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Comparison of gold extraction yields by cyanide treatment vs. bioleaching procedure

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Comparison of gold extraction yields by cyanide treatment vs. bioleaching procedure

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A thesis submitted to the Faculty of Earth and Atmospheric Sciences, Liberal Arts and Sciences of the City College of the City University of New York, in partial fulfillment of the requirements for the degree of

Master of Science

Department of Earth and Atmospheric Sciences

The City College of the City University of New York

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Abstract

Samples from the tailings of the Picacho mine in California were leached using NaCN and native bacteria, *Alcaligenes faecalis*, in column experiments to compare the gold recovery yields in the effluents. Four columns were treated with a different liquid: water, bacteria, NaCN and a mixture of cyanide and bacteria. After they were leached the effluents were analyzed to determine gold yields. During experiment I the total Au recovered in the effluent in the column treated with bacteria was only 10.09% less than those treated with cyanide (C2) and 81.7% more than the control. In experiment II, Au values measured were below detection limits. In experiment III Au recovered in the effluents for the Bacteria Column was 18.51% less than the Cyanide (C2) column and 34.27% more than the control. Mass balance calculations for experiment I show that the column treated with bacteria has a higher percentage recovery yield of the Au recovered in both the effluent (18%) and in the two columns treated with cyanide (13% and 15%, respectively). In experiment III, mass balance calculations show that the percentage of Au recovered in the effluents of the columns treated with the bacteria was 15% and the other two columns treated with cyanide had 25% of Au from the effluents. These findings indicates that the use of *A. faecalis* as an encouraging option for Au leaching of mine tailings which requires little supervision that translates in lower costs. The leaching treatment with bacteria produced favorable results that challenge cyanidation.

Keywords: gold recovery, mining, bioleaching,
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Introduction

In 2011, a new gold rush began in California. Closed mines were reopened to extract gold from remaining ore, driven by a price of $1300/oz, which could make mining profitable again. Sutter Gold, a mining company, estimates over $800 million in unrecovered gold in their closed mines. However, closed mines harbor environmental hazards that need to be mitigated and reopening them creates new environmental concerns. (McKinley 2011). One way to minimize the cost involved with renewed mining operations is through biomining techniques pioneered in the 1950’s. Biomining is used to recover gold from low-grade ores (mines with less than 0.2 mg kg\(^{-1}\)) that cyanidation cannot extract. Much of that gold is strongly sorbed/bound to sulfides, silicates, carbonates, and sulfates, among others (Gasparrini 1993, chap. 6) and is therefore difficult to extract. Biomining techniques have gained popularity due to lower environmental impacts and low cost. Typically, biomining is applied as a pretreatment to cyanide leaching to increase yields from gold mine tailings. However, the effectiveness of the treatment may be limited by the size of the mineral grains associated with refractory gold. While bioleaching has been employed extensively the process usually involves pretreatment followed by conventional cyanide leaching. In fact, *Alcaligenes faecalis*, the organism used in this study, has not been previously considered for pretreatment or main leaching treatment for low-grade ores. The purpose of this work is to compare conventional direct cyanide leaching to bioleaching with *Alcaligenes faecalis* using column experiments.

Purpose of the Study

In this study, the microorganism *Alcaligenes faecalis*, isolated from mining wastewaters from the Picacho, CA gold mine, was used to determine its effectiveness in mobilizing refractory gold. A.
A. faecalis is known to produce gold nanoparticles by reducing gold (Au$^{3+}$) ions for use in nanotechnology applications (El-Deeb et al. 2014). This suggests that A. faecalis can also metabolize and produce gold nanoparticles from mine tailings.

The purpose of this study is to compare the recovery yield between leaching of a low-grade ore by Alcaligenes faecalis and cyanide treatment. We performed a series of column experiments to determine Au yields.

**Background**

Gold [Au] is a noble, malleable, and precious metal that is highly ductile, reflective and with an opaque, metallic, dark yellow color. It is a metal that forms alloys easily with common metals, has a high electrical and thermal conductivity. Gold has six oxidation states (+1, +2, +3, +4, +5, and +7) which provide it with a low reactivity with most anions (Clemente 2013). However, the most common states of gold are aurous (+1) and auric (+3). Although auric compounds are more stable than aurous, the aurous state is more prevalent in fluids that form ores (Clemente 2013).

The geochemical properties of gold dictate how it is formed and where it is found. Gold can be detected in the Earth’s crust with an average concentration of 0.004 mg kg$^{-1}$, but its concentration can vary from 0.2 mg kg$^{-1}$ to around 30 mg kg$^{-1}$; in certain places called bonanzas gold concentration can reach the hundreds of mg kg$^{-1}$. Gold also can be detected in natural waters with a concentration that ranges from 0.02 to 0.20 μ L$^{-1}$. Due to its scarcity on Earth throughout history, it has been highly valued by past and present civilizations. The value that has been given to this noble metal varies from civilization to civilization and is based on religious, artistic and economic custom. In modern times, due to its physicochemical properties, gold is used more broadly, spanning the fields of telecommunication, dentistry, photography, electronics, and medicine, among other uses (Clemente 2013).
Gold is found on the Earth’s crust as flakes, scales or crystals of native gold or as native alloys with Ag, Cu, Al, Fe, Bi, Pb, Zn, Pd, or Pt. Gold can also be a limited substitute for metals in mineral structures (Clemente 2013). There are two types of gold. The first kind is known as primary gold. This type of gold is precipitated during chemical reactions between hydrothermal solutions, containing heavy metals, and the rocks in the earth’s crust. In this environment gold is part of as chloride, thiosulfate, bisulfide and sulfide complexes. The second kind of gold is known as a secondary gold. The origin of this type of gold is debatable but some scientists believe that it is formed from the chemical and mechanical weathering of primary gold particles, however, some of the particles secondary gold appears to have different morphologies that are not observed in primary gold sources. According to Southam et al., morphologies of gold, such as wire, dendritic, octahedral, imply that secondary gold is the result of microbial weathering (Southam et al. 2009). The interaction of gold complexes with the surrounding microorganisms allow gold migration and accumulation in the formation of supergene gold deposits (M. Lengke and Southam 2006). One of such complexes produced by microbiological reaction is gold (I)-thiosulfate (Au(S₂O₃)₂⁻) as sulfur compounds are oxidized (M. Lengke and Southam 2006).

Sulfate-reducing bacteria (SRB) are microorganisms responsible for the bioaccumulation of some supergene gold deposits. These types of bacteria oxidize gold (I)-thiosulfate by utilizing it as a source of energy and initially storing the precipitated gold throughout the cytoplasm. According to Lengke and Southam, thiosulfate and gold (I)-thiosulfate “enter the bacteria through the pores in the cell surfaces” (M. Lengke and Southam 2006), once in the cytoplasm they are metabolized and reduced. The effect of SRB can be seen by the production of hydrogen sulfide and elemental gold precipitated in the form of nanoparticles. Lengke and Southam suggest that the pathway to the reduction of thiosulfate and gold (I)-thiosulfate is not straight, but that there are intermediate
steps that are necessary to their degradation (Fig. 1). However, they are certain that gold (I)-thiosulfate can only start to be consumed for metabolism when the oxidation of sulfur is complete (M. F. Lengke and Southam 2005). Furthermore, the presence of “gold nanoparticles inside the cytoplasm”, Au+ is presumably reduced to Au0, could be achieved by “an intracellular electron donor or exported by a membrane transporter system” (M. Lengke and Southam 2006). In some cases, gold nanoparticles have been found along the cytoplasmic membrane (M. F. Lengke and Southam 2005).

![Cellular model of a SRB using Thiosulfate and Gold(I)-thiosulfate as energy source and the production of hydrogen sulfide and elemental gold particles.](image)

After precipitation, gold particles formed “aggregates of octahedral gold having a micrometer size range” extracellularly and nanoparticles intracellularly (M. Lengke and Southam 2006).

