CHAPTER 10.7

Bioleaching

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Bioleaching, also termed *biohydrometallurgy*, *biomining*, *minerals biooxidation*, and *biooxidation pretreatment*—the latter two terms when employed for precious metals ores and concentrates in which the gold is embedded in a sulfide mineral—is a hydrometallurgical process that uses certain types of microorganisms as catalysts to produce the chemical lixiviant for leaching sulfide minerals. Bioleaching is one of several commercially employed technologies for processing sulfidic ores and concentrates. The decision as to which technology is best suited for a mine site is based on many factors, including test results, mineralogy, ore grade, mine-site topography, and economics.

Bioleaching, which is a naturally occurring process, has inadvertently been used for more than 2,000 years to leach copper (Rossi 1990), but not until the late 1940s and 1950s was it recognized that microorganisms were involved (Colmer and Hinkle 1947; Colmer et al. 1950; Temple and Colmer 1951; Bryner et al. 1954). The first bioleach patent, issued in 1958 to Kennecott Copper Corporation (Zimmerley et al. 1958), claimed the use of bacteria for a cyclic process of ferric sulfate oxidation of sulfide minerals wherein the ferric sulfate served as the lixiviant for oxidation of the sulfides, and bacteria regenerated the ferric sulfate by oxidizing ferrous sulfate. Since these discoveries more than half a century ago, much has been learned about the microorganisms involved and the interaction between the microorganisms and minerals. Bioleaching is now commercially applied for leaching base and precious metals in heaps and stirred-tank reactors with approximately 20% of the world's mined copper and about 3% of the world's mined gold produced by bioleaching technology (Schippers et al. 2014).

This chapter summarizes fundamental microbial and chemical aspects of bioleaching and biooxidation pretreatment, describes test work and scale-up essentials, and reviews commercial operating factors for heaps and stirred-tank reactors.

MICROBIOLOGY AND CHEMISTRY OF BIOLEACHING

Extensive studies of the microorganisms involved in bioleaching began in the late 1960s, followed by recognition of their catalytic role coupled with the process chemistry. The discovery and characterization of robust suites of microorganisms tolerating high concentrations of dissolved metals (30–50 g/L), low pH values (pH <1), high ionic strengths (7.6 M), and populating temperature habitats from less than 15°C to about 90°C contributed significantly to the design of bioleach systems and amplified the types of minerals that could be processed by bioleaching (Watling 2015).

Bioleaching Microorganisms

Early studies by Beck (1967) identified the bacterium *Thiobacillus ferrooxidans*, renamed *Acidithiobacillus ferrooxidans* by Kelly and Wood (2000), as a microbial catalyst for the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}). Sulfur oxidation by *Acidithiobacillus thiooxidans* was first reported in the early 1920s; by the 1930s this organism's function in acid rock drainage formation was known (Ehrlich 2004). The occurrence of *A. thiooxidans* in copper leaching environments was reported in the 1950s by Bryner and colleagues (1954). The *Acidithiobacillus* species described in these publications are mesophilic bacteria—bacteria growing best between 25° and 40°C—that oxidize ferrous iron and sulfur.

Thermophilic microorganisms involved in bioleaching were discovered in the 1960s and 1970s. The moderately thermophilic *Sulfobacillus* species were discovered in base metal heap leach operations (J.A. Brierley and LeRoux 1977; J.A. Brierley and Lockwood 1977; Golovacheva and Karavaiko 1978). The extremely thermophilic and acidophilic archaea, first found to flourish in acid hot springs (J.A. Brierley 1966; Brock et al. 1972), were demonstrated to have a role in bioleaching by J.A. Brierley and Brierley (1986); these archaea, commonly referred to as "extremophiles" or "extreme thermoacidophiles," are genetically distinct from bacteria and classified as Archaebacteria (Woese 1982). The extreme thermoacidophiles are of specific interest for bioleaching primary sulfide minerals such as chalcopyrite, molybdenite (C.L. Brierley 1974; J.A. Brierley and Brierley 1978), and enargite (Lee et al. 2011). Mesophilic and

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Table 1 Bioleaching microorganisms and their temperature ranges for growth

Microorganism	Temperature Range, °C
Mesophilic and Moderately Thermophilic Bacteria	
Acidimicrobium ferrooxidans	<30–55; optimum 45–50
Acidithiobacillus caldus	32–52; optimum 45
Acidithiobacillus ferrooxidans	10–37; optimum 30–35
Acidithiobacillus thiooxidans	10–37; optimum 28–30
Leptospirillum ferrooxidans	Not available; optimum 28–30
Sulfobacillus acidophilus	<30–55; optimum 45–50
Sulfobacillus thermosulfidooxidans	20–60; optimum 45–48
Sulfobacillus thermotolerans	20–60; optimum 40
Mesophilic and Moderately Thermophilic Archaea	
Ferroplasma acidiphilum	15–45; optimum 35
Ferroplasma cupricumulans	22–63; optimum 54
Extremely Thermophilic Archaea	
Acidianus brierleyi	45–75; optimum ≈70
Acidianus infernus	65–96; optimum ≈90
Metallosphaera sedula	50–80; optimum 75
Sulfolobus metallicus	50–75; optimum 65
Sulfurococcus yellowstonensis	40–80; optimum 60

moderately thermophilic archaea have also been isolated and identified (Golyshina et al. 2000) since the discovery of the extremely thermophilic and acidophilic archaea.

Bioleaching microbes are customarily grouped according to their temperature ranges of growth: mesophilic bacteria and archaea, 5° to ≈50°C; moderately thermophilic bacteria and archaea, 45° to ≈60°C; and extremely thermophilic archaea, 60° to 80°C. Table 1 shows examples of bacteria and archaea involved with metal sulfide bioleaching (Schippers 2007; Schippers et al. 2014). The overlapping temperature ranges for the different groups of microorganisms illustrate their capacity to share bioleach environments as a group or consortium. Research and development in the late 1990s and first decade of the 2000s demonstrated that bioleaching of sulfide minerals was accomplished not by just a few genera and/or species of bacteria, as earlier believed, but rather by consortia of different genera of bacteria and archaea spanning a range of temperatures and physical conditions. Optimization of these microbial consortia in bioleach operations remains a goal of scientific and engineering research (Rawlings 1997; Watling 2006; Norris 2007; Schippers 2007; Schippers et al. 2014).

Microorganisms involved in bioleaching have minimal requirements for reproduction and catalytic functions. Carbon for cellular synthesis is provided by CO_2 available in air. Their energy sources for growth derive from oxidation of ferrous iron and sulfur. The terminal electron acceptor for the energy yielding oxidation reactions is O_2 in the atmosphere. Soluble phosphate (PO_4^{3-}), potassium (K^+), ammonium ion (NH_4^+), and a few trace elements, also required for growth of these organisms, are available from the ore and gangue minerals in their habitat. In heap bioleach operations, phosphate can originate from a variety of gangue and ore minerals, and potassium comes from clay minerals. Ammonium ion, which is the organism's nitrogen source, is typically available in sufficient quantities as a residue of blasting (Zaitsev et al. 2008).

Bioleaching microorganisms are acidophilic (acid-loving), requiring a sulfuric acid environment of less than pH 2.5, with some of these organisms functioning actively at pH values less than 1. The acidic environment ensures that ferrous iron and certain reduced sulfur compounds, serving as energy sources for the organisms, remain in solution and available for oxidation. Metals of value, such as copper, zinc, nickel and others extracted from sulfide minerals during the bioleach process, also remain soluble in the acidic solution, making them readily recoverable by conventional metallurgical methods. Although the solution in which the bioleach organisms thrive is acidic (usually pH 2 and lower), the intracellular portion of the microorganisms remains at a neutral pH, resulting in a huge proton gradient across the microorganism's cell membrane.

