Acid and ferric sulfate bioleaching of uranium ores: A review

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Abstract

This review examines the acid and ferric sulfate bioleaching of uranium from low grade ores. The review traces back the progression of the technology from the time the role of microorganisms was recognized in the 1950's and 1960's. Some past and present uranium mining operations with active or potential microbial contribution are summarized. Experimental techniques and laboratory bioleaching experiments are described. Choice microorganisms have been iron- and sulfur-oxidizing acidophiles, comprising bacteria and archaea with mesophilic and thermophilic temperature ranges. Uranium is bioleached from ores in acidic ferric sulfate lixiviant. Ferric iron oxidizes tetravalent uranium to the hexavalent form and is thereby reduced to ferrous iron in this redox reaction. Microorganisms in the bioleaching process oxidize ferrous iron to the ferric form and thus regenerate ferric sulfate. Iron oxidation requires oxygen as the electron acceptor in the leach solution. Acidity ensures that ferric iron is soluble in the lixiviant and protons increase the solubilization of the oxidized, hexavalent uranium. Ancillary sulfide minerals such as pyrite enhance the bioleaching because their oxidation releases ferrous iron and reduced sulfur compounds for biological ferric iron and sulfuric acid generation. The main mining engineering approaches used for uranium leaching are heap, dump, stope, *in situ*, and in-place leaching. The efficiency of uranium bioleaching is affected by a number of mineralogical, physicochemical, microbial and process factors. Bioinformatics and synthetic biology are progressing the research on bioleaching microorganisms but these developments have not been materialized in the industrial practice of uranium mining. New applications of uranium bioleaching may focus increasingly on deposits where other products such as rare earth elements or base metals can be recovered in addition to uranium.

Keywords: acidophilic microorganisms; bioleaching; iron oxidation; sulfur oxidation; uranium mining

Contents

1. Introduction

Uranium is an important strategic element, mostly used as enriched ^{235}U for nuclear power generation and nuclear arsenal. Depleted uranium is used as ballast (density 19.1 g cm^{-3}) in vessels and in some instances as aircraft counterweights, in armor and as penetrators in ammunition (Betti, 2003; Bleise et al., 2003). The quantity of uranium in the Earth's crust varies considerably between different rock types, while the average level of uranium is approximately 2.7 mg kg^{-1} (Majumder and Wall, 2017), making it a more abundant element than for example gold or silver.

Uranium occurs largely as oxide and silicate minerals and in phosphate rocks in association with many different metals in the Earth's crust. The most common mineral of economic importance is uraninite (*i.e.*, the black botryoidal form pitchblende), ideally UO_2, which occurs in a variety of geological settings. Over 200 minerals containing uranium have been described in the literature but approximately 20 of them are of economic importance (Edwards and Oliver, 2000; Pohl, 2005; Pownceby and Johnson, 2014; Bhargava et al., 2015). Table 1 lists uranium minerals in ores that have been beneficiated or tested for bioleaching. The general formula of uranium content in oxide minerals is often presented as U_3O_8 (triuranium octoxide). U_3O_8 is the main component (70-90% w/w) in yellow cake, which is a composite of uranyl hydroxide ($UO_2(OH)_2 \cdot nH_2O$, uranyl sulfate $(UO_2)_x(SO_4)_y(OH)_{2(x-y)}$, sodium *p*-uranate ($Na_6U_7O_{24}$), uranyl peroxide ($UO_4$), uranium trioxide ($UO_3$) and minor amounts of various other uranium oxides (Hausen, 1998). Yellow cake is the stable chemical and physical form of uranium handled by mills and refineries.

The mining of uranium ores over the years has resulted in progressive exhaustion of high-grade uranium reserves (Mudd, 2009). As the grade of uranium in ores is on decline, the industry is looking for alternative processes that can be used to recover uranium from low-grade or complex ores that are difficult to process with existing techniques. Bioleaching may be a suitable alternative when low-grade and complex ores are uneconomical for conventional processes (Kaksonen et al., 2018; Yang et al., 2019). With declining ore grades, the concomitant or sequential leaching of uranium and base metals (Lecomte

et al, 2014), rare earth elements (Nancucheo et al. 2017), thorium (Desouky et al., 2016) and phosphate (Mäkinen et al., 2019) has gained recent attention.

Uranium is usually finely disseminated in ore bodies, making the leaching the most common process to recover it instead of ore dressing for concentrate production. Many uranium ores processed via bioleaching have contained significant amounts of pyrite and other Fe-sulfides, which are oxidized to acidic ferric sulfate. The hydrolysis of ferric iron in bioleach solutions releases protons, which together with biogenic sulfuric acid contribute to satisfy acid demand. Alkaline accessory minerals (e.g., carbonates and some silicates) may have high acid consumption, which can be neutralized with sulfuric acid titration in the pre-leaching stage. Carbonate bearing uranium can be processed with alkaline leaching (pH ~11) that involves, in principle, sodium bicarbonate and sodium carbonate (soda) solutions to precipitate sodium diuranate $Na_2U_2O_7$ (Gow and Ritcey, 1969; Butler, 1972). Alkaline leaching is not usually practiced with ores that have pyrite or other sulfide phases as main minerals because of their high carbonate consumption. Some studies have addressed the alkaline bioleaching (e.g., Fekete et al., 1980; Zakó-Vér et al., 1980; Cecal et al., 2000), but research in this area has been scarce. Lacking documented data, microbes seem to have no known, specific role in the process and the prospect for alkaline bioleaching is in doubt.

The solubility of uranium – and other metals – is influenced by changes in pH and redox conditions which are subject to microbial activity. In addition, the formation of metal-complexing ligands such as organic acids formed by heterotrophic microorganisms including actinomycetes and fungi enhances the solubility of uranium as well as other metal and metalloids (Burgstaller and Schinner, 1993; Hefnawy et al., 2002; Mishra et al., 2009; Patra et al., 2011; Abhilash and Pandey, 2013c; Anjum et al., 2015; Abdulla et al, 2017; Amin et al., 2018; Ghazala et al., 2019; Harpy, 2019). However, to our knowledge heterotrophic bioleaching has not been practiced in commercial scale and the role of heterotrophs in acid bioleaching involving sulfuric acid and ferric iron is low because the available organic substrates are mainly limited to small quantities of residual chemicals from solvent extraction and carry-over soil borne organic compounds as well as those excreted by autotrophic acidophiles in the leach circuit (Johnson and Roberto, 1997).

The objective of this review is to examine the current knowledge of the acid bioleaching of uranium ores. Historical perspectives of past operations are discussed as they have provided impetus to test and develop the technology since the 1950's and 1960's. This review underscores the link between experimental approaches in the bioleaching of uranium ores and the underlying biology of microorganisms. The bioleaching mechanisms and the roles of microorganisms in acid- and ferric sulfate-based processes are appraised in the light of the past and recent advances published in the literature. This review also covers the unique traits and requirements for function of microorganisms in bioleaching processes, with emphasis on tolerance to toxic metals, low pH, and the ability to oxidize reduced sulfur compounds and ferrous iron, which are their only substantially available energy sources in uranium bioleaching operations.