The presence of gold as an alloy with other elements and minerals allows it to move through the regolith across earth’s reservoirs through chemical weathering of the host mineral. The mobility of gold in some cases can be affected by its interaction with microorganisms which can dissolve
and precipitate it (Clemente 2013). Thus, microorganisms can affect the location and concentration of gold presence. Typically, microbes can mobilize gold by excreting metabolites such as thiosulfate, amino acids, and cyanide, which dissolve the metal thereby facilitating its transport. Microbial diversity allows for certain species to have adapted to Au complexes that would otherwise be toxic by precipitating intra- and extra-cellular Au, and in metabolic products such as sulfide minerals. Nonetheless, the bio-assimilation and, therefore, solubilization depends on factors such as climate, soil geochemistry, the quality of the substrate quality (Clemente 2013).

*Economic Impact*

The worldwide production of gold from 2003 to 2009 varied from 2,540 to 2,330 t year\(^{-1}\) (Clemente 2013). The leading commercial producers of Au are currently the People’s Republic of China, the Republic of South Africa, the United States, Russia, Canada, Australia and Peru. The total global gold supply is nearly 3,900 t year\(^{-1}\) which encompasses mined, outflow from above ground bullion stocks and recycled sources. In some instances gold has been detected in secondary sources such as sewage sludge (Clemente 2013). In 2016, the gold supply was nearly flat, compared to 2015. However, it saw a global total increase of 17% of gold recycling. The demand for gold, in 2016, increased 92.9 tons, driven mainly by the finance sector since all other sectors saw decreased demand (Street et al. 2016). According to the World Gold Council, in 2016, 66 % of the world’s total gold supply was obtained from mining and 34 % was obtained from recycled sources (Street et al. 2016). Although mined gold is the primary source of the global supply, swelling global demand is projected to drive the market toward recycled sources and low-grade ores. One of those drivers will be the technology sector which is seeing aggressive demand of gold to be used in gold bonding wire and Printed Circuit Boards that are used in sensors for fingerprint readers and iris sensors (Street et al. 2016)
Recognized Extraction Techniques

The recovery of primary gold is very expensive and produces harmful effects on the environment. At different stages of the mining process the risk to the environment increases. Due to low concentrations of gold in the crust, to make the extraction of gold economically feasible large quantities of ore must be excavated. Open-pit mining is one of the techniques used in ore extraction and can cause atmospheric contamination and changes to the landscape. Chemical treatment of the excavated ore also causes environmental hazards.

Gold ore extraction is a process that is affected by the ore’s mineralogy. It is imperative to know the different kinds of minerals in the ore and how they interact with gold particles since this interaction will dictate the different methods for gold extraction. Most of the gold that is recovered from mining is obtained by using hydrometallurgical processes where gold is extracted using aqueous systems. Figure 2 shows a simple path that gold extraction follows, however, there are steps that are taken to ensure the maximum gold recovery. Often low gold recovery by cyanidation in sulfide, carbonaceous, and tellurite ores leads to the implementation of pretreatment procedures. Low gold recovery may be attributed to gold that is immobilized due to its association with reactive gangue minerals, gold being attached to minerals that consume the reagent solution, and gold sorption to carbonaceous materials during the leaching procedure (House and Marsden 2006; chap 5).

After ore rocks have been crushed and reduced to a specific size in a mill, visible gold can be removed physically using gravitational and centrifugal forces. For particles that are less visible, the ore can be leached or be pretreated (Gasparrini 1993). Pretreatment is a stage in the gold recovery process that is undertaken in sulfide ores and in ores containing carbonaceous materials to lessen the interference of some minerals that consume oxidizing reagents that host gold particles.
during the leaching stage thereby liberating the gold particles strongly sorbed to the interfering minerals (House and Marsden 2006; chap. 5).

Figure 2. Gold Hydrometallurgical Extraction Process

The pretreatment methods employed in gold extraction include pyrometallurgical oxidation, hydrometallurgical oxidation, and microwave exposure. The pyrometallurgical oxidation is a roasting procedure involving oxidation of rock material in oxygen or air under controlled reaction time and temperature (Gasparrini 1993)(House and Marsden 2006; chap 5). This procedure liberates the gold particles from the host mineral by a series of steps (Figure 3) which include, in the case of auriferous arsenopyrite, three phase changes involving arsenic volatilization as gold enters a liquid phase. The arsenic is then removed, and gold remains in the solid phase.
For complex ores that require the separation of Au from other metals processes such as: chlorination, sulfation, reduction, are implemented (Gasparrini 1993).

Environmental regulations set in laws such as the Clean Air Act enforced by the United States Environmental Protection Agency require treatment of major gases emitted: sulfur dioxide, arsenic trioxide, as well as carbon dioxide, carbon monoxide, mercury, tellurium oxides, selenium and antimony oxides that are also a byproduct of the pretreatment method. The treatment of these gases has increased the cost for gold extraction, recovery and refinement resulting in an industry shift to hydrometallurgical pretreatments (House and Marsden 2006).

Hydrometallurgical pretreatments increase the oxidizing potential of certain minerals in solutions, especially sulfides. The type of oxidant used depends on factors such as ore composition, cost of equipment and reagents, and safety and environmental considerations, among others. Oxygen is one of oxidants used in ores containing sulfides. It is used as a pretreatment technique where oxygen is applied in low-pressure procedures or in high-pressure with acidic or non-acidic media. In refractory pyritic and arsenopyritic ores, nitric acid is used as an oxidant. This process is used mainly for silver and copper recovery from refractory concentrates; however, it can be used for
pretreatment of refractory gold ores, albeit not commercially. For carbonaceous and sulfides ores, aqueous chloride solutions are used to alter the preg-robbing or pre-borrowing nature of carboneous matter which affects the efficiency of cyanidation. Chlorination for the oxidation of gold-bearing sulfide ores is not used because of the chlorine consumption is very high. Another way to oxidize gold-bearing sulfides ores is by using bacteria. In bacterial oxidation uses bacteria, such as SRB, which catalyze mineral oxidation and metabolize sulfur and iron species to obtain energy (House and Marsden 2006, chap. 6)

Microwave exposure is used as a pretreatment stage for sulfide and carbonaceous materials. Sulfide minerals have dielectric properties, and therefore react by heating up and causing a direct oxidation of the sulfide to sulfur dioxide, thus allowing the exposure of gold particles (House and Marsden 2006, chap 5) Although carbonaceous material reflects microwaves microwave energy generates temperatures that cause the material to be oxidized (House and Marsden 2006, chap 5).

Cyanide (CN⁻) leaching is an inexpensive technique where a dilute aqueous solution is used to extract Au from ores by forming Au(CN)₂⁻, which can be extracted by dilute aqueous solution. After cyanide is used for the extraction of gold, it is discarded in effluents and solid mine tailings which could reach waterways (groundwater, river systems), potentially killing organisms. Cyanide is used due to its affinity for gold particularly in complex ores that contain other metals. This technique is called cyanidation. There are different procedures that are used during cyanidation which are employed depending on the ore’s mineralogy, porosity, permeability, grain size, pH and mine characteristics. Some of those techniques are: agitation leaching, heap leaching, and intensive cyanidation. In locations where cyanide cannot be used due to environmental regulation and the ore’s characteristics, other leaching procedures such as chlorine-chlorine leaching have been applied and others such as thiosulfate, ammonia, alkaline sulfide have been
investigated.

Some of the advantages that they pose over cyanidation are mainly justified because some can be applied in an acidic media, have faster gold leaching kinetics, or some readily form gold and silver complexes over other metals than cyanide, however, these procedures have been investigated but not implemented on a commercial scale due to their chemistry (House and Marsden 2006, chap.6).

