AN/FO) to blast 6 million tonnes of copper ore on the side of a hill for in-place leaching at the "Old Reliable" project, located 75 km (45 miles) NNE of Tucson. At that time it was the largest conventional explosives blast in history. Following fracturing, sulfuric acid lixiviant was applied and copper was leached and recovered. The project has been described by Scheffell (2004, personal communication) as "an economic success but a technical failure". Apparently the oxide copper leached well, but copper occurring as the sulfide mineral chalcocite did not leach. This led Scheffell (2004, personal communication) to believe that chalcocite could not be leached in-situ or on heaps, which of course is known today not to be the case. It is probable that chalcocite failed to leach due to inadequate oxidant being present in the fractured rock. An abandoned mine drift into the side of the hill at the water table provided a means of collecting pregnant leach solution. To this day a trickle of ARD is believed to flow from the adit, and the site has been the subject, reputedly, of litigation. Here is an example of a project that could well have benefited from the new ISL concept described in this white paper. Improved oxygenation and the constantly changing flow pattern of solutions would likely have improved leach recovery. The ability to inject air or oxygen into the leaching column of fragmented rock would likely have leached copper from the chalcocite too.

Ranchers Exploration "blew" low-grade oxide copper bearing material into the pit at a worked out copper mine near Winnemucca, NV. The fractured rock was then leached in-place. Submersible pumps in the pit bottom brought pregnant leach solution to the surface for copper recovery.

Copper, according to Stano (2004, personal communication) occurs in porous sandstone deposits at the Naciomientos property in Cuba at depths of 30 to 46 meters (100 ft to 150 ft) within a water table. A pilot ISL test consisting of ten wells was performed there reputedly during the 1990s in virgin ground, not impacted by earlier mining operations. Copper leached well and was produced in solutions from which copper was recovered by cementation. The ore contained some carbonates that consumed sulfuric acid lixiviant. Stano (2004, personal communication) recalls that the reagent dosing and consumption needed "trimming", but that the under-funded test project was regarded to have been a technical success. The copper grade is irregular, but averages around 0.3% Cu. The near surface oxide deposit is underlain by sulfides containing up to 0.5% to 1.0% Cu with silver values that had been mined previously. Flotation concentrates were produced at some time in history at an old National Lead Company pit which remains on the property. The Naciomientos copper deposit, hosted in a porous sandstone environment within an aquifer, may possibly be more amenable to the uranium ISL approach than the new ISL concept presented in this white paper, but this has yet to be established.

Occidental Minerals, a Division of Occidental Petroleum, in the 1970's and early 1980's carried out an in-situ leaching project to extract copper from oxide ores at Miami, Arizona. This in-situ leaching project operated for 5 to 7 years and closed down in 1982 when Occidental Petroleum got out of the minerals business.

Metall Mining, a predecessor of INMET Mining, carried out large scale in-place copper leaching demonstration activities in an underground copper mine in Michigan but the degree of success achieved is not widely known, nor is the degree to which the site could be reclaimed on completion.

During the 1970's, Kennecott Copper undertook in-situ leaching tests in a deep copper porphyry deposit near Safford, Arizona. Wayne Henderson, a pioneer in in-situ leaching, relates that in-situ leaching was tested on porphyry ore that had been hydro-fractured as well as explosively fractured. Apparently Kennecott invested around $20 million in these tests that operated for some three years or so. In current dollars that would be a very significant commitment. The in-situ leach test was carried out at a depth of around 762 meters (2,500 ft) below surface in a porphyry deposit located close to leases owned by Phelps Dodge in the Safford area. Wayne recalls the name "East Man Deposit". He believes that Phelps Dodge ultimately acquired the property. Acid proofed submersible pumps with titanium impellers and 1500 psi pressure capability were used. Key to success in extracting copper from this chalcopyrite deposit was the ability to make an emulsion of very small pure oxygen bubbles in solution that created a powerful oxidant under the significant hydrostatic head. The porphyry ore tended to be fractured and there were challenges in avoiding fugitive loss of solutions. Ray Huff of Colorado was involved in this interesting project about which little appears to have been published.

Huff and Associates completed a successful in-situ leaching project test at Chuquiquamata, Chile at CODELCO's Mina Sur copper deposit. The mineralization consists of 100 percent exotic oxide copper mineralization cementing together alluvial gravels in a tilted paleo channel. The field test achieved good permeability of the copper bearing formation and was able to recover copper bearing pregnant solutions.

ISL trials were carried out at several sites near Mt. Isa, Queensland in the late 1960's and 1970's according to Bell (1984) and Mudd (2001a). More recently, a variant of stope leaching was tested at the Gunpowder (Mammoth) copper mine also in Queensland (Landmark, 1992; Middlin and Meka, 1993; Mudd, 2001a). A small

experimental acid ISL copper project was conducted at the old Mutooroo mine, 100 km south of Honeymoon, South Australia during 1981-1982 according to Bampton et al. (1983). On a 2001 trip to Australia, von Michaelis (2004 personal communication) learned from Electrometals Technologies Ltd. that EMEW (Electrometals electrowinning) cells had been used on a small commercial basis for copper recovery from a small in-situ leach operation in Queensland during the late 1990's.

In-situ bioleaching of copper was pilot tested at San Valentino di Predoi mine in northern Italy according to Rossi et al. (1986). Important ore minerals were pyrite, chalcopyrite and pyrrhotite. The mine closed in 1972. This was not a true "in-situ" bioleaching application. Rather, "in place" bioleaching had begun on 10,000 tonnes of <0.5% copper waste backfill and low-grade ore that had been stored underground. Copper was collected by cementation. Little data was given as the test had just begun at the time the paper was written. The unmined part of the lens contained at least 40,000 tonnes grading 1% to 2% copper. The plan was to expand the operation to the unmined ore by fracturing it with explosives.

Numerous large copper heap leaching operations have been successfully brought into production since the 1980's particularly in Chile, the USA, Mexico and Peru. Some of these operations treat oxide ores, whereas several others treat chalcocite ores. These heap leaching successes are relevant to this white paper on in-situ leaching, because much of the same leach chemistry that has been successfully applied in heap leaching, would become directly applicable to in-situ leaching where the new concept described in this white paper finds application.

Increasingly, bioheap leaching processes are being applied for the extraction of copper from sulfide ores. Brierley & Brierley (2000) summarized the operational parameters and techniques involved. They concluded: "Copper bioheap leaching is considered an important processing tool by the mining industry because of its low costs, short construction times, operational simplicity, good performance, and environmental advantages." The innovation that is the focus of this white paper promises to extend the applicability of bioleaching to ore deposits that are not economically viable for mining by conventional techniques.

Noranda conducted tests and took a copper bioleaching project to feasibility at the Geco mine in Ontario. According to Ismay et al. (1986), a reserve of 2.5 million tonnes of low grade (0.5%) massive sulfide copper bearing rock was developed. Access to this ore deposit was from an adjacent underground mine area.

The technology focus is aimed at leaching the bulk base metal ore deposits of copper porphyries and breccias, and volcanogenic and sediment hosted massive sulfides.. Bio-oxidation technologies for base metal sulfides are all based upon sulfuric acid leaching systems. A benefit is that the oxidation of sulfide minerals produces sulfuric acid for use as a reagent, as well as liberating the valuable metal ions.

Gold

During the late 1980's water pumped from underground workings of a gold mine in the Northwest Territories, Canada was found to contain low levels of gold. Seepage of very weak cyanide solution from the overlying tailings impoundment had found its way into the mine workings and resulted in leaching of some gold. The water was pumped from underground and used as process water in the mill resulting in incremental gold recovery. So, the first known commercial in-situ leach of gold was involuntary.

The western world's first deliberate in-situ gold mine was proposed at Eastville, central Victoria in the early 1980's by CRA Ltd. as described by Bell (1984) and Hore-Lacy (1982). The project planned to inject alkaline cyanide into the "deep leads", an underground porous paleoalluvial formation. von Michaelis (personal communication, 2004) discussed this and other potential in-situ leach gold projects at length with the CRA project manager at that time. The in-situ leaching project in principle was viable and unlikely to result in any damage to the aquifer as long as production pumping exceeded injection volumes. The deep leads lie beneath grazing lands in Victoria. Both the community and regulators expressed concerns about the possibility of groundwater contamination arising from the use of cyanide in the rural farming area. After some initial pump and dye tracer tests (Hore-Lacy, 1982), regulatory approvals were denied and CRA later abandoned the project. The new concept described herein incorporates a unique solution collection system that virtually eliminates the potential for ground water contamination. CRA or BHP subsequently also evaluated an in-situ gold leaching project in Western Australia, but it is believed that it never advanced to a full-scale test.

More recent advances in gold leaching using thiosulfate, hypochlorite, or acidified chlorine solutions may possibly provide a future means of exploiting gold production from the deep leads avoiding the concern that arises anytime anyone proposes to pump cyanide into an aquifer. Huff and Associates developed and patented a chlorine dioxide-chloride lixiviant for gold that is effective in the pH range 6 to 7.

The configuration of the deep leads gold occurrences would probably not be suitable for application of the new ISL concept that is reviewed in this white paper. The deep leads are buried deeply and they also do not have adequate thickness to benefit from the new concept. Porosity and configuration of the deep leads gold deposits makes conventional ISL more applicable.

4. BENEFITS OF IN-SITU LEACHING

The benefits of in-situ leaching are summarized as follows:
- Applicability: In-situ leaching may be applied to lower grade ores and/or smaller ore deposits where conventional mining is uneconomic.
- Aesthetics and land use: Smaller footprint of the operation, i.e. absence of waste disposal sites, large open pits, and huge ore processing facilities.
- Energy efficiency: Much less energy is required for in-situ leaching than other forms of mining.
- Efficient use of water: In-situ leaching can reduce water consumption compared with conventional mining as a result of reduced evaporation and elimination of water content in conventional tailings.
- Process conditions: When it is possible to apply solutions under a hydrostatic head, leach rates may be enhanced due to, for example, enhanced oxygen concentration in solution.

• Hydrometallurgy: In-situ leaching employs hydrometallurgy which offers several advantages over older pyrometallurgical processes (e.g. smelting). In-situ leached metals are mostly recovered from pregnant leach solutions by processes such as ion exchange, cementation, and electrowinning.

• Economic: Avoiding the need to mine, handle, crush, grind, treat and transport large quantities of ore, waste and concentrate potentially improves the economics of metal production.

• Environmental: Elimination of huge open pits, ore and waste stockpiles of conventional mining and mineral processing. Better control of acid rock drainage may be possible (however, great care needs to be taken to protect water quality in aquifers that may potentially be impacted by ISL).

• Dust control: Almost total elimination of dust emissions.

• Reclamation: In-situ leaching projects should be faster and less costly to reclaim on completion of the operation. This benefit must still be proven in practice.

In-situ leaching operations may comprise six main components:

a) Physical aspects
b) Leach chemistry
c) Solution injection and recovery system
d) Bioleaching of metals
e) Metal recovery system
f) Environmental management and reclamation

These six operations are discussed more fully below.

a) Physical Aspects of In-situ Leaching

Two different approaches appear to be required depending on whether the ore deposit is porous or non-porous, i.e. not adequately porous for solutions to permeate at an adequate rate to access metal values.

For effective in-situ leaching it is important that the ore body be adequately permeable, and that channeling of leach solutions be minimized within the ore zone. Unwanted plugging of lixiviant flow patterns needs also to be prevented.

Physical aspects account for two major problems that need to be solved before in-situ leaching can be applied on a widespread basis:

1) Adequate lixiviant solution contact with the minerals containing the metal values is essential for efficient metal leach recovery. Adequate fracturing and prevention of plugging are key aspects.

2) A means must be found to contain leach solutions to the ore body (Batterham, 2004). This is essential for environmental protection, product recovery and lixiviant reagent conservation.

Permeability and Porosity

A National Academy of Sciences Foundation Committee on Evolutionary and Revolutionary Technologies for Mining, chaired by Milton Ward, a leader in the copper mining industry, issued a report (2002) that states: **"The chief hurdle to using in-situ leaching for mining more types of mineral deposits is permeability of the ore,"** and later: **"New technologies for the in-situ fracturing or rubblization of rocks could be**

extremely beneficial." Technologies that could fracture and rubblize ore in such a way that fluids would preferentially flow through the orebody and dissolve ore-bearing minerals (although this would be difficult in competent rocks with high compressive strengths) is, therefore, a high priority need for in-situ mining.

The report compiled a list of Opportunities for Research and Technology Development in In-Situ Mining. It includes the item: **"New mining technologies for increasing permeability for in-situ leaching, particularly of base metals."**

It stands to reason that unless the leach solution can be brought into intimate contact with the valuable mineral particles within the ore, the valuable components will not be leached efficiently.

It is important to learn and understand the distinction between permeability and porosity of the rock and the formation to be leached. Porosity is inherent in the rock and is not really enhanced by rubblization, rather distribution of solution is enhanced so that solutions need to

travel shorter distances into the mineral bearing rock in order to gain access to the minerals to be leached. Hydrofraccing has a similar effect. Formation permeability can be reduced as a result of the migration of fines. The new concept described in this white paper provides a means of improving distribution of lixiviant throughout the ore column, i.e., making the ore formation more permeable to lixiviant solutions.

Porosity of an ore can be improved by leaching out sulfides and leaving voids, but can be reduced by precipitation of jarosite and/or reactions with gangue such as gypsum precipitation.

In-situ leaching is currently limited to low-grade deposits in highly permeable (hundreds to thousands of millidarcies), essentially horizontal sandstones, such as uranium in porous sandstone deposits such as occur in Wyoming and Texas. Attempts have been made in the past to design in-situ leaching wellfields in formations with just a few millidarcies permeability, but none is known to have been commercially successful.

Transfer of mass from solid to solution is a consequence of simultaneous processes such as advection, diffusion, dispersion, and sorption (Liu and Brady, 1998). Macro distribution of solution through the ore mass is as important as migration of solution into and out of discrete ore fragments on a micro scale.

When leaching uranium in porous sandstone deposits, the challenge is to ensure that the leach solution comes into contact with the mineralization. The mechanism by which the mineralization was precipitated from solution in the pores of the formation, inherently implies reduction of porosity. Fracturing that type of ore may be counter-productive.

For the in-situ leaching of more "massive" gold, silver, copper and nickel ores with low porosity, the challenge is to get solutions to permeate through "solid" rock. Here, fracturing of the ore may be essential for success. Several approaches to fracturing rock underground have been proposed. Some of the ideas are summarized below and in the section on "Patents". None have thus far found successful application in commercial practice because none was able, in practice, to achieve adequately uniform fineness of fragmentation. This is likely due to inadequate void space to accommodate the swell factor.

The National Academy of Sciences Foundation Committee report (2002) concluded: "Although lixiviants are available to leach various copper oxide and copper sulfide minerals, attempts at in-situ leaching of copper in pristine formations have not been very successful because the lixiviants have not been able to adequately contact the

ore minerals in the rock". The most successful in-situ copper leaching has been in ore bodies that had been previously mined; after the high-grade ores were removed open stopes remained with rubble of lower grade wall rock that could be contacted by lixiviants. **New technologies for the in-situ fracturing or rubblization of rocks could be extremely beneficial. Increasing permeability in the rocks to allow lixiviants to contact ore minerals is the biggest challenge for the in-situ leaching of metals**. One promising approach to increasing permeability, as has been done for copper, is to rubblize rock using conventional mining techniques, thereby taking advantage of the open spaces created to achieve better distribution of lixiviant solution through the mineralized mass.

It is precisely this need that the new ISL concept presented in this white paper seeks to address--rubblization of ore in-situ--but in virgin deposits, and in an engineered manner. The new technology goes further, by introducing the means to further improve contact between ore, injected and dissolved air, and lixiviant. In principle, recoveries should be better than can be achieved in any run-of-mine heap leach operations.

Rubblization and Solution Wetting

Girard & Hart (1974) proposed setting off a nuclear blast underground to create 'nuclear chimneys' to collect pregnant leach solutions introduced through injection wells into the surrounding rock.

Jacoby (1974) advocated drilling two bore holes to the base of the deposit followed by hydraulic fracturing of the formation to create a 'base fracture zone' to interconnect the lower ends of the boreholes. Explosives could then be pumped into the base of the deposit and, it was proposed, detonated to fracture the overlying ore body.

Simply drilling into a deep (30 to 914 meters or 100 ft to 3,000 ft) underground ore body and blasting will create fractures, but leaves large unbroken fragments between the fractured zones. Some of the fractures will be closed or plugged with fines. Coursen (1976) proposed a "blast-flush" process to improve permeability. In this invention, explosive charges are detonated sequentially in separate cavities in the ore body producing a cluster of overlapping fracture zones. Each detonation takes place after liquid has entered the fracture zones produced by the previous detonations. Permeability could be maintained by flushing the fractured rock with liquid to remove fines.

Jones and Greene (1986) proposed a process designed to introduce parallel fractures in ore deposits of low permeability. After hydraulically fracturing a well bore, the principal fracture direction is determined and is used to position production wells to collect leach solutions along the lines of the induced fracturing.

Graves (1985) proposed the creation of a 'pancake' or horizontally oriented fracture pattern in the upper zone of an ore body. The fracture pattern is connected to the injection well drilled into the ore body. "Proppants" are introduced to keep the fractures open as is done in oil fields. Then leach solutions are introduced that flow first horizontally and then vertically to recovery wells drilled deep into the periphery of the ore body.

Considerable technology has been developed in the uranium in-situ leach (ISL) industry for bringing solutions into contact with uranium that has become concentrated in porous sandstone hosted ores, and then for collecting pregnant leach solutions (PLS) containing uranium. The famous "five spot" patterns employing four solution injection wells positioned around each extraction well have typically been employed in uranium ISL projects in Wyoming, Nebraska, Texas and elsewhere. Typically there is an average of 1.0 to 1.3 injection wells for each production well in the overall wellfield.

Successful uranium ISL leaches uranium in porous sandstone ores located in aquifers, i.e. below the water table. Although there are successful rubble leaching operations (e.g. for copper at Silver Bell copper mine, Arizona) in which ore is leached in an unsaturated mode, ISL experts point out that they have yet to come across a successful ISL operation where solution is applied to "dry" ore. There are variations in opinion with respect to the best way to leach uranium in porous sandstone ores. David Miller of Strathmore Minerals in Riverton, Wyoming points out that it is best when leach solutions are injected on the reducing side of the sickle shaped uranium roll front, and collected on the oxidized side of the roll front, thereby forcing solutions to flow through the roll front where uranium is concentrated and avoiding re-precipitation of uranium once leached as would be the case if solutions again contacted the reducing zone.

The design of the leach field as well as the rate of solution injection into the ore is a function of hydraulic models that have been developed over several decades of trial and error, as well as being based on engineering principles. Huff and Associates suggests that rather than 23 attempting to modify the permeability of an ore, a better approach is to design the well field to cater to the ore characteristics and to "live with" its permeability.

In-situ leach operators advise that in practice in-situ leaching does not always follow as models would predict. Apparently there is as much art as science and one optimizes the performance of an in-situ leaching operation by learning empirically from previous results on that particular project.

Solutions that permeate through ore in an underground ore deposit logically follow the path of least resistance. If there are open fractures in the ore, solution will most likely find its way along the fractures thereby reducing the degree of contact with the ore, except at the fracture surfaces. Even if the ore is blasted to enhance fracturing before solution is applied, solution flow will follow patterns dictated by the physical nature of the fractures. Some ore particles will be exposed to solution, whereas other parts of the ore may be left unleached because no lixiviant solutions reached that zone. As a result leaching can be far from uniform through the mass of the ore.

Although the above patents outline several possible methods to fracture ore bodies, none provides a means of generating a widely and uniformly distributed pattern of interconnected fractures likely to bring extensive leach solution contact with the ore. None of the above makes adequate provision for the "swell" in the ore deposit brought about by introduction of widely disseminated void spaces and fracture openings. The new concept described in this white paper provides such a system.

This white paper describes a new technology designed to optimize contact between ore and leach solution thereby making it possible to improve overall leach recoveries. The new ISL system is novel in that it proposes ISL of less permeable ores that can be above or below the water table.

Typically around 95% to 99% of the matrix remains physically unchanged after ISL. In the new ISL system proposed here ore is fragmented by blasting in-situ, and solution then applied to the top of the column of fragmented ore and pumped from collection points located below the column of ore. A major difference between conventional ISL and the new system described in this white paper is that the flow patterns of solution percolating through the column of in-situ fragmented ore are deliberately modified on a regular basis to ensure that all ore fragments become "wetted".

<u>Containment of Solutions</u>

The National Academy of the Sciences (2002) report states: **"Confinement of lixiviants and mobilized metals to the mining area is another major challenge."**

Unless the "pregnant" leach solution (PLS) can be collected with minimum fugitive losses, and pumped to the surface for recovery of the economically valuable components, the efficiency of metal recovery will be adversely impacted. An efficient means of recovering the pregnant leach solution after contact with the ore is therefore equally important to bringing the leach solution in contact with the ore in the first place.

Recovery of leach solutions was a challenge at Kennecott Copper Company's deep high pressure in-situ leach project near Safford, AZ in the early to mid-1970's. Solutions, it is believed, tended to escape along fracture zones in the ore.

within each ore deposit is different. The innovative technology described in this white paper provides a means of controlling the hydrology of a deposit that is undergoing in-situ leaching.

Attempts to in-situ leach residual gold left in underground gold mining stopes on the Witwatersrand failed on account of being unable to adequately recover solutions after they had been brought in contact with gold particles that had migrated deep into fractures in the mine footwall during the mining process. In this case finely divided free gold was located in fractures in the footwall.

During the 1990's several experiments were carried out with a view to in-situ leach gold located in microfractures in the footwall of Witwatersrand gold mines in South Africa. In particular, Thiotek Inc. tested various leaching approaches at West Driefontein Gold Mine. MINTEK carried out tests along similar lines. Unfortunately, although gold leached, solutions escaped through cracks and no effective way could be found to efficiently collect the pregnant leach solutions. Ammonium thiosulphate lixiviants were tested (use of cyanide was not considered wise in an operating mine with low pH waters) and discontinued due to miners at different levels, reputedly complaining about the odor of ammonia in their work environment.

b) In-situ Leach Chemistry and Lixiviants

The National Academy of the Sciences (2002) report: **"Lixiviants are available for leaching not only uranium and copper, but also gold, lead, and manganese, to name a few. Nevertheless, cheaper, faster reacting lixiviants would increase production and could also increase the number of metals that could be considered for in-situ leaching. At the same time, lixiviants need to suppress the dissolution of undesirable elements, such as arsenic and selenium, which have geochemistries that are significantly different to uranium, would be helpful, as would additives that lower concentrations of those elements during reclamation."**

The lixiviant reagent should ideally be selective in its leaching of a specific metal in a given ore body. The mass transfer from solid to solution is the result of simultaneous processes such as advection, diffusion, dispersion and sorption (Liu and Brady, 1998). This white paper considers leaching agents from two perspectives: 1) chemical leaching, and 2) biological leaching. The innovative physical approach to in-situ leaching that forms the prime focus of this white paper provides a realistic way to enhance leach conditions.