2. Methods

This review is based on an in-depth literature search and review of peer review journal papers supplemented with reports, conference publications and information available on company web sites when required. The review covers publications that span over 70 years, from 1950 to 2020. Peer reviewed literature was searched through data bases and search engines such as Web of Science and Google Scholar using keywords including, but not limited to biomining, biohydrometallurgy, bioleaching, ferric leaching,

acidophilic, uranium, and decommissioning. The quality of the selected literature was ascertained by carefully analyzing the content, and publications with relevant information were cited. The information gathered from the literature was analyzed and organized to discuss acid and ferric sulfate bioleaching of uranium ores, effective microorganisms and their underlying biology, experimental approaches**,** and examples of commercial uranium mines and potential microbial involvement. Bench-scale feasibility and optimization experiments were reviewed to discuss mineralogical, physicochemical and microbiological factors that affect uranium bioleaching. Additionally, approaches for the control of microbial processes and uranium contamination after mine closure were briefly discussed.

3. Mechanisms of acid and ferric sulfate bioleaching of uranium

The choice of lixiviant for uranium leaching depends on the valence of the uranium, the composition of the matrix, the solubility of contaminants, economic feasibility, the mill tailings management and other environmental considerations. Several chemical oxidants have been tested for the oxidation of uranium in minerals to the hexavalent form in order to maximize the acid leaching (e.g., Haque and Ritchie, 1982). In the ferric sulfate leaching, these oxidants also regenerate ferric iron if it has been reduced to ferrous iron by contact with U^{4+} and sulfide minerals in the ore. Bioleaching-based processes with acidic ferric sulfate and iron- and sulfur-oxidizing bacteria do not require additional chemical oxidants such as chlorate ($NaClO_3$), pyrolusite (MnO_2), or hydrogen peroxide (H_2O_2) to achieve adequate ferric iron regeneration rates (Muñoz et al., 1995a, 1995b, 1995c; Nemati and Webb, 1996; Venter and Boylett, 2009).

Both tetravalent and hexavalent U occur as admixtures in many uranium ores (examples given in Table 1). Hexavalent uranium (U^{6+} as in paraschoepite $UO_3{\cdot}xH_2O$) is solubilized to uranyl ion (UO_2^{2+}) in dilute sulfuric acid without the need of an oxidant (Reaction 1):

$$UO_3 + 2H^+ \rightarrow UO_2^{2+} + H_2O \quad (1)$$

Tetravalent uranium is not soluble in acidic sulfate-rich solutions. Its dissolution requires the oxidation of the tetravalent uranium to the hexavalent form (Reaction 2). This is greatly enhanced with a chemical oxidant such as Fe^{3+} leading to the formation of uranyl ions (Reaction 3):

$$2UO_2 + O_2 + 4H^+ \rightarrow 2UO_2^{2+} + 2H_2O \quad (2)$$

$$UO_2 + 2Fe^{3+} \rightarrow UO_2^{2+} + 2Fe^{2+} \quad (3)$$

Acidophilic Fe- and S-oxidizing microorganisms catalyze uranium leaching by generating soluble ferric iron and sulfuric acid from Fe^{2+} (Reaction 4), and reduced sulfur compounds such as elemental S (Reaction 5), respectively (Figures 1 and 2):

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

$$2S^0 + 3O_2 + 2H_2O \rightarrow 2SO_4^{2+} + 4H^+ \quad (5)$$

The major steps in uranium bioleaching are the lixiviant production and its contact with the ore for the solubilization of uranium. Both steps can be improved through testing and optimization. In the two-stage bioleaching (or indirect bioleaching) the generation of bioleach solutions (acidic ferric iron-based lixiviants) is separated from contact with the ore. The biological oxidation of Fe^{2+} and the regeneration of

Fe^{3+} in sulfuric acid-containing solutions requires the supply of dissolved O_2 and CO_2 for the microbes.

In addition to the ferric-iron mediated oxidation, tetravalent uranium can also be oxidized directly to the hexavalent form by *Acidithiobacillus ferrooxidans* (Reaction 6) (DiSpirito and Tuovinen, 1981). DiSpirito and Tuovinen (1982a, 1982b) demonstrated O_2 uptake and CO_2 fixation by washed cell suspensions of *A. ferrooxidans* with uranous sulfate [$U(SO_4)_2$] as the substrate in the absence of iron.

$$2U^{4+} + O_2 + 2H_2O \rightarrow 2UO_2^{2+} + 4H^+ \quad (6)$$

Metallosphaera prunae, a thermophilic acidophilic archaeon first isolated from a smoldering refuse pile of a uranium mine (Fuchs et al., 1995), has been reported to solubilize U_3O_8 to the soluble hexavalent form (Mukherjee et al., 2012). The octaoxide is a mixture of tetravalent and hexavalent uranium. It is not clear whether biomolecules are involved in the direct oxidation of U^{4+} to U^{6+} or whether it is indirect chemical action. The facultative anaerobe *Thiobacillus denitrificans* has been shown to oxidize uraninite as an electron donor (coupled with nitrate consumption) and at least c-type cytochromes seem to be involved (Beller 2005, Beller et al., 2009). These findings come from pure culture experiments with limited uranium amounts and it is inexplicable to perceive their significance to the bioleaching of uranium ores.

Sulfuric acid is the most widely used lixiviant for uranium leaching and dissolved uranium forms soluble uranyl sulfate complexes (Tuovinen and Bhatti 1999; Bhargava et al., 2015). In acid uranium leaching systems where bacteria may be involved, iron is invariably in the solution. The source of dissolved Fe is the sulfidic accessory minerals and silicates which may dissolve partially or completely with bacterial action and acid attack. The rate of the biological oxidation of Fe^{2+} is considerably faster (approximately 10^5-10^6 times) than that of the chemical oxidation at pH values $\leq$2 (Lacey and Lawson, 1970; Meruane and Vargas, 2003; Rao et al., 1995).

The formation of secondary mineral phases such as jarosite-type precipitates and elemental S layers is inevitable in bioleaching processes. The solubility of the oxidant Fe^{3+} is greatly influenced by pH, iron concentration, temperature and ionic composition of the leach solution and the precipitation products are strongly dependent on solution conditions. Ferric-iron in acid, sulfate-rich solutions forms mainly jarosite type precipitates ($AFe_3(SO_4)_2(OH)_6$ where A is a structural monovalent cation, usually Na, K, NH_4, H_3O or a 0.5 equivalent of a divalent metal) at ambient temperatures and pressures and at pH values <3.5 (Reaction 7). Depending on the concentration and composition of monovalent cations in bioleaching systems, the precipitates occur as solid solutions of potassium jarosite, ammoniojarosite, and natrojarosite, often with hydronium jarosite making up for the charge deficiency (Jones et al., 2014, 2018). End members of jarosite minerals are not found in leaching systems or mine tailings. For the first three jarosite precipitates, their formation involves the highest affinity for K^+ followed by NH_4^+ and Na^+ (Gramp et al., 2008).

$$3Fe^{3+} + A^+ + 2SO_4^{2-} + 6H_2O \rightarrow AFe_3(SO_4)_2(OH)_6 + 6H^+ \quad (7)$$

In the absence of monovalent alkali ions, the hydrolysis of Fe^{3+} in acidic sulfate-rich environments at ambient temperatures also produces Fe(III)-hydroxysulfate complexes, of which poorly crystallized schwertmannite ($Fe_8O_8(OH)_6SO_4$) is the predominant form at pH 2 to 3.5 (Bigham and Nordstrom, 2000) (Reaction 8).

$$8Fe^{3+} + SO_4^{2-} + 14H_2O \rightarrow Fe_8O_8(OH)_6(SO_4) + 22H^+ \quad (8)$$

Hydrolysis of ferric iron in sulfate-rich solutions at higher pH values initially leads to poorly crystallized ferrihydrite and acid formation (Bigham and Nordstrom, 2000) (Reaction 9).