Gold cyanidation issues
Gold cyanidation has many iterations such as carbon-in-leach and carbon-in-pulp, which produce very high gold recoveries when they are applied to high grade ores. However, when cyanidation is applied to low grade ores, gold recovery starts to decline. The issue rests with the fact that gold occurs in alloys that react with cyanide. Some of the factors that inhibit a high gold recovery are: (1) the association of colloidal gold with sulfide minerals; (2) sparingly soluble gold minerals; (3) reaction of cyanide with carbonaceous matter rather than with gold (“preg-robbing”); (4) scavenging of cyanide by clay minerals, micas, pyrite, and ferrihydrite (Venter, Chryssoulis, and Mulpeter 2004). When gold is in locked with sulfide minerals gold extraction is affected by the consumption of oxygen and cyanide and by providing sulfide ions (arsenite/arsenate, antimonite/antimonate) that form sorption complexes or insoluble compounds on gold particles. Copper inhibits gold recovery through cyanidation because it is found in minerals such as azurite and malachite and forms stronger complexes with cyanide than gold, causing cyanide to be consumed (Venter, Chryssoulis, and Mulpeter 2004).

Biomining
The discovery of bacteria in association with acid rock drainage in 1947 and the characterization and naming of the bacteria *Acidithiobacillus thiooxidans* (A.K.A. *Thiobacillus ferrooxidans*) in 1951 encouraged research on the role of microorganisms in the oxidation of mineral sulfides. New
findings in the 1950’s led the way for the industrial scaling application of the findings such as copper dump leaching by Kennecott Copper Corporation. This new technique for mining was called biomining (Rawlings 1997).

Biomining is a general term for mining techniques that use microorganisms to disassociate economically relevant metals from other minerals such as insoluble sulfides and oxides (in the case of uranium) (Schippers et al. 2014; Rohwerder 2003). There are two categories that make up biomining: bioleaching and biooxidation. Biomining techniques are used to improve environmental biotechnologies, to degrade cyanide, and to remove metals from water and mine tailings. Moreover, it is being used for metal removal from industrial and waste residues (Schippers et al. 2014).

The use of bacteria in the mining industry began in the 1960s when bioleaching and biooxidation were accepted as viable cost-effective commercial practices that maximized metal recovery in mines. The use of bacteria became an environmentally friendly alternative for the mining industry. By 1986, 11 mines were employing commercial bioleaching or biooxidation worldwide (Rawlings 1997, chap. 1). Today, there are companies such as Outotec that provide services to improve mining projects and offer patented biomining processes. One example is BIOX ®, first developed and commercialized by GENCOR S.A. Ltd. in South Africa in the 1970s (Rawlings 1997, chap. 3). BIOX ® is a biooxidation pre-treatment that optimizes gold recovery by liberating gold encased in minerals such as pyrite, thus allowing gold to be recovered through cyanidation. The bacteria culture for BIOX ® uses a mixture of Acidithiobacillus thiooxidans, Acidithiobacillus ferrooxidans, and Leptospirillum ferrooxidans (Rawlings 1997, chap. 3). These bacteria are used almost exclusively in sulfide ores with refractory gold (Rohwerder 2003; Curreli et al. 1997; M. F. Lengke and Southam 2007). During the bioleaching process many factors must be monitored
and corrected such as: pH, temperature, oxygen to guarantee optimal oxidation rates and to keep the bacteria alive. Other bacteria often used in bioleaching are *Desulfovibrio sp.* which are SRB.

Bioleaching as explained by Schippers et al (2014) is the process in which an insoluble valuable metal is transformed into a soluble form using microorganisms. In bioleaching the solutions are conserved and the solids are discarded (Rawlings 1997). On the other hand, Biooxidation refers to the pretreatment processes that are applied to unlock valuable metals from mineral sulfides using microorganisms. However, in biooxidation, metals are left in the solid phase to be processed, and the solution collected is discarded (Rawlings 1997). Biomining is used to recover valuable metals from low-grade ores and it is also used as pretreatment of low-grade ores to unlock metals such as Au from insoluble matrixes and later be subjected in the case of Au to cyanidation. The importance of biomining in the pretreatment of refractory Au ores is very high. The yields for a refractory Au ore using cyanidation is only of 50%, after the bioleaching pretreatment, in some cases, more than 95% of Au is extracted (Bosecker 1997). Biooxidation is preferred for refractory Au ores because of the lower capital and operational costs that it incurs because it can be performed at ambient temperatures, require fewer highly skilled operators, lower safety precautions and minimal supervision (Rawlings 1997, chapter 6).

Biomining is used worldwide mainly for processing sulfidic low-grade ores because it is ecologically acceptable and economically viable. Advances in biomining techniques have allowed biomining to be considered among hydrometallurgical and chemical procedures as a viable option for recovering base metals (Schippers et al. 2014).

The bioleaching and biooxidation techniques are similar to the leaching techniques used in mining except that microorganisms are used as part of the lixiviant. There are four main bioleaching techniques that are used: dump bioleaching, heap bioleaching, *in situ* or underground bioleaching
and tank bioleaching. For biooxidation among the techniques that are used are stirred tank biooxidation, heap biooxidation and biooxidation with thin layer technology (Schippers et al. 2014; Rawlings 1997, chapter 1). Stirred tank biooxidation is a technique that involves many stages using aerated tanks where mine tailings are treated with bacteria and other lixiviants. The tanks are made of high-grade stainless or rubber-lined to avoid corrosiveness, they also have cooling coils to maintain ideal temperature for the bacteria. For refractory Au, the effluent from the tanks is subjected to a series of treatments such as water washing, solid/liquid separation in thickeners, limestone to stabilize arsenic and iron. The solids are water washed to remove any soluble metals and acid than can consume cyanide and lime. The final step is to apply cyanidation to recover gold form the washed solids. This process takes from 4 to 6 days (Rawlings 1997, chapter 1,6). Heap biooxidation is a technique that requires the ore to be crushed and piled up in a heap which is inoculated with bacteria. In this technique oxygen is often scarce at deeper portions of the heap, to avoid this, pipes are placed in places within the heap to provide extra oxygen. The heap biooxidation period ranges from 9 to 13 months, at the end of which the heap is treated to neutralize its acidity and then cyanide to recover Au. In the case of the thin layer technology for gold, a host or support rock is coated with refractory gold concentrate that is crushed and sieved with a mesh size 65 – 400. The coating is applied in slurry via tumbling or spraying or by wetting the host rocks and using dry coating. According to Rawlings, the hydrophobic nature of the sulfide material forms a thin film of approximately 0.0.5 inches on the surface of the host rock. The coated rocks are piled up on a heap and prepared for heap biooxidation. This process may take from 30 to 90 days. At the end of this period the cyanidation process can start for Au recovery. (Rawlings 1997, chapter 6).
**Bacteria: *Alcaligenes Faecalis***

*Alcaligenes faecalis* bacteria is a heterotroph species found in soil, water, and in the intestinal tract of vertebrates. Therefore, it is found in places inhabited by humans. It is a Gram negative organism that moves with the aid of flagella and is shaped like rods and coccoid rods. The bacterium is aerobic and uses oxygen as the terminal electron acceptor, however, some strains have anaerobic respiration when they are surrounded by nitrate or nitrite. They grow optimally in a temperature between 30 and 37 °C and have a fruity odor (Gonzalez 1998). A study in the production of gold nanoparticles concluded that *Alcaligenes faecalis* produced gold nanoparticles of different shapes and sizes extracellularly, which shows its capability of metabolizing gold.(El-Deeb et al. 2014)

**Study Site: The Picacho Gold Mine**

The Picacho gold mine is located the south-eastern California near the Arizona border, less than 16 km northwest from the Little Picacho Wilderness Area and 29 km north of Yuma (Fig. 4). The ore was discovered in 1862 and it was in operation until 2002. Through the years the mining operation methods have changed from underground mining to shallow open pits (Losh et al. 2005). From 1980 to 1999 mined “15.5 Mt of ore at an average grade of 1.0 g/ton, and recovered 12.1 Mt of gold” (Losh et al. 2005). Historically the mine’s gold production is estimated to be 18.6 Mt (Losh et al. 2005).
The Picacho gold mine is recognized as a low-grade gold deposit and it is” characterized by a gold-
arsenic-antimony geochemical signature consistent with bisulfide complexing of gold in reducing fluid” (Losh et al. 2005). The gold formation at the Picacho ore is said to be a consequence of
“faulted and fractured Mesozoic leucogranite and schist as well as from Tertiary postore conglomerate that was derived from the mineralized crystalline rocks” (Losh et al. 2005). It is also
associated with the Chocolate Mountains Detachment Fault, which is a low-angle normal fault. Detachment faults or Denudational faults hold gold deposits (Losh et al. 2005).