In addition to lixiviants, oxidants are often required, typically oxygen, peroxide, ferric ions, chlorine, chlorate or nitrate. Henderson (2004, personal communication) mentioned an important technology whereby an emulsion of oxygen microbubbles with leach solution is created using an ultra-sonic or sonic generator under pressure in order to

achieve greatly elevated oxygen levels. This emulsified solution provides significant oxidizing capacity. Enough, it is believed, even to oxidize chalcopyrite.

Numerous large copper heap leaching operations have been successfully brought into production since the 1980's particularly in Chile, the USA, Mexico and Peru. Some of these operations treat oxide ores, whereas several treat chalcocite ores. These heap leaching successes are relevant to this white paper on in-situ leaching, because much of the same leach chemistry that has been successfully applied in heap leaching, can be applied directly also in in-situ leaching of copper.

A sound knowledge of chemistry is essential to achieve successful ISL. Different leach solution chemistries are needed for different types of ores depending on their composition. For example, sulfuric acid is a commonly used lixiviant for oxide copper ores. However, all too often, real life observations depart from what one might expect after conducting a desk study.

Several patents specific to in-situ leaching of copper focus on ways to improve the introduction and reactivity of oxygen with copper sulfides.

Van Poolen and Huff (1975) pointed out that the complexity and cost of two phase injection of water and oxygen may be reduced by using steam instead of water. Thus it was conceived that copper may be leached in-situ from several of its sulfides by injection of a gaseous mixture of oxygen and steam into the wellbore. Additionally, the oxidation produces sulfur trioxide and/or sulfur dioxide producing an acidic solution. The advantage of using steam and oxygen would be that it permits one-phase injection.

Injecting oxygen with the lixiviant can cause problems when gas bubbles coalesce and blind off the pores of the rock in the ore deposit. Surfactants can be added in small quantities to avoid coalescence of the bubbles and to enhance production of very small microbubbles (< 10 μm) that are able to pass through the formation.

Henderson (2004, personal communication) relates various efforts to demonstrate a method of sonically (or ultra-sonically) generating an emulsion of oxygen microbubbles in lixiviant solution that provided a greatly enhanced oxidizing capacity for in-situ leaching.

Huff and Associates, a firm based in the Denver area, patented a chlorine dioxide-chloride lixiviant for gold that works well in the pH 6 to 7 range. This solution is believed to offer advantages for leaching gold associated with oxide copper minerals.

Dr. Jaime Arias, in Chile, was developing the use of a combination of nitric acid and sulfuric acid to oxidize chalcopyrite ores. He presented a paper at one of the Randol Copper Hydromet conferences, and is currently believed to be promoting his chemical oxidation of sulfides under the name "Metex".

Beane (1999) described his perception of some of the solution chemistry challenges that need to be overcome to achieve successful in-situ copper leaching. His conclusions were drawn from personnel experience with in-situ leach solution chemistry at the San Manuel Oxide Operation. Based on a study carried out on leach solution compositions at numerous operations in the Western Hemisphere, it was apparent that many of the features described by Beane (1999) for San Manuel in-situ solutions extended more generally to heap leaching of copper as well.

Beane (1999) showed that typical sulfuric acid lixiviants used in in-situ leaching as well as heap leaching of copper, are magnesium, aluminum, and sulfate brines typically with sulfate at 1 mole/liter and the remaining three each at around 0.1 mole/liter. PLS has a titrated free acid concentration around 2 grams/liter corresponding to a pH of 1.4. However, measured pH is 2.1 being higher on account of much hydrogen ions being

tied up as HSO_4^-. He showed that acid consumption for the leaching of copper was only 25% of the total acid consumption at San Manuel Oxide ISL leach, the balance being consumed by reactions with gangue. He showed that dissolution of gangue minerals provides the large concentrations of aluminum and magnesium, in addition to iron, manganese and other cations in leach solutions. However, these components do not increase in mature leach solutions through continuous recycling. The only concentration changes observed during an injection-production cycle is an increase in copper and a decrease in hydrogen ions. Because of the decrease in hydrogen concentration from injection to production wells, leach solutions become saturated with, and deposit, new minerals along their flow paths.

A study at San Manuel (Beane and Ramey, 1995) showed that although overall permeability in a leach field increased with increasing leach time, permeability adjacent to the production wells decreased. This results from acid raffinate dissolving material near injection wells and then redepositing it further along the flow path.

Precipitation of gypsum reduces permeability (Beane, 1999). Aluminum precipitation is a bigger factor in fracture filling than ferric iron precipitation. Precipitation of Fe results in reduced chalcocite leaching because ferric iron is needed as an oxidant. Both Fe and Al are kept in solution by maintaining low pH. High Al concentrations also prevent copper sorption by clays.

Beane (1999) points out that a challenge remains to develop an economically viable lixiviant that reacts only with copper bearing minerals. That would yield optimal acid consumption and prevent aperture clogging by materials dissolved from gangue. In the absence of such a panacea, however, a reasonable goal would be a "soft leach" which maximizes copper dissolution while minimizing reactions with gangue. He suggests that this might be attained either by direct modification of leach solution composition, or by a pre-leach conditioning step to modify gangue mineralogy so that it reacts minimally with the lixiviant. Possibly the first step that would be achievable is lixiviant modification by optimization of acid content with regard to reactions with copper and gangue minerals.

In the USA, commercial uranium ISL projects today typically use alkaline chemistry employing sodium carbonate, and bicarbonate or ammonium carbonate solutions with sodium peroxide, hydrogen peroxide or oxygen addition as an oxidant. Alkaline carbonate leaching produces the soluble uranyl tricarbonate complex (actually a series of complexes).

Conversely, ISL uranium leaching projects in Eastern Europe, Russia and Australia use acidic chemistry employing sulfuric acid with oxygen, ferric ions and/or nitric acid added as oxidant (Mudd, 2001b). Sulfuric acid leaching produces soluble uranyl sulfate complexes.

In general, the uranium deposits in Wyoming were formed as a result of natural uraniferous solutions coming in contact with reducing agents, e.g. carbonaceous material or pyrite, that caused the uranium to precipitate out along with other metals such as vanadium. In Texas, uranium was precipitated out of underground water by reduction caused by methane gas. Consequently, the in-situ leachable uranium deposits in Texas tend to be "cleaner" than those in Wyoming according to Miller (2004, personal communication). Oxygenated leach solutions convert insoluble U (IV) into readily soluble U (VI) species that generally are present in solution as inorganic anionic complexes.

In recent times, particularly in the USA, injecting ammonia into groundwater has become less desirable, and sodium carbonate is preferred to ammonium carbonate. In

Eastern Europe and Russia, sulfuric acid has been extensively used as a lixiviant for uranium, often with nitric acid as the oxidant.

Gold and silver can be leached using cyanide in alkaline solutions with oxygen or low levels of peroxide. The cyanide performs the role of complexing agent to keep the oxidized gold and silver ions in solution. Gold can also be leached using acidic chloride solutions or ammonium thiosulfate at near neutral pH. Auric gold in chloride or thiosulfate complexes is less stable and generally more readily reduced back to gold metal than auric ions complexed by cyanide. Cyanide is generally a more selective lixiviant for gold than thiosulfate or chloride/chlorine.

Chloride/chlorine solutions at low pH can be considered, in principle at least, for the leaching of sulfidic base metal deposits and also gold. However, chlorine/chloride leach solutions are much less selective and reagent consumptions may be uneconomically high due to proprietary concepts as to how chlorine/chloride lixiviants could potentially be more economically regenerated.

The innovation that is the focal point of this white paper relates to the physical aspects of bringing leach solution in contact with mineral values in the deposit. The benefits of this physical innovation would be applicable regardless of the lixiviant used since bringing solutions into more intimate contact with ore would benefit any leach chemistry system.

This focuses on a means of improving the fragmentation of ore for in-situ leaching, describes a way to improve the distribution of lixiviant solution through the ore mass, provides a low cost and reliable means of oxygenating the process and provides a means of effectively collecting pregnant solutions and preventing fugitive losses. This technique will most likely find the most immediate applicability in the extraction of base metals from porphyry deposits. With that in mind, the most likely lixiviant will be sulfuric acid.

Sulfuric acid in-situ leaching has been used in the USA for copper at BHP's test site in Florence, AZ; at BHP's San Manuel in-situ leach project and at ASARCO's Silver Bell rubble leach project outside Tucson, AZ, all of which continued into the late 1990's. It has also been used for underground stope leaching of uranium at Denison Mines in Elliott Lake, Ontario.

Van Poolen and Huff (1975) proposed in-situ leaching of ore bodies containing copper even at moderate and great depths by injecting a gaseous mixture of oxygen and steam and producing a lixiviant optionally with the addition of sulfur trioxide or sulfur dioxide.

In the case of certain types of chemical leaching (e.g. gold by cyanide; uranium by sodium carbonate; and bioleaching of sulfide ores) introduction of oxygen into the ISL environment may be required. The novel method described herein provides a low cost, reliable means of doing this.

Over the last three decades or so, the technology of heap leaching (particularly for the extraction of copper, gold and silver, and more recently also from sulfidic copper ores) has advanced significantly. Heap leaching was originally developed for the treatment of oxidized copper, gold and uranium ores. More recently, heap leaching has been applied also in the treatment of sulfide ores, particularly chalcocite, with increasing success. This has been made possible by the advancement of bioleaching and bioheap leaching technologies.

The in-situ leaching concept that is the subject of this white paper creates a leaching environment similar to heap leaching, but offers better solution-ore contact and oxygenation than conventional run-of-mine heap leaching.

Acetic acid has been proposed by Geisler and Puddington (1996) as a lixiviant possibly used in conjunction with an oxidant to leach lead from lead sulfide minerals. The high concentrations of reagent required would however probably detract from the immediate economic viability of this technology.

Halogens, i.e. chloride/chlorine and bromide/bromine solutions and admixtures of halogens are less selective lixiviants, however, and reactions with gangue minerals are likely to result in high reagent consumptions for most ore types.

c) Leach Solution Injection, PLS Recovery and Hydrology

Single well leach tests are often carried out using a "push-pull" system in which leach solution is first injected (pushed) into the formation, and then the pregnant solution is pumped (pulled) out of the same well.

In the late 1980's a Texas company by the name of Solution Engineering, Inc. developed and installed a commercial scale in-situ uranium leaching operation that recovered uranium from unconsolidated uranium mine tailings impoundments. von Michaelis (2004) recalls more than one paper presented on the subject by Joseph R. Stano.

In-situ leaching of uranium roll fronts in porous sandstone deposits has been the most widely practiced form of in-situ leaching. Considerable knowledge exists about the hydrology of such systems and about well field design and operation.

Typically, in-situ leach wells are arranged in a grid consisting of the well known "five spot" patterns with one injection well in the center of four production wells. When arranged in a grid this typically works out to 1.2 to 1.3 production wells per injection well. Well spacing depends on the porosity and permeability of the deposit (measured in millidarcies) but 30 to 40 m spacing is not uncommon. The general practice is to complete injection and production wells through the entire vertical dimension of the formation of interest, (Graves, 1985). Monitor wells, employed to monitor fluid flow and containment, are distributed around the periphery of the injection-production well field.

Well completions are similar to water wells, with casings perforated in the permeable, ore-bearing aquifers. The use of polyvinyl chloride casing, which is considerably cheaper than steel casing, limits depths of economic drilling to within 270m of the surface. (Dennis Stover, VP Engineering and Project Development, Rio Algom Mining Corporation, as reported in National Academy of Sciences, 2002) The development of inexpensive casings that could withstand higher pressures would permit leaching of known deposits located at greater depths.

Leach solution travels in a radial pattern horizontally from the points of injection to the production wells. It must be remembered that uranium precipitation in a roll front results in a localized reduction in permeability of the formation right where it's needed the most. Depending on the degree of fracturing, the area within the mineralized zone of the formation that is actually contacted by leach solution can be quite small. Solutions tend to follow fissures and high permeability streaks. "Thief zones" of higher permeability surrounding the mineralized sections of the formations can cause substantial losses of injected fluids (Graves, 1985).

When pregnant leach solution (PLS) is pumped via production wells from an aquifer it can be relatively easy to engineer a cone of depression such that excursions of leach solutions to the rest of the aquifer are avoided or at least minimized.

The National Academy of Sciences (2002) pointed out that because development of ISL depends heavily on drilling and completion of the well field, improvements in drilling efficiencies (faster, cheaper drilling) would increase the productivity of in-situ mining. Directional drilling with sensors, for example, was indicated as one possible improvement.

Uranium in "dry formations" cannot be extracted by conventional ISL practices. However, minerals in "dry formations" may become leachable when the new ISL concept is employed, since it provides a means whereby a very high percentage of the ore zone can be contacted by leach solution.

The new concept for in-situ leaching presented in this white paper provides a practical means of generating a much more uniform fragmentation of the ore deposit and the overlying rock column with introduction of significant void space between the ore fragments. Solution will be introduced at the surface by spraying it on to the top of the column of fractured rock and ore. This ensures that a very much greater part of the ore gets wet by the leach solution. A limitation of the system is that the top of the orebody needs to be at or near the surface. The system can be conceptually applied also to mineralized pit bottoms.

Leach solutions permeate the fractured ore body even more efficiently than in a heap leach operation, for reasons provided in the description, and pregnant leach solutions are then pumped from collection points located beneath the column of fractured ore as described. More ideally, the base of the leaching column of ore will be located within or above, but close to, the water table from which pregnant leach solution can be drawn creating a cone of depression as a means of preventing losses to the environment.

d) Bioleaching of Metals

The Committee on Evolutionary and Revolutionary Technologies for Mining (National Academy of the Sciences, 2002) reported: "The committee also rates as a high priority development of lixiviants and microbiological agents that can selectively dissolve the desired elements and leave the undesired elements in the rock."

Bioleaching of sulfide minerals involves the generation of acid and oxidant (ferric iron). Consequently, in-situ bioleaching can be viewed as a process by which both generation of the leaching agent and reaction of the leaching agent with the mineral of interest take place in-situ. Typically, bioleaching processes require the injection of air or oxygen, or are enhanced thereby.

The science and art of bioleaching has advanced significantly over the last twenty years with numerous commercial operations and several very promising demonstration plants testing new applications of bioleaching and biooxidation for the extraction of metals from sulfide ores. Specifically, biooxidation of sulfides technology has been applied to copper, refractory gold ores and is being extended to other metals such as nickel, cobalt, and zinc. Bioheap leaching has become well established on a large-scale commercial basis for copper. This technology appears readily transferable to the new concept for in-situ leaching described in this white paper.

The new in-situ leach system concept appears to provide an excellent environment for the application of bioleaching processes. Reaction temperatures can readily be controlled by the addition rate of aqueous solutions and by controlling the rate of oxidation through adjustment of the rate of air ingress. The column of ore will have

adequate void space to allow the upflow of air through it in a well distributed and controlled manner. The configuration of the ore column will also provide a chimney effect when heat is released as result of biooxidation of sulfides. Nutrients in solution can be introduced in a controlled manner. Solution flow patterns will constantly be changing thereby eliminating blinding or dry spots within the ore column. Solution composition and application rates can be readily adjusted.

True "in-situ" bioleaching of uranium, analogous to chemical in-situ leaching described above, has not yet been developed. However, "in-place" bioleaching of uranium has been practiced in the Elliott Lake, Ontario region of Canada. Acidic mine water was sprayed onto mined-out stopes, and uranium leached by flooding the stopes. Additional in-place leaching was practiced on blasted, rubblized ore according to McCready and Gould (1990). Ontario experiences cold winters and a distinct improvement in uranium recovery was observed during the warmer months. von Michaelis (2004, personal communication) visited Elliott Lake in the 1980's and observed the in-place bioleaching of uranium in operation.

Elliott Lake uranium ores consist of Archean quartz pebble conglomerates with some pyrite contained in the matrix. Biologically induced oxidation of this pyrite generates sulfuric acid in place that in turn leaches uranium in the presence of an oxidant, namely ferric ions generated from biooxidation of pyrite. Similar processes are known to occur naturally on the Witwatersrand where some mine waters can contain moderately elevated uranium levels. These

acid rock drainage generating processes could possibly be harnessed for uranium recovery in selected Witwatersrand mine stopes and waste dumps. This deserves further evaluation, notwithstanding the relatively lower grades of uranium in Witwatersrand ores, and constraints due to the need to protect aquifer water quality.

Acidophilic iron-oxidizing bacteria are able to leach uranium by oxidizing U(IV) to U(VI) in dilute sulfuric acid solution. The mechanism is generally considered to be indirect, i.e. the organisms maintain a high solution redox potential through oxidation of ferrous ions derived from iron sulfides in the ore. Ferric ions oxidize uraninite UO_2 to UO_2^{2+}. The UO_2^{2+} forms soluble $[UO_2(SO_4)_n]^{2-2n}$ species.

Heap leaching of chalcocite copper ores involves a bio-oxidation step that generates sulfuric acid and liberates copper ions. The importance of injecting air (oxygen) under the oxidizing chalcocite ore heaps has been demonstrated over and again in commercial chalcocite heap leach operations. The innovation that forms the focus of this white paper provides a realistically possible way to enhance the leaching of chalcocite ores with sulfuric acid, by introducing appropriate bacterial inoculum, injecting air and through the creation of optimal temperature, pH, nutrient, and moisture environments. Dissolution of copper should logically be greatly enhanced by the improved contact between leach solutions and in-situ ore fragments.

Hydrometallurgical research is progressing to extend the biooxidation of copper sulfide minerals to the effective treatment also of chalcopyrite, a copper mineral that oxidizes far less readily than chalcocite, for example. The bioleaching of chalcopyrite involves the application of thermophilic bacteria. The innovation that is the focus of this white paper will conceptually create an environment that is conducive to the application of thermophilic bacteria for bio-leaching. Therefore a summary of the latest developments of bioleaching chemistry and technology is relevant here.

Bioheap leaching of copper from sulfide ores has also made significant advances. Brierley & Brierley (2000) summarized the results for ten commercial operations treating chalcocite copper ores. Eight of theses are in Chile and two in Australia.

Table 6.1 Commercial Copper Bioleach Operations

Plant Size (tonnes/day) Years in Operation
Lo Aguirre, Chile 16,000 1980-1996
Gunpowder Mammoth, Australia in-situ 1991-present
Mt. Leyshon, Australia 1,370 1992-closure (1997)
Cerro Colorado, Chile 16,000 1993-present
Girilambone, Australia 2,000 1993- 2003?
Ivan-Zar, Chile 1,500 1994-present
Quebrada Blanca, Chile 17,300 1994-present
Andaquollo, Chile 10,000 1996-present
Dos Amigos, Chile 3,000 1996-present
Zaldivar, Chile ~20,000 1998-present

Bruynesteyn (2000) reported progress in developing technology for bio-leaching chalcopyrite. Olson et al. (2000) reported on test results showing that low-grade ores containing

chalcopyrite bio-leached faster in column tests when subjected to thermophilic bacteria. Uhrie (2000) reviewed the chemistry of secondary sulfide copper ore leaching and some of the bio-oxidation mechanisms.

Brierley & Brierley (2000) report costs of extracting and recovering copper by bioheap leaching between US\$0.40 to US\$0.65 per pound copper cathode. Given that a substantial part of these costs is for mining, leach pads, and heap stacking, it can be expected that in-situ leached copper will likely be producible using the innovative technology described in this white paper at a substantially lower cost.

Copper can be recovered from higher grade chalcopyrite ores by flotation, but lower grade ores cannot be economically leached because chalcopyrite tends to passivate after only partial extraction of copper. The extent of passivation varies with the ore and with the leaching conditions. Early studies attributed the passivation to a layer of elemental sulfur formed as oxidation proceeded. However, more recent work suggests passivation results from a refractory, iron deficient copper sulfide layer that forms on the surface or the mineral. Only extreme fine grinding or use of thermophilic biooxidation seems to prevent passivation. Recently it has been suggested that high solution redox potentials cause passivation of chalcopyrite and that maintenance of a slurry "redox window" of 350 mV to 450 mV (versus standard calomel electrode, or 595 to 695 mV versus standard hydrogen electrode) permits improved biooxidation (Pinches et al., 2001). However, this concept has not been shown to apply to a wide variety of chalcopyrite ores. We found biooxidation of a chalcopyrite ore proceeded readily at relatively high redox potentials (800 mV, SHE) in the presence of extremely thermophilic microorganisms at 65°C (unpublished results). Additionally, ore that passivated at 35°C resumed bioleaching when heated to 65°C.

The main roadblock to applying thermophilic microorganisms for biooxidation of chalcopyrite in heaps is establishing and maintaining temperatures conducive to their growth. Sufficient "fuel" (i.e., pyrite) must be present in the ore to be oxidized and heat the heap, and a means of temperature control is required. In this regard, ISL offers

exciting possibilities. First, the insulating conditions present during biooxidation of chalcopyrite in-situ would permit the development of warm temperatures even at relatively low rates of sulfide biooxidation. Deeper ore deposits may already be at temperatures permitting thermophilic biooxidation. Reaction rates could be controlled by the rate of introduction of air which is facilitated by the new concept described herein. This "chimney effect", whereby air enters through the constructed decline, drawn in by the upward flow of heat convection, offers an interesting prospect for controlled temperature thermophilic bioleaching.

General Issues Relevant to Using Microorganisms In-Situ

Use of microorganisms for in-situ leaching of metal ores has not been developed, though for many years there has been great interest in the potential for this technology. A number of exciting possibilities exist for combining the new ISL concept described in this white paper with biooxidation of minerals in the subsurface.

Bioleaching likely would be initiated by introducing organisms, pregrown on the surface and injected into the deposit. However, deep ore bodies are not necessarily devoid of microbial life. The occurrence of thermophilic, anaerobic microorganisms in deep subsurface environments has been known for a long time (ZoBell, 1958; Olson et al., 1981). As in bioheap leaching, introduced organisms may initiate a process but with time selection will occur for

organisms, introduced or naturally occurring, best suited for growth under conditions present in the ore body.