$$5Fe^{3+} + 12H_2O \rightarrow Fe_5HO_8{\cdot}4H_2O + 15H^+ \quad (9)$$

With time, ferrihydrite is increasingly converted to well crystallized goethite (α-FeOOH) (Bigham et al. 1996a, 1996b; Bigham and Nordstrom, 2000) (Reaction 10):

$$Fe^{3+} + 2H_2O \rightarrow FeOOH + 3H^+ \quad (10)$$

In bioleaching systems without pH control, jarosite-type precipitates may accumulate on mineral surfaces, decreasing the level of dissolved Fe^{3+} that acts as an oxidizing agent. The precipitation creates diffusion barriers for fluxes of reactants and products on mineral surfaces and impacts negatively the leaching rates of mineral surfaces (Nemati et al., 1998; Stott et al., 2000; Watling, 2006; Petersen and Dixon, 2007; Pradhan et al., 2008; Abhilash and Pandey, 2013b, 2013d). Sorption of hexavalent uranium on Fe-precipitates can negatively impact the concentration of dissolved uranium and affect adversely the movement of reactants and products on mineral surface (Vuorinen et al., 1986; Duff et al. 2002). Precipitates can also physically occupy space within a heap or an *in situ* fractured ore body and block solution flow paths or pipelines, pumps and valves. Thus, uncontrolled ferric iron precipitation can create major challenges to bioleaching operations. A separate ferric iron regeneration step in the leach circuit (Nurmi et al., 2009, 2010; Kaksonen et al., 2014a, 2014b, 2014c) could, therefore, also facilitate the removal of excess iron before contact with uranium ore.

Oxidative dissolution of U^{4+} is favored at high Fe^{3+}/Fe^{2+} ratios, which largely determine the redox potential of the leach solution. The redox potential should be above +400 mV vs. standard hydrogen electrode for efficient uranium oxidation by ferric iron (Muñoz et al., 1995a, 1995b, 1995c). In addition to UO_2, Fe^{3+} in acid solution oxidizes sulfide minerals (Reaction 11), releasing Fe^{2+}, and SO_4^{2-} and H^+, shown as an idealized example for pyrite:

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (11)$$

Pyrite is a common sulfide mineral in many uranium ores and constitutes a natural source of ferric sulfate and acidity via microbial oxidation. Pyrrhotite ($Fe_{(1-x)}S$, x = 0-0.2) is also present in many sulfidic ores and is readily oxidized in acid leaching systems (Reaction 12). Pyrrhotite is Fe-deficient and is oxidized chemically faster than pyrite, up to two orders of magnitude by some accounts (Nicholson and Scharer, 1994). Partial oxidation of pyrrhotite can also produce elemental S or sulfur-enriched pyrrhotites (Janzen et al., 2000), all of which can be oxidized in bioleaching systems (Reaction 12).

$$Fe_{1-x}S + (2-0.5x)O_2 + xH_2O \rightarrow (1-x)Fe^{2+} + SO_4^{2-} + 2xH^+ \quad (12)$$

Pyrrhotite is also dissolved reductively under anaerobic conditions in acid solutions (Chiriţâ and Rimstidt, 2014) (Reactions 13 and 14).

$$Fe(1-x)S + 2(1-x)H^+ \rightarrow (1-x)Fe^{2+} + (1-x)H_2S + xS^0 \quad (13)$$

$$FeS + 2H^+ \rightarrow Fe^{2+} + H_2S \quad (14)$$

The subsequent reaction with Fe^{3+} can form additional elemental sulfur (S^0) and polysulfides (S_n-S-S-S_n), which may precipitate on mineral surfaces. Sulfur-oxidizing bacteria are an integral part of the microbiome in uranium bioleaching systems. Bioleaching studies have addressed their role in sulfur oxidation by using *A. thiooxidans* in mixed inocula (e.g., Abdollahy et al., 2011; Umanskii and Klyushnikov, 2013; Li et al., 2017). Pyrrhotite oxidation can generate thiosulfate ($S_2O_3^{2-}$) and polythionates *(*e.g., tetrathionate $S_4O_6^{2-}$ and trithionate $S_3O_6^{2}$), which are corrosive in contact with iron and steel structures (Newman et al., 1982; Horowitz, 1983; Abd Elhamid et al., 2001; Yonezu et al., 2013). Acid ferric sulfate solutions are also corrosive and thus the selection of corrosion-resistant materials for leach circuits is particularly important.

4. Bioleaching in uranium mines

4.1 Bioleaching microorganisms

Historically, acidophilic iron-oxidizing bacteria were enriched and isolated from metal and coal mine impacted water, sediment, and tailings samples and recognized to contribute to acidity of coal mine drainage (Colmer et al., 1950; Leathen et al., 1953a, 1953b; Temple and Delchamps, 1953). Iron-oxidizing acidophilic bacteria obtained from coal mine samples were named as *Thiobacillus ferrooxidans* (renamed *Acidithiobacillus* (*A.*) *ferrooxidans* in the late 1990's) (Temple and Colmer, 1951). Sulfur-oxidizing bacteria were also described (Leathen et al., 1953a) and identified as *Thiobacillus* (*T.*) *thiooxidans* (renamed *Acidithiobacillus thiooxidans*). However, the original description of *A. thiooxidans* dates back to the 1920's as elaborated by Harrison (1988). *Ferrobacillus ferrooxidans* was a novel bacterial isolate that could only oxidize Fe^{2+} but not inorganic S-compounds (Leathen et al., 1956). It was subsequently renamed as *T. ferrooxidans* (and later as *A. ferrooxidans*; Kelly and Wood, 2000) when it was recognized that its lack of growth on sulfur compounds was due to poorly designed experimental conditions (Kelly and Tuovinen, 1972). *Ferrobacillus sulfooxidans* was also described from coal mine drainage (Kinsel, 1960) but the nomenclature was surpassed when the two *Ferrobacillus* species were renamed as *T. ferrooxidans*. In general, the two physiological groups of bacteria, acidophilic iron- and sulfur-oxidizers, have been of primary interest but they were not well characterized in the 1950's and 1960's investigations of bioleaching. Over the succeeding decades, many new genera and species were discovered and characterized including *Acidithiobacillus ferrivorans*, *A. ferridurans, A. ferriphilus, A. ferrianus*, *A. sulfuriphilus, Leptospirillum* spp., moderately thermophilic *Acidithiobacillus caldus*, *Acidimicrobium* and *Sulfobacillus* spp. as well as mesophilic and thermophilic archaea such as *Ferroplasma, Sulfolobus*, *Acidianus*, *Metallosphaera* spp. and many others including iron-oxidizing heterotrophs (Johnson and Hallberg, 2008; Schippers et al., 2010, 2013, 2014; Johnson, 2012; Mahmoud et al., 2017; Falagán et al., 2019; Norris et al., 2020).

The initial impetus for the bioleaching approaches was the discovery in laboratory studies that the yields of leaching of metals from sulfide minerals were always higher in the presence of iron- and sulfur-oxidizing bacteria as compared to the corresponding abiotic chemical controls (Bryner et al., 1954, 1967; Bryner and Anderson, 1957, 1958; Razzell and Trussell, 1963; Duncan et al., 1968). When the potential application of bioleaching of sulfide ores was better recognized, these findings were extended also to uranium leaching from ores. These acidophilic bacteria could be used to produce lixiviants for uranium leaching with ferric iron and sulfuric acid from Fe-sulfides and elemental sulfur (Miller et al., 1963; MacGregor, 1966, 1969a, 1969b; White and Smith, 1969; Gow et al., 1971; Manchee and Garrett, 1974; Manchee, 1977). The early trials and findings in the 1950's and 1960's have been summed up by Hamidian et al. (2009) and Hamidian (2012).