Losh et. al (2005), explain that the Picacho Mine is considered mineralogically simple since gold found only in association with pyrite and it precipitated during the final stages of faulting. Gold mineralization occurred from the mixing of a hydrothermal fluid that carried gold as a sulfur ligand with high pH and specular hematite-precipitating fluid which acted as oxidizing fluid that lead to the precipitation of gold. The fluid circulation was started by a magmatic heat source that is associated with volcanic rocks in the area and was guided by the Chocolate Mountains/Gatuna Fault system to the Picacho ore deposit (Fig. 5). The source of the hydrothermal fluids is meteoric fluid interacting with minerals, primarily Mesozoic rocks, in the area (Losh et al. 2005). According to Losh et al. (2005), the hydrothermal were low in salinity and a temperature of about 200 °C.

Figure 5. Schematic Fluid Flow (Losh et al. 2005)
Materials and Methods
This work compares the gold recovery yield from the tailings of a low-grade ore mine in Picacho, CA using three leaching agents: native bacteria (*Alcaligenes Faecalis*), 200 ppm NaCN solution (95% min by Alfa Aesar), and a control (Milli-Q Ultrapure Type 1 water). The following section describes three aspects of the experiment: inoculation of the tailings over a period of three weeks, followed by the stepwise preparation of samples for chemical analysis. A description of the method for determination of metal concentration by atomic absorption spectrometer (AAS) follows, along with an explanation of the technique, along with the sequence of steps taken to operate the AAS, including preparation of standards and parameters set on the instrument to acquire gold concentrations. The gold recovery concentration from effluents (leachates) and solids (tailings) was used to determine a mass balance in the system.

Sample Description

Tailings from the Picacho mine were sampled/obtained from between 2008 and 2010. The grain size range for the tailings varied from coarse silt to very coarse pebble on the Udden-Wentworth Scale (Appendix A). Samples were not processed prior to column experiments and are therefore the columns are non-uniform with respect to grain size. Solid samples from the control columns and untreated samples were analyzed using X-ray powder diffraction (XRD) to identify minerals present.

Microbial Cultures

The bacterium *Alcaligenes faecalis*, from mining wastewaters at the Picacho mine was employed in this work. Bacteria were grown over a period of 2 days in modified (glucose-free) M9 media. The culture was then used without dilution during the column treatment as described below.
Experimental Setup
Four Chromaflex standard chromatography columns (Kimble Chromaflex Borosilicate Glass, Volume 543 ml, inside diameter 4.8 cm, length 30 cm) with PTFE fittings with 20 μm porosity polyethylene bed to avoid clogging were used during the leaching process. The columns were filled with 1000 g of tailings and placed upright and secured with clamps (Fig. 6). The leachate liquid was introduced through the bottom of the columns and extracted through the top. The bottom part of the columns was connected to a peristaltic pump, Masterflex L/S made by Cole-Parmer (Model # HV-07523-70), using a Masterflex Norprene tubing of size 14 (inside diameter 1.6 mm). The top part of the columns was connected to an automated fraction sample collector model CF-1, made by Spectra/Chrom with tubing size 14.

![Experimental Setup Diagram](image)

Figure 6. Inoculation set up; A) Beakers containing the inoculating liquid; B) Peristaltic Pump; C) Columns; D) Automated fraction sample collector
**Leaching Procedure**

Each column was treated for five consecutive days with 200 mL of a leaching agent regulated by a peristaltic pump over a period of 3 weeks. The volume rate at which all the columns were treated is 0.3 mL/min (Appendix B). The resultant effluent was collected daily for five days using the automated fraction sample collector with 20 mL glass tubes (Appendix C) and then transferred to 50 mL centrifuge test tubes to be stored for further analysis. The peristaltic pump was connected to a timer to enable shutting off the instrument before the total aliquot was added to the column to prevent introducing air into the columns.

Each column is labelled and treated with specific liquid agents. Column 1 is labelled Bacteria (B<sub>c</sub>) and is treated with bacteria, Column 2 is labelled Cyanide (C1) which is treated with NaCN, Column 3 is labelled Water Column (W<sub>c</sub>) and it is treated with Milli-Q Water (control), and column 4 is labeled Cyanide (C2) which is treated with NaCN. The following table shows the inoculating schedule for each column:

*Table 1. Inoculating Schedule of the Columns*

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<tr>
<th></th>
<th>WEEK 1</th>
<th>WEEK 2</th>
<th>WEEK 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>W&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Water</td>
<td>Water</td>
<td>Water</td>
</tr>
<tr>
<td>B&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Water</td>
<td>Bacteria</td>
<td>Water</td>
</tr>
<tr>
<td>C1</td>
<td>Water</td>
<td>Sodium Cyanide</td>
<td>Water</td>
</tr>
<tr>
<td>C2</td>
<td>Water</td>
<td>Sodium Cyanide</td>
<td>Water</td>
</tr>
</tbody>
</table>
Preparation of Tailings for Au Analysis
After three weeks, the columns were sampled by dividing the soil content of each column into three parts: top, middle and bottom, and storing them in separate containers. The soil was air dried (Appendix D) and split into 15 g subsamples placed into individual containers. The subsamples were finely ground and placed in 250 mL centrifuge bottles. To separate the metals from the mineral matrix, the samples were mixed in a nickel-sulfide fire assay flux (Appendix E) and shaken vigorously before transferring to a fire clay crucible, which was covered and placed into a furnace at 400 °C for 75 minutes to fuse the mixture. The furnace temperature was increased at 200 °C intervals over 10-20 minutes until reaching 1000 °C to prevent explosions and spillage from volatile release.

The fire assay fractionates the mixture into two phases: a NiS phase that aggregates Au and PGEs into a NiS button that sinks to the bottom of the crucible and a siliceous phase that appears as black-glass slag at the top of the cooled fusion product (Juvonen et al. 2002). To recover the NiS button, the crucible is crushed using a hydraulic press and the button is removed from the slag (Appendix F).

Determination of Au Concentration To prepare samples for determination of Au concentration remaining in the leached tailings by AAS, the NiS button is transferred to a 500 mL Erlenmeyer flask and dissolved in 300 mL of 37% HCl. The flask is covered with a watchglass and heated on a hot plate at 90 oC in a fume hood overnight to exhaust evolved H₂S (Juvonen, Lakomaa, and Soikkeli 2002) (Appendix G) flask is subsequently cooled and diluted with 150 mL of Milli-Q water (Juvonen, Lakomaa, and Soikkeli 2002). To separate gold from PGE’s and other metals 5 mL of 1000 ppm Te and 7 mL of 1000 ppm Sn solution (Tin (II) chloride dihydrate 98+%%) were added to the flask. Upon addition of Te a black precipitate containing AuTe begins to form.
(Appendix H). The solution is reheated on a hot plate for 1 h at 90 oC to promote further precipitation of AuTe. To remove the AuTe from the acid solution, the solution is vacuum pumped through a 0.45 μm pore size Whatman cellulose nitrate membrane filter (Appendix I). The filter containing AuTe is removed and transferred to a 15 mL centrifuge test tube to which 5 mL of aqua regia (37% HCl and 70 % (HNO3) are added. The test tube is stoppered lightly to allow the gases generated from this reaction to escape. The test tubes are placed in a water bath for 1 h at 70-80 ºC inside the fume hood and left overnight. The color of the solution changes from dark orange/copper to a light orange/yellow and has a strong odor.