Two reactions (1 and 2) embody the microorganism's task in bioleaching. The microorganisms oxidize ferrous iron as an energy source for biosynthesis and growth under acidic conditions in the presence of oxygen supplied by air. The end product, ferric iron, is an effective oxidant for sulfidic minerals:

$$2Fe^{2+} + 2H^+ + 0.5O_2 \rightarrow 2Fe^{3+} + H_2O \quad \textbf{(EQ 1)}$$

Sulfur is also an energy source for some bioleaching microbes:

$$2S^0 + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 \quad \textbf{(EQ 2)}$$

The end product of this oxidation is sulfuric acid, which maintains acidic conditions to keep metal ions in solution and reduces the amount of acid that may need to be added to meet acid demand of the ore or concentrate. Microbial sulfur oxidation also requires oxygen derived from air.

Bioleaching microorganisms tolerate most heavy metal cations to 50 g/L or even higher concentrations (Watling 2011) via natural adaptation or intentional adaptation in the laboratory. There are, however, a few cationic metals/metalloids that can be toxic, and for these constituents to be toxic, they must be soluble. Mercury and silver, generally thought to be toxic, are usually not problematic because silver has a low solubility in acidic leach solutions and mercury adsorbs to gangue materials, mitigating its toxic effect. Arsenic in the form of arsenate (AsO_4^{3-}) anion has low toxicity; bioleaching microorganisms are resistant to 20 g/L arsenate (Van Aswegen et al. 2007). Arsenic as arsenite (AsO_3^{3-}) elicits a toxic effect; 1.5 g/L As^{3+} retards microbial activity with 5g/L As^{3+} completely inhibiting the organisms (Breed et al. 2000). Arsenite is effectively oxidized to arsenate by ferric iron (Reaction 3), which is present in the bioleach environment, thus mitigating potential As^{3+} toxicity. However, attention is necessary to ensure that the ferric iron concentration is sufficient and conditions are suitably oxidizing for this reaction to proceed.

$$H_3AsO_3 + 2Fe^{3+} + H_2O \rightarrow H_3AsO_4 + 2Fe^{2+} + 2H^+ \quad \textbf{(EQ 3)}$$

Nitrate (NO_3^-) at concentrations of 1.25–2.5 g/L slows microbial cell division and suppresses ferrous iron oxidation by bacteria and archaea. There is some evidence that mesophilic and moderately thermophilic bacteria exhibit some adaptation to NO_3^- at these concentrations and resume ferrous iron oxidation, but iron oxidation by archaea remains suppressed (Shiers et al. 2014; Watling 2015).

Chloride (Cl^-) inhibits iron oxidation and growth of biomining microorganisms (Watling 2011) with inhibition occurring in the range of 5–7 g/L NaCl. The search for salt-tolerant biomining organisms has been ongoing for at least 20 years. Norris et al. (2010) discovered a bacterium capable

of iron oxidation in 20 g/L NaCl at a temperature just below 50°C; however, growth of this organism was difficult to establish on copper sulfide ore in column tests. More recently, organisms capable of tolerating 70 g/L of sea salts have been obtained from acidic saline drains, lakes, and sediments in Western Australia (Rea et al. 2015); research to determine these salt-tolerant organisms' effectiveness for bioleaching is ongoing. Chloride toxicity is a significant problem for biomining in regions of the world where the water supply is brackish or only seawater is available for processing. In some mining areas, atacamite, an acid-soluble copper chloride mineral, is prevalent. Care must be taken to ensure that atacamite is excluded from ore destined for commercial bioleaching circuits; otherwise, chloride concentrations in the leach solution can easily exceed the tolerance level of the bioleaching organisms.

Fluoride (F^-) can be problematic in bioleaching, but not because the anion is toxic. Below pH 3.45, fluoride occurs predominantly as hydrofluoric acid (HF), and HF crosses the cell membrane of the acidophilic microorganisms as an uncharged molecule. When it is inside the microbial cell, HF dissociates because, as noted earlier, the intracellular portion of the microbes is a neutral pH. HF dissociation releases protons acidifying and killing the cell (J.A. Brierley and Kuhn 2010). F^- toxicity levels are directly related to the presence of other ions, particularly aluminum, in solution. Fluoride complexes with aluminum, significantly reducing the toxicity of F^- to the biomining microorganisms, because the complexed molecule is too large to cross the organism's cell membrane (J.A. Brierley and Kuhn 2010; Watling 2015).

Sulfate (SO_4^{2-}) concentrations in stirred-tank and heap leach solutions easily reach concentrations of 150 g/L, and although high sulfate concentrations have been implicated with the slowing of iron oxidation by the bioleaching bacteria (Watling 2013), there is little research data to support this contention.

Chemistry of Sulfide Mineral Bioleaching and Microorganism–Mineral Interactions

Bioleaching is based on oxidation–reduction reactions. Microorganisms oxidize ferrous iron and sulfur compounds, as expressed in Reactions 1 and 2, with the ferric iron and sulfuric acid providing the oxidizing agent for dissolution of sulfide minerals and acid maintaining metals in solutions, respectively. From ferrous iron oxidation, the microorganisms benefit by obtaining energy for their cell division and biochemical functions. Therefore, microorganisms serve as catalysts generating and regenerating the oxidant (Fe^{3+}) and supplying acid. In purely chemical systems, a chemical oxidant, such as hydrogen peroxide, ozone, or oxygen (usually under pressure), must be added to oxidize iron and sulfur, and the chemical oxidant is not regenerated, requiring recurrent addition of the oxidant.

Oxidation–reduction (redox) potential is controlled by the ratio of ferric iron to ferrous iron (Fe^{3+}:Fe^{2+}) in solution; the greater the Fe^{3+}:Fe^{2+}, the higher the redox potential. Different minerals go into solution at differing redox potentials, referred to as the rest potential of the minerals. The secondary copper sulfide mineral, chalcocite, is oxidized rapidly by the microbially generated ferric iron at a relatively low redox potential (~550–600 mV vs. SHE [standard hydrogen electrode]). Consequently, chalcocite is often considered to be acid soluble. Nevertheless, ferric iron is consumed in the oxidation reaction (4), and this consumption must be accounted for in the process design of commercial operations.

$$Cu_2S + 2Fe^{3+} \rightarrow Cu^{2+} + 2Fe^{2+} + CuS \quad \textbf{(EQ 4)}$$

One of the products of the kinetically fast oxidation of chalcocite is blaubleibender covellite (CuS). The oxidation of CuS (Reaction 5) requires a much higher redox potential, that is, a higher Fe^{3+} to Fe^{2+} ratio (~850–870 mV vs. SHE), and is kinetically slow (Bustos et al. 1999).

$$CuS + 2Fe^{3+} \rightarrow Cu^{2+} + 2Fe^{2+} + S° \quad \textbf{(EQ 5)}$$

The resulting elemental sulfur is then oxidized to sulfuric acid by microorganisms according to Reaction 2.

Ferric iron oxidation of pyrite (Reaction 6) yields substantial acid and ferrous iron, with the ferrous iron serving as a source of energy for the microorganisms as it is reoxidized to ferric iron:

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad \textbf{(EQ 6)}$$

Pyrite oxidation produces 0.41 kg acid/kg FeS_2 oxidized and generates significant heat (12,884 kJ/kg pyrite oxidized). In some commercial applications, the acid generated from pyrite oxidation offsets the purchase of acid; in other operations, particularly stirred-tank bioleaching/biooxidation of concentrates, pyrite oxidation may require the addition of a neutralizing agent (typically limestone, $CaCO_3$) to maintain an optimum pH for the microorganisms. Heat generation in heap leach operations is usually beneficial, because increasing the temperature improves microbial activity, increases chemical rates of reaction, and enhances primary copper sulfide mineral leaching, if these minerals are present. However, pyrite oxidation in stirred-tank reactors necessitates cooling to ensure that the temperature does not rise above the maximum temperature tolerated by the consortium of microorganisms used in the reactor system.