Microbial diversity in active and abandoned uranium mine sites has been examined with cultivation-dependent and molecular ecological methods. Dhal (2018) summarized several studies on cultivable and uncultivable bacteria in uranium mine tailings and concluded that microbial diversity entails many physiological and ecological groups of aerobes and anaerobes. These analyses have uncovered not only the ubiquitous presence of acidithiobacilli but also considerable microbial diversity (e.g., Silver, 1987; de Silóniz et al., 1991; Berthelot et al., 1993, 1997; Schippers et al., 1995, 2013; Selenska-Pobell et al., 2001; Selenska-Pobell, 2002; Coral et al., 2018). Proteobacteria is usually the dominant phylum and Acidobacteria and Firmicutes are also invariably present. At the genus level, dominant microorganisms usually represent *Acidithiobacillus*, *Leptospirillum*, *Sulfobacillus*, *Alicyclobacillus*, and *Ferroplasma*. *Alicyclobacillus* and *Sulfobacillus* spp. have been found to dominate some microbial communities in laboratory column leaching of a weathered low-grade uranium ore from the Ranger Uranium Mine (Vázquez-Campos et al., 2017). Fungi have also been found in uranium mine water and raffinate samples **(**Mishra et al., 2009; Vázquez-Campos et al., 2014, Coelho et al., 2020). Microbial composition varies with the specific location at the mine; *i.e*., heap interior layers, leach solution and raffinate can have very different microbial populations.

Open pit and underground uranium mines practicing leaching operations have naturally, over time, enriched for microorganisms by virtue of being open processes exposed to air, moisture and rain. This is analogous to sulfide ore mines and tailings that undergo environmental, chemical, and microbiological changes over time. It is inevitable that microbes have some role in redox and chemical reactions leading to changes in the solubility of mineral constituents. It is not possible to separate microbial and abiotic effects on the solubilization in uranium leaching operations. Thus uranium-bearing ores and mines with exposed mineral surfaces have microbiomes that include indigenous acidophilic bacteria and archaea. These communities have developed under selective environmental conditions, including selection pressure by low pH, dissolved metals, and usually insignificant levels of organic compounds. The predominant energy sources for microbial life at mine sites are reduced compounds of iron and sulfur. Several reviews have addressed the microbial diversity associated with bioleaching and sulfide mine water impacted environments (Schippers et al., 2013, 2014; Mahmoud et al., 2017).

Anecdotally, some large scale pilot and commercial scale trials have involved inoculation of uranium bioleaching operations with laboratory-grown bacteria. Native microbes, selected over decades of exposure to ambient conditions, may be the best acclimated to the specific environmental conditions at the mine site but not necessarily to bioprocess conditions when a leaching operation is initiated in an open or closed system (Holmes, 1998). To date, there are no reports that external introduction of laboratory-grown inocula would have replaced the native microbiome in uranium bioleaching mine sites. In general, laboratory and pilot-scale bioleaching studies have used iron- and sulfur-oxidizing acidophiles as inocula from a broad range of sources that may have had no previous association with the specific ore material or the location. Given the scale of commercial leaching operations with leach solution volumes in millions of cubic meters, it is uncertain how quickly and efficiently the external addition of microbes could impact the operation in an open system. Some commercial-scale heap bioleaching operations have initially inoculated leach solutions with a wide spectrum of laboratory-grown microbes from various sources with a view to promoting early establishment of diverse microbes in the heap, but technical information and monitoring of these endeavors remain undocumented. This is a gray area in permitting and environmental regulations in many countries, but the general interpretation is that genetically modified microbes must not be released with such inoculations.

4.2 Lixiviant generation and leaching systems

The main mining engineering approaches used for uranium leaching are heap, dump, stope, *in situ*, and in-place leaching (Figures 3 and 4). Pachuca and stirred tank leaching have also been practiced in the industry. Livesey-Goldblatt et al. (1977) developed a pilot-plant unit for the continuous oxidation of recycled ferrous sulfate leach solution from a uranium ore processing plant. The process was called BACFOX, and utilized iron-oxidizing *Acidithiobacillus ferrooxidans* bacteria, which were retained as a film on corrugated packing media. The process achieved oxidation rates of 7.5 g Fe^{2+} m^{-2} h^{-1} (Livesey-Goldblatt et al., 1977). The BACFOX system was tested for instance in conjunction with a Pachuca tank leach circuit at the Buffelsfontein uranium plant (closed in the mid-1990's) in South Africa (Ring, 1980). The system also performed successfully in a pilot-scale Pachuca bioleaching of a low-grade uranium ore in India (Mathur et al., 2000). Murayama et al. (1987) reported a large-scale application of bacterial oxidation of iron as part of the acid mine water treatment process. Since then various types of bioreactors, including fluidized-bed reactors, packed-bed reactors, trickle-beds, circulating-beds, agitated reactors, airlift reactors and rotating biological contactors, have been tested for their potential for high-rate Fe^{2+} oxidation by acidophilic prokaryotes (Nurmi, 2009; Nurmi et al., 2009, 2010; Kaksonen et al., 2014a,b,c). Biofilm-based reactors allow faster oxidation rates than suspended cell systems due to greater biomass density and mass transfer rates (Jensen and Webb, 1995). The fastest iron oxidation rates (52 and 26 g Fe^{2+} L^{-1} h^{-1}, respectively) have been achieved in packed-bed and fluidized-bed reactors (Grishin and Tuovinen, 1988; Kinnunen and Puhakka, 2004). Granular activated carbon has been the best support material for fixed film bioreactors (van der Meer et al., 2007; Nemati et al., 1998; Özkaya et al., 2019). Other tested solid matrices for biomass retention have included sand, glass and resin beads, polyvinyl chloride and ore particles (Tuovinen and Bhatti 1999). Precipitation of jarosite type secondary phases has been reported to promote biomass retention as the precipitates serve as a porous matrix for bacteria (Nikolov and Karamanev, 1987; Karamanev, 1991; Kinnunen and Puhakka, 2004).

Reactor or tank leaching (Figure 3A) has been used mostly for concentrates whereas heap (Figure 3B), dump, stope (Figure 3C) and *in situ* leaching (Figure 3D) methods are more suitable for low-grade ores (Figure 4). Vat leaching allows the processing of larger particle size materials than reactors, and thus can also be used for low grade ores. In heap leaching, the ore is crushed, often also ground, and agglomerated with H_2SO_4, and piled on a water-impermeable leach pad. The heaps are aerated from the bottom. The leach solution is sprayed or irrigated on the top and allowed to percolate to the bottom of the heap where it is collected as a pregnant solution for the recovery of dissolved metals (Figure 3B). Dump leaching is similar to heap leaching but the dumps are often not aerated and the process is less controlled. In stope leaching a stope is filled with broken ore and sealed with concrete bulkheads (Figure 3C). The stope is flooded, drained and allowed to rest periodically. Flooding, draining and rest cycles are repeated (Chien et al., 1990). In the *in situ* leaching process the ore is not removed from the ore body. Leaching solution is injected into the subsurface ore body, which is usually not fractured, and pregnant leaching solution is collected from production wells for recirculation or metal recovery (Figure 3D). In-place leaching is similar to *in situ* leaching but the ore body is fractured by blasting to improve the permeability before the leaching (Wadden and Gallant, 1985).