The aqua regia treatment is also applied to the leachate gathered from the columns to dissolve any leached Au. 10 mL of the leachate is placed in a 50 mL centrifuge test tube followed by 5 mL of HCl and 5mL HNO3. The same procedure as before is followed. After the aqua regia treatment, the two sets of samples are placed in coated cuvettes for analysis by AAS.

Atomic Absorption Spectrometry

Atomic Absorption spectrometry (AAS) is an analytical technique used to determine the concentration of metals in solution. It derives from the studies of the Fraunhofer’s lines in the visible region of the solar spectrum formed by the components of the sun’s atmosphere which absorb the sun’s radiation. In the 20th century, AAS began to be used to study the atmospheric composition of other celestial bodies. Adam Walsh was the first to start using AAS for chemical analysis (Morton and Roberts 2003, chap.1). Elements are identified using AAS by measuring the absorbance of energy with a specific wavelength (λ) when it interacts with the electrons of an atom. Electrons in atoms have a unique energy levels, and the spacing between those energy levels can be quantified by measuring the energy absorbed by an electron in a ground state (E₀) until it becomes excited (Eₙ). In order for the electron to become excited, the energy provided by a photon
(\(E_p\)) must be equal to the energy gap (\(E\)) between an electron in ground and excited state (Morton and Roberts 2003, chap. 1)

\[
E= E_n - E_0 \\
E_p = h \times v
\]

Where \(h\) = Planck’s constant  
\(v\) = Frequency  
\(E_p = E_n - E_0\)

\[
v = c / \lambda
\]

\(c\) = speed of light in vacuum  
\(\lambda\) = wavelength.

\[
\lambda = h c / (E_n - E_0)
\]

In order for the photons to interact with the atoms in a sample solution, the sample solution must be heated until the sample is atomized (Morton and Roberts 2003, chap. 1) and the photons can be sent through it. This process occurs inside an instrument called Atomic Absorption Spectrometer. There are two options to heat up the sample solution. The first option is by using a acetylene flame and the second option is by using a graphite furnace. A spectrometer that uses a flame system, heats up the solution to 2000 – 3000 °K. During this process the solvent in the solution evaporates, leaving behind the solid particles in the solution. The particles are melted and subsequently vaporized. At this point the atoms become excited by absorbing energy from the photons. As temperature increases the atoms are ionized. In a graphite furnace spectrometer the solution is injected into a cylindrical tube (furnace) which is heated gradually up to 3000 °C by resistive heating when a high current is sent through the furnace. The sample solution follows the same steps as the flame system until its remaining contents are ionized (Morton and Roberts 2003, chap.
2). Atomic absorption spectrometers have a basic layout regardless of how the sample solution is heated: line source, atomizer, monochromator, detector, readout device (Fig. 7):

![Diagram of Basic Flame Spectrometer](image)

*Figure 7. Basic flame spectrometer (Morton and Roberts 2003, chap 3)*

A line source is where photons are generated in the spectrometer. A hollow cathode lamp is used to produce the photons with a narrow absorbing spectral line which is important for atomic absorption to occur and to be measured. There are element specific lamps that can be used such as for gold and silver. Inside the lamp there is an inert gas that is ionized to allow the gas atoms to interact with the metallic atoms. The metallic atoms become excited as a result of the interaction and emit energy of that reflects their own wavelength before they return to the ground state. The photons then are directed to the atomizer which is where they interact with the atomized sample (Morton and Roberts 2003, chap. 3). For large sets of samples the graphite furnace is more appropriate since it can be automated, less opportunity for cross-contamination, and temperature control is more precise. This last feature of graphite is very important since by being able to increase the temperature of the graphite tube at different rates, the atom production rate will be affected as well. Boris L’vov, who pioneered graphite tubes, predicted that to achieve maximum sensitivity “the sample would have to be atomized within less than 0.1 seconds” (Morton and
Roberts 2003, chap 10). As the energy in the form of photons passes through the atomizer, it is absorbed proportionally to the concentration of the sample solution. The photons that pass through the cloud reach a monochromator whose function is to reduced any background noise that could originate from the line source that can interfere with the result. A monochromator has slits that allow the light to enter. The smaller slits yield more accurate recordings. Inside the monochromator there may be other pieces that manipulate the light: prisms, grating. These devices help disperse the wavelengths. After the light has been manipulated inside the monochromator, it passes to the detector (Fig 8) which is where the intensity of the light radiation is measured by amplifying the signal coming from the monochromator (Morton and Roberts 2003, chap 3). The results are finally transferred to the computer where they are compared against the spectrometer’s calibration.

![Figure 8. Ebert Configuration of Optical Layout in a Single Beam AAS (Morton and Roberts 2003, Chap 5)](image)

Au concentrations were obtained on a Thermo Electron Corporation atomic absorption spectrometer (AAS) M5 series with SOLAAR data station software for Windows OS in graphite furnace mode which allows a large number of samples to be analyzed without cross contamination.
The samples are placed in the autosampler outer rings. In the inner ring a 0.01 ppm Au stock standard is placed in R1 position and blank samples are placed in R2 and R3 position. After opening the Ar gas parameters are set on SOLAAR specifying the cathode tube to be used (Au hollow) and the sample sequence. Three replicates of each sample and standard were run for 3 s, with the following settings: lamp current of 70%; wavelength of 242.8 nm; and the bandpass of 0.5 nm. Four calibration standard concentrations were entered in the calibration tab. For Runs 1 and 2 effluent and Runs 1, 2, and 3 tailings, calibration measurements were collected for 0, 25, 50, 75, and 100 ppb. After Runs 1 and 2 yielded low Au concentrations in the effluent, the calibration was adjusted to capture a lower range with standard concentrations at 0, 5, 10, 20 ppb for Run 3 effluent. All calibration standards were obtained by instrument autodilution from the 0.1 ppm stock Au standard. The calibration was set to normal linear least squares fit. In the furnace tab the cuvette was set to “coated” and the temperature and duration parameters for the furnace were set as follows:

Table 2. Furnace Heating Parameters

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (s)</th>
<th>Ramp (°C/s)</th>
<th>Gas</th>
<th>Gas Flow</th>
<th>RD</th>
<th>RS</th>
<th>TC</th>
<th>HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 100</td>
<td>30.0</td>
<td>10</td>
<td>2 inert</td>
<td>0.2 L/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 800</td>
<td>20.0</td>
<td>150</td>
<td>2 inert</td>
<td>0.2 L/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 1900</td>
<td>3.0</td>
<td>0</td>
<td>2 inert</td>
<td>Off</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>4 2500</td>
<td>3.0</td>
<td>0</td>
<td>2 inert</td>
<td>0.2 L/min</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

After all the parameters were set and the instrument was warmed up for > 20 min, the physical parts of the AAS have to be calibrated and cleaned using commands in the SOLAAR software: 1)
the optics system is aligned, cleaned and calibrated; 2) the graphite cuvette and the pipette tip is cleaned. Upon completion of this procedure, the AAS begins measurement of all the samples and data is output in ASCII format.

X-ray diffraction

XRD analysis was performed to determine the mineralogy of an untreated sample as well as samples taken from the bottom sections of \( W_c \). The samples were prepared by powdering in an agate mortar with methanol. The slurry was transferred to a glass slide using the bottom tip of the pestle pressing once against the glass and moving to an area next to the previous application, as to avoid the accumulation of slurry in one area, until the glass slide is covered in a flat film.