The microbes in bioleach/minerals biooxidation plants are both present in the leach solution (referred to as planktonic cells) and attached to mineral surfaces. The number of organisms attached to the minerals far exceeds the number in solution, and techniques for enumerating attached microbes are limited and often fraught with error. Attachment also has serious implications when using microbial counts in solution as an indicator of microbial "health" of an operating bioleach plant; the number of microbes in solution is often far lower than the actual number of microbes present in the leach system that includes the ore or concentrate. Microbes favor attachment to sulfur-rich zones and dislocation sites, such as cracks, mineral grain boundaries, and crystal defects. Initial attachment is likely because of physicochemical factors, such as electrostatic and hydrophobic forces between mineral particles and the microorganisms. After microbes are attached to the minerals, a biofilm is formed consisting of extracellular polymeric substances. The conditions and chemical reactions occurring within and at the biofilm–mineral interface remain under investigation, but the prevailing theory (Rohwerder and Sand 2007) is that high concentrations of ferric iron under the biofilm react with the sulfide minerals. Metal sulfide oxidation occurs via two pathways mediated by microbial catalysis of the ferrous-ferric cycle: the "thiosulfate pathway" for acid-insoluble metal sulfides, such as pyrite and molybdenite; and the "polysulfide pathway" for acid-soluble metal sulfides,

such as sphalerite, arsenopyrite, and chalcopyrite. For the latter pathway, the metal sulfides are dissolved by the combined action of electron extraction by ferric iron and proton attack (Schippers et al. 2014).

Advances in understanding the complexity of microbial communities in bioleaching processes are facilitated by developments in modern molecular biology techniques that allow analysis of complex microbial populations in commercial bioleach plants. Methods to accomplish this have been described by Roberto (2008), Chávez et al. (2011), Watling (2011), and Demergasso et al. (2011). These protocols lead to a comprehensive definition of the diversity of the microbial populations present in mineral biooxidation and bioleaching operations. Genetic manipulation has been considered for improving the bioleach microorganisms. To date this has not succeeded in demonstrable improvements in leaching. However, there is significant value in understanding the genetics of the bioleaching microbes. In the near term, these findings are important in the indirect improvement of bioleaching through enhanced understanding of the physiology of the microbes rather than by developing and adding genetically manipulated microorganisms to commercial processes (Gericke 2011).

More detailed information on the microbiology and chemistry of bioleaching can be found in the following publications: Rossi 1990; Watling 2006, 2015; Donati and Sand 2007; Rawlings and Johnson 2007; Gericke 2011; Sobral et al. 2011; Vera et al. 2013; C.L. Brierley and Brierley 2013; Schippers et al. 2014; C.L. Brierley 2016.

BIOLEACHING/MINERALS BIOOXIDATION TESTING

Testing to evaluate bioleaching/biooxidation requires the same level of metallurgical rigor that is applied to the evaluation of alternative technologies such as pressure oxidation and chemical leaching. Bioleaching is unique only in using microbial agents as catalysts for the extraction process. All other aspects of the technology entail classic extractive metallurgical considerations. This section is a guide to evaluate bioleaching for treating sulfidic ores and concentrates.

Analyses

Implementation of a successful bioleach project is dependent on comprehensive geometallurgical characterization of the ore (McFarlane et al. 2011), the concentrate (Dew et al. 1997; Van Aswegen et al. 2007), or both. Geometallurgical characterization must be carried out on representative samples over life-of-mine, and extensive analyses are of paramount importance to preclude poor and misleading interpretation of the test results. Reliable geological, mineralogical, chemical, and metallurgical data are used for process design and economic modeling. Table 2 outlines key parameters to be determined using the best possible representative ore and concentrate samples for analyses.

Detailed mineralogical evaluation is essential. QEMSCAN (Quantitative Evaluation of Minerals by Scanning Electron Microscopy) is a fully automated microanalysis system for quantitative chemical analysis and generation of high-resolution mineral maps and images and porosity structure. The data are used to determine the size, shape, mineral associations, and liberation of mineral particles (Greenwood-Smith et al. 1985). Mineral liberation analysis (MLA) establishes mineral quantity and mineral phases (Miranda and Seal 2008). MLA and QEMSCAN are powerful analytical tools that can be utilized for samples of ores, concentrates, tailings, and leached

Table 2 Key analyses of ores and concentrates in preparation for bioleach testing

Chemical Analyses
• Metals: Identify possible toxic metals/metalloids—inductively coupled plasma (ICP) spectrometry; other analyses for anions of concern
• S species: Total sulfur, sulfide-sulfur, sulfate-sulfur—LECO combustion analysis
• Carbon: Total carbon, carbonate, organic carbon—LECO combustion analysis
Mineralogy
• Characterize ore and gangue constituents.
• Identify and quantify metal associations with sulfides or other constituents (e.g., organic carbon).
• Ascertain and thoroughly characterize any encapsulation of ore minerals in gangue or other sulfides—ore microscopy, petrographic analyses, X-ray diffraction (XRD), X-ray fluorescence (XRF).
• Use sophisticated analytical techniques for metals dissemination in sulfide minerals—QEMSCAN, mineral liberation analysis (MLA), scanning electron microscopy (SEM), mass spectroscopy, other spectroscopic analyses for speciation determination.
Metallurgical
• Sequential leach procedure
• Size fraction determinations
• Bulk density, etc.

samples. Other sophisticated analytical techniques are available for assessing mineral and chemical structure, morphology, surface and bulk impurities, and particle identification: time-of-flight laser ionization mass spectrometry (TOF-LIMS), time-of-flight secondary ion mass spectroscopy (TOF-SIMS), X-ray photoelectron spectroscopy (XPS), electron probe microanalysis with automated image analysis (EPMA-AIA), and others. With comprehensive mineralogical information of representative samples, the variables for process development, including bioleaching, become defined and manageable.

Mineralogical and chemical analyses are not limited to head samples of ores and concentrates. These analyses are conducted on bioleached residues to assess the effectiveness of leaching, determine which minerals may not have been effectively leached, and identify secondary mineral formation (e.g., jarosite precipitation).

A useful tool in metallurgical amenability evaluation is the semiquantitative sequential (diagnostic) leach procedure (Parkinson and Bhappu 1995), which entails partial dissolution of copper minerals in sequential solutions of sulfuric acid, cyanide, and aqua regia. Copper oxide species have approximately 100% dissolution in acid and cyanide solutions. The secondary copper sulfide minerals have little dissolution in acid with approximately 100% dissolution in cyanide. Primary sulfides (e.g., chalcopyrite) have no dissolution in either solution. The sum of copper dissolved in the sulfuric acid and cyanide steps indicates the amount of copper that may be leachable (recoverable). A ferric sulfate solution step can be added as an indicator of enhanced copper extraction by bioleaching. However, this ferric leach step associated with the sequential (diagnostic) leach procedure will not reveal potential microbial toxicity issues that might be associated with the sample.

Preg-robbing is the adsorption of gold–cyanide complexes by carbonaceous material in the ore or concentrate; certain sulfide minerals (e.g., pyrrhotite, chalcopyrite, and pyrite) have also been implicated in the adsorption of gold from solution (Rees and van Denter 2000; Zhao et al. 2016).

It is prudent to determine whether preg-robbing is a factor with gold ores and concentrates, because this phenomenon can result in substantially lower than expected recoveries of gold in cyanide leaching. Simple and widely used tests for determining preg-robbing have been described by Hausen and Bucknam (1985) and Schmitz et al. (2001).

Bioleaching/Biooxidation Amenability Determination in Stirred-Tank Reactors

Amenability testing assesses whether a particular metal sulfide ore or concentrate is likely to undergo dissolution at a rate and to an extent that justifies consideration of bioleaching as an option for a commercial metal extraction process (Olson and Harvey 2011). Amenability testing is conducted in well-aerated, stirred batch reactors typically 5 L or less in size with a solids concentration of 15%–20% (w/v) in a nutrient solution (Lee et al. 2011). Stirred-tank batch reactor testing has advantages over simple flask reactor testing, which is sometimes used for amenability testing, because greater liquid volumes and mass of ore or concentrate provide more bio-oxidized material for subsequent analyses and process evaluation. Solution samples can be withdrawn during the test to monitor the progress of the bioleach process without interfering with the test. Stirred-tank reactors also provide excellent mixing and good aeration for microbial activity during the evaluation phase.