A schematic flowsheet of possible contributions of microorganisms in uranium solubilization in the indirect *in situ* leaching with the two-stage approach is shown in Figure 4. The two-stage process allows separate optimization of the conditions for both stages and facilitates the removal of excess iron and sulfate should that be desired. *In situ* leaching is especially suitable for ore bodies which are not economic to mine by conventional open-pit or underground methods. It does not usually require extensive mine infrastructure and may reduce the visual impact of the mining operation. However, *in situ* leaching

requires very long contact times and well developed and characterized permeability of the ore body. For both *in situ* and in-place leaching especially, extensive knowledge of the hydrology and geology of the underlying and adjacent area is needed, and careful control and volumetric mass-balance of leaching solutions are required to prevent contamination of underlying aquifers.

In situ leaching is an established technology in many uranium producing countries (Mudd, 2001a, 2001b; Lottermoser, 2010; Campbell et al., 2015). The mines use either dilute sulfuric acid or alkaline solutions (carbonate or bicarbonate) for uranium extraction depending on the accessory minerals in the fractured ore body. Pyritic uranium ores with <2% carbonate content are typically subject to sulfuric acid leaching in the initial range of 15-25 g acid/l according to some estimates (IAEA, 2001, 2016). Ferric iron in acid *in situ* leach solutions are regenerated with oxidants such as nitric acid, hydrogen peroxide or pyrolusite. There is no specific information on the presence or use of iron and sulfur-oxidizing bacteria in *in situ* uranium leaching operations (Zammit et al., 2014). Microbial community analyses of acidic mine waters from *in situ* uranium leaching indicate resemblance to bacteria commonly found in acid mine drainage (Coral et al., 2018). In the past, *in situ* acid leach uranium mines in Eastern Europe especially were controversial and abandoned as the Cold War ended. They have left an environmental legacy and liability of surface contamination and polluted groundwater that extend for decades because of poor practices, lack of regulation, and indifference toward environmental protection during active mining.

4.3 Examples of commercial uranium mines and potential microbial involvement

4.3.1 Australia

In the early 1950's, there was a push to develop uranium production in Australia because of increased demand (Stewart, 1967a, 1967b). The Radium Hill uranium plant (South Australia) was the first uranium plant in Australia, starting in 1906, operating intermittently, and commencing once more in 1954-1961. The ore contained uranium mainly as davidite, which is a complex oxidic mineral containing some rare earth elements (Ce, La, and Y) in addition to uranium. The Rum Jungle uranium plant (Dyson's ore, White's ore, and Rum Jungle Creek South ore, all in Northern Territory) operated from 1954 to 1971. Leach piles were constructed for low-grade uranium ores and they were leached with sulfuric acid (Andersen and Allman, 1968; Lowson, 1975). No effort was made to direct the sulfuric acid leaching toward a bioleaching type circuit. Pitchblende was the main uranium mineral, associated with carbonaceous slate while the overburden contained pyrite mineralization. Copper was also recovered from most ore deposits. South Alligator River Valley (Northern Territory) had 13 uranium mines in 1954-1965. Pitcblende was the main source of uranium in the mined areas and gold was recovered as a by-product. The Mary Cathleen uranium plant in Queensland operated in 1958-1968 and 1972-1982. The ore contained uraninite as the main mineral, which was altered to various other uranium phases in the oxidized zone. Uranium was mined with the open-cut method, which amplified subsequent pollution problems because of the site exposure to acidophilic bacteria and monsoon climate. Sulfuric acid leaching of uranium was practiced in all plants. Mine closures became inevitable in the face of prominent environmental pollution problems (Davy, 1975).

Although bioleaching trials at the mine sites were not initiated, the subject has been under scrutiny for decades in Australia (Lowson, 1972, 1975). Bioleaching of samples from a Rum Jungle uranium mine (Dyson's ore) and Mary Cathleen mine was tested in 6 kg batches in column systems (effective depth 61 cm) (Miller et al., 1963). Up to 80% uranium was leached from the samples with 10% pyrite addition. Ferrous sulfate supplementation also resulted in similar yields. Samples from the Rum Jungle site especially yielded acidophilic iron- and sulfur-oxidizing bacteria in later investigations (Babij et al., 1980; Goodman et al., 1981a, 1981b), which were largely prompted by the environmental problems resulting

from mine closures. Native microorganisms at the mine sites produced acid and dissolved metals from exposed sulfide minerals in flooded open cuts, tailings and overburden heaps (Babij et al., 1981). The monsoon climate promoted the seepage, acid mine water formation, and drainage problem (Davy, 1975; Harries and Ritchie, 1981). Initial attempts to mitigate the Rum Jungle site and acid drainage were inadequate and the Federal Government instituted a more comprehensive rehabilitation program in the 1980's (Allen and Verhoeven, 1986; Mudd and Patterson, 2010). To date, all the uranium plant sites from the 1950's are still under reconnaissance to continue monitoring and abatement.

None of the three currently operating uranium mines in Australia intentionally uses bioleaching for uranium extraction (Mudd, 2014; Pizarro et al., 2017; Energy Resources of Australia Pty Ltd, 2018a, 2018b). The Ranger Uranium Mine in Northern Territory commenced operation in 1981 (Energy Resources of Australia Pty Ltd, 2018a) and is facing closure within the next few years. The mine utilizes ore from an unconformity deposit, containing uraninite (UO_2), with minor coffinite ($U(SiO_4)_{1-x}(OH)_{4x}$) and brannerite ($(U,Ca,Ce)(Ti,Fe)O_6$) (Pownseby and Johnson, 2014; Skirrow et al., 2016). Ground ore is leached in tanks with sulfuric acid, and uranium is recovered by solvent extraction with kerosene tertiary amine, followed by stripping with ammonia and precipitation as ammonium diuranate. The precipitate is converted in a furnace to uranium oxide U_3O_8 (Energy Resources of Australia Pty Ltd, 2018b).

The Olympic Dam Mine in South Australia produces copper (chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4), and chalcocite (Cu_2S)) and uranium (pitchblende (UO_2)) as well silver and gold. Copper is primarily recovered by copper sulfide flotation of finely ground ore (Edwards and Oliver, 2000). Silver and gold are separated from the waste before production of uranium concentrate. Uranium and remaining Cu are leached in sulfuric acid and further separated by solvent extraction. Uranium is converted to yellow cake and uranium oxide, while copper is recovered by electrowinning (Edwards and Oliver, 2000).

Beverley Mine in South Australia has a sandstone-hosted deposit with predominantly coffinite and minor amounts of uraninite (Pownseby and Johnson, 2014) confined by clays above and below (Taylor et al., 2004). Pyrite and other sulfides are less abundant (Märten, 2006). The mine commenced Australia's first *in situ* recovery operation in 2000. The process utilizes sulfuric acid lixiviant with hydrogen peroxide as a chemical oxidant for ferric iron regeneration (Taylor et al., 2004). Uranium is captured with anionic ion exchange, eluted with sulfuric acid and salt, and precipitated with hydrogen peroxide after neutralisation with caustic soda. The precipitated uranium is then thickened and washed to remove impurities, dewatered and dried (Taylor et al., 2004; Märten, 2006).

Australia's second uranium *in situ* recovery operation was commissioned at Honeymoon Mine in South Australia in 2011 (Mudd, 2014; Boss Resources Limited, 2019), but in 2013 the mine was placed in care and maintenance due to low uranium prices and high operating costs (World Nuclear News, 2013). Similar to Beverley, Honeymoon also has a sandstone-hosted deposit, but aquifer salinity in Honeymoon is considerably higher (Taylor et al., 2004; Pownseby and Johnson, 2014). The mine used sulfuric acid leaching followed by solvent extraction with a tertiary amine extractant in an organic diluent (Taylor et al., 2004; Mudd, 2014).