Encouragement of microbial growth in-situ is not without potential problems. In particular, solution flow may be adversely affected by microbial growth or oxidation products. For example, growth of microorganisms during in-situ chemical leaching of uranium is believed to be one of the causes of flow path plugging in the ore body. A laboratory study showed inoculation of mixed cultures of bacteria into a uranium core specimen reduced permeability. The reduction was overcome by hydrogen peroxide which killed the organisms (Yates et al., 1983). Injection of bacteria into rock with very small pores results in little penetration; the cells are filtered out close to the injection well (McInerney and Westlake, 1990). In the case of acidophilic iron- and sulfur oxidizing microbial processes, precipitation of jarosites from biogenic acid mine drainage solutions can impede solubilization of uranium minerals and should be minimized (McCready et al., 1986). However, the proposed new process should create sufficiently fractured ore permitting good solution flow. Indeed, it may be closer in situation to the microbiologically-assisted in-place leaching of uranium and copper (Sand et al., 1993) in rubblized stopes that has been practiced. Movement of ore within the ore column also is envisioned in the new process and is likely also to cause some surface attrition of the ore particles which should be beneficial with respect to reduction of the plugging effects described above.

Thermophilic microorganisms especially may have significant applications with in-situ leaching, particularly in deeper deposits. Again, solution chemistry must be evaluated with respect to the potential for precipitation of minerals and its resultant effect on solution flow (Jacobson et al., 1989). For example, jarosite precipitation is much more significant at elevated temperatures (75^oC to 80^oC), particularly at pH values above 1.3 (Norton et al., 1991). Calcium sulfate (gypsum) is less soluble at elevated temperatures and its precipitation in-situ could severely restrict solution flow, as has been observed in thermophilic bioleaching columns (Olson et al., 1998). Again, the new concept described

in this white paper provides movement and attrition within the ore column that could mitigate these effects.

Sulfide mineral biooxidation in-situ will require introduction of oxygen. In the new process envisioned in this white paper, a significant flow of air is expected from the chimney effect of a heating ore body. The flow rate of this air can be controlled which in turn will control biooxidation rate, heat production, and leaching rate.

Anaerobic processes might also be considered where bioreduction might be advantageous. For example, a number of oxidized metal species can be bioreduced using hydrogen or simple organic compounds as reductants. These reductions include U(VI) to U(IV), Fe(III) to Fe(II), V(V) to V(III), Cr(VI) to Cr(III), among others (Lovley, 2000). These reductions generally lead to metal precipitation, not mobilization. However, the reduction of Fe(III) to soluble Fe(II) might be considered in cases where ferric precipitates (iron hydroxides or jarosites) coat reactive minerals or block solution flow.

Finally, microbial in-situ formation of organic acids or chelators able to mobilize certain minerals also might be considered. Many neutral pH microorganisms produce such leaching agents from the oxidation of organic substrates. Again it must be kept in mind that excessive amounts of introduced organic materials can lead to significant biomass formation and plugging.

In-situ biooxidation of refractory sulfidic gold ores may be a pretreatment step to increase subsequent leaching of gold, as is practiced commercially in stirred tank and bioheap processes (Olson et al., 2003). Gold locked in pyrite or arsenopyrite is made available for subsequent extraction by biooxidation of the sulfides. The basic requirements for this process would be similar to those required for bioleaching of copper ore.

As discussed above, lixiviants for gold other than cyanide are sought. Among biogenic agents, hydrosulfide anion is a lixiviant for gold. Hydrogen sulfide production from microbiological sulfate reduction underground is a common and widespread process. Although hydrogen sulfide is no less toxic than cyanide, there may be situations in which it could be safely used to extract gold. Furthermore, in the presence of air it is readily oxidized biologically or chemically.

Microorganisms are able to produce cyanide from certain substrates and leach gold (Campbell et al., 2001). Such a process potentially could be performed in-situ. However, there could be problems related to biomass plugging of the deposit. Additionally, a strategy would have to be developed to ensure indigenous microorganisms might not outcompete cyanide-producing bacteria for the substrate. Furthermore, the potential problems related to cyanide use in-situ would remain. In any case, cyanide is also readily biodegradable. The in-situ leaching system described in this white paper lends itself to biodegradation of cyanide in-situ on completion of gold leaching by simply changing the solution chemistry and by inoculation with appropriate bacteria during the reclamation stage.

Cyanide is the best lixiviant for gold and silver that has been developed to date. It is selective, applied in very low concentrations and degradable on completion of the leaching task. Objections to cyanide as a gold lixiviant are mostly emotional and based largely on perceptual psychological grounds not supported by scientific fact. The new in-situ leaching technique described here may prove ideally suitable for use of cyanide in conjunction with biodegradation.

Of course, many of the considerations discussed above for chalcopyrite and refractory gold ores apply to bioleaching of other minerals. Sulfide mineral biooxidation

rates increase with temperature. Consequently, leaching will be increased in warm underground ore deposits.

The in-situ leach technology that is the focus of this white paper lends itself to the application of the same leaching technology used for heap leaching and bioheap leaching, but applied to deposits that cannot be economically mined by conventional techniques. In addition the new in-situ leach technology promises to create a means of optimizing the leach process in a manner that is not possible in conventional heap leaching or bioheap leaching.

e) Metal Recovery from Leach Solutions

Once valuable metals have been leached in-situ, the pregnant leach solution will be collected and pumped to the surface for metal recovery. Various recovery techniques have been used in the past. These include:

• Cementation. Examples include cementation of copper using iron cans, or cementation of gold from solution using zinc dust or zinc shavings. Cementation of copper on iron was used in Cuba to recover copper from PLS generated at the Naciomientos copper ISL test project. Cementation was also used in a primitive way in the first informal experiments in ISL in ancient times.

• Solvent extraction and electrowinning (SX-EW). SX-EW is widely used for the recovery of copper from acid heap leach solutions. Cupric ions are extracted by organic amines from leach solutions. Generally, for SX-EW to be cost-effective, the tenor of the leach solution needs to be quite high. Ness (2000) provides basic principles, processes, chemistry and hardware for copper SX-EW and Spence (2000) presented insights into extractant considerations in copper SX-EW.

SX not only concentrates the ions ahead of electrowinning but reduces the concentration of ions that are deleterious to EW.

Solvent extraction and precipitation. SX has been successfully used for uranium recovery from leach solutions in uranium milling circuits treating higher-grade uranium ores following leaching, solid liquid separation and clarification steps. After being extracted by an organic solvent (typically kerosene) containing organic ingredients (such as tertiary amines), the loaded organic solvent is then recovered in mixer-settlers and then stripped to yield a concentrated aqueous pregnant solution from which uranium is chemically precipitated using ammonium or peroxide solution additions.

Solid Ion Exchange. Dilute pregnant leach solutions such as those likely to be generated by in-situ leaching are generally more amenable to metal recovery using solid anion ion exchange. Various ion exchange resins are available. Fixed bed ion exchange systems require prior clarification of the pregnant solution. A benefit of upflow fluid-bed ion exchange metal recovery systems is that the relatively expensive and operator intensive pregnant leach solution clarification step can be eliminated. Loaded ion exchange resins are then transported to an elution cell where the metal is eluted (stripped) from the resin beads to form a concentrated solution from which metals (e.g. copper and nickel) can then be electrowon, or in the case of uranium can be precipitated to make yellowcake.

Recently the successful new "Pumpcell" technology developed for carbon-in-pulp recovery of gold has found growing application in South Africa and elsewhere. Pump cells (applied in a resin-in-solution mode) appear eminently suitable for the

recovery of uranium, gold and/or copper by adsorption from pregnant leach solution onto solid ion exchange resin beads.

Uranium is stripped from loaded anion exchange resin, and the uraniferous eluate is separated from the base anion exchange resin, and thereafter is generally recovered by treating the uraniferous eluate by first acidifying it and then treating it with ammonia to produce a relatively pure ammonium diuranate.

South Africa's MINTEK has developed and demonstrated ion exchange resin processes for the recovery of gold from cyanide leach solutions.

Direct Electrowinning. New direct electrowinning technology and hardware systems have been developed that make it possible to selectively electrowin metals such as copper, nickel and cobalt from acidic solutions. One such system is the Electrometals Technology Ltd. EMEW cell which claims to be able to achieve improved current efficiencies at lower metal concentrations in the electrolyte. In general, the current efficiency for metal recovery by direct electrowinning improves as the concentration of the metal to be recovered increases. Around 1999, on a trip to Queensland, von

Michaelis (2004, personal communication) recalls being told by Electrometals Technologies Ltd. that EMEW cells were being used to recover copper at a small semi-commercial scale in-situ leach operation in Queensland. Lately the application of EMEW cells to the direct electrowinning of silver from cyanide solutions is attracting industry attention.

• Reverse Osmosis has evolved to a stage where it can be applied to recover water while concentrating reagents and metal ions in the concentrate. Reverse osmosis can now be considered in lieu of SX ahead of electrowinning or precipitation for metal recovery.

• Ultrafiltration and cross-flow filtration can be considered as a potential means of dewatering yellowcake precipitated from pregnant aqueous eluates after stripping loaded SX solutions and or after eluting loaded resins.

• Electrowinning of base metals from chloride solutions offers distinct fundamental advantages over electrowinning from sulfate solutions.

f) Environmental Management and Reclamation

The Committeee on Evolutionary and Revolutionary Technologies for Mining of the National Academy of the Sciences (2002) wrote: **"Key environmental and health concerns raised by in-situ leaching are the possibility of potentially toxic elements being brought into groundwater. For example, selenium, arsenic, molybdenum, and radioactive daughter products of uranium are concerns in mining sandstone-type uranium deposits. Therefore, the committee also rates as a high priority development of lixiviants that can selectively dissolve the desired elements and leave the undesired elements in the rock."**

Post-mining water quality is the major environmental concern of in-situ leaching. Again, quoting from the National Academy of the Sciences committee report: **"The closure of in-situ leaching facilities raises an additional environmental concern, especially in the copper industry where large-scale in-situ leaching of oxide ore bodies above sulfide workings and leaching of sulfide (particularly chalcocite) ores have been conducted. During operations the maintenance of a cone of depression around these ore bodies and the continuous extraction of product solution limits the release of lixiviants and mobilized metals to the surrounding aquifer. However, once**

mine dewatering and solution recovery are completed, there may be a significant potential for the transport of metals and residual leaching solution. To the extent that the ore body is again totally immersed in the water zone, metals will be in a reduced state, and their mobility will be limited" (…last sentence questionable?)…**However, if leaching has taken place above the water table, metals may continue to leach if meteoric water penetration and bacterial activity are sufficient to produce acid conditions. Research should, therefore, also include the evaluation of how these facilities can be closed without long-term adverse impacts to groundwater quality."**

Subsurface environmental aspects of in-situ leaching are today recognized as being of critical importance. Preserving groundwater quality is vitally important and is an increasingly critical issue all over the world. This aspect of in-situ leaching is of such critical importance that it must receive significant attention in the further development of n-situ leaching.

Uranium in-situ leaching extracts metal from porous sandstone rollfront deposits located within aquifers. In most cases in the USA the mineralized deposits that are in-situ leached are in aquifers that are sandwiched between impermeable rock, shale or clay layers, such that contamination of lixiviant or leachate metal ions is isolated from overlying and underlying aquifers.

As described above in section (b), extensive uranium in-situ leaching has been practiced in Eastern Europe and Russia where no advance consideration was given to protecting water quality in the aquifers. This has resulted in extensive environmental damage as described by Mudd (2001b). Repairing the damage retroactively will be very expensive, and may not be affordable to an extent that prevents future beneficial use of the impacted aquifers.

The fact that environmental damage to aquifers as a result of ISL of uranium in the eastern Europe and Russia, where acid leach systems are employed should not be the cause for a prejudicial stigma against acid ISL systems.

From the beginning there was a marked difference in concern for and attention to the environment between ISL uranium leaching projects in the USA and Australia, on one hand, and those in eastern Europe and Russia (Mudd, 2001a,b). In US uranium ISL projects significant care has been taken to avoid damage to aquifers. Test ISL projects have been required to demonstrate that the project design and reclamation techniques can avoid contamination prior to going into commercial operation, and all projects are required to be carefully monitored.

In the case of uranium, concentrations of uranium, and its associated radioactive daughter products and, in some cases potentially toxic elements, such as arsenic, vanadium and selenium could become elevated (National Academy of Sciences, 2002). Site reclamation has been successful at several south Texas sites where ISL of uranium was first undertaken in the 1970s. In-situ uranium leaching has advantages in terms of health and safety because the leaching process selectively removes uranium and leaves most of the dangerous radioactive daughter products in the ground. Also, little heavy machinery is employed to remove the large volumes of rock that would have been processed in a conventional mining operation. Dust generation, a growing concern at open pit operations, is also not an issue in ISL operations.

Mudd (2001a,b) has undertaken a careful study of acid ISL of uranium around the world. His papers provide excellent insights into the critical importance of designing ISL

projects in such a manner that contamination of groundwater aquifers is avoided. They also provide

excellent insights into the damage to groundwater that has resulted in Eastern Europe and Russia when such precautions were not taken. Mudd (2001a,b) clearly shows that repairing damage to groundwater after the fact is generally much more costly than avoiding the problem in the first place.

Hunkin et al. (1979) outlined a method of reclaiming uranium deposits after the uranium in the ore has been removed by ISL. Once uranium has been recovered to the extent that is economically possible, the aqueous ammonium carbonate plus peroxide oxidizing leach solution is replaced by an aqueous reducing solution that is passed through the ore deposit to precipitate and render insoluble any residual uranium and metals such as vanadium, molybdenum and selenium. This process produces a very low volume of above ground impurities and waste solutions requiring disposal, and causes no significant contamination of the underground deposits or any aquifer associated therewith.

T.W. Arman proposed to implement bioleaching technologies at Iron Mountain Mines, Inc. as early as 1983, but the EPA prevented it, and instead spent $250 million making sludge. Now we have to clean up the sludge, thanks a lot.

DETAILED DESCRIPTION OF THE CONCEPT

Summary

The winning of metals from the earth has historically involved mining the ores using either underground or open-pit mining methods followed by processing of the ores to concentrate or extract the metals in some type of above ground treatment facility. In-situ extraction of some highly soluble or easily liquefiable minerals (such as phosphates, salt and sulfur) has been commercialized. The recent advent of heap leaching technology and large scale earth moving equipment has resulted in making large tonnage low grade deposits economically viable due to economics of scale and/or production of refined metals on-site (thereby circumventing cost prohibitive concentrate shipping, handling and treatment charges). The emphasis over the last 20 years has leaned towards developing increasingly larger deposits due to improved economics of scale. Small, low-grade deposits may not be large enough to realize sufficient benefit from economics of scale to render them viable.

In-situ leaching of metals has been developed and undergone experimentation in the past few decades and has been tried in a number of different formats for a variety of metals. These efforts have been generally directed towards extraction of the metals from the rocks using some type of chemical solvent or collector coupled with collection and pumping of the pregnant solvent from within the deposit to an on-site treatment plant for refining. In almost all of the literature, the solutions are injected into the ore deposit through drilled wells and subsequently extracted using these same wells or other wells drilled for the expressed purpose of extracting the pregnant solution. Various patents deal with ways in which the "porosity" or "percolation rate" of the deposit are increased by fracturing the deposit using various types of explosives (including thermonuclear devices), hydro-fracturing or actual dissolution of some or all or the host rock to increase the solution flow rate and/or wetted surface area and thereby the extraction rate and/or ultimate recovery of in-situ metals or minerals. Other patents deal with methods of

aerating the rock mass by injecting compressed air, oxygen or oxygenating chemicals into the deposit. Oxygen is needed for metal dissolution and acid formation.

The mining and extraction process proposed herein contemplates establishing a system of underground draw points beneath a leachable deposit from which a slot raise (or raises) is driven to surface to act as a primary opening for blasting the entire deposit as a single event and undertaking in-situ leaching of the entire deposit over a very long time period. It is envisaged that the leaching process would continue for many years until either all leachable material has been extracted or the daily recovered metal revenue decreases to a point where it is less than operating costs.

The sequences of events for this new concept are as follows. The surface expression of the deposit is stripped of topsoil which is stockpiled for future replacement as a capping on the deposit when extraction is complete. The entire ore body is then drilled off from surface for blasting. In practice, multiple slots will be established and the deposit may be blasted in multiple events over a short time period of a few weeks to months. Concurrently, a decline is driven to establish the draw points and undercut system through which the leach solution is collected and mucking of broken ore is undertaken to keep the broken rock in continual motion. A slot and undercut openings equivalent to nominally 10% of the total deposit volume is first blasted and withdrawn to provide room for expansion to permit blasting the entire remainder of the deposit thereby rubblizing it for leaching. The degree of fragmentation achievable is, in general, a function of the quantity of explosive used. The slot muck removed from the draw points is hauled to surface and temporarily stockpiled prior to being eventually dumped on top of the blasted deposit.

The top of the entire fragmented deposit is then sprayed with leaching solution using high pressure nozzles to spread the leachate over the entire surface of the blasted deposit. The solution percolates through the rock mass down to the draw points which act as collection points to direct the pregnant solution to sumps. The pregnant solution is then be pumped to a surface recovery plant from which the desired metals are recovered. Barren solution is recycled to the top of the deposit. Blasted rock is continually drawn from the draw points throughout the life of the operation but at a very slow rate. Only sufficient rock is removed to keep the rock mass in a "constant" state of mixing. Over the life of the operation it is anticipated that only about 10% to 15% of the total rock mass will be mucked and hauled to surface and placed on top of the muck pile. The above sequences of activities are illustrated as Stages 1 to 4. Four figures are provided for each stage depicting the ongoing activities in plan, cross-section and vertical longitudinal projection with explanatory comments annotated on the sketches to highlight the state of events for each particular stage (see the appendix for the complete set of 16 figures).

By establishing a system of draw points beneath the leachable deposit, capital and operating costs are dramatically reduced in comparison with a conventional heap leach operation and the drawbacks associated with other proposed in-situ leaching methods are overcome. Specifically, aeration, solution channeling, solution collection, environmental contamination by leach solutions and porosity issues are alleviated by the continual movement of the blasted rock due to mining extraction through the draw points. Oxygenation of the rock mass occurs naturally due to the exothermic nature of the leaching process which draws air upwards by convection through the broken rock thereby aerating the mass while simultaneously providing ventilation throughout the underground workings.

The environmental disturbance using this methodology is minimal as there is very little need for surface disturbance initially and ultimate reclamation only requires replacing the original topsoil back on top of the leached deposit, removing the extraction plant and placing a small concrete plug in the decline collar. If warranted, all facilities could be located underground.

Heap Leaching Technology

Heap leaching technology has advanced rapidly in recent years. However, standard heap leaching operations are still plagued by a number of limiting technical factors. The leachability of any particular deposit is unique to that deposit and is a function of rock porosity and mineralization type. Some deposits are refractory and do not leach well under any conditions while others contain metal hosted in unoxidized sulfides or encapsulated in silica (quartz). Notwithstanding the above, many deposits are leachable and technology has been developed to predict the leachability and leach rates of any given deposit. The leachability of any deposit is usually only affected by the particle size being leached, which defines that amount of desired metal that can be leached over a given period of time, and the presence or absence of clay minerals that cause blinding in the heaps. Ore may be heap leached as run-of-mine or after crushing to optimize the economics of the operation. In practice, heap leaching operations typically are designed to extract approximately 70% of the contained metal over about a 12 month period. Heap leaching pads must be designed around the above parameters. A particle size is selected as optimal for the operation and the run of mine ore may need to be crushed in order to reduce it to a size that will permit a reasonable extraction over a 9 to 12 month period. Leach pads must be designed so that minimal compaction occurs as compaction hampers aeration within the heap as well as being one cause of solution blinding, pooling, and channeling. When the ore needs to be finely crushed prior to leaching or contains sufficient clay minerals to hamper leaching, the ore is frequently agglomerated to overcome these obstacles.

Heap leaching test work can predict, with reasonable accuracy, how quickly a deposit will leach. Usually this test work is only undertaken to project leach rates over a maximum one (1) year period. It is known that leaching follows an exponential decay curve and that leaching will continue theoretically until all the metal has been leached. The time frame involved for complete leaching to occur is very long. The new concept presented herein will take advantage of very low operating costs to be able to undertake continuous leaching of the ore for a period that may stretch to 20, 30 or more than 50 years.

Fragmentation

As mentioned above, leaching rates are generally a function of ore characteristics, particle size and leach time. Heap leaching operations are usually large operations using big equipment to handle material. Drilling and blasting is undertaken to reduce the ore to a size that can easily be handled by the equipment fleet. When big open pit equipment is used, the largest boulder that can be handled can weigh many, many tonnes so drilling and blasting is undertaken with this in mind. Open pit blasting usually employs a powder factor of around 0.20 pounds of explosive per ton of ore to achieve the desired level of fragmentation. Underground mining faces different operating conditions and must undertake drilling and blasting operations to reduce the ore size to "hat size" so that it will not hang up in ore passes and chutes. In order to do this, underground operations employ a higher powder factor in the order of 0.40 to 0.60 pounds per ton. Drilling and blasting technology has advanced to the point that combined with geotechnical and

electronic advances, it is possible to predict with a high degree of accuracy the particle size distribution that will result under a given set of drilling and blasting conditions. Recent advances in the ability to embed computer chips in blasting caps have enabled technicians to undertake very precise blasting that produces very fine fragmentation with very little oversized material. Agnico Eagle's LaRonde Mine employs this technology and has been able to reduce their stope blasting to only two (2) blasts per complete stope. The resulting fragmentation is exceptional.

The new in-situ leaching concept described herein will make use of this technology in combination with leachability test work to fragment the ore body in-situ to the desired particle size distribution. It should be noted that the use of explosives energy is a very inexpensive means of breaking rock. AN/FO costs about $0.35 per pound. Using a powder factor in the order of 1.0 pounds per ton would result in very fine fragmentation of the in-situ ore so that in-situ leach rates could be comparable to or better than heap leach rates. The degree of fragmentation is simply a function of drill hole spacing and powder factor.

Most of the previous approaches to fragmentation for in-situ leaching attempted to increase permeability and leachability by creating fractures in the rock mass through hydrofracturing, explosives and even thermonuclear devices to fracture and heave the rock mass. The new concept employs standard underground mining practices to create a void representing about 10% to 15% of the total volume of rock to be broken. This provides sufficient void space for the ensuing mass blast to expand during the blasting process. The resulting broken rock will heave on the surface of the blasted area by approximately 6.1 to 9.1 meters (20 to 30 feet) while creating a void space within the broken material of about 35% to 45%. The particle size distribution will be predictable.

The ore removed through underground mining activities undertaken to prepare the void space needed to produce the desired fragmentation will be emplaced on top of the heaved muck pile on surface and the total deposit will then be leached.

The conceptual deposit used herein assumes for simplicity's sake that the deposit bottoms out at the 91 to 152 meter (300 to 500 foot) depth. Present drilling technology is capable of drilling to this depth with minimal deviation. Deeper drilling would require specialized drill control techniques or multiple phases of drilling and blasting coupled with the establishment of one or more intermediate drilling horizons which are entirely possible but would unnecessarily complicate the simple concepts this paper is attempting to present.