The selection of microorganisms for amenability testing is dependent on the mineralogy of the ore as well as conditions anticipated for application. For example, a heap with sulfidic refractory gold ore in which the gold is embedded in pyrite would be expected to heat. Therefore, amenability testing should include evaluation of consortia of mesophilic, moderately thermophilic, and extremely thermophilic (archaea) microorganisms. With the exception of the extremely thermophilic archaea, microbial consortia used for amenability testing are often cultured from the ore samples to be evaluated and adapted to increasing solids concentration through subculturing. For amenability tests run at temperatures between about 49° and 65°C, enrichment cultures of moderately thermophilic microorganisms can be obtained from mine sites that have stockpiles or heap leach operations close to these temperatures (Norris et al. 2012). Enrichment cultures are obtained using a growth medium containing ferrous iron, elemental sulfur, or both at pH ~1.5 and incubated at temperatures suitable for growth of the desired group of microorganisms (i.e., 30°–45°C for mesophilic microorganisms, with 55°C preferable for moderately thermophilic microorganisms). The extremely thermophilic archaea typically are not naturally occurring in base and precious metal ores and therefore cannot be cultured from ore samples. The extremely thermophilic archaea are available from type culture collections, such as the American Type Culture Collection or the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, which is the German type culture collection; the extremely thermophilic archaea can also be cultured from hot acid springs and solfatara. Laboratories that routinely perform amenability testing usually maintain consortia of mesophilic, moderately thermophilic, and extremely thermophilic microorganisms and adapt these consortia to the ore and/or concentrate undergoing evaluation through a subculturing process.

Amenability tests in batch reactors can be conducted in a relatively short period (several months). These tests allow for evaluating several variables (e.g., different temperature groups of microbes) and are fairly inexpensive yet provide critical information as to whether next-stage testing (continuous stirred-tank reactors for concentrates and column tests for ores) should be pursued or whether the ore/concentrate has characteristics (e.g., excessive amounts of acid-consuming gangue minerals, or excessive cyanide and/or lime consumption for refractory gold ores) that preclude bioleaching/biooxidation as a process option. Table 3 lists the purposes of amenability testing. Careful interpretation of amenability test results is imperative, because the testing is conducted on ground ore and concentrates under batch conditions that do not reflect the conditions of whole ore heap leaching or concentrate leaching in continuous stirred-tank reactors (Olson and Harvey 2011). Interpretation of acid production and consumption and rates of oxidation of ores and concentrates in amenability tests needs particular attention because of the particle size and because oxidation rates are usually slower in batch reactors.

Table 3 Laboratory bioleach batch reactor amenability testing with concentrate and pulverized ore

1. Determine amenability of feed to bioleaching.
2. Identify potential toxicity of ore/concentrate feed and site water.
3. Determine ultimate metal extraction.
4. Approximate oxidation rates of various sulfide minerals.
5. Quantify gold recovery vs. percent sulfide–sulfur oxidation for precious metal ores/concentrates.
6. Obtain order-of-magnitude reagent consumptions.
7. Ascertain performance of different suites of microorganisms:
   - Mesophilic microbes for low-temperature conditions
   - Thermophilic microbes for high-temperature conditions
8. Estimate acid consumption/production.
9. Determine mass loss.

Continuous Stirred-Tank Reactor/Mini Pilot-Plant Testing of Concentrates

The next stage of evaluating concentrates for bioleaching/biooxidation after aerated and stirred batch reactor amenability testing is continuous stirred-tank reactor (CSTR) testing. For CSTR tests involving microorganisms, typically three to four aerated and agitated reactors in series are employed. The first stage is usually larger in size or involves two reactors in parallel for retaining solutions for a longer period to allow approximately 60% or more of the sulfide-sulfur to be oxidized in this stage. CSTRs should minimally be several liters in size; small CSTRs are usually constructed of glass and are water-jacketed to allow temperature control for testing various suites of microorganisms. Small CSTRs are used to confirm amenability of the concentrate to bioleaching, adapt the microbial consortium to higher metal and total dissolved solids (TDS) concentrations, confirm metal recovery versus percent sulfide-sulfur oxidation, and quantify residence time in each reactor stage for the bioleaching/biooxidation process to achieve acceptable metal recoveries.

Mini-CSTR pilot plants are usually stainless-steel (316 L), temperature-controlled reactors equipped with agitators and sparged with air. Mini pilot plants of 120-L size can be used to design industrial plants for biooxidation of sulfidic refractory gold concentrates. These mini pilot plants are composed of one 60-L primary reactor and three 20-L secondary reactors in series. A continuous mini pilot-plant run of four to six months can require nearly 1 t (metric ton) of concentrate.

CSTR runs of this duration confirm amenability of the concentrate sample to biooxidation pretreatment; adapt the microbial culture to improve oxidation performance; obtain process data for design of the industrial plant including residence time of the concentrate in each reactor stage; ensure that waste products are environmentally acceptable; establish optimal cyanidation conditions for gold recovery from the biooxidized product; and establish optimal reagent consumptions during biooxidation and subsequent countercurrent decantation, neutralization, and cyanidation of the biooxidized product.

CSTR campaigns are run on concentrate samples representative of life-of-mine. Among the many considerations are the types and amounts of sulfide minerals, the total sulfide-sulfur content, the percentage of sulfide-sulfur that must be oxidized to achieve acceptable metal recoveries, optimal particle size, ideal solids density, and concentrate throughput. These factors, among others, influence the retention time of the solids in each stage, the amount of oxygen required, and the amount of heat generated. These in turn determine the design of the aeration and cooling systems, the reactor sizes and configuration, and ultimately the capital and operating costs of the full-scale industrial plant. The ultimate plant design is based on a combination of factors including the highest sulfide-sulfur content coupled with tonnage throughput (i.e., the maximum metric tons of sulfur loading per day), loading of ions such as arsenic, and variations of feed through life-of-mine.

Performing successful mini-CSTR pilot-plant campaigns requires extensive sampling and analysis over a period of months. Each reactor is analyzed daily for total iron, iron speciation, redox potential, pH, dissolved oxygen, and temperature; solution from the last-stage reactor is analyzed for a suite of metals. The feed to the CSTR is assayed for metals, total iron, total sulfur and sulfur species, total carbon and carbon species, silica, and specific gravity. The oxidized solids and leach liquor from the final reactor are assayed daily to complete a mass balance. A mass balance is performed on each concentrate tested. A key microbiological element of CSTR operation and design of the commercial-scale plant is determining optimum solids retention time in the various stages, particularly in the first-stage reactor, which sees the highest sulfide-sulfur concentrations. Avoiding microbial "washout" is critical to CSTR performance. Washout occurs when the solids retention time in a reactor stage is less than the time required for the microorganisms to divide (i.e., divide by binary fission to produce two daughter cells). Biooxidation/bioleaching ceases if washout occurs and is manifested almost immediately by a rapid decline in the redox potential of the reactor suffering the washout.

Laboratory Column Reactor Testing of Ores

The next step for evaluating ores for heap bioleaching/biooxidation is column testing, after batch reactor tests are completed and the ore is shown to be amenable to the process. Column tests can be carried out in sizes ranging from small columns that may be 1 m high and 15 cm in diameter to 10 m high and more than 1 m in diameter. Column diameters should be a minimum of 4×, preferably 6–10×, the diameter of the largest particle size to avoid wall effects and solution channeling in the column. The objectives of column testing are summarized in Table 4.

Column tests are performed on different crush sizes of ore, which have been thoroughly analyzed for metal concentrations. It is advisable to agglomerate the ore with a microbial culture, adapted to the ore being tested. It is advantageous to use the mesophilic, moderately thermophilic, and extremely thermophilic cultures that have been adapted to the ore during the batch reactor amenability tests.

Table 4 Purpose of bioleach laboratory column testing

1. Determine optimum ore particle size for maximizing extraction.
2. Verify acid consumption for respective particle sizes.
3. Ascertain rates of oxidation for sulfide minerals by particle size.
4. Understand iron balance.
5. Reveal potential toxic conditions.
6. Evaluate rates and effects of bioleaching at different temperatures.
7. Assess competence of ore.