The Yeelirrie deposit in Western Australia has vanadium-rich potassium carnotite ($K_2(UO_2)_2(VO_4)_2$ 1-$3H_2O$) present in calcrete material associated with clay-rich carbonated rocks (Pownseby and Johnson, 2014). The deposit changed ownership from BHP Billiton to Cameco in 2012 (Cameco, 2018a) and plans are underway to utilize open cut mining with alkali leaching and direct precipitation alkaline leaching, counter current decantation, and direct precipitation of U_3O_8 (Cameco, 2018a, 2018b).

4.3.2 Brazil

Uranium production in Brazil was started in 1982 at the Poços de Caldas mine (Amaral et al., 1985; Fernandes and Franklin, 2001). Uranium was associated mostly with pitchblende, coffinite, autunite, and tobernite and was extracted from the ore at 70 °C with sulfuric acid and MnO_2 as an oxidant (Fernandes *et al*., 1996). The mine was closed in 1997 and has since been decommissioned. As with sulfide and coal mines, acidophilic iron- and sulfur-oxidizing microorganisms can be readily enriched from mine waters in Brazilian uranium mines and deposits (Garcia, 1991, 1993; Campos et al., 2011). Such acidophiles are a resource for bioleaching experiments with uranium ores but also a cause of concern for the long-term remediation of the tailings area. Laboratory bioleaching experiments with uraninite-containing ore from the Figueira mineralization were piloted with 800 tons of crushed ore (-10 in.) and 200 m^3 leach solution (Garcia and de Brito, 1984; Garcia, 1993). The experiments demonstrated positive results for acid bioleaching of uranium and enhancement by ferric sulfate, but the bioleaching project was not sustained to commercial output. Uranium production in the Caetité mine (Bahia) was started in 2002 and the ore is leached in heaps with sulfuric acid (Fernandes et al., 2006). Processing of uranium ore by heap leaching was started at the Lagoa Real (Bahia) mine in 2000 (Carvalho et al., 2005). In efforts for self-sufficiency, work at the new Engenho uranium mine (Bahia) started in 2017 and is now online for yellowcake production. Another one, Santa Quitéria (Ceará), is to start in 2020 for phosphate and uranium production (Filho et al., 2009). Sulfuric acid continues to be the lixiviant of choice.

4.3.3 Canada

In the early 1960's, bioleaching processes were applied to the commercial-scale extraction of uranium by heap, dump and stope leaching of mine waste rocks and worked-out stopes in uranium mines in the Elliot Lake area, Ontario, Canada (Campbell et al., 1985, 1987; McCready and Gould, 1990). Uranium-bearing ore bodies in this region are pyritized quartz pebble conglomerates. Uranium mineralogy is variable, comprising brannerite, uraninite, pitchblende, some monazite, and minor coffinite, thucolite, uranothorite, and uranothorianite (MacGregor, 1969a, Robertson and Gould, 1983). Underlying test work was undertaken in several research laboratories (Fisher, 1966; Harrison et al., 1966a, 1966b; MacGregor 1966, 1969a, 1969b; McCreedy et al., 1969; Duncan and Bruynesteyn, 1971a, 1971b; Manchee and Garrett, 1974; Derry et al., 1976; Manchee, 1977). The surge in uranium demand during the Cold War in the 1950's resulted in the construction of 12 uranium mines in the Elliot Lake area between 1955 and 1958 (Goode, 2013). Subsequently the demand slowed down and several uranium mines were phased down and eventually closed. In the 1970's the international oil crisis shifted energy production to nuclear power plants, leading transiently to increased demand from the Canadian uranium mines (Goode, 2013). Among the uranium leaching operations in the Elliot Lake area were the Stanrock Mine, Denison Mine and, later, Agnew Lake Mine. In the 1980's it was recognized that the demand for nuclear fuel was overestimated, causing global uranium supply to exceed the demand. Other uranium mines with chemical processing were also started, for example in Saskatchewan (e.g., the McArthur River and Cigar Lake uranium mines). Eventually the remaining uranium mines in the Elliot Lake area could not economically justify their operation, leading to mine closures. Some ore deposits in the Elliot Lake area also contained thorium and rare earth elements (yttrium especially), and their recovery afforded additional operation and production in several mines (Goode, 2013). Saskatchewan is now the only province in Canada that has uranium mining ongoing.

The Stanrock Mine initiated a bacterial leaching circuit in 1964. This involved washings of underground stopes and other workings on shifts as well as sprinkling of waste heaps (MacGregor, 1969a). The operation tested nutrient effect by adding mineral salts to the leach solution, but no enhancement in

uranium dissolution was noted (Duncan and Bruynesteyn, 1971a). Following clarification to remove suspended fines, the pooled solutions were routed to ion exchange followed by elution and precipitation as yellowcake. The Stanrock Mine also recovered yttrium from the leach solution. The mine closed in 1985 (Goode, 2013).

The Denison Mine in the Elliot Lake area, Ontario, practiced underground stope bioleaching in the 1980's and early 1990's. Prior research with pyrite oxidation (Napier et al., 1968) and column leaching with regenerated Fe^{3+} in small scale and in 330 kg batches of ore (Derry et al., 1976) suggested that more uranium can be dissolved using acid ferric sulfate and bacteria than by purely chemical acid leaching. The bioleaching process at the mine employed fracturing of the ore followed by construction of a concrete bulkhead across the opening of a horizontal shaft. The ore behind the bulkhead was flooded with leach solution and, after a period of up to three weeks (at 12-15 ºC), the leach solution was drained and pumped to the surface for uranium recovery by ion exchange (McCready and Gould, 1990). This cycle was repeated until the amount of uranium leached declined to a level that was no longer economically viable (Wadden and Gallant, 1985). Numerous iron-oxidizing bacterial isolates have been obtained from Denison mine samples and their characterization has revealed variation in metal resistance and suboptimal temperature for iron oxidation (Ferroni et al., 1986; Berthelot et al., 1993, 1997; Leduc et al., 1997).

The Agnew Lake Mine in Ontario, Canada, was considered to be the first operation where the uranium bioleaching process was applied to a virgin ore body (Figure 6). Uranium was mineralized as uranothorite [$(Th,U)SiO_4$] and monazite [$(Ce,La)PO_4$], with lesser amounts of uraninite, in pyrite- and pyrrhotite-rich conglomerate quartz (SiO_2) pebbles (McCready and Gould, 1990). The ore also contained cubanite ($CuFe_2S_3$) and pyrrhotite ($Fe_{(1-x)}S$), and rare earth element mineralization has also been reported. The mine started production in 1971 and employed surface heap leaching of the swell from ore breakage and a circuit for underground stope leaching (Edwards, 1992). The mine did not have a mill for the comminution of the blasted ore particles. Ore fracturing was moderately orchestrated because of concerns on escape of the leach solution to groundwater. Stope leaching was not sustainable because the fractures in the underground stopes were insufficient to promote contact between uranium mineralization and leach solution. The surface heap leaching did not have sufficient contact time because of large ore pieces. Subsequent tests with flood leaching underground did not justify continuation. The height of the operation was from 1977 to the closure in 1983, which was dictated by low uranium recoveries. The site was decommissioned during the ensuing five years and turned over to the province of Ontario in 1988 (Edwards, 1992; Goode, 2013). Subsequently the ownership has changed with renewed interest in the site because of prospects for additional metals in the mineralization.