Leaching

Leach solutions will be sprayed over the top of the broken material and be allowed to trickle through the blasted rock leaching metals encountered along their path. Channeling will occur and not all of the rock mass will be whetted initially. Knowing the leach rates from the leaching test work and the predicted particle size distribution, one will be able to assess the extent of channeling during the initial leaching of the deposit.

While leaching is under way, the blasted rock mass will be slowly moved downward by withdrawing broken material from the draw points on a daily basis. Over the life of the operation it is anticipated that only about 15% of the broken rock will need to be withdrawn to be able to create sufficient movement with the blasted material to permit all of the broken ore to be wetted. The draw point system array is similar to those used for underground block caving operations. Draw control at underground block caving operations is practiced with a great deal of care in order to draw down the ore evenly so that intermixing of waste with the ore and loss of ore intermixed within the waste is

minimized. Even with careful draw control, ore recoveries in the 85% range accompanied by about 15% dilution are the norm for block caving operations. Considerable work has been done to identify the conditions under which intermixing occurs during block cave mining and considerable effort is expended to avoid creating these conditions. The slow removal of material envisaged in this new in-situ leach concept does not employ the same draw control parameters. Instead, intermixing of the material is preferred. Draw points will be mucked in a sequence that causes the most intermixing. The ore will also undergo further fragmentation during the process of movement within the column. The constant movement taking place within the rock mass will reroute solutions and minimize channeling and also serve to flush out fine material to the draw points located at the bottom of the zone. While the actual leaching rates cannot be predicted at the moment, the process can eventually expose all of the broken rock to leaching solutions.

The new in-situ leaching concept presented herein solves the problems of solution channeling that has plagued previous attempts and also addresses the problem of blinding due to fines. The fines that are flushed out will eventually settle in the underground sumps and be pumped to surface where they will be held until the end of the mine life whereupon they will simply be added to the top of the ore pile as part of the final mine rehabilitation activities.

Aeration

The presence of approximately 40% void space in the broken material coupled with the heat generated through exothermic reactions will produce natural ventilation through the rock mass that will provide oxygen to aid in reaction kinetics. The dissolution of gold requires oxygen while copper leaching requires oxygen to generate acid from sulfides contained in the ore. The airflow through the rock mass can be controlled because it must enter through the decline access which can be restricted to reduce airflow or pressurized using air fans to increase the airflow. Aeration within heap leaching pads is an issue that restricts the thickness one can emplace upon a given pad and has been the subject of many attempts to improve oxygenation of leach pads through injection of oxidizing chemicals or air through drill holes and pipes as well as using explosives to heave leach pads to alleviate blinding, channeling and improve oxygenation. This new concept provides a simple, low cost and effective means of solving another of the major problems encountered during heap leach operations.

Leach Solution Collection and Containment

Previous in-situ leaching attempts usually envisaged collection of pregnant solution via drill holes drilled beneath or adjacent to the material being leached. In this new concept, the deposit is completely undercut during the preparation of the draw points and undercuts. Assuming that the deposit is nearly vertical, all solutions must flow by gravity ultimately to the draw points at the bottom of the deposit. All of the underground tunnels will be driven at a slight uphill slope from the collection sump so that all of the pregnant solutions will drain to the sump. This new concept initially envisages locating the draw points at or slightly below the oxide/sulfide interface which is generally at the water table. With the draw point located below the water table and no static head being developed in the leach column, any fugitive flows should be INTO the draw point system from the surrounding groundwater. Solutions should not be able to enter the groundwater if the draw points are located below the water table. Similarly, the blasted perimeter walls of the in-situ leach column actually will form the containment and reaction vessel in which leaching takes place. It is envisaged that the walls of the vessel will be vertical or inclined slightly outward as it progresses to depth so that gravity effects will naturally

force any leach solutions to shy away from the walls. In this way, any ground water flows will be inflows into the leach column not leach solution outflows into the surrounding environment. Inflow of ground water could result in dilution of the pregnant leach solution and water balance modeling will be an important aspect of more detailed design of the new in-situ leaching system.

ECONOMICS OF THE CONCEPT

Summary

Table 9.1 illustrate the comparable capital and operating costs associated with in-situ leaching for a typical 20 million tonne heap leachable oxide gold deposit. The capital costs to undertake in-situ leaching are roughly 40% to 60% of the capital costs required establishing a standard heap leaching operation and the operating costs are very low as very little material is moved on an annual basis.

Although existing technology can predict leaching rates and recoveries for run of mine ores and heap leaching in pads, little data is available to accurately predict in-situ leaching rates and recoveries using the proposed system. The economics of this in-situ leaching method is based on an estimation of how much recovery is needed from the overall deposit in order to recoup capital costs and how much recovery is needed annually in order to cover operating costs. Recovery of less than 10% of the in-situ contained metal will pay for the initial capital costs while operating costs can be recouped from as little as 1.0% to 1.5% annual recovery of the in-situ contained metal.

Rapid payback is anticipated as all of the deposit is brought under leach within a short time frame as opposed to heap leaching where the ore is mined and treated over a 7 to 10 year mine life. The up front capital costs are estimated to be recovered by leaching only 10% of the contained metal in the case studies.

Capital Costs

Capital costs have been estimated based on known costs for other projects with which we have been involved. The mine development costs assume that the operator provide the equipment and facilities and contracts the labor and materials component. The capital costs for this new concept are much lower than for a conventional heap leaching operation primarily due to the minimal infrastructure required for the concept. Based on a comparison to the capital requirements for a known 50 million tonne heap leach gold deposit, the capital costs are in the order of 40% of that needed for conventional heap leaching. Based on less than 10% recovery of the contained metal over a one year operating period, all capital would be repaid.

The capital cost estimate for the metal recovery plant is based in the example cases on the estimated cost for a carbon adsorption-desorption-regeneration (ADR) plant for gold. An SX- plant for copper recovery and water purification willcost more.

Operating Costs

Operating costs for this new in-situ leach concept are expected to be very low. Operating costs have been estimated based on standard US wage rates for mining and equipment operating costs. Very few people are needed to operate once the project is in production. One man per shift is needed to operate the processing plant and 2 men are needed on only one shift per day, five days per week to muck the draw points and service the topside dump and spray nozzles. The operating costs to keep the ore in motion and operate the gold recovery plant will be in the $3 to 4 million per year range and require less than 1% recovery of the contained metal to cover operating costs.

<h2 style="text-align:center">Table 9.1 IN-SITU LEACHING</h2>

<h3 style="text-align:center">20 MILLION TONNE SULFIDE GOLD DEPOSIT</h3>

PROJECT PARAMETERS

Deposit is oxide gold nominally 20 Million tonnes grading 1.5 gm/T Au
Roughly 200m wide by 300m long by 150m deep

CAPITAL COSTS			**$ X 1000**
Mine Preparation			
Decline	1000m @ $1,500/m=		1,500
Undercuts & Mucking Drifts		3,500m @ $1,000/m=	3,500
Draw Points	75 @ 10m ea @ $1000/m=		750
Slot Raise	150m @ $400/m=		60
U/G Drill & Blast Undercuts		1 Million Tonnes @ $2.00/T=	2,000
Slot Drill & Blast		1 Million Tonnes @ $0.50/T=	500
U/G Mucking & Hauling		2 Million Tonnes @ $2.00/T=	4,000
Move Surface Stockpile		2 Million Tonnes @ $0.50/T=	1,000
Sub-Total			13,310
Drill & Blast Remainder of Orebody		18 Million Tonnes @ $0.40/T	7,200
Sub-Total			7,200
Surface Facilties			
Surface Stripping		500,000 cu. m. @ $2.00/m=	1,000
Surface Plant (CIL) & Facilities			4,000
Misc. Facilties & Equipment			2,000
Sub-Total			7,000
TOTAL CAPITAL COSTS			27,510
Contingency			2,490
TOTAL PROJECT CAPITAL			**30,000**

ANNUAL OPERATING COSTS		**$000/YR**
Reagents and pumping costs		1,000
ADR Plant Operation		1,500
UG Mucking	250,000T/yr @ $2.00/T	500

Supervison, Maintenance, G & A	1,000
TOTAL OPERATING COSTS	**4,000**
<u>**RECOVERIES REQUIRED**</u>	**$ X 1000**
Deposit Grades 1.5 gm/T Au or Roughly $15/T	
Deposit Gross Value 20 Million Tonnes @ $15/T=	300,000
Capital Costs as % of Gross Value (Break even Recovery Needed)	**10.0%**
Operating Costs as % of Gross Value (Break even Recovery Needed)	**1.33%**

Economic Viability

Revenue is difficult to accurately predict given that this concept has never been attempted. In order to gain an insight into the economic potential of the concept, a reverse economic approach has been used. Based on the capital and operating costs estimated, the recovery needed to offset these costs was estimated. As can be seen from Tables 9.1 and 9.2, since the whole orebody is under leach at the outset of production, very little metal need be recovered to recover capital and operating costs.

Assuming a 20 year operating life and an ultimate overall recovery comparable to heap leaching of 70% (7% recovery per year), the 20 million tonne deposit would generate a total life of mine revenue of approximately $210 million to offset an estimated capital cost of $30 million and 20 years of operating costs that would total about $80 million for a net operating profit of about $100 million or about $6.5 million per year of operating profit, (revenue $10.5 million less operating costs of $4 million) which is a healthy revenue to cost ratio. Similarly, the 40 million tonne deposit would generate a total life of mine revenue of approximately $420 million to offset an estimated capital cost of $47 million and 20 years of operating costs that would total about $100 million for a net operating profit of about $320 million or about $16 million per year of operating profit (revenue $21 million less operating costs of $5.25 million).

BENEFITS OF THE CONCEPT

The benefits that arise from application of the concept are summarized as follows:
- The new system promises to improve the economics of treating mine waste.
- The new ISL concept will likely accelerate the leach rate of the deposit resulting in an economic benefit.
- This system overcomes many of the challenges of in-situ leaching hard rock ores, e.g. copper porphyry deposits, oxide gold and silver deposits, volcanogenic massive sulfides and sediment hosted ores, namely: poor metal recovery due to inadequate fragmentation, inadequate leaching due to fines migration and blinding, inadequate leaching due to solution channeling, poor oxygenation, gas bubble exsolution and blinding of pores, etc.
- By excavating a slot through the ore deposit prior to blasting, provision is made to accommodate the swell factor that occurs during blasting. This results in improved fragmentation of the ore to the point that the fragmentation can be engineered to produce the fragmentation required for optimal metal leach recovery.
- Fines migration within a blasted ore zone subjected to in-situ leaching can result in blinding off zones within the ore deposit. The innovative system described here

prevents blinding from occurring, as the entire column is moved and new solution flow patterns are constantly being created.

• When air, oxygen or sulfur dioxide are injected into an in-situ leach system it is possible for gas pockets to form that blind off portions of the deposit preventing solutions from reaching or penetrating into the ore particles immediately adjacent to the gas bubbles. This phenomenon can be expected to be overcome by the new technology since the entire column of blast-fragmented ore is moved on a regular basis thereby establishing a constantly changing flow pattern of oxygenating air and solution through the ore.

• Gases, e.g. air and oxygen are pumped into the leach system via the access decline and introduced via the bottom of the column of ore undergoing leaching. The rate of oxidation and bio-oxidation of sulfides within the ore being treated can be controlled by 1) the rate of oxygen or air injection, and 2) the rate at which lixiviant solution is applied to the top of the column of fragmented ore.

• The new concept provides a much more controllable environment for bacterial oxidation of sulfide minerals, and for solution and oxygen contact with the fragmented material in the ore column.

• The new concept configuration, with the ability to cause movement within the ore column, provides a means of more efficient rinsing of the spent ore column on completion of the project, if rinsing is a requirement for reclamation and closure.

11. WHAT NEEDS TO BE DONE NEXT?

These are key steps to be taken in order to further develop the new concept.

1. Proceed with in-situ leach concept development and new water treatment.

2. Carry out a conceptual design study of how the new in-situ leach concept will be applied.

3. Future development work.

4. In order to advance the new concept, it will be helpful to enlist the assistance of engineers and scientists who have prior experience in in-situ leaching and metal recovery from leach solutions.

5. Prime focus should be given to at least the following aspects seeking to apply the new concept in-situ leach system to a real life ore deposit:

• Geometry of the system
• Applying the new concept to improving permeability of the ore to be leached
• Lixiviant and mobilized metal ion containment
• Solution application system
• Solution collection and pumping system
• Air injection system
• Air flow control system
• Metallurgical balance management system
• Reagent balance management system
• Practical aspects of the solution flow pattern adjustment system.
• Closure and reclamation aspects.
Purchase new solvent extraction technology, build facilities.

The following articles by professor Jill Banfield of U.C. Berkeley explain the microbes.

MICROBIAL COMMUNITY STRUCTURE AND FUNCTION: A GENOMICALLY-ENABLED CASE STUDY IN AN ACID MINE DRAINAGE SYSTEM

"In the surreal world of Iron Mountain, creeks run a brilliant red, sterile from high doses of heavy metals. Dark-green water, as caustic as battery acid, pours from underground caverns, and poisoned springs bubble up as if from a steaming caldron."
-The Los Angeles Times Apr.10,1993

As dangerous and uninviting as the conditions may seem based on the quote above, the solutions draining fom Iron Mountain mines are not sterile. In fact, flourishing communities of microorganisms populate this habitat. These microbial communities, and their role in the generation of acid mine drainage, are the focus of DOE (Microbial Genome Program) and NSF (Biocomplexity Program)-funded work at Iron Mountain.

Previous studies have demonstrated that microorganisms promote acid mine drainage (AMD) formation by oxidizing ferrous to ferric iron. Ferric iron is a much more effective oxidant of pyrite surface sulfide groups than O2. Thus, understanding of AMD formation requires understanding of microbial activity.

The goal of these projects is to obtain a molecular-level understanding of the metabolism of organisms involved in AMD formation. Because so few organisms can be cultivated for detailed study, our approach has been to develop cultivation-independent methods to identify the organisms present and determine their roles.

This web page provides basic project information and describes genomic and genomically-enabled studies of microbial biofilms that populate the Richmond Mine, the source of much of the acid mine drainage (AMD) at Iron Mountain. The genome sequencing is being conducted at the Joint Genome Institute under sequencing awards from the DOE's Microbial Genome Program.

THE SITE

Iron Mountain Mine is located approximately 9 miles northwest of Redding, CA in the foothills of the Kalamath Mountains. Mining operations at Iron Mountain began in the late 1800's and continued intermittently until 1962. The 4,400 arce property contains many miles of underground tunnels and workings, an open-pit mining area, waste-rock dumps, and tailings piles. The ore body is a massive hydrothermal sulfide deposit in rhyolite host-rock and was mined for iron, silver, gold, copper, and zinc. Blasting and tunneling have made the sulfide ore permeable to air and water which results in the generation of vast quantities of metal-contaminated sulfuric acid solutions. It has been estimated that there is enough remaining ore in the disused mines at Iron Mountain to allow the acid mine drainage process to continue for about 3,000 years.

The biofilms under investigation are growing in regions within the ore body where acid mine drainage is currently forming. The sampling sites are accessed by a recently renovated horizontal entrance tunnel that is maintained as part of the Superfund site remediation effort. The ore deposit is encountered about 460 meters (1,500 feet) inside

the mountain. The entrance tunnel meets with 4 other tunnels in a region referred to as the five-way (see figure below). In the first years of our work at Iron Mt. (starting in 1994), our sampling was conducted within ~20 m of the five-way. Recently, we have been working in three areas in the A drift, AB-B drift junction area (AB muck dam) and C drift. Here, temperatures are high due to the exothermic nature of pyrite oxidation, varying between 35-50 degrees C (95-120 F). Drainage waters which are collecting and flowing through the tunnels have a pH between 0-1, which is indeed as acidic as battery acid.

Map of the field site used in this study.
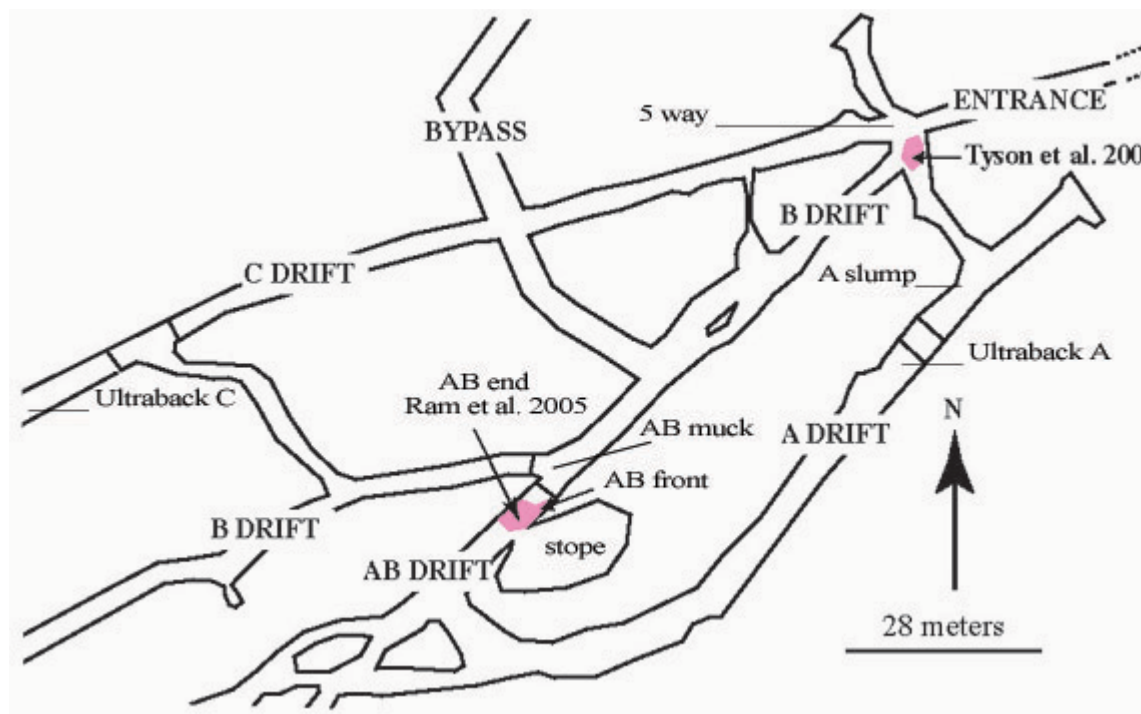
Molecular Microbiology

Of special importance to understanding acid mine drainage and for the development of predictive models, remediation strategies, etc., is an assessment of the importance of known and new microbial species and the determination of their role in the generation of AMD.

Typically, it had been assumed that the microbiology of acid mine drainage environments is well represented by a few readily cultured bacteria species (Acicithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, and Leptospirillum ferrooxidans and related species). However, results from our early studies showed that extreme pH and temperature conditions at acid generating sites are dominated by other unknown or little studied organisms.

We have documented the microbial species involved in metal and sulfur cycling at our field site using an array of molecular techniques. These include DNA sequence analysis and fluorescence in-situ hybridizations (FISH). A FISH image of a biofilm is shown below.

FISH image of a biofilm. Cells shown in yellow are Leptospirillum group II, those in white are Leptospirillum group III, blue cells are a variety of archaea.
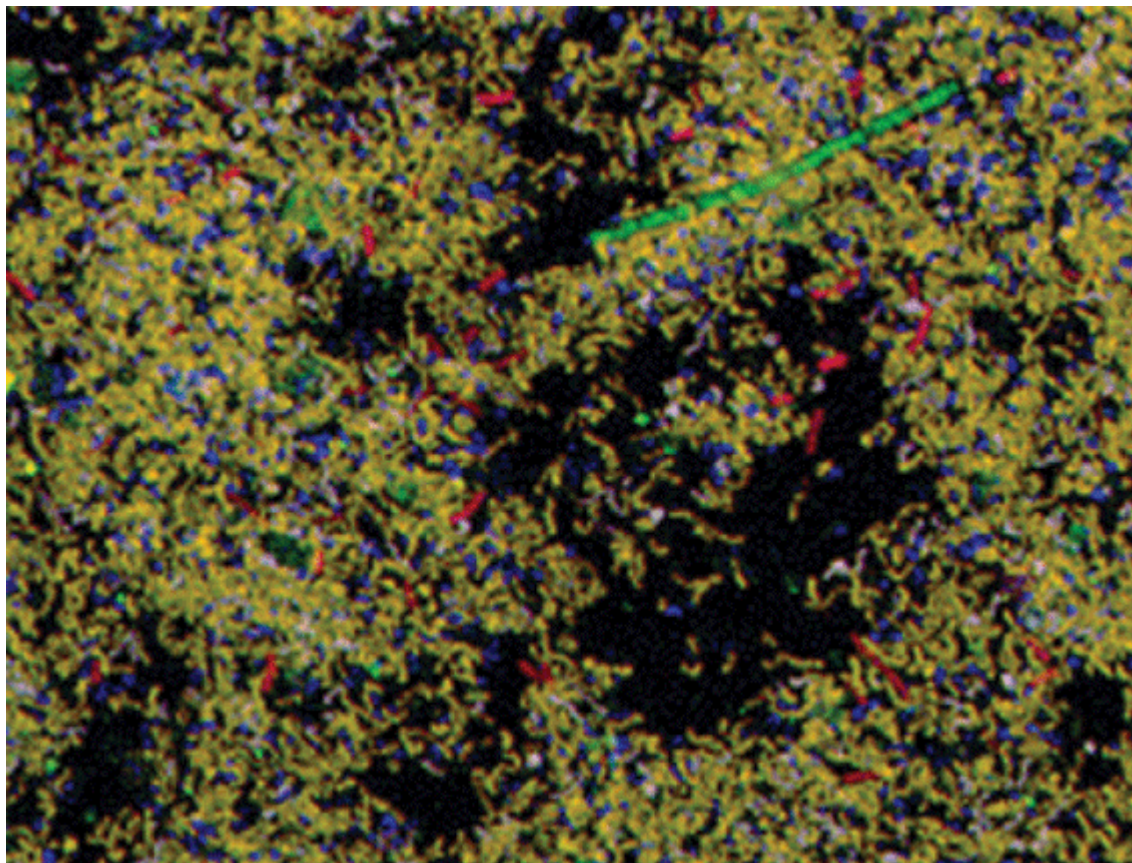
DNA extractions and sequence analyses can answer "who's out there" but offer limited information about the abundance, in-situ metabolic activity, and ecological niche of individual organisms. Therefore, we have used FISH to label cells on mineral surfaces and in suspension to quantify cell type distribution and correlate the distribution of organisms (species level) with geochemical conditions (Edwards et al. 1999).