Columns are aerated from the base and may be irrigated initially with a microbial nutrient solution (Lee et al. 2011). Barren raffinate from downstream solution processing is often used for irrigation of columns when copper ores are being column leached. Columns should be operated in closed-loop solution circulation, which allows constituents to build up in solution; the purpose is to evaluate microbial tolerance to increasing ion concentrations and to identify constituents that may be problematic to the microorganisms. pH control is often necessary to maintain the pH in the desired range. When testing copper sulfide ores, the effluent solution, referred to as pregnant leach solution (PLS), is processed periodically with a small solvent extraction plant to remove dissolved copper; the resulting raffinate is recycled for column irrigation. Column tests are used to compare metal recoveries and rates of leaching at different particle sizes, evaluate leaching under different degrees of acidity, and assess rest–leach cycles. Column tests are useful in assessing the performance of different microbial populations (see the "Case Study 1" section), such as thermophilic microbes (Norris et al. 2012), when heating of the proposed ore heap is predicted based on sulfide grade of the ore and rates of sulfide oxidation and for primary copper sulfide ores (e.g., chalcopyrite and enargite). Such tests are performed using temperature-controlled columns (Acar et al. 2005).

Column testing of sulfidic refractory precious metal ores requires additional consideration. The microorganisms oxidize the sulfide minerals, typically pyrite and arsenopyrite, releasing iron and arsenic into solution. The gold and most of the silver, if present, remain with the solids. It can be difficult to determine when sufficient sulfide has been oxidized to achieve maximum gold recoveries, because there are no methods of determining this effectively by assaying the effluent solution. Dissolved iron and arsenic are not good indicators because of precipitation in the column of jarosite, possibly scorodite and other ferric arsenate compounds. Consequently, replicates of the column test are set up and periodically a column is terminated and the leached ore analyzed for sulfide-sulfur and other constituents, and the oxidized ore is subjected to bottle-roll cyanidation testing to determine gold recovery. For very large column tests, provisions are made for periodic sampling, preferably through portals constructed on the side of the column at various depths.

Head samples and leached residues of column tests are fully analyzed mineralogically and chemically. Effluent solutions are analyzed systematically for redox potential, pH, metal/metalloid concentrations, total iron, iron species, and TDS; effluent solution volumes are measured. Makeup water must be added because of evaporation. A mass balance that

considers the solution volumes and metals concentrations of samples removed for analyses and solvent extraction removal of copper is completed on termination of each column test (Olson and Harvey 2011).

Lee (2011) outlined testing for a sulfidic refractory whole ore biooxidation pretreatment. The ratio of sulfide oxidation to increased gold extraction along with metal extraction achievable at different crush sizes are fundamental to evaluating the potential for practical application of a whole ore biooxidation pretreatment process. Other factors include cyanide consumption for leaching gold after biooxidation pretreatment as well as reagents required for neutralization of the ore prior to gold leaching and disposal of acidic solutions generated in the process.

Iron precipitation as jarosite is often extensive in column tests and can be particularly voluminous when column leaching is at elevated temperatures with moderately thermophilic and extremely thermophilic microorganisms. Jarosite precipitation is of no concern because it is fine grained and does not impact solution percolation. It does increase acidity, as jarosite precipitation is an acid-producing reaction and it affects the iron mass balance. Care must be exercised when interpreting iron assays in the leached residues because precipitated iron will be measured.

Hydraulic testing of the ore should also be an imperative during column leaching. Hydraulic testing involves compression tests to ascertain the ore dry bulk density and hydraulic conductivity as a function of ore stacking depth. Other hydraulic tests determine moisture retention and agglomerate quality, ore porosity, pore pressure, percent saturation, and drain-down curves. These data are useful to predict full-scale heap behavior and metal recovery (Robertson et al. 2013).

The modeling and predicting of column bioleach/biooxidation results to performance of full-scale, crushed ore heap leach operations requires applying scale-up factors. Scaling crushed ore column tests to run-of-mine (ROM) stockpile bioleaching is difficult, but column tests do provide useful information on the feasibility of ROM leaching.

Case Study 1: Temperature Selects Predominant Groups of Microorganisms

Column testing using a pyritic ore has demonstrated the variability of the microbial populations with change of temperature (J.A. Brierley 2003). A stepwise increase of temperature from 22°–60°C resulted in decimation of mesophilic iron-oxidizing bacteria with an increase of moderately thermophilic microbes, followed by the thermophilic archaea iron-oxidizing microbes. Reversal of the temperature series from 60°–22°C resulted in return to dominance of mesophilic bacteria with a concurrent decrease in the populations of moderately thermophilic microorganisms and the thermophilic archaea. This column test demonstrated that temperature was the overriding factor that selected for the dominant groups of microbes.

Pilot/Demonstration-Scale Heaps

The type of ore being considered for heap bioleaching/biooxidation often determines whether pilot or demonstration heaps are operated. Secondary copper sulfide ores may undergo crib tests at the mine site. These are essentially large-scale column tests, and the same protocols applied to laboratory column tests pertain to crib tests. Often, secondary copper sulfide ores are not subjected to pilot- or demonstration-scale testing after successful column and crib tests, principally because secondary copper sulfide heap bioleaching is considered a mature technology for porphyry-type supergene ores. It is prudent, however, to pilot-test secondary copper sulfide ores that differ in ore genesis from porphyry-type supergene ores as these ores may possess very different hydraulic characteristics and vary significantly from porphyry-type supergene ores in their response to heap bioleaching. Primary copper sulfide ores, such as chalcopyrite and enargite, and sulfidic refractory gold ores should be evaluated in pilot- or demonstration-scale tests at the mine site. Primary copper sulfide ore heap and ROM stockpile bioleaching is an emerging technology; the precise conditions and the engineering of the heap/stockpile to effect primary copper ore leaching are still under development. Heap biooxidation of sulfidic refractory gold ore was commercialized (Logan et al. 2007) at one mine site after pilot- and demonstration-scale testing. Sulfidic refractory gold ores from other sources may perform differently, which necessitates pilot- or demonstration-scale testing. An important benefit of mine-site pilot or demonstration plant testing is to provide a learning opportunity for mine operators in handling microorganisms and conducting the bioleach process.

J.A. Brierley et al. (1995) described pilot- and demonstration-scale testing of a sulfidic refractory gold ore that entailed bioleaching pyrite to expose encapsulated gold. Six pilot plants varying in size from 432–25,900 t were used to test different ores and gold leach methods after biooxidation pretreatment of the ore. After the pilot-plant test, a much larger demonstration plant of 708,000 t ore was constructed and operated (Shutey-McCann et al. 1997). Inoculation of the ore was found to decrease the time required for natural microbes to establish an active population in the ore. Also, the heap temperature increased substantially because of oxidation of pyrite, which required the addition of moderately thermophilic and extremely thermophilic microbes to the ore heap, as the succession of microbes in heaps that heat is important and complex (C.L. Brierley 2001). A process for microbial inoculation of ore was developed (J.A. Brierley and Hill 1993). Other processes for inoculating heaps are described by Gericke (2011).

Laboratory amenability and column tests, pilot- and demonstration-plants, and commercial bioleach plants are always subject to colonization by naturally occurring (transient, wild-type) microbes. Therefore, trying to control conditions for specific microbes, which may be intentionally added to these systems, is difficult, if not impossible, given current understanding and technology know-how. The physicochemical environment (e.g., pH, TDS, temperature, metal concentration, and mineralogy) of bioleach systems selects the resident microflora. Inoculation of a heap by a select monoculture or a specialized laboratory consortium can initiate a bioleach process, but ultimately process conditions will select the best microbes for the operation. The only known exception is the inoculation of a heap with the extremely thermophilic archaea, when the heap temperature exceeds 60°C (Logan et al. 2007).

Solution and solids analyses performed for column tests are also carried out for pilot- and demonstration-scale plants. Probes to measure various parameters (e.g., pH, redox potential, temperature, and moisture) should be placed at various depths and locations in the pilot or demonstration heap. Periodically, the heap should be cored to obtain samples at different depths and locations for chemical, mineralogical, and microbial analyses. Samples can be examined for direct

counts of microorganisms, analyzed for the numbers of iron- and sulfur-oxidizing microorganisms at different temperature regimes, and subjected to metagenomics sequence analysis to determine the distribution of different types of organisms.