The X-ray diffractometer used was an X’pert Pro by Malvern-Panalytical with Pixcel1D detector and a copper X-ray source. The machine’s tension and current are set to 40 kv and 40 mA, respectively. At the start and to reduce the scattering and noise slits are used. The first slit is used before the X-ray hits the sample on the flat stage and its size is 1° and the second slit known as the antiscatter slit is 5.7mm which is placed before the rays reach the detector. X’Pert Data Collector software was used to control the XRD. The parameters used in the software are shown in Table 3. The results are saved automatically and are subsequently viewed and analyzed using X’Pert HighScore Plus, which allows for removal of background noise and search-match of diffraction peaks against the IPCC Powder Diffraction File database to determine minerals present. The parameters used for data collection is shown in Table 4.
Table 3. Parameters of Program for XRD

<table>
<thead>
<tr>
<th>Scan Properties</th>
<th>Continuous Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scan Axis</td>
<td>Gonio</td>
</tr>
<tr>
<td>Start angle (°)</td>
<td>10</td>
</tr>
<tr>
<td>End angle (°):</td>
<td>80</td>
</tr>
<tr>
<td>Step size (°)</td>
<td>0.026 ° per step</td>
</tr>
<tr>
<td>Time per step</td>
<td>117.30 seconds</td>
</tr>
<tr>
<td>Net time per step</td>
<td>114.24 seconds</td>
</tr>
<tr>
<td>Scan speed (°/s)</td>
<td>0.057008</td>
</tr>
<tr>
<td>Pre set counts</td>
<td>10000</td>
</tr>
<tr>
<td>Number of steps</td>
<td>2666</td>
</tr>
<tr>
<td>Total time (m)</td>
<td>21:27</td>
</tr>
</tbody>
</table>

Table 4. Search Peak Parameters

| Minimum Significance             | 0.75            |
| Minimum tip width                | 0 (°2th)        |
| Maximum tip width                | 1 (°2th)        |
| Peak base width                  | 2.00            |
| Method                           | Top of smoothed peak |
RESULTS

Appendix J shows the calibration curves for low and high concentrations used in the AAS to get the Au concentration in the effluents and tailings.

Tailings
The samples from the tailings were taken from three different sections (top, middle, bottom) of the column.

The results from each section was averaged to get the recovered gold of each column for each experiment.

Table 5 shows the mass of Au recovered from tailings for each treatment in all three iterations.

Table 5: Average Au Mass recovered from Tailings in experiment I, II and III

<table>
<thead>
<tr>
<th>尾矿Au质量 (μg)</th>
<th>Water</th>
<th>Bacteria</th>
<th>Cyanide (C1)</th>
<th>Cyanide (C2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp 1</td>
<td>98.22</td>
<td>54.84</td>
<td>59.17</td>
<td>75.43</td>
</tr>
<tr>
<td>Exp 2</td>
<td>47.04</td>
<td>65.5</td>
<td>47.67</td>
<td>45.27</td>
</tr>
<tr>
<td>Exp 3</td>
<td>97.84</td>
<td>96.05</td>
<td>63.75</td>
<td>63.59</td>
</tr>
<tr>
<td>Average</td>
<td>81.03</td>
<td>72.13</td>
<td>56.86</td>
<td>61.43</td>
</tr>
</tbody>
</table>

Figure 9: Average mass of Au recovered from tailings samples for experiment I, II and III.
Figure 9 shows that the Water Column ($W_c$) has the highest average amount of Au recovered with an total of 81.03 µg, followed by the Bacteria Column ($B_c$) with 72.13 µg, the Cyanide Column (C2) with 61.43 µg and the Cyanide Column (C2) with 56.86 µg. When the total yield for each column is compared to the total Au collected in $W_c$, the results show that: C1 yielded 35.06 % less than $W_c$, C1 yielded 27.52% than $W_c$ and $B_c$ yielded 11.62% less $W_c$.

**Effluents**

The samples of each columns were collected for five days for three consecutive weeks and the total recovery for each experiment was calculated by adding them. Table 6 shows the mass of Au recovered from tailings for each treatment in all three iterations. Figure 10 shows the average mass of Au recovered from the effluents for all three treatments.

*Table 6. Average Au Mass recovered from the Effluent in experiment I, II and III*

<table>
<thead>
<tr>
<th></th>
<th>Total Au Mass Effluent (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>Exp 1</td>
<td>4.9</td>
</tr>
<tr>
<td>Exp 2</td>
<td>0</td>
</tr>
<tr>
<td>Exp 3</td>
<td>12.38</td>
</tr>
<tr>
<td>Average</td>
<td>5.76</td>
</tr>
</tbody>
</table>
The results for the effluent measurements show that C2 had the biggest average amount of Au mass leached out with 17.03 µg followed by Bc with 12.94 µg, C1 with 11.37 µg and Wc with 5.76 µg. The results also show a great difference in Au mass recovered between Wc and the other 3 columns. It is notable that even though C1 and C2 were inoculated with the same leaching agent, the effluent from C2 had higher Au mass leached out. When Bc is compared to C2, it can be see that Bc recovered 27.29% less Au than C2. Furthermore, when Bc is compared to C1, Bc recovered 12.91% more Au than C1, which shows that bacteria produced higher Au recovery yields in the effluents than C1 which was treated with NaCN.

**T-test**

The Student t-test was performed on the average of the amount of Au recovered in the effluents of the columns for the three experiments. The test was performed to check whether the results of the Bc, C1, C2 are statistically different from Wc. The t-test was calculated with 90% certainty using the values from table 6.
Table 7. T-test for the effluents for the Bacteria and Water Columns

<table>
<thead>
<tr>
<th></th>
<th>Bacteria</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>12.80</td>
<td>5.76</td>
</tr>
<tr>
<td>Variance</td>
<td>18.02</td>
<td>38.87</td>
</tr>
<tr>
<td>Observations</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>Hypothesized Mean Difference</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>t Stat</td>
<td>5.88</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>1.89</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>2.92</td>
<td></td>
</tr>
</tbody>
</table>

The t-test shows that B_c and W_c are statistically different with 90% certainty. This can be seen because the p-value (0.028) is significantly smaller than the alpha value (0.10). Also, the table shows that the t-value (5.88) is significantly larger than t-critical (2.92) with two tails.

Table 8. T-test for the effluents for the Cyanide and Water Columns

<table>
<thead>
<tr>
<th></th>
<th>Cyanide (C1)</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>11.21</td>
<td>5.76</td>
</tr>
<tr>
<td>Variance</td>
<td>84.67</td>
<td>38.87</td>
</tr>
<tr>
<td>Observations</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>Hypothesized Mean Difference</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>t Stat</td>
<td>3.06</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>1.89</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>2.92</td>
<td></td>
</tr>
</tbody>
</table>
Table 8. shows that C1, and Wc, are statistically different with 90% certainty. This is shown by the p-value (0.09) which is smaller than the alpha value (0.10). Also, the table shows that the t-value (3.06) is larger than t-critical (2.92) with two tails.

Table 9.  T-test for the effluents for the Cyanide and Water Columns

<table>
<thead>
<tr>
<th></th>
<th>Cyanide (C2)</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>17.03</td>
<td>5.76</td>
</tr>
<tr>
<td>Variance</td>
<td>16.65</td>
<td>38.87</td>
</tr>
<tr>
<td>Observations</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Hypothesized Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>t Stat</td>
<td>3.85</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>1.89</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>2.92</td>
<td></td>
</tr>
</tbody>
</table>

The t-test in in table 9. shows that C2, and Wc are statistically different with 90% certainty. In this table p-value (0.06) is smaller than the alpha value (0.10) and the t- value (3.85) is larger than t-critical (2.92) with two tails.

The t-test values for the previous three tables show that the results obtained using different leaching treatment in the Bc, C1 and C2 columns are different from the Wc and therefore confirming that they are statistically different.
Experiment I

Effluent

Table 10. Weekly Au Recovered from Effluent for Experiment I

<table>
<thead>
<tr>
<th></th>
<th>Week 1 Au in Effluent (μg)</th>
<th>Week 2 Au in Effluent (μg)</th>
<th>Week 3 Au in Effluent (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>2.00</td>
<td>1.37</td>
<td>1.53</td>
</tr>
<tr>
<td>Bacteria</td>
<td>9.87</td>
<td>1.00</td>
<td>0.81</td>
</tr>
<tr>
<td>Cyanide (C1)</td>
<td>6.67</td>
<td>1.27</td>
<td>0.86</td>
</tr>
<tr>
<td>Cyanide (C2)</td>
<td>12.08</td>
<td>0.50</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Figure 11 juxtaposes the total amount of Au recovered in the effluents each week.