4.3.4 Finland

Uranium containing accessory minerals and phases occur in many sulfidic ores that are mined for other metals. An example is the heap bioleaching operation at the Terrafame Mine (formerly known as Talvivaara Mine) in Finland (Figure 7). It was started in commercial scale in 2008 for Cu, Co, Ni, and Zn production from black schist, but has since expanded to Mn recovery (Riekkola-Vanhanen, 2010, 2013) and the mine has plans to commence uranium production in the near future. The main sulfide phases hosted by black schist are pyrrhotite ($Fe_{1-x}S$), pyrite (FeS_2), sphalerite ((Fe,Zn)S), pentlandite ($(Fe,Ni)_9S_8$) and altered pentlandite ($(Fe,Ni,Co)_9S_8$), chalcopyrite ($CuFeS_2$) and alabandite (MnS) (Luukkonen and Ruokola, 2011). External Fe is not supplied because the Fe-sulfides in the black schist dissolve to produce acidic ferrous sulfate.

Uranium in the black schist ore is associated with thucholite, which usually has a core of uraninite

599 flanked by pyrobituminous hydrocarbons. The black schist contains on average 17 ppm U (0.0017% U)
600 (Pohjolainen, 2015), with estimates ranging between 10 and 30 ppm and 19 and 50 ppm U (Luukkonen
601 and Ruokola, 2011; Lecomte et al., 2014). Although the concentration in the ore is low, dissolved uranium
602 concentration increases due to recirculation of the leach solution. Thus, uranium can be economically
603 recovered from the leach solution as part of the process cycle. Uranium recovery would also benefit
604 environmental management because it would decrease the concentration of uranium in discharge water.
605 Terrafame Ltd. applied for a permit for uranium recovery in October 2017 and a permit was issued by the
606 Finnish Government in February 2020 (Terrafame, 2020). Plans are in place to commence uranium
607 recovery at the Terrafame Mine within one year, but the Government decision is still subject to appeal at
608 the Supreme Administrative Court of Finland, which may cause additional delay.
609
610 Finland has several ore deposits that contain uranium, but the deposits are low grade and relatively
611 small (Pohjolainen, 2015). Uranium in these low-grade rocks and deposits are primarily associated with
612 apatite and other phosphoritic mineralizations with predominance of various silicates such as quartz and
613 quartzite, muscovite, and plagioclase gneiss. Iron sulfides are usually rare. Uranium bioleaching has been
614 tested in shake flasks with samples of rock and ore deposits from four locations (Kesänkitunturi,
615 Nuottijärvi, Temo, and Vihanti). In general, uraniferous phosphorites requite additional iron source, either
616 in the form of pyrite or ferrous sulfate, to sustain microbial activity (Tuovinen et al., 1983). Most uranium
617 in these samples dissolve via acid attack that can employ biologically produced sulfuric acid (Tuovinen and
618 Hsu, 1984). Because much of the uranium in these samples was already in the oxidized form, the contact
619 time needed for uranium dissolution was as short as 24 h cycles for finely ground samples (-200 mesh).

620 *4.3.5 Portugal*

621 Commercial processes for the leaching of uranium from low-grade ores have been operated in
622 Portugal since the 1950's (White and Smith, 1969). More than 60 uranium mines, mostly small, were in
623 operation as open pits and underground mines or their combinations at the height of the national
624 program in Portugal, largely credited to the contract with the U.K. Atomic Energy Authority to provide
625 uranium for nuclear armament as the period of the Cold War was beginning in the late 1940's (White and
626 Smith, 1969). Some Portuguese uranium was also used in the U.S. nuclear industry.
627
628 In Portugal, some uranium heap leaching processes with dilute sulfuric acid involved conditions that
629 were conducive to auxiliary enhancement due to bioleaching. The Urgeiriça uranium mine had the largest
630 mill and chemical plant in Portugal, and uranium ores from small nearby mines were also transported to
631 Urgeiriça mill for processing (Pereira et al., 2014; Abreu and Magalhães, 2017). The mine was initially a
632 ^{226}Ra plant operated from 1913 to 1944. Uranium mining with chemical processing commenced after
633 World War II (Pereira et al., 2014). Heap leaching of uranium was practiced at the Urgeiriça mine in the
634 mid-1950's but the yields of uranium dissolution remained lower than expected. Subsequently it was
635 discovered that some uranium had already leached from the stockpile before the leaching process was
636 initiated, and this was attributed to exposure to rainwater (Cameron, 1963; Lowson, 1975; Carvalho et al.,
637 2016). Consultant research at the National Chemical Laboratory in the U.K. showed that the rainwater
638 effect in stockpiles was due to the action of iron- and sulfur oxidizing bacteria, which increased the yields
639 of uranium dissolution multifold in column leaching experiments (Miller et al., 1963). Thus, research in
640 the U.K. with Portuguese uranium ore samples (Urgeiriça, Bica, and Valhos mines) provided preliminary
641 information on enhancing effects of bacteria in the leaching process (Miller et al., 1963).
642
643 In the chemical leaching circuit for finely ground ores, uranium was mainly associated with
644 pitchblende and uraninite and the ore (particle size 50% -70 μm) was processed with sulfuric acid leaching

(pH 0.5-0.9) at 40 °C with MnO_2 as the oxidant. Since the 1970's the Urgeiriça mine intensified the effort on the heap leaching operations with sulfuric acid for low-grade uranium ore, and *in situ* leaching (also termed static leaching) by injecting sulfuric acid into low-grade ore was also introduced in the late phase to recover uranium from abandoned galleries. No information is available on bacterial activities or distribution at the Urgeiriça mine. The mine phased down the production in 1991 for the final closure and decommissioning (Pereira et al., 2014; Pinto et al., 2016; Abreu and Magalhães, 2017), and the mine ceased the operation completely in 2001.

The Bica mine in Portugal practiced underground leaching of uranium between 1951 and 1977, followed by the preparation of the mine for sulfuric acid leaching of the underground excavations. The excavations were leached *in situ* by the injection of acidic leach solutions, which were collected in the bottom floor (-250 m). This static method of leaching was continued until the late 1980's. The mine was subsequently flooded and the treatment of mine water now includes neutralization and barium precipitation of ^{226}Ra (Carvalho et al., 2011, 2016).

4.3.6 South Africa

Uranium processing in the Witwatersrand Basin, South Africa was started in the 1950's as a by-product from gold mining (Finney, 1971; Kenan and Chirenje, 2016). The deposit is relatively abundant with pyrite in conglomeration with uraninite (Pretorius, 1974). The uranium plant employed chemical leaching with sulfuric acid, ion exchange, solvent extraction and ammonium precipitation and thermal conversion to produce U_3O_8 (Matic and Mrost, 1964). Uranium levels were in the range of 100 to 300 ppm. For Au-U ores, uranium was leached first before the ore was processed for gold recovery (Matic and Mrost, 1964). The production of uranium declined substantially in the 1980s, eventually with only three mines left producing uranium (Kenan and Chirenje, 2016). Samples of tailings (slimes) from Witwatersrand have been evaluated over the years for acid bioleaching of uranium (Matic and Mrost, 1964; Mrost and Lloyd, 1971). The oxidation of pyrite in the slimes was key to producing acidic ferric sulfate for contact with low-grade uranium bearing minerals. Uranium recoveries from the slimes using the bacterial leaching with ferric sulfate were in excess of 90% at a hydraulic loading rate of 10 cm/month, but uranium dissolved from the slimes could not be captured quantitatively from the solution because of the vertical movement of water in the dams during wet and dry months (Matic and Mrost, 1964). Heap leaching of Witwatersrand tailings in a small scale (1 m^2, 0.3 m depth) yielded recoveries of 87% in 60 days (Livesey-Goldblatt, 1986). Bioleaching was not commercialized for uranium production in the advent of the world-wide decline in uranium demand and the overstocking in South Africa in the 1980-1990's (Kenan and Chirenje, 2016). Present plans are underway to increase uranium production in the Witwatersrand Basin operations and to open new production in the Karoo Uranium Province and the Namaquala region (Kenan and Chirenje, 2016). The new production sites are based on chemical processes and no specific bioleaching plans are in place at this time.