Culturing and Metabolic Analysis

Cultivation-based methods have been used in order to obtain organisms for physiological characterization and for experiments designed to evaluate the impact of microbial activity on pyrite dissolution. To date, we have isolates of Leptospirillum group II, Leptospirillum group III (obtained only after genomic data provided clues to an isolation strategy),

and Ferroplasma acidarmanus.

We are continuing to enrich for and isolate bacteria and archaea from within an extreme acid mine drainage environment. Microbial samples are collected from different environments, including sediments, pore fluids, free-flowing waters, and subaerial biofilms. These field samples are used as innoculum in a variety of different media which are incubated under aerobic, microaerophillic, and anaerobic conditions. As isolates are obtained, metabolic and biochemical characterizations are being carried out, including assessments of their growth rates, optimal growth conditi ons (pH, temperature, ionic strength) and their range of metabolic capability. New and unknown isolates are being identified through DNA sequence analyses.

Although these isolates are important for some aspects of our work, our primary goal is to investigate entire natural communiites in situ, without cultivation biases and with the ability to understand the roles of closely related organism types (i.e., without assuming that isolates are representatives of their species or that laboratory conditions adequately represent those in the field).

Acid Tolerance Mechanisms

A very important and fundamental question is "How do microorganisms survive (and thrive) in highly acidic and metal-rich solutions?"

The abundance and diversity of microbial life at pH < 1.0 (extreme acidophiles) raises interesting questions about microbial adaptions to the environmental extremes of acidity and dissolved metal concentrations. Microoragnsims thriving in acid mine drainage must have systems for protection from their environment . Of particular interest is the ability of microorganisms to maintain internal pH homeostasis against enormous pH gradients. The only well studied acidophilic chemolithotroph is Acidithiobacillus ferrooxidans. Optimal growth of A. ferrooxidans occurs between pH 1.5 to 3.5, yet cytoplasmic pH remains near neutral. Internal pH maintenance in T. ferrooxidans has been attributed to a reversed membrane potential. However, the mechanisms of pH homeostasis operating in extreme acidophiles are unknown. It is likely that the system(s) differ from those previously identified in A. ferrooxidans and the well-studied neutrophiles, such as Escherichia coli and Salmonella.

Our investigations of pH tolerance have focused on the archaeal inhabitants. We have analyzed the lipids of F. acidarmanus and shown that the cells are bounded by predominantly tetraether-linked membranes that prevent proton penetration so that the cytoplasm can be maintained at

~ pH 5.3 (Macalady et al. Extremophiles, 2004).

More recently, our focus has been on protein stability at low pH. The results of this work are noted below.

Enzymology of Iron Oxidation

Biologically-mediated pyrite oxidation has probably exerted a fundamental geochemical control on the global iron and sulfur cycles for a significant fraction of geologic time. Iron oxidation occurs in the bacterial and archaeal domains, and in both of the two, early diverging archaeal kingdoms. An important challenge is to relate metabolic innovations to the geological record, and place these on an absolute time scale. Evolution of iron oxidizing metabolisms may have been coupled to the build up of atmospheric oxygen. This capability may be distributed through the prokaryotes because it is a characteristic of an ancester to both domains, or it may have multiple evolutionary origins. Diversity or conservation of metabolic pathways provides key information to constrain these possibilities.

Despite the diversity of iron oxidizing organisms, the vast majority of research has been on the respiratory chain of A. ferrooxidans. However, studies have shown that many different types of iron oxidizing pathways exist throughout the Bacterial and Archeal domains. Further characterization of the functional diversity of iron oxidation in these two domains and groups within these domains may reveal evolutionary relationships between them.

Our team is trying to determine the mechanisms for iron oxidation in microbial communities at very low pH. Our primary approach has been to use genomic sequence information to identify candidate proteins that may be involved in the electron transport chains of the different organisms. This information can be used to identify proteins that occur abundantly in the biofilm (e.g., that are extracted from the biofilm, purified, and N-terminal sequenced). More recently, our focus has been on using proteomic methods to identify those candidate molecules that exist in high concentration and occur in the membrane of periplasm (see below). The pink color of many biofilms is due to a high concentration of a red heme-based cytochrome.

Mineral Dissolution Rates

The debate surrounding the exact mechanisms and rates of microbial sulfide mineral dissolution continues. Attempts to clarify the role and relative importance of the two proposed mechanisms, "direct" and "indirect" attack, have not been conclusive. A new model for biofilm function is currently being developed. This may change our view of how organisms function in communities and how energy metabolism is structured.

In prior work we conducted experiments to determine the rates of oxidation and the surface morphologies of different iron sulfide minerals that are oxidized abiotically and in the presence of bacteria and archaea. The correlation between attached cells and surface "pitting" has been evaluated. Results indicate that although many pits form inorganically, some cell-mediated local pitting can occur (e.g., F. acidarmanus on arsenopyrite and Leptospirillum cells on pyrite). Dissolution rates for attached and unattached microorganisms have been measured for a number of species with different metabolic capabilities (Edwards et al. 1998, 1999, 2000).

Speciation and Release of Arsenic

Large doses of arsenic can be fatal and chronic, repeated exposure results in body-wide effects including skin lesions, cancer of the internal organs, heart and blood vessel damage, and liver and/or kidney failure. Water supplies in many areas of the world are contaminated with high levels of arsenic. While some arsenic contamination can be attributed to industrial and agricultural sources (e.g. tanneries and pesticides), a significant fraction is liberated through the alteration of arsenic-bearing minerals. In general, the mineralogical, biological, and geochemical factors leading to arsenic contanimation are not well understood.

The central goal of this part of our research is to understand the chemical, structural, and microbial factors that control the speciation and release of arsenic into the environment. In prior work we conducted experiments to understand the reactions that result in arsenic release from sulfide minerals and evaluated the processes that determine its form and distribution in water, secondary minerals, and organic materials. The ways in which both biological and inorganic processes control the rates of dissolution of arsenic-bearing minerals in acid mine drainage conditions has also been studied. In addition, we are determining which microorganisms in acid mine drainage environments have the ability to utilize or transform arsenic and what the mechanisms are. Most recently, this work has involved testing of a very novel archaeal gene with similarity to arsenate reductase by inserting the gene in E.coli cells that lacked this capacity (Flanagan et al. in prep.).

Mine Water Chemistry and Modeling

Waters from the Richmond 5-way at Iron Mountain Mine are being collected in parallel with microbial studies at the site. The interactions between microbial organisms and the minerals and waters they are associated with is being investigated. Complete chemical analyses of these waters using a variety of techniques (including - ion chromatography, atomic absorption spectrophotometry, colorimetric spectrometry, and inductively-coupled plasma mass spectrometry) are coupled with field measurements (pH, Eh, T, cond, D.O.) to rigorously define the aqueous chemistry at the site both spatially and temporally. Waters at the Iron mountain Mine contain extremely high concentrations of Fe (15,000 - 80,000 mg/l), SO4 (100,000 - 500,000 mg/l), Zn (1,000 - 6,000 mg/l), and other metals (including As and Cu). Very low acidities (pH -3 to 1) and high concentration of metals and sulfur give these waters ionic strengths from over 1 to up to approximately 7 m. Speciation of the waters is accomplished in part by using the PHRQPITZ model, but there are limitations in the utilization of that model with respect to redox chemistry due to the lack of information on Fe3+ specific interaction parameters in the current PHRQPITZ database. Aqueous geochemical changes correlated to changes in the microbiological community are being coupled with both forward and inverse modeling codes (PHRQPITZ, PHREEQC, CCBATCH, and an in-house mass balance spreadsheet) to understand the role of microbes in the overall generation and maintenance of a highly acidic environment. Mass balance modeling will be coupled with several laboratory column experiments, a set of in-situ field experiments at Iron Mountain, and finally on observed geochemical changes in acid mine drainage waters to assess relative reaction rates of sulfide minerals in the field and the effect of microbial catalysis and oxygen infiltration.

Table 1 provides geochemical information from a series of sampling time points to illustrate the range in pH, temperature, redox potential, and metal concentrations.

TABLE 1 : Geochemical data (from Druschel, Baker et al. 2004).

Water chemistry of samples collected within the study site at the Richmond Mine 5-way area (at the 5-way, A, B, C weirs, and at the A-slump and red pool in 2... Concentrations are reported in millimolar units.

sample name	date	T (°C)	pH	total H⁺	Fe²⁺	Fe³⁺	Fe_T	Eh (mV)	Cu	Zn	SO₄²⁻
99IM25-way	May-99	45	0.71	740	344	31	375	708	6.2	22.4	786
00IM15-way	June-00	36	0.60	761	159	23	182	720	3.2	19.4	743
01IM15-way	January-01	29	0.84	558	64	165	229	794	2.0	16.5	657
02IM15-way	March-02	42	0.83	587	297	20	317	700	4.0	14.3	684
99IM2A1	May-99	38	0.85	566	360	27	387	703	6.4	16.4	824
00IM1A1	June-00	38	0.60	688	107	9	115	705	3.1	11.5	606
01IM1A1	January-01	30	0.89	441	81	177	258	790	2.1	11.3	560
02IM1A1	March-02	42	0.83	579	269	50	319	727	4.1	14.0	665
99IM2B1	May-99	38	0.52	1167	380	41	420	713	5.4	27.9	1161
00IM1B1	June-00	31	0.60	1000	209	61	271	738	3.5	25.3	1020
01IM1B1	January-01	27	0.75	715	55	257	312	810	1.4	25.5	929
02IM1B1	March-02	47	0.78	582	261	13	274	692	2.2	16.8	550
99IM2C1	May-99	48	0.76	805	372	30	403	705	6.0	26.8	1020
00IM1C1	June-00	44	0.60	858	170	25	196	721	3.3	23.8	756
01IM1C1	January-01	32	0.85	503	47	195	243	806	2.0	24.1	635
02IM1C1	March-02	50	0.76	800	281	7	288	676	4.7	14.3	651
02IMM B-back	March-02	45	0.83	544	240	1	241	638	2.3	17.2	586
02IM1 C-back	March-02	50	0.82	583	274	12	286	689	4.5	14.2	656
00IM1 Aslump	June-00	32	1.10	190	26	21	46	764	0.9	1.3	149
01IM1 A red pool	January-01	30	1.38	115	10	25	35	793	0.3	2.7	142
02IMM A-back	March-02	42	0.83	675	363	16	380	690	6.0	18.4	892

Organism and Community Genomics: Ecology and Lateral Gene Transfer

Isolate genomics

The genome of Ferroplasma acidarmanus, an iron-oxidizing extreme acidophile from the site, was sequenced at the Joint Genome Institute and at UW Madison. The genome (with manual annotation) is available at:

The extraordinary simplicity of AMD microbial communities (compared to other communities in less extreme environments) makes it possible to go beyond use of analysis of isolates for genomic characterization. Our approach is to sample the genomes of an entire microbial community, without cultivation.

Community genomics for study of lateral gene transfer (LGT)

Our approach has been to simultaneously characterize the genomes of organisms that represent a natural microbial community. This is feasible because prior work has demonstrated that the communities have low species-level diversity.

An advantage of acidophilic communities for study of LGT is that the microbes have been largely isolated from most organismal diversity over geological-evolutionary time scales due to their extreme habitat. A hierarchy of LGT probabilities may exist, based on the likelihood of interactions with organisms in other environmental niches. Our goal is to identify the genes that have been transferred, to determine whether these are peripheral or central to the survival of the host organism, and to assess the predicted relationship between LGT frequency and the probability of inter-species interaction.

AMD community genomics

Samples for the first community genomic study were collected in March 2002. Several 50 ml tubes of biofilm were collected from six of the seven previously sampled locations (pH ~ 0.6- 1.2, 45-50 Â°C). Water samples were also collected for geochemical analysis. A pink biofilm was selected for detailed study. Macroscopically, the community formed a mm thick biofilm that grew at the air-AMD solution interface in a small stream running through fine-grained pyrite sediment.

Using FISH, it was determined that the 5-way community contained approximately 75% Leptospirillum group II, 10% Leptospirillum group III, and 10% Ferroplasma-like archaea (specifically 3 distinct types within this group, including the Ferroplasma acidarmanus population), and 5% eukaryotes (not sampled genomically). Two PCR-clone libraries were constructed using Archaea- and Bacteria-specific 16S rDNA primers applied to DNA extracted from the target community to verify the simplicity of the sample.

Small insert libraries were constructed from DNA extracted from the biofilm sample, and ~ 100 Mb of sequence obtained. We obtained >95% coverage of the genomes of all 5 dominant organism types. The results of this study were reported in an Article in Nature by Tyson et al. (2004).

New sequencing of four additional biofilm samples is planned in 2005.

Post-genomic studies

Ongoing work is focusing on using the existing genomic data to evaluate microbial function in the environment. The first approach has been to combine genomics and mass spectrometry-based proteomics to identify the

abundant proteins in a biofilm. The paper reporting results of this work is currently in review (Ram et al.). The second phase of this work will involve microarray-based experiments.

Visit Jill Banfield's Home Pages

Visit UCB EPS Home Pages

Protein study finds clues to microbes' survival techniques

LIVERMORE, Calif. — When humans gather in communities, they specialize and adapt. Farmers grow crops and raise animals for food based on the area's climate and soil. Builders fashion structures engineered to keep their inhabitants warm in winter and cool in summer. Physicians tend to the sick; police and firefighters protect the public.

Terry Johnson /UC Berkeley

Acidophilic microbes thrive in this biofilm growing inside an abandoned mine at Iron Mountain, Calif. The microbes' metabolism creates highly toxic acid mine drainage, a major environmental problem associated with the mining of coal and uranium.

Communities of microorganisms, researchers are finding, exhibit very similar behavior – genetically evolving, specializing and cooperating in ways that allow them to adapt to extreme conditions of temperature, acidity, toxicity and pressure.

In the first comprehensive study of gene expression in a microbial community from an "extreme" natural environment, scientists from the University of California, Berkeley, Lawrence Livermore and Oak Ridge national laboratories, and Xavier University in New Orleans have identified more than 2,000 proteins produced by five key species in the community. More than 500 of the proteins – chains of amino acids linked together in an order specified by a gene's DNA sequence – appear to be unique to the community, which thrives in hot, highly acidic conditions in an Environmental Protection Agency Superfund site at an abandoned mine at Iron Mountain, Calif. A report on the research, "Community Proteomics of a Natural Microbial Biofilm," appears online today in *Science* Express.

"This is the first effort to accumulate information on genes that are expressed within a natural community, and one that has a major environmental impact," said Michael Thelen, a protein biochemist at Lawrence Livermore. "One of the most interesting things we found is that there are large numbers of proteins that don't resemble any other proteins we know about. Many are enzymes that function to maintain the correct structure of other proteins that are exposed to the unusually harsh acidic environment."

The Iron Mountain microbial community, which consists of bacteria and archaea, has been under study for nine years by a research team led by Jill Banfield, professor of earth and planetary science and of environmental science, policy and management at UC Berkeley (Racha Ram, first author of the *Science* paper, is a postdoctoral researcher in Banfield's laboratory). Last year the Iron Mountain microbes became the first microbial community characterized at the genetic level through large-scale genome sequencing at the DOE Joint Genome Institute in Walnut Creek, Calif.

"This work illustrates the power of the genome sequencing done at the Department of Energy's Joint Genome Institute to contribute to understanding the microbiological communities living at contaminated sites," said Dr. Raymond L. Orbach, Director of DOE's Office of Science. "Now scientists can investigate not only the 'community genome,' but also the resulting 'community proteome' for enzymes and pathways that can help clean up some of the worst environmental sites in the nation. This underscores the value of basic research carried out by the DOE Genomics:GTL Program that can develop novel approaches and solutions to national challenges."

The microorganisms, known as "extremophiles" for their affinity to harsh environments, grow as a biofilm at the Richmond Mine near Redding in Northern California. The complex interaction of microbes, water and exposed ore at the mine has generated dangerously high levels of sulfuric acid and toxic heavy metals, known as acid mine drainage.

The microbes are densely packed in the pink biofilm, which is just a few millimeters thick and floats on the surface of the mine water. The biofilm is a self-sustaining system, using carbon and nitrogen from the atmosphere within the mine and deriving

Rudy Carver /UC Berkeley

Close-up view of biofilm growing onto the water from the pyrite surface of the Richmond mine.

energy from iron that has been leached out of the iron sulfide rock, also known as pyrite or "fool's gold."

Acid mine drainage biogeochemistry at Iron Mountain, California

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Abstract

The Richmond Mine at Iron Mountain, Shasta County, California, USA provides an excellent opportunity to study the chemical and biological controls on acid mine drainage (AMD) generation *in situ*, and to identify key factors controlling solution chemistry. Here we integrate four years of field-based geochemical data with 16S rRNA gene clone libraries and rRNA probe-based studies of microbial population structure, cultivation-based metabolic experiments, arsenopyrite surface colonization experiments, and results of intermediate sulfur species kinetics experiments to describe the Richmond Mine AMD system. Extremely acidic effluent (pH between 0.5 and 0.9) resulting from oxidation of approximately 1×10^5 to 2×10^5 moles pyrite/day contains up to 24 g/1 Fe, several g/1 Zn and hundreds of mg/l Cu. Geochemical conditions change markedly over time, and are reflected in changes in microbial populations. Molecular analyses of 232 small subunit ribosomal RNA (16S rRNA) gene sequences from six sites during a sampling time when lower temperature (<32°C), higher pH (>0.8) conditions predominated show the dominance of Fe-oxidizing prokaryotes such as *Ferroplasma* and *Leptospirillum* in the primary drainage communities. *Leptospirillum* group III accounts for the majority of *Leptospirillum* sequences, which we attribute to anomalous physical and geochemical regimes at that time. A couple of sites peripheral to the main drainage, "Red Pool" and a pyrite "Slump," were even higher in pH (>1) and the community compositions reflected this change in geochemical conditions. Several novel lineages were identified within the archaeal *Thermoplasmatales* order associated with the pyrite slump, and the Red Pool (pH 1.4) contained the only population of *Acidithiobacillus*. Relatively small populations of *Sulfobacillus* spp. and *Acidithiobacillus caldus* may metabolize elemental sulfur as an intermediate species in the oxidation of pyritic sulfide to sulfate. Experiments show that elemental sulfur which forms on pyrite surfaces is resistant to most oxidants; its solublization by unattached cells may indicate involvement of a microbially derived

electron shuttle. The detachment of thiosulfate ($S_2O_3^{2-}$) as a leaving group in pyrite oxidation should result in the formation and persistence of tetrathionate in low pH ferric

iron-rich AMD solutions. However, tetrathionate is not observed. Although a $S_2O_3^{2-}$ - like species may form as a surface-bound intermediate, data suggest that Fe^{3+} oxidizes the majority of sulfur to sulfate on the surface of pyrite. This may explain why microorganisms that can utilize intermediate sulfur species are scarce compared to Fe-oxidizing taxa at the Richmond Mine site.

Introduction

Relevance of the study site

The enhanced oxidation of sulfide minerals principally pyrite (FeS_2), by mining activities is a worldwide problem of significant environmental interest because it leads to the generation of acidic, metal-rich waters. The Richmond Mine, at Iron Mountain in northern California, USA, represents a rare opportunity to study the processes of acid mine drainage underground within an actively oxidizing pyritic body. Where many AMD sites are characterized by precipitation of iron oxyhydroxides, this site is characterized by the dissolved chemical species resulting from microbial pyrite oxidation. Specifically, this field site permits investigation of chemical and microbial factors important in the oxidation reactions that form acidic mine drainage isolated from the part of the system where precipitation of secondary oxyhydroxides occurs. An access tunnel intersects four mine tunnels within the Richmond ore deposit at a junction referred to as the "5-way" (Fig. 1). Essentially all solutions draining from the mine are collected at the 5-way, making it possible to determine and monitor the flux of metals and sulfur from the system. Previous studies of the geology, water chemistry, and microbial communities in the vicinity of the 5-way [1-5] provide the basis for ongoing work at the site.

Figure 1
(Color) Location map of field site at the Richmond Complex 5-way area at the Iron Mountain Superfund Site, northern California, USA. Size of enlarged area is approximately 30 meters in diameter.

Site location and history

Iron Mountain is located approximately 9 miles northwest of the city of Redding, California (Fig. 1). Access to the Richmond Mine is provided by a 430 m long horizontal access tunnel that is maintained as part of the United States Government Superfund program remediation effort. The area of the 5-way is located at the edge of the main body of the Richmond deposit, a large, lenticular body that was originally over 800 m long, 60 m wide, and 60 m thick. The body contains approximately 90–95% pyrite (FeS_2), locally enriched with ore minerals. The mine is within a Kuroko-type volcanogenic massive

sulfide deposit that contains chalcopyrite ($CuFeS_2$), sphalerite (ZnS), galena (PbS), bornite (Cu_5FeS_4), arsenopyrite (FeAsS), and tetrathedrite–tennantite ($Cu_{12}Sb_4S_{13}$–$Cu_{12}As_4S_{13}$), as well as massive pyrite and late-stage veins of fine-grained pyrite.[6] The ore is hosted in Devonian-age Balaklala Rhyolite composed of quartz, albite, chlorite, and sericite,[7] characterized by very limited acid-buffering capacity. Assays of the pyritic material at the Richmond Mine Complex average 1% copper and 2% zinc.[8] Secondary sulfate minerals associated with the deposit include a variety of Fe^{2+}, Fe^{2+}–Fe^{3+}, and Fe^{3+} sulfates (for details see Alpers *et al.*[2,9]). Several mines were operated intermittently between the 1860s and 1962 for Au, Ag, Cu, Fe, Zn, and pyrite. Of the original reserve estimated at 11.5 million tons, approximately 3.5 million tons were removed.[2] Acidic effluent from the mine was blamed for significant fish kills in the Sacramento river before treatment began.[2] The site has been listed under the National Priorities list for EPA Superfund since 1983[8] and all effluent from the mine is now collected and treated on site by addition of lime (CaO) to remove metals to below the U.S. E.P.A. drinking water standards for Cu (Joe Cobliati, private communication).

Pyrite oxidation and AMD solutions

The oxidation of pyrite proceeds by a transfer of 15 moles of electrons per mole of FeS_2 (converting pyritic sulfide, S^{1-}, to sulfate, $S^{6+}O_4^{2-}$ and Fe^{2+} to Fe^{3+}). Redox reactions occur in increments involving 1 or 2 electrons.[10] Thus, the overall pathway of sulfide oxidation must be composed of many steps. All of the electrons are lost via a series of anodic (oxidizing) half reactions, the sum of which may be represented by:

$$FeS_2 + 8\,H_2O \rightarrow Fe^{3+} + 2\,HSO_4^- + 14\,H^+ + 15e^-, \quad (1)$$
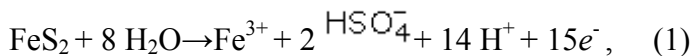
where the product bisulfate is used to represent this reaction occurring below pH 2 [pK_2 of sulfuric acid = 1.99 (Ref. 11)] as it likely does at the site. Anodic reactions must be coupled to a cathodic reaction (in the absence of an applied potential). A subset of possible anodic and cathodic reactions describing pyrite oxidation are given in Table I.