Pilot and demonstration heaps offer opportunities to evaluate seasonal effects, precipitation events, rest periods, irrigation rates, aeration, evaporation rates and effects, ore competency under extended leach times and lift heights, irrigation methods, heap covers, and other factors. The extensive data obtained from these tests can be used to develop models to predict performance and economic viability (Marsden and Botz 2017).

Successful testing and the decision to commercialize a bioleach operation is a lengthy process. The time required from initiation of testing at laboratory scale to commercialization can be about 10 years. Regardless of the time required, thorough testing is mandatory to prevent possible failure.

ROM STOCKPILE LEACHING AND COMMERCIAL HEAP

Run-of-Mine Stockpile Bioleaching

Stockpile bioleaching in which ROM ore is used has been referred to as "dump" leaching. The mining industry has employed this process for about 50 years to recover copper from oxides and secondary sulfide, submarginal-grade ROM ores (Sheffer and Evans 1968). Initially, dissolved copper was recovered by the process of cementation using scrap iron (Rossi 1990) until solvent extraction technology became widely used in the 1970s. Most early operations employed surface solution ponds where bacteria oxidized acidic ferrous iron. The resulting ferric iron percolated into the ore bed. Limited oxygen reached the bacteria in the ore bed, because of the solution impoundments on the surface. Early investigation (see the "Case Study 2" section), of the bioleaching bacterial population indicated a limited distribution of the microbes within a sulfidic copper leaching operation (Bhappu et al. 1969).

ROM stockpile leaching of submarginal-grade copper ores remains an economically vital practice at many open pit copper mining operations. Some stockpiles have been under leach for decades with new ore continually being added. As mines become deeper, new ore added to stockpiles has trended increasingly toward copper sulfides, including chalcopyrite. Although little or no engineering efforts were undertaken in the early history of ROM stockpile leaching to enhance bioleaching activity, this has changed in recent years, principally to enhance the bioleaching of primary copper sulfide minerals.

Recognizing that ROM stockpiles were oxygen limited, which significantly diminishes microbial development and activity, techniques have been introduced to increase natural air convection, and some operations have instituted forced aeration. These include (Schlitt 2006)

- Finger dumps, which entail stacking ROM ore in patterns that allow maximum exposure of stockpile slopes to the atmosphere;
- Angled holes drilled into the stockpiles through which pressurized air is introduced;
- Placement of perforated pipes under newly stacked ore through which pressurized air is introduced;
- Air injection through vertical drilled holes; and
- Extended rest periods, which promote stockpile drainage, allowing more air to enter the pore spaces.

As early as the 1960s, heating of stockpiles was known (Beck 1967) with temperatures of 60°–80°C recorded. Higher temperatures in portions of the heap induced convective airflow, particularly in stockpiles under rest.

Engineered stockpiles are becoming increasingly common with primary copper sulfide ores being placed in stockpiles and the recognition that moderate and extreme thermophiles at temperatures higher than 50°C are required to leach these hypogene ores. Engineered stockpiles at Morenci, Arizona, United States (Ekenes and Caro 2012), incorporate aeration lines under newly stacked ore, and forced aeration is employed to ventilate the heaps. Microorganisms native to the ore are introduced onto newly stacked ore via raffinate irrigation to kick-start leaching. Extensive sampling and analysis of solutions and solids have been instituted on engineered stockpiles to evaluate the effectiveness of chalcopyrite bioleaching (Ekenes and Caro 2012). Engineered stockpiles have shown increased microbial pyrite oxidation, which has resulted in higher stockpile temperatures; increased acidity reducing external acid addition to maintain an acceptable operating pH; and, most importantly, improved copper extraction from primary copper sulfide minerals (Ekenes and Caro 2012).

Microbial populations in the PLS from the commercial-scale, engineered ROM stockpile leach facility at Escondida, Chile, were monitored over a period of six years using culture-dependent and independent approaches. The Escondida stockpile, which incorporates forced aeration, contains copper sulfide ores including chalcopyrite. As the height of the stockpile increased, microbial populations changed from predominantly mesophilic to thermotolerant and moderately thermophilic microorganisms—with *Leptospirillum ferriphilum*, a thermotolerant iron-oxidizing bacterium, and *Sulfobacillus thermosulfidooxidans*, a moderately thermophilic, iron- and sulfur-oxidizing bacterium—predominating the microbial population (Acosta et al. 2014).

Extensive microbial analyses are limited on commercial-scale stockpiles under leach, particularly on leached solids from these operations, so it is somewhat premature to draw conclusions about operating conditions in relation to the identified microbial populations. The numbers of microorganisms attached as biofilms to the ore within the stockpile far exceed those in the PLS, and the microbial composition may differ as well. Nevertheless, continuing to identify microorganisms and determine their distribution on the ore within the stockpile and in the PLS will eventually lead to a better understanding of the dynamic changes in microbial populations over time and to correlating changes in populations with chemical and physical changes within the stockpile.

Case Study 2: Early Investigation of Bacterial Distribution in an ROM Stockpile Under Leach

Samples of the stockpile were collected from the surface and at depth using a pneumatic drill providing mixed samples from 8-ft intervals within the stockpile. Iron-oxidizing bacteria were present at the surface in concentrations from 10^3 to 10^7 cells/g dry weight. Table 5 shows the decline of iron-oxidizing bacteria with depth. No bacteria were present below 88 ft.

The bacterial population was likely affected by lack of moisture or limited wetting of ore in some areas of the stockpile and lack of oxygen within the stockpile. PLS samples from the stockpile contained 10^6 cells/mL of iron-oxidizing bacteria suggesting that (1) portions of the stockpile were

Table 5 Iron-oxidizing bacteria detected at depth

Interval Below Surface, ft	Bacteria Concentration, cells/g dry weight
8–16	10^3
24–32	10^2
40–48	10^3
56–64	10^1
72–80	10^1
88–144	None detectable

favorable for growth of the bacteria, or (2) the bottom area of the stockpile, where large boulders segregate as a result of the way stockpiles are constructed, allowed air to enter, resulting in good bacterial activity at the base of the stockpile.

Commercial Heap Bioleaching of Base Metal Sulfides and Biooxidation of Sulfidic Refractory Gold Ores

Commercial-scale heap bioleaching, also referred to as percolation leaching, of secondary copper sulfide ores began in the mid-1990s. The technology was a merger of sulfuric acid leaching of copper oxides, developed in the 1960s; ROM stockpile leaching of copper sulfide ores; and "thin-layer" leaching with ferric iron (Bustos et al. 1993). Today commercial-scale heap bioleaching of copper sulfide ores is widely practiced in many locations throughout the world and has been developed for other sulfide minerals as well (Saari and Riekkola-Vanhanen 2011).

Biooxidation pretreatment of sulfidic refractory gold ores, developed in the 1990s, has also been practiced commercially (Logan et al. 2007). Biooxidation pretreatment is a process for enhancing gold recovery by oxidizing sulfide minerals, principally pyrite and arsenopyrite (Reactions 7 and 8), in which gold is embedded. Biooxidation pretreatment exposes the gold for subsequent extraction by conventional metallurgical technology, typically cyanide leaching. Significant improvements in gold recovery are achievable using biooxidation pretreatment.

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad \textbf{(EQ 7)}$$

$$FeAsS + 5Fe^{3+} \rightarrow As^{3+} + 6Fe^{2+} + S^\circ \quad \textbf{(EQ 8)}$$

Heap "reactors" are technically and economically (Acevado and Gentina 1993) suitable for pretreating refractory, lower-grade (e.g., 1.0–2.4 g Au/t) sulfidic whole ores that may not be amenable to flotation; are too low-grade to economically process by roasting, pressure oxidation, or stirred-tank biooxidation processes; or have mineralogical characteristics that preclude other processing methods. Biooxidation heap pretreatment, requires a period of 270–380 days depending on sulfide-sulfur grade, particle size, and other factors. After biooxidation pretreatment, the heap can be neutralized with lime/limestone and gold is extracted using conventional cyanide leaching practices. Alternatively, ammonium thiosulfate can be used for gold extraction after biooxidation of double-refractory sulfidic ores containing preg-robbing carbon (Wan and Brierley 1997).