Figure 11. Weekly Au Recovered from Effluent Experiment I
Figure 11 shows a markedly larger difference in recovery yields when comparing week 1 to week 3. In the case of Bc there was a decrease of 91.79% of Au yield, C1 experimented a decrease of 87.10% and C2 yielded 97.27% less in week 3 than it did in week 1. In the case of Wc, Au leached out from this column only fluctuates 23.5% when comparing yields from week 1 against week 3 which is only 0.47 µg. This figure also shows that there is no significant change in Au recovery for Bc, C1, C2, from week 2 to week 3.

Mass Balance

![Mass Balance Diagrams]

Figure 12 shows the percentages of total mass recovered from the effluents and tailings. Blue represents the percentage of Au recovered from the tailings and the orange the percentage of Au recovered from the effluents. The greatest percentage of gold remaining in the tailings was seen in Wc with 95% of the Au. Bc
recorded the lowest percentage of gold in the tailings with 82%. C2 and C1 recorded 85% and 87% of the total Au collected from the tailings, respectively. When compared to all the other columns Bc recovered the highest percentage of Au in the effluent with 18% compared to C1 and C2 that only recovered 13% and 15%, respectively, of its total Au recovered from effluents.
Experiment II

Effluent

Table 11. Weekly Au Recovered from Effluent for Experiment II

<table>
<thead>
<tr>
<th></th>
<th>Week 1 Au in</th>
<th>Week 2 Au in</th>
<th>Week 3 Au in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effluent (µg)</td>
<td>Effluent (µg)</td>
<td>Effluent (µg)</td>
</tr>
<tr>
<td>Water</td>
<td>B.D.L.</td>
<td>B.D.L.</td>
<td>B.D.L.</td>
</tr>
<tr>
<td>Bacteria</td>
<td>9.24</td>
<td>B.D.L.</td>
<td>B.D.L.</td>
</tr>
<tr>
<td>Cyanide (C1)</td>
<td>3.46</td>
<td>B.D.L.</td>
<td>B.D.L.</td>
</tr>
<tr>
<td>Cyanide (C2)</td>
<td>17.11</td>
<td>B.D.L.</td>
<td>B.D.L.</td>
</tr>
</tbody>
</table>

Figure 13. Weekly Au Recovered from Effluent for Experiment II
Figure 13 shows only the results from week 1 since they produced values above detection limits. Results from week 2 and 3 are labelled B.D.L. because the Au concentration corresponding to each column was below or too close to the limits of detection for low level concentrations (<25 ppb) in the AAS which has a < 1 ppb. detection limit.

**Mass balance**

Figure 14 shows that the Water column mass balance is limited since the all the Au concentration recovered in the effluent was below the limit of detection of the AAS. Although the Au recovered in the effluent in the other three columns was only measured for week 1, because the values of week 2 and 3 were below the detection limit. The graph shows that C2 has 27% of its total Au recovery coming from the effluent.
Figure 14. Experiment II Mass Balance

The column Bc showed that 12% of its total Au recovery collected in the effluent In C1, 93% of Au was collected in the tailing and 7% in the effluent. It is important to note that column C1 and C2 are treated with the same inoculating agent, however, both show different percentages of Au recovered in the effluent.

Experiment III

Effluent

Figure 15 shows that for week 3 the amount of Au recovered is much lower, in some cases, by as much as 9x than in the previous weeks. Comparing week 3 to week 1: Wc in week 3 showed an increase of 8.42%, while Bc, C1, and C2 recorded a decrease of 90.3%, 91.45%, and 92.55%, respectively. Comparing week 3 to week 2, Wc in week 3 recorded a decrease of 90%, while Bc, C1, and C2 showed a decrease of 94.1%, 90.29%, and 92.55%, respectively.

Table 12. Weekly Au Recovered from Effluent for Experiment III

<table>
<thead>
<tr>
<th></th>
<th>Tailings Au Mass (μg)</th>
<th>Week 1 Au in Effluent (μg)</th>
<th>Week 2 Au in Effluent (μg)</th>
<th>Week 3 Au in Effluent (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>97.84</td>
<td>0.89</td>
<td>10.40</td>
<td>1.03</td>
</tr>
<tr>
<td>Bacteria</td>
<td>96.05</td>
<td>6.38</td>
<td>10.51</td>
<td>0.62</td>
</tr>
<tr>
<td>Cyanide (C1)</td>
<td>63.75</td>
<td>10.88</td>
<td>9.58</td>
<td>0.93</td>
</tr>
<tr>
<td>Cyanide (C2)</td>
<td>63.59</td>
<td>11.14</td>
<td>9.09</td>
<td>0.83</td>
</tr>
</tbody>
</table>
Figure 15. Weekly Au Recovered from Effluent for Experiment III

Overall, there is no clear pattern in the accumulation of Au for each week to determine which week produces the most Au. However, Fig. 15 shows that during week 3 all columns recorded the lowest levels of Au recovered. Furthermore, this graph shows that most of the Au in the effluents was recovered during week 1 and 2 for C1, C2, Bc; in the case of Wc, most of the Au was recovered during week 2.
This figure shows the percentage of Au recovered in the effluent and mine tailings. For $W_c$ the amount of Au recovered from the effluent was 11% of the total amount recovered leaving 89% of Au in the tailings. In column $B_c$ the ratio between Au found in the tailings and effluent changed such that the amount of Au in the effluents increased to 15% and the amount of Au in the tailings was reduced to 85%. In the case for columns C1 and C2 the amount of Au in the effluent increased to 25% and the Au in the tailings decreased to 75%.

The graph depicts that the even though the most Au can still be recovered from the tailings, it also shows that the columns treated with NaCN are more efficient at recovering Au in the effluents.
The second most efficient way to recovered Au is by treated the columns with bacteria. The least efficient way to leach out Au is by treating the columns with water only.

**Mineralogy**

The results of X-ray diffraction analysis of the control sample and \( W_c \) for experiments I, II and III are shown below.

### Mineralogy of tailings Control

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz</td>
<td>Q</td>
</tr>
<tr>
<td>Albite</td>
<td>A</td>
</tr>
<tr>
<td>Muscovite</td>
<td>M</td>
</tr>
<tr>
<td>Feldspar</td>
<td>F</td>
</tr>
<tr>
<td>Microcline</td>
<td>X</td>
</tr>
<tr>
<td>Clinochlore</td>
<td>L</td>
</tr>
<tr>
<td>Zeolite</td>
<td>Z</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>K</td>
</tr>
</tbody>
</table>

### Mineralogy of tailings Exp. I [Wc]

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz</td>
<td>Q</td>
</tr>
<tr>
<td>Albite</td>
<td>A</td>
</tr>
<tr>
<td>Muscovite</td>
<td>M</td>
</tr>
<tr>
<td>Feldspar</td>
<td>F</td>
</tr>
<tr>
<td>Microcline</td>
<td>X</td>
</tr>
<tr>
<td>Clinochlore</td>
<td>L</td>
</tr>
<tr>
<td>Zeolite</td>
<td>Z</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>K</td>
</tr>
</tbody>
</table>
Figure 17. Mineralogy of tailings used in controls and Water Column in experiments I, II, III.

Minerals present: clinochlore (L), zeolite (Z), albite (A), muscovite (M), feldspar (F), microcline (X), kaolinite (K) quartz (Q).