Table I
Compilation of selected inorganic anodic and cathodic reactions potentially involved with the oxidation of pyrite.

Not all coupled redox reactions in the pyrite oxidation pathway generate protons. The reaction producing elemental sulfur, a commonly observed partial product of pyrite dissolution, [12-14] generates no protons:

$$FeS_2 + 2\,Fe^{3+} \rightarrow 3\,Fe^{2+} + 2\,S^0. \quad (2)$$
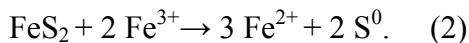
This reaction represents formation of elemental sulfur directly through the sulfidic sulfur in pyrite. It has been suggested that thiosulfate production at low pH can yield significant elemental sulfur,[15] but recent studies[16] concerning thiosulfate and tetrathionate transformations in acidic waters with excess ferric iron clearly indicate that significant elemental sulfur cannot form from thiosulfate under those conditions. The observation of up to 20% elemental sulfur product in laboratory studies of pyrite oxidation in acidic

conditions with excess ferric iron therefore indicates a reaction generating elemental sulfur through another source such as via oxidation of pyritic sulfide.

Thiosulfate has been invoked to be the first product of pyrite oxidation by several researchers. [17-21] Reaction of pyrite over several steps to form thiosulfate (outlined by Luther[17]), which subsequently forms tetrathionate via reaction with Fe^{3+},[16,22] may be represented by the overall reaction:

$$FeS_2 + 7\ Fe^{3+} + 3\ H_2O \rightarrow 8\ Fe^{2+} + 0.5\ S_4O_6^{2-} + 6\ H^+. \quad (3)$$
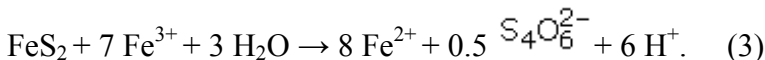
Reaction of pyrite to form tetrathionate, another intermediate species detected in laboratory pyrite oxidation experiments over a range of conditions,[18,19,21] generates 6 moles of protons per mole of pyrite.

The reaction for the total oxidation of pyrite, assuming that ferric iron is the electron acceptor for all steps, is typically written (for reactions occurring below pH 2):

$$FeS_2 + 14\ Fe^{3+} + 8\ H_2O \rightarrow 15\ Fe^{2+} + 2\ HSO_4^- + 14\ H^+. \quad (4)$$

There is no direct source of ferric iron in AMD systems, so ferric iron must be regenerated from oxidation by oxygen according to the reaction:

$$14\ Fe^{2+} + 3.5\ O_2 + 14\ H^+ \rightarrow 14\ Fe^{3+} + 7\ H_2O. \quad (5)$$
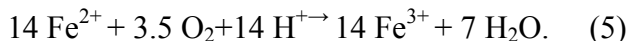
Some reservoirs of Fe^{3+} exist, as pools of dissolved Fe^{3+} and as different ferric sulfate and oxide minerals, which may essentially store this oxidant for varying times before movement/dissolution carries the Fe^{3+} into contact with pyrite surfaces. If reactions (4) and (5) are summed, the overall reaction for pyrite dissolution is given by

$$FeS_2 + 3.5\ O_2 + H_2O \rightarrow Fe^{2+} + 2\ HSO_4^-. \quad (6)$$
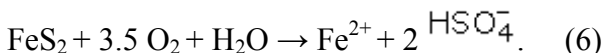
If reactions (4) and (5) are exactly balanced to yield reaction (6), the ratio of total iron:protons:sulfate should be 1:2:2. The molar ratio of iron:protons:sulfate will reflect complete oxidation of pyrite if the iron and sulfur are properly accounted (i.e., if no sulfate minerals or iron oxyhydrox-ide minerals precipitate before sampling the water) and if protons can be properly accounted (i.e., if speciation of pro-tonated forms of carbon, silica, iron, sulfate, etc., can be accounted for). That minerals with mixed-iron valences can precipitate in these waters and that variations in iron oxyhy-droxide precipitation can also affect S molar ratios (through precipitation of schwertmannite or sorption of SO_4^{2-} on goe-thite, for example) emphasize the care required to interpret compositional data.

Although it is clear that proton production is coupled to sulfur oxidation, solution pH is determined by the excess of protons over that required to complex anions (primarily sulfate). If the system is described by reaction (6), sufficient protons would be available

to speciate sulfate as HSO_4^-, implying a pH around the pK_1 for HSO_4^-, i.e., ~pH 2. However, not all protons generated by reactions (5) and (6) are required to complex sulfate due to formation of other sulfate complexes (notably Fe^{2+}–sulfate species). Evaporative concentration and precipitation of certain sulfate minerals [e.g., jarosite, $KFe^{3+}_3(SO_4)_2(OH)_6$] drives the pH down whereas iron oxidation drives the pH up[23] for solutions at low pH. Modeling results indicate pyrite oxidation can account for lowering pH to approximately 0.0.[24] Under some conditions, extremely low pH values may be attained by evaporative concentration. The lowest pH ever recorded for an environmental sample (-3.6) came from an evaporative pool within the Richmond deposit.[25]

Although oxygen supply ultimately controls pyrite oxidation in the environment, ferric iron is the most efficient oxidant. The lowest unoccupied molecular orbital (LUMO) of Fe^{3+} is lower in energy than the LUMO of O_2 (which typically yields a lower activation energy barrier), which explains why ferric iron is the most effective oxidant.[26] Additionally, O_2 is not as efficient an oxidant due to the spin-restriction of reacting paramagnetic O_2 (2 unpaired electrons) with diamagnetic pyrite (all electrons paired).[18] Because the oxidation of pyrite by Fe^{3+} is so much faster than by O_2, and in view of slow inorganic Fe^{2+} reoxidation rates at low pH, it was proposed that Fe^{2+} reoxidation limits the rate of pyrite oxidation in AMD solutions.[27] Since microorganisms catalyze the oxidation of ferrous iron it was hypothesized that they control pyrite oxidation rates in the environment.[27] Organisms which utilize intermediate sulfur species for metabolic energy are also active in pyrite-oxidizing environments.[28] Microbial populations may change significantly in response to the local environment,[4,29] and between sites of varying acidity.[3] The intermediate reactions governing oxidation of sulfur species may vary with pH, O_2, and Fe^{3+} concentrations as the kinetics of competing reactions depend on the availability of oxidants or the pyrite surface composition. Therefore, understanding differences in the pyrite oxidation pathway as a function of pH may yield better understanding of the interdependence between micro-bial activity and redox kinetics in acidic environments.

Previous studies of the microbiology of the Richmond deposit
The importance of microbial activity in pyrite dissolution and AMD formation is well documented.[1,19,23,27,30-33] To date, the majority of microbiological studies of AMD systems have been based on physiology and characterization of cultivated organisms. Nordstrom and Southam[32] and Johnson[33] review the concepts that have emerged from these studies. The key finding is that microorganisms oxidize ferrous to ferric iron, increasing the rate of supply of this important oxidant to the surfaces of dissolving sulfide minerals.

Nordstrom[1] first noted the potential importance of microorganisms at Iron Mountain. Several subsequent studies at the site have applied molecular biological methods to describe the microorganisms present and to correlate microbial community structure with the geochemistry of their environments. Rodgers et al.[34] created the first small subunit riboso-mal RNA (16S rRNA) gene library from the site (see Edwards[35]). Subsequently, a fluorescent in-situ hydridization (FISH)-based study by Schrenk et al.[3] determined the relative abundance of archaea, bacteria, and eukaryotes. Schrenk et al.[3] also confirmed the findings of Rodgers et al.[34] (that Acidithiobacillus ferrooxidans is not a significant organism in active AMD generating regions of the ore deposit).

Edwards *et al*.[4] tracked microbial populations over one year and further established the restricted distribution of *At. ferrooxidans*. This study also suggested that another well known Fe-oxidizing acidophile, *Leptospirillum ferrooxidans*, could not account for the majority of microorganisms in most communities sampled. Edwards *et al*.[29] established that archaea are abundant, especially in low-pH, high-ionic strength environments.

A novel, microaerophilic iron-oxidizing archaeon was isolated from the site by Edwards *et al*.[36] This organism comprises up to 85% of the microbial cells in some samples. An isolate, referred to as *Ferroplasma acidarmanus* (strain ferl), is the subject of current detailed physiological (Bond *et al*.[37]) and genomic-based (Allen *et al*., in prep.) studies. Bond *et al*.[37] analyzed 16S rRNA gene diversity of several specific microenvironments within the Richmond mine. In contrast to Rodgers *et al*.,[34] who targeted planktonic species in AMD solutions and sediments, Bond *et al*.[37] primarily sampled biofilms. The diversity of known archaea, specifically within the order *Thermoplasmatales*, was broadened by this study. Members of the *Thermoplasmatales*, which include clones from the public databases, have been named the "alphabet plasmas.[38] Bond *et al*.[37] also identified three distinct phylogenetic groups of *Leptospirillum* (groups I, II, and III). Group II was subsequently named *Leptospirillum ferriphilum*.[39] These findings laid the groundwork for more detailed studies.

Methods

Geochemical analyses

Field work at the Richmond Mine at Iron Mountain in northern California was conducted over 4 years in the present investigation, as part of a 10-year effort to study the microbial activity associated with metal-sulfide oxidation at this site. Water at the Richmond Mine offers some interesting challenges to the analytical techniques employed in conventional water analyses. Notably, pH standards require special attention to the activity coefficient of H^+ in samples of high ionic strength[25] and oxygen concentration measurements are virtually impossible using standard membrane electrodes or titration methods. On-site analysis of water samples for Eh, conductivity, and temperature were conducted routinely, after established USGS protocols.[40] Standards for pH measurements were prepared from H_2SO_4 stock after the method for measuring extremely low-pH waters developed at the same location by Nordstrom *et al*.[25] A ZoBell's solution was prepared immediately before analysis for a check of the platinum electrode. As noted by Nordstrom,[23] Eh measurements were highly consistent with the Eh calculated from the Fe^{2+}/Fe^{3+} couple (typically within 5%).

Water samples were collected with sterile 60 ml syringes and filtered through 0.2 μm syringe filters into several splits for later analyses. Samples were stored in 15 ml Falcon tubes, filled to occlude any headspace gas, and stored on ice and in a refrigerator until

analyses were complete. Ferrous iron was measured on site using a 1,10 phenanthroline method and total iron was measured using a FerroZine method with a Hach portable digital spectrophotometer. In the lab, duplicate splits were measured for Fe^{2+} and Fe^{3+} directly, using the method of To *et al.*[41] and an Ocean Optics UV-Visible spectrophotometer (2000S series). Iron measurements using both methods were consistent within analytical uncertainty, which is primarily due to the high level of dilution necessary for spectrophotometric analyses of these samples. Chromatographic analyses of collected samples for anion determinations were performed on a Dionex Series 500 ion chromatograph equipped with a conductivity detector and UV-Vis detector collected at 230 nm. Sulfate, sulfite, thiosulfate, dithionate, and sulfide were detectable at tens of micromolar levels using an isocratic 5 mM $NaHCO_3$/5 mM Na_2CO_3 eluent at a 1.0 ml/min flow rate through an IonPac AS16 column. Although chloride, phosphate, nitrate, nitrite, and fluoride are also resolvable using chromatographic methods, the extremely high sulfate concentration made quantitative analysis of these ions impossible, given the relatively low concentrations of these species compared to sulfate.

Attempts to measure polythionates were made using an isocratic 30% acetonitrile:H_2O eluent containing 2 mM tetrabulylammonium hydroxide, buffered with 3 mM $NaHCO_3$/3 mM Na_2CO_3 and run through an IonPac NS1 column, modified after several advances in individual polythionate detection by chromatography.[42] Tri-, tetra-, and penta-thionate were all resolvable down to 5 μM concentrations using a 25 μl sample loop. Water samples collected directly from mine effluent, supernatants of samples centri-fuged (~4000 rpm) in the field, and water samples preserved on an anion exchange resin after the method of Druschel *et al.*[43] were all analyzed for polythionates and thiosulfate. Elemental sulfur was extracted and analyzed by HPLC using a Shimadzu system UV-Vis at 254 nm, an Alltech C18 column, and an isocratic 95% Methanol:H_2O eluent at 1.0 ml/min after the method detailed in McGuire and Hamers.[44] Trace metals, cations, and silica were analyzed by ICP-MS (VG PlamsaQuad 3), operated in peak jumping mode at the University of Windsor Dept. of Geology. All standards, blanks, and samples were prepared in ultrapure (distilled) 2% $HN0_3$. Multiple internal standards were used to correct for machine drift that was less than 2% across the mass range.

Mineralogical analyses
Mineral samples from the Iron Mountain Mine site were analyzed by XRD using a Scintag PadV x-ray diffractometer (XRD). Peak matching utilizing the JCPDS Powder diffraction file was used to identify minerals in samples analyzed by XRD. Selected aliquots of cleaned minerals were also analyzed by a LEO 1530 scanning electron microscopy (SEM) and a Cameron SX50 electron microprobe (EMP). Back-scattered electron images and energy-dispersive spectrometer analyses in EMP were utilized to determine enrichments of As, Cu, and Zn in pyritic material.
Geochemical modeling
Geochemical modeling using the Geochemist's Workbench suite of programs[45] was used to calculate speciation in the solutions. Waters from the Richmond Mine have a very high ionic strength. Therefore, proper calculation of the activity coefficients for species of interest requires application of models able to account for interactions beyond simple bimolecular electrostatic interactions, such as the Pitzer equations or HMW models.[46] Speciation calculations of the AMD solutions were carried out using the PHRQPITZ database, which is more accurate in the calculation of activity coefficients for the high-

ionic strength solutions at Iron Mountain. However, this database does not include all appropriate ion pairs, especially $FeSO_{4(aq)}$ species. Speciation calculations using PHRQPITZ were carried out using thermo-dynamic data including available virial coefficients. In an attempt to assess what the errors are with using a database which is missing potentially important species (especially $FeSO_{4(aq)}$), additional and separate calculations were made on these waters using the thermo.dat database provided with the React code of the GWB 3.1.

Particular attention to the total moles of iron, sulfur, and protons in the solution was paid in order to assess iron and sulfur transport and potential sinks relative to the pathways controlling pyrite oxidation and microbial activity in the mine. Analytically, the measured amounts of iron and sulfate accurately reflect the amount of each element in solution. Total H^+ in solution generated from pyrite oxidation is derived from both the free H^+ in solution (from pH and H^+ activity coefficient) and the molar concentrations of proto-nated species such as bisulfate (which is strongly affected by the abundance of the $FeSO_{4(aq)}$ complex). Bisulfate available to complex H^+ is also affected strongly by iron speciation, as ferrous iron more strongly complexes SO_4^{2-} than does H^+. Results reported for total H^+ are calculated based on the speciation results and are functionally equivalent to the sum of free H^+ and HSO_4^- in solution. There are other species in these solutions, but over 99% of the H^+ is contained in these two species. As such this calculation is slightly different than would be found from an acidity titration because cations which would contain or take up OH^- are not explicitly considered.

Use of GWB 3.1 and the thermo.dat thermodynamic data will result in errors due to extrapolation of equilibrium constants well outside their useful range of ionic strength. There is no current modeling protocol to arrive at a better solution, however. We use H^+ values resulting from these calculations in a strictly qualitative sense, as the error can be greater than 50%, based on comparison of activity coefficients for H^+, HSO_4^-, and $FeSO_{4(aq)}$. Errors in the PHRQPITZ calculations in which total H^+ is comparable to 30–50% of the sulfur species present. Caution should be applied when attemping to use these numbers in anything but a qualitative sense.

Arsenopyrite dissolution and surface colonization studies
The surface colonization and dissolution of pyrite and arsenopyrite by iron- and sulfur-oxidizing micro-organisms from the Richmond Mine have been reported (e.g., Edwards *et al.*[47,48]). Surface-sensitive spectroscopic studies using Raman spectroscopy revealed that elemental sulfur is an important product of the dissolution of pyrite and arsenopyrite.[49,50] In the case of abiotic arsenopyrite dissolution, the majority of the sulfide oxidized is converted to highly insoluble S_8, which accumulates at the mineral surface.[50] Thus, in this study, we conducted experiments designed to explore the interaction between sulfur-oxidizing micro-organisms and the dissolving arsenopyrite surface. These experiments used *At. caldus* because this sulfur-oxidizing organism cannot impact sulfide dissolution via Fe^{2+} oxidation, and thus its primary biogeochemical effect is on the oxidation of intermediate sulfur species such as S_8. An important question to be resolved is whether microbial utilization of sulfur influences sulfide dissolution rates by removal of a diffusion-limiting S_8 coating. Cylindrical Teflon chambers were assembled

for three parallel experiments. Chamber 1 was configured with a 0.22 μm pore-size polycarbonate membrane dividing the chamber into two compartments. Each end of the chamber was fitted with a port for removal and addition of material to the compartments. Chambers 2 and 3 were configured in the same manner as Chamber 1, but with no membrane partition in place.

Arsenopyrite preparations and surface-area measurements were carried out as described by McGuire *et al.*[12] Equal portions (by mass) of crushed arsenopyrite were added such that each chamber contained approximately 0.015 m^2 of material. Sections of arsenopyrite polished and cut into 3 × 3 × 1 mm coupons were added to Chamber 2 for cell attachment studies. These slabs were placed in perforated 0.5 ml microcentrifuge tubes attached to a Teflon wire extending through the chamber port. The crushed arsenopyrite and the polished coupons were placed into the experiment chambers prior to sterilization by autoclaving. The sterilization techniques likely oxidized the surface of the minerals, but the long-term nature of these experiments with microbial cultures incapable of reduction should not be significantly affected by this.

Each sterilized chamber containing arsenopyrite was filled to half capacity with a total of 210 ml of growth medium. We prepared 9 K medium,[51] modified by omission of FeSO$_4$ and amended with 0.01% (w/v) yeast extract. The medium was adjusted to pH 1.6 and was autoclaved prior to use. Cultures of *At. caldus* TC1 were prepared as previously described[31] to serve as innoculum. The compartment of Chamber 1 isolated from the arsenopyrite material was inoculated with *At. caldus* cells. Chamber 2 was also inoculated with the same volume of culture (in contact with arsenopyrite material). Chamber 3 was left sterile to serve as an abiotic control. The chambers were incubated in a horizontal position at 37°C.

Samples for total dissolved iron and cell density measurements were taken from each chamber at prescribed time intervals. One arsenopyrite coupon was retrieved from Chamber 2 during each of the 23, 67, 161, 286, 384, and 477 hour sampling sessions. The coupons were fixed in 3% (w/v) paraformaldehyde for 4 h, rinsed with PBS (phosphate buffering solution), and stored in a 1:1 PBS:ethanol solution at -20°C.

Total dissolved iron was measured by the FerroZine method. Direct counts of cells in suspension were performed by light microscopy using a Petroff-Hausser counting chamber. Cells attached to arsenopyrite coupons were visualized by staining with DAPI and were viewed using an epifluores-cence microscope. Digital images were captured using a CCD camera and the NIH Image software. The area of one image at 1000× was calibrated and 15 random images were counted and averaged for cell density determinations.

Microbial sampling at Richmond Mine
In order to extend prior studies of correlations between microbial populations and geochemical habitats, samples were collected from six sites in the A-, B-, C-drifts and 5-way area (Fig. 1) in January 2001. These were taken from the following locations in the mine; a damp biofilm growing on the surface of a pyrite pile in the A-drift referred to as the "A-drift Slump" (0lIMlAslump, pH 1.1), the "A-drift Red Pool" (01IM1red pool, pH 1.4), "A-drift Slime Streamers," below the waterfall that separates the "A-drift Weir"

(01IM1A1, pH 0.9) from the "5-way" (01IM1A1, pH 0.89), and biofilms on the "B-drift Weir" (01IM1B1, pH 0.75) and "C-drift Weir" (01IM1C1, pH 0.85).

Microbiological cultivation

All enrichments contained a basal media consisting of 0.8 g/l $(NH_4)_2SO_4$, 0.4 g/l KH_2PO_4, and 0.16 g/1 $MgSO_4$. As carbon sources 0.25 g/1 trypcase soy broth (TSB) and 0.2 g/1 yeast extract were supplemented to 20 g/1 $FeSO_4$ and incubated in 250 ml flasks with vented covers. To enrich for members that can utilize intermediate sulfur compounds 5 mM, thiosulfate, tetrathionate, and S° with 10 mM glucose were added to screw-cap tubes and sealed (with air head-space). The enrichments were incubated at a range of temperatures commonly seen in the mine (24°C, 37°C, 45°C, and 60°C). All media were pH adjusted to 0.8–1.0. Once growth was observed, < 10[10] dilution series were made to isolate novel organisms.

Construction and analysis of 16S rRNA gene libraries

In January 2001, samples from six sites were collected into sterile containers, placed on ice, and transported back to the laboratory. Samples stored frozen in 25% glycerol were washed in PBS buffer (at pH 1.2 to prevent lysis of obligate acidophiles) to remove extracellular ions. The samples were then resuspended in pH 7.0 PBS, and DNA extractions were carried out as previously described[37] with an additional chloroform wash to remove residual phenol that might inhibit PCR reactions. A suite of domain level primers (27F5' -AGAGTTTGATCCTGGCTCAG-3'; 21Fa5' -TTCC GGTTGATCCYGCCGGAA-3′; A16F235' -TCYGGTTGAT CCTGCC-3'; and 1492R5'-GGWDACCTTGTTACG ACTT-3') were utilized to amplify 16S rRNA genes from total genomic DNA extract of each of the samples. Specifics of 16S rRNA gene amplification, cloning, sequencing, and analyses of the libraries are provided elsewhere.[52] A few chimeric sequences were found via dual tree comparisons and not included in the final phylogenetic analyses. Sequences were deposited in GenBank under the accession numbers AF543496 through AF543512. Phylogenetic trees were generated using ARB software package and the topologies were confirmed using bayesian inferences as described previously.[52]

FISH (fluorescence *in situ* hybridization) analyses

Samples collected from the same sites, and at the same time as those used for clone library construction, were washed with pH 1.2 PBS buffer and fixed using 4% paraformaldehyde within 12 hours of collection. Hybridization was done following protocols previously reported.[53] Details of all probe synthesis, sequences, and specificity are described elsewhere.[53] Background staining was done to all FISH slides with a DNA stain, DAPI (4′,6-Diamidino-2-Phenylindole Dihydrochloride). Slides were viewed using a Leica LEITZ DMRX epifluorecence microscope. Images were captured with a Hamamatsu digital charge-coupled-device camera using Axiovision software.