One reported project used a heap approach for biooxidation pretreatment of refractory gold ore. The Newmont Biopro technology (Logan et al. 2007; Roberto 2017) pretreatment heap was operated using the same principles as a bioleach heap for base metal ores. The goal is to maximize the biooxidation of pyrite (Reaction 7) and arsenopyrite (Reaction 8) and other sulfide minerals that occlude gold. Inoculated and agglomerated ore is stacked on a high-density polyethylene-lined pad with a base layer of crushed rock placed on the liner. Within the crushed rock layer is an array of perforated pipes for forced aeration of the ore pile and a system of pipes for collecting the circulating leach solution. Low-pressure fans provide air for the ore heap. The heap is subjected to leach–rest cycles to conserve heat within the heap and to ensure drainage of the heap to allow void spaces to fill with air.

The refractory sulfidic ore is inoculated and agglomerated with an acidic solution containing mesophilic, moderately thermophilic, and extremely thermophilic microorganisms. A consortium of microbes for inoculation is required because the ore heats during the biooxidation process as a result of pyrite and arsenopyrite oxidation. Initially, the stacked ore is at ambient temperature favoring the growth of mesophilic microbes. As the temperature increases in the ore bed, those microbes active at higher temperatures dominate the population. Eventually, the extremely thermophilic archaea dominate in those areas of the heap reaching temperatures of more than 55°–60°C (J.A. Brierley 2003). Metallurgical processing of the biooxidized gold ore entails either neutralization of the heap followed by cyanide leaching of the heap or dismantling the heap, and neutralizing the biooxidized residue and leaching the product using carbon-in-leach cyanidation. The Biopro technology processed 8.8 Mt of sulfidic refractory gold ore, resulting in recovery of 120,000–180,000 oz/yr of gold between 2000 and 2005 (Roberto 2017).

The commercial success of heap bioleaching/biooxidation requires attention to physical design factors based on ore characteristics and operating methodology to ensure optimal permeability for solutions and air (John 2011). These factors are critical to favorable solution chemistry and microbial development and activity. Based on successful column testing, ores are crushed, typically to 12 mm or larger, agglomerated with acid and in some cases with an acidic microbial culture, and the ore is stacked on prepared pads. Depending on the ore permeability, forced aeration is implemented with placement of perforated air pipes beneath the ore in a gravel bed to provide oxygen and carbon dioxide to the microorganisms. Some agglomerated ore heaps are sufficiently permeable that forced aeration is not necessary, relying on natural air convection through the top and exposed slopes of the heap. In implementing forced aeration, it is important to make sure the air pipes are not in the phreatic head to avoid the air pipes filling with solution. This is usually managed by placing drain lines in the gravel bed beneath the air lines (Schnell 1997; Schlitt 2006). Air addition is based on the amount of sulfide to undergo oxidation, sulfide mineralization, oxidation rates of the sulfide minerals, heap height, elevation of the plant, and efficiency of oxygen utilization. Copper sulfide heap leach operations are drip-irrigated with raffinate, intermediate leach solution—often with additional acid added to meet demand—and solution application is controlled to ensure wetting of the heap without saturation and ponding; this facilitates airflow throughout the heap. Copper sulfide heaps of porphyry-type supergene ore do not generally heat; sulfide grades are typically low, and pyrite, although present, does not effectively oxidize because the redox potential required to oxidize pyrite is seldom achieved in the heap. Consequently,

these commercial secondary copper heap bioleach operations in high desert regions often suffer from cool temperatures that slow microbial and chemical reaction rates. Thermal covers are sometimes employed to reduce evaporative losses and minimize solution cooling because of evaporation. Rest cycles are typically instituted to enhance microbial oxidation as the heaps drain of solution and to mitigate heat loss through solutions exiting the heap. Other measures that have been used to increase heap temperature are heating the raffinate and heating the ore.

Ores that contain higher sulfide grades or necessitate the microbial oxidation of pyrite or arsenopyrite to enhance gold recovery do require the use of forced aeration (Logan et al. 2007) and can benefit from implementation of other approaches to improve natural ventilation, including "kit-kat" structures, whereby heaps are separated by valleys or ditches to expose larger areas of the heap to the atmosphere (Saari and Riekkola-Vanhanen 2011).

Effective wetting of heaps undergoing bioleaching biooxidation is often a problem because of the stacking of clay materials or ores that create excessive fines during leaching, compaction of heaps by truck stacking of ore rather than automated stacking devices, compaction from multiple lift emplacement, and solution channeling because of poor agglomerate formation. Solution channeling can result in significant metal reserves left in the leach pad because of large volumes of under-leached ore. Subsurface leaching is now being employed to deliver leach solution to these areas resulting in recovery of metal inventories from under-leached ore volumes (Rucker et al. 2017). Compaction can lead to saturated conditions within the heap; solution saturation prevents effective aeration of the heap resulting in limited ferrous iron oxidation by the microorganisms and ultimately poor extraction of the metals of value.

Total iron concentrations in the PLS of many commercial heaps bioleaching supergene copper ores from porphyry-type deposits are often low; 1–2 g/L in solution is common. The pH is maintained by acid addition in the 1.2–2 range so metals, including iron, remain in solution. These operations are dependent on naturally occurring microorganisms, principally mesophilic bacteria and archaea, developing throughout the heap and maintaining the iron in the ferric form. Nutrients for the microorganisms are not added in commercial heap leach operations, as sufficient nutrients are generally available from the ore. The success of microbial development and activity in the heap is ascertained by measuring the redox potential and the ferric-to-ferrous iron ratio in the PLS. The first mole of copper from chalcocite (Cu_2S) is readily leachable at a low redox potential and occurs rapidly in the heap. The second mole of copper from the blaubleibender covellite product of chalcocite leaching requires a higher redox potential and leaches slowly. The redox potential in the heap will not increase to the millivolt level required for dissolution of blaubleibender covellite (CuS) until all available Cu_2S is oxidized to Cu^{2+} and CuS (Reaction 4).

In heap leach operations with arsenic minerals as a component of the ore, it is particularly important that the redox potential is sufficiently high and adequate ferric iron is present to ensure that As^{3+} is oxidized to As^{5+}, because the former is toxic to the microorganisms. Some arsenic minerals, such as realgar and orpiment, are acid soluble and arsenic will go into solution as arsenite (AsO_3^{3-}); ferric iron readily oxidizes this to arsenate (AsO_4^{3-}). Special attention during commercial plant start-up is necessary to accomplish this oxidation reaction.

Microbial population developments in heap leaching of supergene porphyry-type copper ores have not been extensively studied. Most microbial evaluations have looked at populations of mesophilic iron- and sulfur-oxidizing bacteria in PLS and raffinate from these operations. Microbial numbers are usually in the 10^4–10^6 per milliliter range in PLS with slightly lower numbers in raffinate. However, it is not clear how these microbial numbers and populations relate to microbial development and activity within the ore bed.

Several microbiological studies have been done on secondary copper operations that are not porphyry ores. The secondary copper ore at Zijinshan mine in China represents a quartz-alunite type epithermal deposit with high pyrite (5.8%) content. A coarse crush (–4 cm) sulfidic ore is stacked, and heaps rely on convective ventilation, not forced aeration. The oxidation of the pyrite results in an estimated internal heap temperature of 70°C, which enhances convective aeration. The PLS has a pH <1, acidity of 30 g/L, total dissolved iron concentration of 50 g/L, and Eh (redox potential) 700–740 mV versus SHE. Reportedly, the severe conditions of the leach solution have limited the microbial population of the PLS to 10^4/mL. The microbial population is dominated by bacteria of the genus *Leptospirillum* near the heap surface with increasing distribution of thermotolerant and moderately thermophilic microorganisms with depth (Liu et al. 2010). Archaea of the genus *Ferroplasma* were also detected (Ruan et al. 2011), which is tolerant to low pH values and high dissolved iron concentrations.