Figure 17. shows that the samples produce patterns consistent with each other both in peak position (2θ angle) and relative intensity. This indicates that most of the same minerals are present in the control sample and the other the Wc. The minerals present in these samples are the silicates albite, potassium feldspar, microcline, quartz, muscovite, zeolite, kaolinite and clinohore. One important finding is the presence of the phyllosilicates kaolinite and clinochlore, both of which are clay minerals.
The identification process was done by visually comparing the location (2θ) of the 100% and lesser intensities in a unique stick pattern graph that each mineral has, against the graphs' peaks. If there was a match then the mineral was flagged as present.

Other minerals were found in the samples but with greater uncertainty since their peaks corresponded or were too close to the peaks of minerals already identified with more certainty. Among the minerals found with lesser certainty are nontronite, which is a clay mineral, and hematite, possibly responsible for the red color of the tailings from the Picacho mine.
Discussion

The results obtained from this experiment were not necessarily the best-case scenario for the use of this bacterium but rather a first attempt to see how the bacteria would perform leaching these mine tailings knowing that it is native to the Picacho mine. In the experiment there were very interesting results that show that A. faecalis is metabolizing gold albeit the exact mechanism is not known and is beyond the scope of this work. This is evident in the total effluent recoveries for column Bc in all experiments where Au was recovered 1.4 to 16.86 times more than the control Wc. This can also be seen when the mass balances are taken into account since the percentage of of the total Au recovered in the effluent is consistently greater in Bc than Wc. This was an indication that use of A. faecalis effectively leaches refractory gold.

One important finding is that most of the Au recovered in the effluents occurred during the first week of leaching, in most columns except for Wc and Bc in experiment III. In those two cases, the largest amount of Au recovered was during week 2. This indicates that running the experiment as it is designed is most efficient at mobilizing Au using A. faecalis when treatment is carried through two weeks.

The results also show that Au recovered in the tailings in all experiments for C1 and C2 is less than what was recovered in the columns that didn’t use CN at any stage of the treatment. In contrast, the opposite happens when the presence of Au is measured in the effluents since it shows that the amounts are larger in C1 and C2. This finding is consistent with what the industry considers the most effective way to recover Au from mine tailings. However, the leaching treatment with bacteria shows interesting results that challenge cyanidation. The columns treated with A. faecalis produce comparable results to columns treated with NaCN. During experiment I the total Au
recovered in the effluent in Bc was only 10.09% less than C2. In experiment III Au recovered in the effluents for Bc was 18.51% less than C2. In experiment II the difference between Au collected in the C2 and Bc effluents was 55.11% which reflects the small amount of Au collected in the Bc effluent.

In experiment I and II Au recovery in the effluents of C1 was lower than for C2 and Bc. However, in experiment III, C1 yielded higher recoveries than the other 3 columns. Since the mineralogy of the sample is consistent, and C1 and C2 are identical treatments in Week 1, the discrepancy can be attributed to heterogeneity in grain size of the tailings subsample resulting in a different yield after one week. The heterogeneity of the tailings grain size could also explain the higher Au recovery in the effluents for Bc than C1 in table 6.

The use of bacteria is often as a pretreatment is aimed to increase the yield of gold recovery and not as a primary mean to recover gold from low-grade mines prior to cyanidation. This is shown by Curreli et. al in a study where grinding and the bacteria leaching were used as a pretreatment for cyanidation in different scenarios. They concluded that although grinding, roasting and cyanidation increased gold recovery by 85% compared to cyanidation alone, it was a bioleaching pretreatment and grinding that increased gold recovery by 77% that suggested the procedure as an environmentally viable option due to a lower cost and environmental impact than roasting.(Curreli et al. 1997)
Conclusion

The purpose of this study was to compare the recovery yield between leaching of a low-grade ore by Alcaligenes faecalis and NaCN treatment. In this study, we were able to show that using bacteria alone as treatment had very similar Au recovery yields than that of NaCN, indicating that the use of A. faecalis is an encouraging option for Au leaching of mine tailings.

In typical biomining applications bacteria are monitored for various parameters that guarantee optimal oxidations rates and growth and survival of the bacteria during the leaching process. Because A. faecalis is not an organism used in mining operations, this information is not readily available in the literature. Other parameters that were not optimized were: grain size of the mine tailings, residence time of the bacteria, ore composition, and type of leaching technique. These results indicate that further optimization may lead to greater yields.
References


Appendix A
Image of samples from the Picacho mine
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Column experiment set up
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20 mL test tubes holding the different effluents coming from the columns. Starting from left to right the effluents are Bacteria, Cyanide, Water and Cyanide
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Appendix G
Dissolution of button
Appendix H
Te Precipitation
Appendix I
AuTe removal from solution
Appendix J

Calibration curves for high concentration and low concentration values

Calibration Curve - Higher Concentration

\[ y = 0.0119x + 0.028 \]

\[ R^2 = 0.997 \]

Calibration Curve - Low Concentration

\[ y = 0.0111x + 0.0018 \]

\[ R^2 = 0.9994 \]
Appendix K

Stacked columns that show the total mass of Au for Experiment I that were collected from the effluents and the tailings. The top part (yellow) of the columns is the amount of Au recovered in the tailings and the bottom part of the columns are the amount of Au recovered from the tailings.

*Total Mass of Au for Experiment I*

<table>
<thead>
<tr>
<th></th>
<th>Tailings Au Mass (μg)</th>
<th>Total Au Mass Effluent (μg)</th>
<th>Total Au Mass (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>98.22</td>
<td>4.90</td>
<td>103.12</td>
</tr>
<tr>
<td>Bacteria</td>
<td>54.84</td>
<td>11.67</td>
<td>66.51</td>
</tr>
<tr>
<td>Cyanide (C1)</td>
<td>59.17</td>
<td>8.79</td>
<td>67.97</td>
</tr>
<tr>
<td>Cyanide (C2)</td>
<td>75.43</td>
<td>12.91</td>
<td>88.33</td>
</tr>
</tbody>
</table>
Appendix L

Stacked columns that show the total mass of Au for Experiment II that were collected from the effluents and the tailings. The top part (yellow) of the columns is the amount of Au recovered in the tailings and the bottom part of the columns are the amount of Au recovered from the tailings.

*Total Mass of Au for Experiment II*

<table>
<thead>
<tr>
<th>Column (Experiment II)</th>
<th>Tailings Au Mass (µg)</th>
<th>Total Au Mass Effluent (µg)</th>
<th>Total Au Mass (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>47.04</td>
<td>&lt;1</td>
<td>47.04</td>
</tr>
<tr>
<td>Bacteria</td>
<td>65.50</td>
<td>9.24</td>
<td>74.73</td>
</tr>
<tr>
<td>Cyanide (C1)</td>
<td>47.67</td>
<td>3.11</td>
<td>50.78</td>
</tr>
<tr>
<td>Cyanide (C2)</td>
<td>45.27</td>
<td>16.72</td>
<td>61.99</td>
</tr>
</tbody>
</table>
Appendix M

Stacked columns that show the total mass of Au for Experiment I that were collected from the effluents and the tailings. The top part (yellow) of the columns is the amount of Au recovered in the tailings and the bottom part of the columns are the amount of Au recovered from the tailings.

Total Mass of Au for Experiment III

<table>
<thead>
<tr>
<th>Column (Experiment III)</th>
<th>Tailings Au Mass (μg)</th>
<th>Total Au Mass Effluent (μg)</th>
<th>Total Au Mass (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>97.84</td>
<td>12.38</td>
<td>110.22</td>
</tr>
<tr>
<td>Bacteria</td>
<td>96.05</td>
<td>17.50</td>
<td>113.55</td>
</tr>
<tr>
<td>Cyanide (C1)</td>
<td>63.75</td>
<td>21.38</td>
<td>85.13</td>
</tr>
<tr>
<td>Cyanide (C2)</td>
<td>63.59</td>
<td>21.07</td>
<td>84.66</td>
</tr>
</tbody>
</table>

![Experiment III: Total Au Collected](chart.png)