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Results

Mineralogy

Much of the pyrite ore is fine grained, thus readily liberated from the ore deposit by dissolution. This fine-grained material accumulates as sediment in slumps and on the tunnel floors in layers up to several meters thick in some areas. Analysis by optical and scanning electron microscopy and electron microprobe analyses indicated the sediment is predominantly pyrite with an average particle diameter of ~10- 1000 μM. The sediment also contains a few percent quartz. Particle surfaces are roughened by dissolution (Fig. 2). Sul-fate minerals (including blue-green melanterite, yellow-brownish jarosite, copiapite, green-blue chalcanthite, pinkish coquimbite, and a compound with cubic crystal form and composition consistent with voltaite) form by evaporative concentration and are locally abundant (inset in Fig. 2).[8] Electron microprobe data indicate that the pyrite particles contain regions that are locally enriched in Zn, Cu, and As. These impurities may account for several percent of the cations in pyrite. No discrete metal sulfide inclusions were observed and no other sulfides were detected in this sediment (although other sulfide phases do occur in the deposit). It is conceivable, however, that the zones of observed Zn, Cu, and As enrichment were small inclusions of ZnS, CuFeS, and FeAsS too small to be imaged in the electron microprobe.
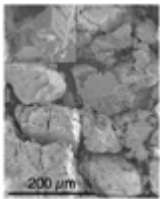
Figure 2
SEM image of secondary sulfate mineral formation in samples collected within the study site at the Richmond Mine 5-way area. Note sulfate mineral formation on cubic pyrite crystal in the inset.

Some surfaces of pyrite from the sediments contain deep euhedral pits (Fig. 3). Occasionally very thin, elongated cells occur on the pit base and pit walls. These pitmicrobe associations are very similar to those noted by Edwards *et al*.[35,54] in a SEM study of polished pyrite surfaces of *in-situ* experiments retrieved from the field. In general, the sediment pyrite surfaces are colonized by cells with a limited morphological diversity, suggesting direct interactions involve only a few species.
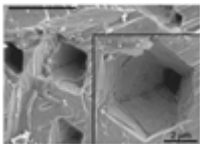

Figure 3
SEM image of pyrite collected with in study site at the Richmond Mine 5-way area. Note pitted texture of the actively oxidizing pyrite.

Arsenopyrite dissolution and surface colonization studies
Acidithiobacillus caldus cells were allowed to directly contact the arsenopyrite in some experiments and were kept separate from the mineral substrate by a 0.2 μM filter in others. As shown by total soluble iron measurements, arsenopyrite dissolution rates were only slightly higher with cells present relative to the abiotic controls [Fig. 4(a)]. Dissolution was most rapid in Chamber 2 where cells were allowed to contact the mineral surfaces. However, the difference in rates between the two biologic and the abiotic reactions is minimal. Given that previous experiments have shown that α-S_8 builds up on the surface during arsenopyrite dissolution,[12,14] the similarity between the abiotic and biotic rates (measured with Fe_T as the reaction progress variable) indicates the absence of dissolution-inhibiting surface products.

At. caldus cells were observed to grow regardless of attachment to the mineral surface [Fig. 4(b)]. This observation suggests that a microbial product may be involved in converting elemental sulfur, which is extremely insoluble under the experimental conditions,[55] into a bioavailable form. This compound may be a shuttle that moves electrons from elemental sulfur to the cell surface. It is unlikely that iron is the electron shuttle as *At. caldus* is unable to oxidize Fe^{2+} and inorganic iron oxidation kinetics are extremely slow under the experimental conditions.

Microbiology
Biofilms, and subaqueous and water surface streamer occur in the 5way area (Fig. 5). Microscopic observations indicate variable numbers of micro-organisms associated with the pyritic sediment. No simple trend between cell numbers and depth of sample site within the sediment was noted.
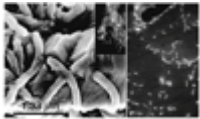
Figure 5
SEM (left) and DAPI-stained epiflourescence microscopic images of biofilms collected within the study site at the Richmond Mine 5-way area. Note the rod morphology of organisms coating actively oxidizing pyrite grains.

Tables II and III list the Fe^{2+}, Fe^{3+}, SO_4^{2-}, Cu^{2+}, Zn^{2+}, Al^{3+}, As_T, Cd^{2+}, K^+, Mg^{2+}, Mn_T, Na^+, Pb_T, and SiO_2 concentrations at the sites where microbial samples were collected. Insufficient fluid was available for analysis at the A-drift slump in 2001, and only one sample was collected from the A-drift samples because they are separated by only a few meters of flow path.

Because of PCR bias, clone abundances cannot be used as a proxy for species abundance. Consequently, *in-situ* hybridization studies using probes designed to bind specifically to RNA of organisms detected via clone library analysis were conducted. Due to the high cell density of most samples, the probe-based studies are only semiquantitative. A representative image is shown in Fig. 6, which highlights the *Sulfobacillus* cells in a biofilm. Results confirm earlier deductions[5] that the number of distinct organism types (a type being a phylotype with generally >97% homology in the sequence of the 16S rRNA) is small.
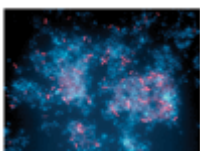

Figure 6
FISH analyses of *Sulfobacillus* rods in B-drift Weir biofilm on January 2001. Sul228 probe (Ref. 53) is shown in red and DAPI in blue.

Cultivation-based studies
After several months of incubation on selected intermediate sulfur compounds (thiosulfate, tetrathionate, and S°) no growth was detected. Enrichments containing yeast extract and TSB often resulted in growth. Dilutions of these cultures largely resulted in isolation of *Ferroplasma*. Some *Sulfobacillus* isolates were obtained on yeast extract and $FeSO_4$. Figure 7 shows the phylogenetic placement of three isolates obtained on heterotrophic (yeast extract as a carbon source) media at 37°C from the mine (SB6 and

SB37) that are very closely related to previously characterized organisms (*S. thermosulfidooxidans* VKM and *S. disulfidooxidans*) and clones (see below, BW7). Attempts to grow isolate SB37 on S° were unsuccessful.

Figure 7
Phylogeny of 16S rRNA gene sequences from the mine on January 2001 sampling. Tree generated using maximum likelihood (FastDNAM1) in ARB package method. Distance bootstraps values are labeled at their corresponding nodes. Bar represents 0.1 changes per (more ...)

Cultivation-independent molecular studies
As shown in Table IV, 232 clones from six libraries were obtained from spatially separate microenvironments sampled in January 19, 2001. No more than six distinct organism lineages were detected in any sample. Previous studies have also shown that AMD communities are characterized by a limited number of taxonomic groups.[38] Clones belonging to *Nitrospira, Bacillales, Alphaproteobacteria, Deltaproteobac-teria, Gammaproteobacteria, Actinobacteria, Acidobacteria*, and the archaeal "alphabet plasma" groups were identified and near complete 16S rRNA gene sequences were obtained (Fig. 7). Some of these lineages are closely related to sequences that were previously published from the Richmond mine[37] and a forested wetland site (U.S. Dept. of Energy's Savannah River Site) impacted by acid solutions derived from coal[56] (see coal refuse clones in Fig. 7).

The novel *Deltaproteobacteria* were found solely in A-drift Slump and Red Pool samples. *Leptospirillum* group III was ubiquitous in the communities sampled in this study. *L. ferriphilum* (group II) clones were only found in the A-drift Slump sample. The result distinguishes the microbial communities present at these locations in January 2001 from those detected in previous work.[5]

Clones significantly divergent from those previously identified (>93% 16S rRNA gene sequence identity) within the order *Thermoplasmatales* were sequenced from A-drift Slump and A-drift Slime Streamer samples. All of these clones fall within the A, B, C, and "Dplasma" subgroups (Fig. 7) as defined by Baker and Banfield.[38] These "alphabet plasma" are restricted to the lowest pH environments (i.e., they were not found in the pH 1.4 A-drift Red Pool). Other novel lineage related to the division *Actinobacteria* were detected, including clone ASL8 which has 91% 16S rRNA gene sequence similarity with that of a Rheims *et al.*[57] clone TM214.

Sulfobacillus acidophilus-like, clones were recovered from all but the A-drift Slump and Slime Streamers. FISH analyses confirmed their presence in several locations, including the A-, B-, C-drift Weir communities (Fig. 6). A lineage not previously associated with the Richmond Mine, closely related to *Sulfobacillus disulfidoxidans* SD-11, was detected in the clone library from the B-drift Weir sample. Furthermore, an isolate belonging to this group was obtained (SB37).

A new group of *Alphaproteobacteria* was identified in the A-drift Red Pool and A-drift Slump libraries. The *Alphaproteobacteria* are directly associated with the protists.[52]

The 16S rRNA gene library from the more oxidized, pH 1.4, A-drift Red Pool is distinct in that it contains a high abundance (21 of 37 total) of *At. ferrooxidans* (Table IV). The Red Pool is also the only location where group I *Leptospirillum*, and *Acidobacteria* were detected. Fluorescent *in-situ* hybridization (FISH) analyses confirmed that *At. ferrooxidans* predominates and that *Acidobacteria* were present in low numbers in the Red Pool (data not shown). Finding *At. ferrooxidans* only in higher pH environments is consistent with oligonucleotide probe-based studies of Schrenk *et al*.[3] Microscopic investigations of the A-drift Red Pool samples also revealed the presence of protists. This sample and others are the focus of current eukaryotic studies.[58]

Water chemistry
Tabulated results of field and laboratory analyses of water samples collected from the Richmond Mine on 05/11/1999, 06/12/2000, 01/19/2001, and 03/12/2002 are presented in Tables II and III. No intermediate sulfur species (including aqueous sulfide, thiosulfate, polythionates, or elemental sulfur) were within detection limits in any of the samples.

Table V lists the Fe:S:H$^+$ ratios calculated using the Geochemist's Workbench for several samples at the mine. Total H$^+$ was calculated using the GWB 3.1 program React, but the ability of the algorithms to accurately represent the H$^+$ load is hampered by errors in the calculation of the activity coefficient for H$^+$ (see discussion in methods section, above). Comparing speciation for calculations using the ther-mo.phrqpitz database and the thermo database indicates that only a first-order determination of the total H$^+$ in the solutions can be made.

Discussion
Microbial communities in the Richmond Mine
The study sites located within the mountain are colonized by microbial communities sustained by iron and sulfur oxidation. The microorganisms derive all essential nutrients (phosphate and other ions), materials (CO_2, O_2, N_2), and energy from air, pyrite, and the acid mine drainage solutions. Although the mixture of organisms in individual biofilms varies, most communities are constructed from the same relatively limited set of organism types, many of which have cultivated representatives. Based on physiological measurements for isolates from the Richmond Mine (*F. acidarmanus*,[36] and Baumler *et al.;* [59]*L. ferriphilum* group II, Smriga, unpublished; Lo *et al.* unpublished; *Sulfobacillus* spp. Baker *et al*., unpublished; *At. caldus*[4]), published measurements for closely related species *(Acidithiobacillus* spp., [60]*Acidimicrobium* spp., [61]*Ferromicrobium* spp., [62]*Leptospirillum ferriphilum* [39,63] and inferences based on phylogenetic placement *(Leptospirillum* group III and the "alphabet plasma"), most of

the prokaryotes contribute to AMD generation, either through regeneration of ferric iron oxidant or via metabolism of intermediate sulfur compounds.

We have documented changes in microbial community structure between locations within the mine, and over time. The structure of communities and levels of activity of members is certainly closely tied to geochemical factors, which vary with the seasons. For example, periodically high temperatures typically correspond with periods of high rainfall whereas high ionic strength correlates with low-flow periods. The community structure can also be shaped by non-geochemical factors related to biomass concentration, including fungal growth, phase predation, and grazing byprotists.[64] These are in turn affected by temperature and solution concentration. In order to evaluate the coupling between the many processes that control AMD generation rates we examine the physical and chemical processes that are the foundation for the biogeochemical system.

The net effect of geochemical and microbial processes

The Richmond Mine AMD effluent is extremely acidic and metal rich because the hydrologic, microbiological, and geologic conditions favor rapid oxidation of large amounts of pyrite. The low pH (~0.5) of the AMD discharge was modeled by Alpers *et al.*,[2] who noted that approximately 2000 tons of pyrite is dissolved from the Richmond deposit per year (~1.6×10^7 moles or a daily average flux of ~4.4×10^4 moles/day). Using the flow data available at the mine flow meter (recorded on days of sampling at the Richmond Mine outflow pipe–collected primarily at the 5-way) and the measured iron concentrations for 5-way samples, the flux of metal coming out of the mine at the 5-way may be determined for that specific sampling period. For the four sampling points in our study, the flux at the 5-way is equivalent to dissolution of between approximately 1×10^5 to 2×10^5 moles FeS_2/day, in good agreement with the average values of Alpers *et al.*[2] considering the significant variability of these data over seasonal scales.

Fe:S:H+ ratio

As noted above and in Eq. (6), the ratio of iron to sulfate to protons predicted for the complete oxidation of pyrite to sulfuric acid is 1:2:2. Using the chemical data in Tables III and IV (where total H^+ was calculated with GWB as noted in the methods section), we calculated the Fe:S:H ratio at the study sites for each sampling trip. The Fe:S:H^+ values in Table V have an error of approximately $\pm$ 0.4 for the H^+ value in the ratio for most samples. It is critical to again note that due to the difficulties of properly defining total H^+ only a qualitative discussion of gross differences in this ratio between different sampling times and between different locales sampled at the same time is possible. As an example, comparing ratios of 1:2.1:2.0 with 1:2.5:2.0 would be inappropriate whereas comparing ratios of 1:2.1:2.0 with 1:4.1:4.2 would be appropriate given the error associated with the H^+ calculations.

For most sites at most times the ratio Fe:S:H^+ is close to 1:2:2. Deviation of this value (outside experimental and cal-culational error) from 1:2:2 may be due to

(1) incomplete oxidation of the pyritic sulfide (e.g., to α-S_8 or another sulfoxyanion less oxidized than SO_4^{2-}),

(2) incomplete reoxidation of Fe^{2+} to Fe^{3+},

(3) net precipitation or dissolution of one of the many iron sulfate minerals found in the Richmond Mine,[9]

(4) dissolution or precipitation of silicates, clays, other sul fate minerals,

(5) precipitation or dissolution of iron oxyhydroxide miner als, or

(6) mixing with another solution affected by (1)–(4).

Incomplete oxidation of pyritic sulfur (e.g., formation of elemental sulfur) can significantly change the H^+ balance in solution [e.g., Eqs. (2) and (3)]. Large amounts of S_8 retained on surfaces may also affect the solution $Fe:S:H^+$ (most other intermediate sulfur species are quite soluble). Incomplete re-oxidation of x Fe^{2+} to Fe^{3+} (as the primary oxidant) increases the amount of H^+ in the system [Eq. (5)], and would raise the H^+ ratio by $1:2:2+x$.

Sulfate mineral precipitation/dissolution may affect Fe, S, and/or H^+ budgets, depending on which sulfate minerals are involved. There are a variety of sulfate minerals that have been observed in the study area (for a thorough review, see Jambor et al.[65]) and each may affect overall solution chemistry very differently. For example, jarosite $(KFe_3(SO_4)_2(OH)_6)$ precipitation will drive pH down, while rhomboclase precipitation $((H_3O)Fe(SO_4)_2.3H_2O)$ will drive pH up. Perturbation of the Fe:S ratio is more significant however, as sulfate minerals are either 1:1 ferrous salts or 1.x: 1 mixed ferrous-ferric sulfate minerals (no combination approaches the 1:2 ratio of pyrite, as the charge of sulfate is twice that of pyritic sulfide).

Other minerals associated with the Balakla rhyolite and mineralization events are also dissolved, as evidenced by the amounts of soluble Al^{3+}, Ca^{2+}, Mg^{2+}, and other ions in these samples. Rhyolite dissolution does affect the proton budget of the solutions; for example, the reaction of anorthite would consume 8 protons per mole, releasing 1 mole of Ca^{2+}, 2 moles of Al^{3+}, and 2 moles of $SiO_{2(aq)}$. The significant amount of Al^{3+} in these waters may impact the $Fe:S:H^+$ ratio by consuming some of the H^+ (up to approximately 200–250 mmolar, which can be up to ~30–50% of the total H^+ in some samples).

Ferric oxyhydroxide precipitation/dissolution will affect the Fe_T reservoir and pH. However, most solutions observed at Iron Mountain are at a pH well below that corresponding to ferric oxyhydroxide solubility, even with the very high levels of iron present. Only in the A-drift Red Pool (01IM1A2; pH 1.4, $Fe:S:H^+$ ratio= 1:4.1:3.3) and in higher pH environments outside the ore deposit was evidence for FeOOH accumulation noted (see below). The mine there has an overlying gossan layer, indicating remobilization of iron into oxyhydroxide phases in times before mining. The 5-way area differs from most AMD sites because sampling can occur before solutions have had a chance to neutralize and precipitate significant ferric oxyhydroxide minerals.

The observation that the predicted ratio of $Fe:S:H^+$ of the AMD solutions generally matches that expected based on the overall stoichiometry suggests that the reservoir of sulfate minerals within the mountain is relatively constant, i.e., oxidation of 2000 tons of pyrite each year is not generating a fast-growing sulfate mineral deposit. Sulfate mineral

deposits are known to exist within the mine, but the mine also significantly floods on a seasonal basis, dissolving at least part of that away.[8,9] The Fe:S:H^+ ratio was significantly different in the 06/12/2000 sampling trip (closer to 1:4:4).

Jarosite precipitation can sequester Fe and S at about a 1:1 ratio, and generate more H^+ to maintain the 1:1 ratio between S and H^+. Yellow materials identified through XRD analysis as jarosite, copiatite, chalcanthite, and coquimbite were found in several places on the floor of the mine in 2000 (data not shown). The 2000 Fe:S:H^+ ratio likely reflects significant sulfate mineral precipitation and indicates that sulfate accumulation is heterogenous over time.

Oxygen flux
Overall Fe:S:H^+ flux out of the mine is ultimately determined by O_2 supply [Eq. (6)]. The requirement for 3.5 moles O_2 per liter of solution to dissolve pyrite and generate 1 M Fe_T solutions puts the oxygen demand in perspective [see Eq. (6)]. The solubility of O_2 at 3000 m elevation, 40°C, in a 1 molal ionic strength solution is approximately 140 μM.[66] This means that over the course of fluid flow from recharge to discharge at the 5-way, every liter of water must be completely re-oxygenated about 7500 times.

Though the fact that Iron Mountain "breathes" is well established and necessary as part of the development of the AMD solutions at the site,[2,25] we know little about the hydrogeology of the Richmond Mine. Certainly there are a number of deficient hydrogeologic domains. Solutions may move along fracture-controlled flow paths, open channels, percolate through the vadose zone in piles of sediment, or move via saturated subsurface flow. The time it takes for a fluid pulse following a rainfall event to be observed at the 5-way was estimated to range from hours to months (P. Eko-niak, private communication) depending upon the saturation state of the cracks through the solid rock and the pores in the pyritic sediment covering the tunnel floors. However, the transit time for individual pulses may be quite variable and the upper limit is possibly on the scale of years to decades for paths through saturated sediment (D. Dodds, private communication).

Based on historical maps, a fluid path length in the hundreds of meters to several kilometers may be approximated. Using this range, we estimate that the solution must be re-oxidized every 1–100 cm along its flow path. Movement of fluids along this path may be very irregular (fluids may sit in pools or pores for long periods of time prior to flushing or move through the deposit quickly), thus the rate and mechanism of reoxidation may vary considerably.

The mechanisms by which O_2 molecules are dissolved into the solution include (i) diffusion: Calculations using estimates of diffusion indicate O_2 penetration distances in the pyrite sediment in the range of mm to cm (the uncertainties are high); (ii) evaporation-condensation. At times of the year when temperatures are high, steam is associated with flowing AMD. Deeper in the mountain where temperatures exceed 50°C, this may represent an effective water reoxygenation mechanism. Additionally, the rate of reoxygenation in any fluid is affected by the flow and turbulence of the fluid, therefore any calculation of diffusion rates would be a minimum (slowest) estimate of rate.

The shortest residence time for water in the mine is probably in the range of a day to a month; the upper limit may be years to decades. For a transit time of one day, reoxidation of the water along the flow path must occur on the order of once every 10 seconds in order to generate a 0.3 M $FeSO_4$ solution. If residence time is on the order of a month, the reoxidation must occur on the order of once every 5 minutes. Assuming a diffusion gradient of 140 μM/mm (assuming the O_2 profile goes from saturated to anaerobic in 1 mm), and taking the diffusion coefficient of O_2 into water as $2.1 \times 10^{-9} m^2 s^{-1}$,[67] Fickian diffusion of O_2 into water would occur at approximately 3 μmol $m^{-2} s^{-1}$. To consider the timing of reoxidation another way, if a liter of water has a surface area of $100^2 cm^2$ (1 liter with geometry 100 cm $\times$ 100 cm $\times$ 1 mm depth), reoxidation of 140 μmol O_2 would require about 45 seconds. A liter of water with a surface area of $10^2 cm^2$ (1 liter with geometry 10 $\times$ 10 $\times$ 10 cm depth) would require over an hour to reoxidize 140 μmol O_2. Oxygen flux into the sediment may then be fast enough to support short residence times only if the gradient is extremely steep, which must then be supported by a process that maintains that steep gradient. Such a process is likely a microbial driven oxidation, though qualitative analysis of this is not possible due to the uncertainties involved with knowing cell numbers, diffusive flux, advective transport effects, and the rates of oxidation.

Heat balance

The temperature of the Richmond solutions at the 5-way typically ranges between 30°C-50°C (Table III). Deep within the mine temperatures higher than 56°C are encountered. It has been suggested previously that the heat is generated as the result of exothermic pyrite oxidation.[8] Using the average $[Fe]_{aq}$ as 0.23 M, ~350 kJ (= 83 calories) of heat is liberated per liter of solution due to dissolution of 0.23 moles of pyrite. Assuming the heat capacity for these waters is not significantly different from that of pure water and that all heat accumulates in solution, pyrite oxidation can easily account for the temperature of the AMD fluids.

There are several other processes which may contribute to the overall heat budget in the mine, and especially to temperature changes observed in the system associated with high rainfall and increased flow within the system. Assuming that it takes at least a year for the bulk of the fluid to move from the surface to the 5-way, the high temperatures at the 5-way soon after high rainfall (without a significant pH change) could simply be attributed to recharge-driven movement of fluids from hotter regions of the system.