The Monywa (Myanmar) secondary copper sulfide ores are from a high-sulfidization system. In the heap bioleach operation, total iron concentrations in the PLS approach 25 g/L with nearly all as ferric iron with a redox potential of 750–775 mV versus SHE and high acidity (~20 g/L). Solid and leachate samples from the Monywa operation were subjected to direct cell counts and polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) analysis of the 16sRNA gene in 2006 (Hawkes et al. 2006). At the time of this analysis, portions of the heap temperature at Monywa reached 46°C and the PLS and raffinate pH ranged from 0.95–1.3. Organisms identified by Hawkes and colleagues (2006) at Monywa were closely related to *L. ferriphilum* and *A. caldus*; a new archaea species, *Ferroplasma cyprexacervatum*, was isolated. *Sulfobacillus* species were also detected.

Bioleaching using a heap reactor has been developed for recovery of nickel, zinc, cobalt, and copper from a black schist ore (Puhakka et al. 2007; Saari and Riekkola-Vanhanen 2011) at the Talvivaara Sotkamo mine located in eastern Finland. The internal heap temperatures range from 10° to 90°C; heating is attributed to rapidly oxidizing pyrrhotite in the ore. A complex consortium of bioleaching microorganisms is present in the heap. The reported microbes span the range of operating temperatures with the mesophilic and moderately thermophilic bacteria and archaea, and the extremely thermophilic archaea.

Crushed Ore Heap Bioleaching of Hypogene Copper Sulfides

A significant challenge for heap bioleaching is effective leaching of primary copper sulfide minerals, principally chalcopyrite and enargite. Effective bioleaching technology to accomplish this is under development.

Lee et al. (2011) compared mesophilic and thermophilic bioleaching of complex sulfidic ore composites containing primary enargite and covellite. The results verified the advantage of high-temperature bioleaching; 80%–95% copper extraction at 65°C compared with 8%–20% copper extraction at 20°–22°C was observed. Advances in high-temperature bioleaching will lead to eventual demonstration of the process using field-site pilot plants.

Instrumented laboratory columns (Robertson et al. 2011) and large laboratory column (6 m) testing have shown the potential for effective high-temperature bioleaching of chalcopyrite (Dew et al. 2011). At temperatures higher than 50°C and up to 80°C, copper recovery from low-grade (0.35%–0.7% copper) primary chalcopyrite ores increased from typical limiting values of about 30% to more than 60% (Dew et al. 2011). Application of chalcopyrite bioleaching in a commercial-scale, crushed ore heap is dependent on autogenous heat generation from oxidation of contained pyrite in the ore. Pyrite content and rates of pyrite oxidation will control which groups of microbes should be tested and considered for bioleaching of primary copper sulfide ores (Dew et al. 2011). Significant engineering considerations are also necessary to maximize and maintain heap temperatures to attain optimal conditions for microbial activity of thermophiles.

COMMERCIAL-SCALE STIRRED-TANK BIOLEACHING/ BIOOXIDATION OF CONCENTRATES

Testing of biooxidation pretreatment used for refractory sulfidic gold concentrates with CSTR systems (Van Aswegen et al. 1991) resulted in development of commercial operating Biox plants throughout the world (Van Aswegen et al. 2007). The first commercial engineered bioreactor system for biooxidation pretreatment of a refractory sulfidic gold concentrate was implemented by Gold Fields in 1986 (Gericke et al. 2009) at the Fairview mine, South Africa; this Biox plant remains in operation today. Thirteen Biox plants have been successfully commissioned (Outotec 2017). In addition, four CSTR plants for biooxidation pretreatment of refractory gold concentrates have been built and operated by other technology developers and one CSTR plant developed by the Bureau de Recherches Géologiques et Minières (BRGM) operated for bioleaching cobalt from pyritic tailings (Morin and d'Hugues 2007).

Based on mini-CSTR pilot-plant runs, a process design package, which includes process specifications, mass and heat balances, flow diagrams, valve and instrument diagrams, equipment list, and detailed equipment specifications, is completed and the plant is constructed by an engineering company. A typical commercial plant has a layout similar to the mini pilot plant with two or more primary reactors in parallel followed by up to four secondary stages. Each reactor is equipped with impellers to disperse air and suspend concentrate in the reactors and cooling coils to maintain reactor temperatures in the 40°–45°C range. The microbial inoculum used in the mini pilot-plant runs is brought to the plant site and scaled up. Each reactor is started in batch and then converted to continuous operation during commissioning. Because of the large amount of sulfide-sulfur present in the typical 20% pulp density used in stirred-tank reactors, many microorganisms (10^8–10^{10}/mL) are present in the reactors. To maintain these organisms and to ensure rapid cell division, nutrients are supplied (Table 6). Limestone is typically added to maintain a pH of 1.1–1.5 across all reactor stages. Air is added to each reactor via a sparge ring located just under the impeller.

Table 6 Nutrients for mineral biooxidation processes

Nutrient	Amount, kg/t concentrate	Source	Reference
Nitrogen	1.7	Ammonium sulfate	van Aswegen et al. 2007
Phosphorus	0.9	Ammonium phosphate	
Potassium	0.3	Potassium salts	
NH_4^+	8.4		Dew et al. 1997
PO_4^{3-}	1.56		
K^+	1.42		

Residence time of the solids across all stages of commercial CSTRs is usually four to five days. After the final stage of biooxidation/bioleaching, the contents of the final reactor are subjected to solid–liquid separation. When bioleaching base metal concentrates, the liquor contains the metals of value and this PLS is processed by conventional metallurgical technologies to recover the metals. In biooxidizing precious metal concentrates, the gold and silver remain with the solids, which are water-washed in countercurrent decantation thickeners and then neutralized with limestone and lime; the precious metals are solubilized with cyanide.

The only commercial CSTR plant for base metals is in Uganda. This technology, developed by BRGM, was first used at the Kasese Cobalt Company in 1988 to bioleach cobalt from pyritic tailings (Morin and d'Hugues 2007).

OTHER BIOLEACH TECHNOLOGIES

Other bioleach technologies have been proposed and described by Carretero et al. (2011) as alternative commercial applications for metals leaching. Several of the process technologies have been field tested. Only one of these technologies, the Bacox process, resulted in construction of commercial plants.

Bioleaching of ore particles sized to 0.95–1.9 cm in a vat reactor has been tested at laboratory scale. No data are available as to the effectiveness of this approach on a commercial scale.

The Geocoat and Geoleach technologies are based on bioleaching of sulfidic concentrates coated on a crushed ore substrate (Harvey and Bath 2007). The Geocoat process was evaluated at pilot-plant scale for biooxidation pretreatment of refractory sulfidic gold concentrates. The concentrate was inoculated with microorganisms and coated on the ore for biooxidation in heaps. The Geoleach technology was designed to bioleach metal sulfide concentrates coated on an ore substrate and stacked in heaps. The basis of this process was to control the heap leach variables to facilitate heating via biooxidation of the sulfide minerals and to maintain elevated temperature within the heap reactor. This process could be used for bioleaching primary copper sulfide concentrates at elevated temperatures with thermophilic microorganisms. Neither technology has been used on a commercial scale.

BioCop is a bioleach technology for copper extraction from primary copper concentrates in a stirred-tank reactor process (Carretero et al. 2011). This technology is based on thermophilic bioleaching at 80°C. A 2,300-m^3 demonstration plant was constructed and operated in Chile. The extremely corrosive conditions of high temperature, acidic pH, and high ferric iron concentration necessitated use of tanks with ceramic lining. Apparently, the technology proved viable for bioleaching, but the plant was closed and the process abandoned. Although the reasons for not implementing a

commercial operation were not disclosed, economic consideration is a probable cause.

BioNic/BioZinc technology is a stirred-tank reactor process for bioleaching nickel and zinc sulfide concentrates. There is no information to indicate the process has advanced to the commercial development stage (Carretero et al. 2011).

The Brisa process has been tested for bioleaching copper from primary chalcopyrite (Carretero et al. 2011). The technology is based on using a microbial ferric iron generator with a separate chemical leach reactor for contacting the chalcopyrite with the ferric iron to which chloride and silver is added to enhance copper extraction. There is no information indicating this technology has been advanced to pilot-scale testing.

BacTech Environmental Corporation (BacTech 2017) developed a biooxidation pretreatment process (Bacox) for refractory sulfidic gold concentrates. This process, similar to the Biox technology, employs a CSTR system. Bacox was advanced to commercial scale with commissioning of three plants (J.A. Brierley 2014).

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