If there are rapid fluid flow pathways that allow mixing between dilute rain water and more acidic solutions, heats of mixing may also be a source of thermal energy. Calculation of the heat generated by mixing of a two different solutions may be estimated by

$$H_{mix}^{(1,2 \to 3)} = H(x^{(3)}) - [X^1 H(x^{(1)}) + X^2 H(x^{(2)})], \quad (7)$$

where the enthalpy of mixing is derived from enthalpies of solutions 1 and 2 of some mole fraction x mixed at some mole fraction X of the total solution, 3 (after Zeleznik[68]). Based on the mole fractions of H_2SO_4 for some of the extremely low pH solutions reported by Nordstrom *et al.*[25] and higher pH solutions more typically found in other AMD areas, the heat from mixing for several hypothetical scenarios can be

calculated. A significant amount of very low pH solution mixing with higher pH solution (50% pH -3 +50% pH 2) would be required to even approach the 20 calories per liter needed to raise the solution to observed temperatures. This level of mixing decreases the resulting solution pH more than that observed for the majority of water flowing out of the mine. An alternative scenario might involve mixing of circumneutral rainwater (transported rapidly from the surface without significant reaction or mixing) with films of very low pH pyrite sediment pore solution. In this scenario, 5% pH -3 solution +95% pH 7 solution results in a solution pH of approximately 0.5, but only contributes 1.7 cal to the solution (i.e., enough to raise 1 liter of water 1.7°C). Therefore, it is assumed that heat from mixing of different solutions at the Richmond Mine is not a significant contributor to the elevated temperatures.

A temperature pulse may also be induced when rainfall which flushes standing pools of oxidized water into adjacent fine-grained pyrite sediment. These pools may be analogous to the red pool (this study) or to evaporative pools, some of which contain 300 mM Fe^{3+}.[25] Flushing of previously stagnant large pools of Fe^{3+}-rich solutions could contribute large amounts of heat to the system through localized, increased amounts of pyrite oxidation. This process may explain the significant temperature differences observed at different times within the Richmond Mine site.

Significant evaporation that occurs within the mine may be a heat sink. At 40°C, it requires 10.36 calories to evaporate 1 mole of pure H_2O. However, as the volume of air in the mine is not well constrained and because we have no idea of the rate of evaporation-condensation in these systems, it is impossible to evaluate the role this process plays on the heat budget within the mine.

Pathways for pyrite oxidation and implications for microbial metabolism
The microbial community structure is impacted by the pathways for pyrite oxidation, as these determine the number and type of substrates for growth. Microbes that utilize Fe^{2+} as a substrate depend on inorganic and biological reactions that reduce Fe^{3+} (by reaction with sulfur or organic compounds) to close the cycle. The reactivity of different intermediate sulfur species with Fe^{3+} will impact the rate at which Fe^{2+} is regenerated for microbial use as a substrate.

The mechanism of pyrite oxidation has been intensively studied over a range of conditions utilizing a wide array of techniques and theory.[17-19,21,31,69] Figure 8 illustrates several potential pathways through which pyritic sulfide may oxidize to sulfate. We will examine sulfur oxidation at low pH and consider the potential roles that microorganisms may play. For reference, Table VI (and Fig. 9) presents inferences about metabolisms for each species/group of organisms detected at the Richmond Mine site.
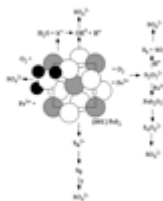

Figure 8
Diagram representing some of the major potential pathways of pyrite oxidation at low pH.
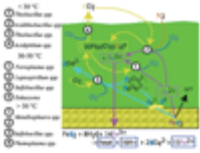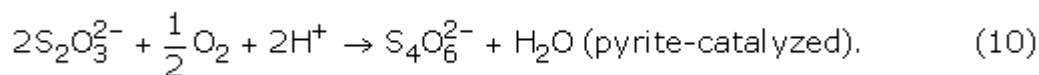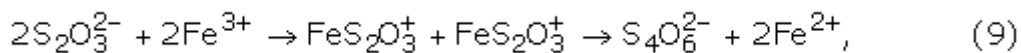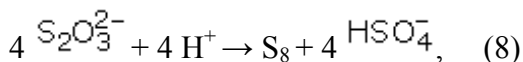
Figure 9
Schematic diagram of microbial metabolisms prevalent at the
Richmond Mine study site.

Several studies have observed the formation of polysulfides (see Fig. 8) on the pyrite surface.[70,71] Hu[71] suggested that polysulfide is a precursor to elemental sulfur formation on pyrite surfaces exposed to ferric iron at low pH. Elemental sulfur has been shown to form in many pyrite oxidation experiments in variable amounts. [12-14] It has also been shown that the surface-normalized rate of elemental sulfur oxidation under low pH conditions is up to several orders of magnitude slower than the rate of pyrite oxidation.[72] Thus, elemental sulfur should be available for microbial utilization.

There is no evidence for the accumulation of elemental sulfur on pyrite surfaces at the Richmond Mine. *L. ferrooxidans* (groups I and II), which comprise the majority of the community, have not been shown to utilize any S species (Table VI). The *Sulfobacillus* spp. isolates utilize a variety of sulfur compounds as well as organic compounds and other substrates. Although there is evidence for the existence of *Sulfobacillus* spp. in biofilms within AMD solutions and at the solution-air interface, FISH work on sediments did not find significant *Sulfobacillus* populations. [5]*At. caldus* has been isolated from the site (although not commonly detected in clone library-based studies). *At. caldus* can oxidize elemental sulfur without contacting the mineral, suggesting that a shuttle mechanism is important in elemental sulfur oxidation. Electron shuttles refer to small, redox-active organic molecules capable of carrying electrons between reduced and oxidized species as a general mechanism by which microbes may derive energy for cell growth and/or maintenance.[73] Druschel[72] reviews the criteria for potential electron shuttles in this system. Ongoing work is targeted toward identifying and characterizing potential shuttles.

Oxidation of pyrite through the leaving group thiosulfate (A in Fig. 8) is one of the most widely accepted models for the oxidation of pyrite, and is based on experimental evidence[18,21] coupled with a molecular orbital theory description.[17] Subsequent work on the stability of thiosulfate in low-pH solutions similar to those generated during the course of pyrite oxidation indicated thiosulfate decomposition through separate pathways:[15,22,74]

$$4\ S_2O_3^{2-} + 4\ H^+ \rightarrow S_8 + 4\ HSO_4^-, \quad (8)$$

$$2S_2O_3^{2-} + 2Fe^{3+} \rightarrow FeS_2O_3^+ + FeS_2O_3^+ \rightarrow S_4O_6^{2-} + 2Fe^{2+}, \quad (9)$$

$$2S_2O_3^{2-} + \frac{1}{2}O_2 + 2H^+ \rightarrow S_4O_6^{2-} + H_2O \text{ (pyrite-catalyzed).} \quad (10)$$

Kinetic modeling of the fate of thiosulfate in solutions analogous to those found at the Richmond Mine indicates that any thiosulfate formed in this environment would oxidize to tetrathionate almost immediately.[38,75] The fate of polythionates in solution was
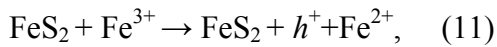
studied in detail by Druschel *et al*.[75,76] Results illustrated that the kinetics of polythionate oxidation coupled to Fe^{3+} or O_2 reduction are slow under acidic conditions. The lack of any observed polythionate species at the Richmond Mine suggests that either pyrite does not oxidize through this pathway, or that microorganisms are present which are able to quickly oxidize the species. Results from the clone libraries (Table IV) and culturing results do not clearly indicate a significant population of microorganisms that directly utilizes intermediate sulfur species. However, it is possible that *Ferroplasma* spp., the "alphabet plasma," and/or *Leptospirillum* group III can couple intermediate sulfur oxidation to reduction of alternate electron acceptors in microaerophilic environments. Based on results for *Ferroplasmsa* strain MT17,[77]*F. acidarmanus* may oxidize intermediate sulfur compounds. Okibe *et al*.[77] suggest that *Ferroplasma* strains MT16 and MT17 are able to utilize tetrathionate as an electron acceptor due to the observation of decreased tetrathionate concentrations after a significant lag phase. However, the growth of one *Ferroplasma* strain in that study ceased at the same time the polythionate concentration decreased and the strains found in the Richmond mine have not demonstrated the ability to utilize intermediate sulfur species as substrate.

The crux of the pyrite oxidation model, where thiosulfate detaches from the surface (Fig. 8), is based on the relative bond strengths of the S–S and Fe–S bonds.[78,79] The comparisons have been made based on bulk values and general observations concerning electron density shifts as a result of oxidation. The strengths of these bonds would certainly be affected by both oxidation processes (bonds affected by loss of *e*- and a decrease in symmetry) and as a result of being at the surface (symmetry decrease). Electron redistribution as a result of these interactions will affect the relative strength of the Fe–S and S–S bonds, which will determine whether $S_2O_3^{2-}$ will detach as a free ion.

Experimental observations of the force required to remove $S_2O_3^{2-}$ from a surface,[18] and the lack of significant observed $S_2O_3^{2-}$ or $S_xO_6^{2-}$ at low pH;[13,18,75] suggest that the Fe–S bond is stronger than the S–S bond at low pH. Borda *et al*.[20] have detected thiosulfate-like surface-bound groups on actively oxidizing pyrite, but found no evidence that the species detaches and oxidizes to sulfate in solution. Thus, it seems likely that although the molecular orbital argument of Luther[17] may be correct in large part, the fate of the S_2O_3-like surface group (Fe–S–S–O$_3$) may not result in appreciable free $S_2O_3^{2-}$ in low-pH conditions. Limited (or no) $S_2O_3^{2-}$ in solution suggests fundamental changes at this step in the oxidation pathway of pyrite as a function of pH. Consequently, microbial utilization of thiosulfate may be affected by whether it is surface bound or released to solution. If thiosulfate remains bound to the pyrite surface (e.g., as Fe–S–S–O$_3$), then either cells must attach or some other step is required to liberate thiosulfate into solution.

Another possible pathway for pyrite oxidation involves the formation of intermediate oxygen radical species (Fig. 8). [18,79-81] Hydroxyl radicals (OH*) form at the pyrite surface due to dissociation of water (present either as a free species in solution or as part of the hydration sphere of iron) following reaction with a hole (vacancy of an *e*- in a filled energy level; the hole is filled by the electron lost from water).[82] The hole may be created by an oxidation reaction (in solution or in air), a defect, or by a dopant in the

mineral. Natural pyrite may include significant impurities, and examples of both *n*-and *p*-type pyrite (*p*-type pyrite has extra holes) are abundant in natural deposits.[79]

Oxidation of pyrite by OH* generates sulfoxy species in which the oxygen is derived from water (consistent with above mentioned isotopic results):

$$FeS_2 + Fe^{3+} \rightarrow FeS_2 + h^+ + Fe^{2+}, \quad (11)$$

$$H_2O + h^+ \rightarrow OH^* + H^+, \quad (12)$$

$$FeS_2 + OH^* \rightarrow FeS_2O^- + H^+ \quad (13)$$

where h^+ is a hole site in the pyrite. In this pathway, oxidation of intermediate sulfur species is rate-limited by the formation of OH*. The formation of OH* is due to intrinsic defects and/or *p*-type dopants is a sulfur oxidation pathway that is independent of oxygen supply.

Most intermediate S species will react with hydroxyl radicals very quickly, with the notable exception of elemental sulfur.[72] The abiotic rate of oxidation of many intermediate sulfur species with OH* is on the scale of microseconds or faster.[76] Thus, the hydroxyl radical pathway in environmental systems may out-compete micro-organisms for the potential energy available from intermediate sulfur species, possibly explaining the apparent scarcity of these organisms within the Richmond Mine system. Generation of additional hydroxyl radicals may be associated with wetting of oxidized surfaces.[72,80] This source of OH* may also affect the distribution of intermediate sulfur species in the mine during times of flooding after dry periods.

As seen in Table I, the source of oxygen in sulfate formed from pyrite-derived sulfide is H_2O for all cathodic reactions except where O_2 is the primary oxidant. Isotopic analyses indicate that the source of most of the oxygen in sulfate derived from pyrite oxidation under laboratory and natural conditions is H_2O and not O_2.[83] However, at low pH sulfite–water isotopic exchange is thought to be very fast and any record of direct O_2 oxidation on previous intermediate sulfur species formed in the course of pyrite oxidation could be erased[84] and is thus not a good indicator of reaction pathways at Iron Mountain.

Toxic metals
Arsenic, Cu, or Zn in the water-transported pyrite accumulations (sediment) in the tunnels may exist as separate mineral grains, small inclusions of discrete minerals within pyrite grains, or as local enrichments. Additionally, these elements are probably heterogeneously distributed in the ore body and may be more abundant in recently exposed ore than in the more weathered sediments.

Galvanic interactions between sulfide minerals lead to rapid loss of arsenopyrite, chalcopyrite and sphalerite.[85] This galvanic coupling would protect the pyrite cathode while selective anodic dissolution of the other sulfides proceeds. Localized As, Cu, and Zn impurities revealed by EMP analysis will change the pyrite reactivity by affecting its local electronic structure. Selective oxidation of inclusions or enrichment areas may lead

to localized pitting, such as observed in Fig. [3], and influence patterns of microbial attachment. The concentrations of toxic metals such as As, Cu, Cd, Hg, and Ag can also influence microbial community structure because different species and strains may have different metal tolerances.

Spatial and temporal variability

Geochemical data from the sampling sites at each weir (A-, B-, and C-drifts) and the 5-way area on four sampling dates are shown in Table [II] and Table [III] and the correlation matrix is represented in Table [VII]. This table lists the Pearson correlation coefficients for each variable vs every other statistically significant variable. Strong correlation of temperature with Fe^{2+}, Fe^{3+}, and Eh illustrates that the heat of pyrite oxidation controls temperature. Strong correlation of H^+ with SO_4^{2-} illustrates the dependence of proton generation on the oxidation of sulfur species in the pyrite-oxidation pathway. Interestingly, Cu correlates with Fe^{2+}, while Zn does not, suggesting different factors control their concentrations. This correlation may indicate release by galvanic reaction, or it could be caused by melanterite dissolution, as the melanterite in this mine is known to contain significant Cu and Zn.[9]

Over time, Eh varies in the drifts in the same manner (Table [II]). This suggests that perturbations due to seasonal changes affect the entire system consistently, and implies that similar processes are occurring in all drifts and associated stopes.

Anomalously high Eh was observed in 2001. The high rainfall one week prior to the sampling trip may have caused flushing of sulfuric acid-rich films formed on pyrite surfaces and of pools created during dry months, dissolution of sul-fate minerals, and introduction of O_2. In time, Fe^{3+} consumption due to pyrite oxidation returned the Eh value to that determined by the balance between O_2 diffusion, surface oxidation reactions, and microbial activity.

The ~0.14 moles/L excess Fe^{3+} observed in solution in January 2001 compared to prior sampling times requires supply of an additional 3.5×10^{-2} moles O_2/L. Even if the entire volume of solution was new rainwater (with 2.5×10^{-4} moles O_2) there is a more than two orders-of-magnitude deficit in O_2 supply. In fact, in order to provide the 3.5×10^{-2} moles O_2/L it is necessary to reoxygenate the solution over 200 times, given the solubility of O_2 in AMD of $<1.40 \times 10^{-4}$M. Thus, rainfall alone cannot explain the high Fe^{3+} compared to other sampling times. Other factors that could raise the Eh of the system include alteration of the ferrous/ferric iron ratio due to changes in the rate of advec-tion through the system or in the activity level of Fe-oxidizing prokaryotes.

Geochemical factors controlling microbial community structure

Iron-oxidizing microorganisms are abundant in all communities studied (Bond *et al.*[5] and this study). Aerobic iron-oxidizing microorganisms form conspicuous biofilms (often dominated by *Leptospirillum* spp.) at the sediment- or solution-air interface. Activity within these biofilms generates solutions in which the aqueous ferric iron concentrations are typically several of orders magnitude greater than oxygen concentrations. These solutions percolate into sediments where they induce anaerobic oxidation of pyrite and associated intermediate sulfur compounds. This supply of Fe^{3+} is important for organisms that couple oxidation of S compounds (or organic carbon) to reduction of Fe^{3+} (Fig. [9]).

The decoupling of oxygen diffusion from oxidation reactions suggests that oxygen-saturated pyrite sediments are probably populated by microbial communities that are distinct from those growing in aerobic or microaerophilic regions.

Some differences in microbial population composition are observed among the different sampling locations for the January 19, 2001 trip (Table IV). Microbial communities within the more oxidized A-drift Red Pool and at the A-drift Slump are associated with higher pH solutions with generally lower metal and sulfate concentrations (up to 5–10 times lower, see Tables II and III). The geochemical and microbiological characteristics of the Red Pool are most distinctive. The A-drift Red Pool contains the only populations of *At. ferrooxidans, L. ferrooxidans*, and *Acidobacteria*, consistent with the known pH and temperature optima for these bacteria.[39,60,86] The A-drift Slump area contains significant populations of "alphabet plasma" (Table IV). These are also present in the A-drift Slime Streamers and the Slump (Table V). As the "alphabet plasma" clones are significantly divergent (>7%) from characterized *Thermoplasma* spp. isolates (Fig. 7), which are thermophilic facultative anaerobes,[87] it is not possible to deduce their metabolic roles based on phylo-genetic position. Factors that may contribute to proliferation of "alphabet plasma" in A-drift Slump slimes are the lower temperatures, higher pH, and high abundance of biofilm polymers. Phylogenetically related *Thermoplasma* have been reported from a wetland impacted by coal refuse with a pH and temperature similar to that of the A-drift Slump.[56]

The clone libraries and FISH results for all sites in the B- and C-drifts are similar, as expected based on general similarities in geochemistry and temperature. Populations are dominated by *Ferroplasma acidarmanus* and *Leptospirillum* (group III) microorganisms. The predominance of *Leptospirillum* group III in January 2001 distinguishes these communities from those previously documented at these sites[5] and Tyson *et a/.*[88]). The most likely geochemical control is the unusually high Fe^{3+}:Fe^{2+} in solutions in January 2001, which may imply that the redox potential for electron transport chain enzymes involved in iron oxidation differs between the *Leptospirillum* groups.

It has been noted previously that microbial populations at the site vary substantially in response to seasonal rainfall, which correlates with changes in ionic strength and temperature. Generally, archaeal populations predominate during the drier months when pH and ionic strength are higher, and bacterial and eukaryotic populations are more significant in wetter months.[29,89] The relatively low abundance of archaeal populations in January 2001 is consistent with this conclusion.

Conclusion

Rainfall, primarily occurring early in the year, introduces a relatively small amount of oxygen that is rapidly consumed by reaction with pyrite within the mine workings and by microorganisms that couple its reduction to oxidation of ferrous iron. The evolving AMD solution must be reoxidized thousands of times along its flow path in order for it to acquire the metal and sulfur load observed as it exits the system. Oxygen diffusion into solution is promoted by microbial respiration. A key role for iron-oxidizing bacteria and archaea in AMD generation is acceleration of the otherwise slow Fe-oxidation reaction (which is particularly slow at low pH), which promotes pyrite dissolution and leads to rapid accumulation of Fe^{2+}, SO_4^{2-}, and H^+ along the flow path. Exothermic pyrite oxidation is the predominant source of heat. However, flushing of very oxidized solutions (formed over the dry summer and fall months) into pyrite sediments may contribute to the heat spike following high rainfall. The ratio of $Fe:S:H^+$ indicates no consistent accumulation of sulfate minerals, elemental sulfur, or other sulfoxy compounds over extended periods of time. However, seasonal variations may be attributed to sulfate mineral accumulation and dissolution, as noted previously.[9] These results indicate that sulfate minerals in the mine precipitate and dissolve over seasonal time frames, but averaged over time, the effluent generally reflects complete pyrite oxidation.

Microbial communities contain a relatively small number of distinct taxa, as discussed previously.[37,38] Cultivation-independent surveys of microbial communities revealed a few newly recognized lines of descent. Much of the novelty lies within the *Thermoplasmatales* order and *Actinobacteria*, detected primarily within the A-drift subaerial habitats. Only in higher pH, lower metal and sulfate concentration environments were groups such as *Acidithiobacillus ferrooxidans, Acidobacteria* sp., and *Leptospirillum ferrooxidans* found. The apparent predominance of Fe-oxidizing organisms suggests that abiotic pathways of sulfur oxidation are rapid due to the high flux of ferric iron and possibly due to formation of oxygen radicals. However, further metabolic characterization of as yet uncultivated "alphabet plasma" and *Leptospirillum* group III species is required in order to test for their ability to oxidize intermediate sulfur species.

At the Richmond Mine, the dominance of iron-oxidizing microorganisms *(Ferroplasma acidarmanus* and *Leptospirillum* spp.) ensures a continual supply of ferric iron, making it likely that all steps along the oxidation pathway of pyrite to SO_4^{2-} involve the oxidation of intermediate sulfur species by ferric iron. The kinetics of thiosulfate and tetrathionate oxidation at these conditions,[22,74-76] suggest that the pathway of pyrite oxidation does not significantly progress through the detachment of thiosulfate in low-pH environments. Bioavailablity of elemental sulfur as an intermediate sulfur species is possible even if the mechanisms of pyrite oxidation include generation of oxygen radicals because elemental sulfur is not readily dissolved via radical pathways tested to date. Some subset of intermediate products (such as elemental sulfur) are resistant to reaction with Fe^{3+} and is open to utilization by *Sulfobacillus* spp. and *Acidithiobacillus caldus*, either through direct contact or through an enzymatic shuttling process that allows the organisms to keep some distance away from the pyrite surfaces. This speculation requires further analysis.
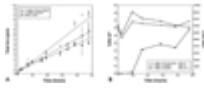
Figure 4
Results of FeAsS oxidation experiments in which *Acidithiobacillus caldus* was added to reaction vessels and allowed either direct contact with the mineral, or was separated from the mineral with a 0.2 μm filter.

Table II
Water chemistry of samples collected within the study site at the Richmond Mine 5-way area (at the 5-way, A, B, C weirs, and at the A-slump and red pool in 2001). Concentrations are reported in millimolar units.

Table III
Water chemistry of samples collected within the study site at the Richmond Mine 5-way area (at the 5-way, A, B, C weirs, and at the A-slump and red pool in 2001). Concentrations are reported in millimolar units. nd = not determined.

Table IV
List of 16S rRNA gene sequences and their taxonomic affiliations (based on BLAST searches of NCBI GenBank database) from the January 19, 2001 sampling. n.i. = not included in phylogenetic analyses.

Table V
Fe:S:H$^+$

Table VI
Microbial metabolisms prevalent in micro-organisms described in the Richmond Mine and their relative abundances defined by FISH and clone library results.

Table VII
Correlation table of selected ions in the 5-way, A, B, and C drifts over the sampling times in Tables II and III. A value of 1.00 is the maximum correlation, and the sign indicates positive or inverse correlation.