4.3.7 Sweden

Sweden has large alum shale deposits that contain uranium up to 100-300 ppm, possibly having 11% of the global U resources (Beeson and Goodall, 2014; Lecomte et al., 2017). These are pyritic black shales that comprise kerogenic organic material, micas, graphite, quartz, and feldspar (Armands 1970, 1972; Andersson et al., 1985; Beeson and Goodall, 2014). Uranium is associated with the kerogenic fraction and micas. In the 1950's, as the country established the Swedish Nuclear Power Program and was planning to develop independent nuclear power, chemical processing of uranium-containing shale was started at a pilot plant scale at Kvarntorp (near the city of Örebro) in 1953. Several leaching techniques were tested

in the pilot system. Commercial scale processing was started at a plant in Ranstad in 1965, using -2 mm shale and leaching with $FeCl_3$ and sulfuric acid (Hormander and Gelin, 1963; Peterson, 1967a, 1967b). The leach solution was reduced with Rongalite (sodium formaldehyde sulfoxylate) in order to precipitate uranium as a $U^{4+}U^{6+}$-phosphate complex, and ferrous iron was re-oxidized with chlorine gas. Uranium was sold in the open market, mostly to the U.S. as the plans to develop nuclear power independence were abandoned. The mine and mill were closed in 1969, but plans were to recommence the processing in the advent of the oil crisis (Andersson, 1976). Eventually, these plans did not materialize due to public opposition and protests locally and nationally. The national referendum in 1980 caused a change of the national policy on nuclear power. Subsequent decommissioning and monitoring of the mine site including the tailings, briefly described by Ehdwall (1996), Ledin and Pedersen (1996), and Sundblad (2000), are ongoing. Tailings at Ranstad are geochemically not stable and continue to release metals due to microbial action (Kalinowski et al., 2004, 2006; Edberg et al., 2010).

Regions of interest in Sweden for further testing of samples and development are Tåsjö and Myrviken in Jämtland and, possibly, still also Ranstad (Mount Billingen) in Västergötland. Preliminary results indicate that up to 90% U can be solubilized in bioleaching experiments (Beeson and Goodall, 2014), but the details have not been documented in public. Acidithiobacilli as well as eukaryotic microbes (fungi) have been enriched from Billingen samples (Napier and Wakerley, 1963). The deposit is also a potential source of Ni, Zn, Mo and V. Very preliminary test work on uranium shale bioleaching in a three-stage experimental design was reported by Björling (1973). The first stage (33-50% pulp density) was the bacterial oxidation of pyrite at pH 2.0 to produce ferric sulfate, which was used as the oxidant in the following two chemical leaching stages. In shake flask and stirred tank bioleaching experiments of a uraniferous black shale sample from Tåsjö (Bhatti, 2015), metals were released primarily due to acid dissolution. The large kerogen fraction (>10% in the Tåsjö samples) is relatively recalcitrant and may be problematic in a large scale leaching system due to its partial dissolution and organic residue. The large organic fraction can act as a major reductant of ferric iron. Prospecting for uranium deposits has continued in the Jämtland and Lapland regions (Beeson and Goodall, 2014).

5. Bench-scale feasibility and optimization experiments

Uranium bioleaching experiments have employed numerous experimental designs to address factors that influence the outcome of the leaching. Most of the experimental techniques for testing the bioleaching of ore samples and microorganisms have summarized by Rossi (1990). Some studies have used response surface methodology for statistical optimization of uranium bioleaching (Jalali et al., 2019; Mo et al., 2019; Zhou et al., 2019b). Examples of shake flask, column and tank bioleaching experiments are summarized in Supplementary Tables S1-S3, and bioleaching yields from the various studies are illustrated in Figure 8. In the literature, the experimental conditions in shake flasks experiments have been variable and have had little commonality for comparative purposes. The particle size fractions have been too fine to represent a commercial scale leaching process. The shake flask technique is usually considered as a screening tool to assess the feasibility of the acid bioleaching. With phosphate minerals the technique can promote uranium dissolution within <24 h, but with other types of uranium mineralization the time course can extend to several weeks (Supplementary Table S1). Museum grade uranium minerals can dissolve much faster than uranium from polymetallic ores or rock samples because of the lack of uranium masking or encapsulation by other minerals.

Column tests have also varied extensively. An example of column bioleaching of a uraninite-containing ore is shown in Figure 9 (Zare Tavakoli et al., 2017a). Laboratory feasibility and optimization studies rarely address nor simulate scaled-up designs and commercial-scale operational parameters. The gap between

735 the laboratory studies and mine practice can be extraordinarily difficult to reconcile. Hence, pilot and
736 demonstration scale experiments are typically required to derive reliable design parameters for large scale
737 processes. Wang et al. (2017) used a 4854 ton heap to explore heap bioleaching of uranium from a low-
738 grade ore (U-content 0.082%). The dominant minerals were quartz, potash feldspar, pyrite, hematite,
739 fluorite and calcite (Wang et al., 2017) (Figure 10). The efficiency of uranium bioleaching can be influenced
740 by a number of process factors, such as leaching method, dimensions of the leach system, irrigation (Zare
741 Tavakoli et al., 2017b), aeration and mixing rate, pulp density and residence time (Wadden and Gallant,
742 1985). The efficiency of uranium bioleaching is also affected by a number of mineralogical,
743 physicochemical and microbial factors (Table 2). Some of the key factors are discussed briefly below.

744 ***5.1 Mineralogical factors***

745 The potential of uranium bioleaching is usually considered particularly amenable to low-grade uranium
746 ores with a threshold content of about <0.05% U_3O_8 (<0.042% U). Uranium mineralizations vary in ores
747 that can be leached with acid and ferric sulfate. In general, oxides, phosphates, sulfates and carbonates
748 are more readily leached in acid solutions as compared to silicates (Muñoz et al., 1995a, 1995b, 1995c).
749 Based on bioleaching tests with synthetic mineral specimens, Yang et al. (2019) reported that the
750 leachability of uranium minerals decreased in the following order: pitchblende ≈ uraninite > coffinite >>
751 brannerite > betafite. Comparative ranking of native uranium minerals for bioleaching is not available
752 from the literature data because they are difficult to standardize due to differences in experimental
753 variables such as surface area, conglomeration, and ancillary minerals. Conglomerates of uranium and
754 other minerals rather than single uranium bearing phases are common in low-grade ores. Although pure
755 uranium minerals have been tested in bioleaching studies, uranium ores in leach mines rarely involve
756 only a single uranium-bearing mineral. The presence of both U^{6+} and U^{4+} in uranium-bearing ores is not
757 uncommon. For example, Abhilash et al. (2010) evaluated the column bioleaching of a low-grade silicate
758 ore of uranium, which contained ferrosilicate and magnetite as the major phases. Uranium was present
759 as uraninite (UO_2) in the ore with 38-40% as U^{4+} and the rest as U^{6+}. Uranium solubilization amounted to
760 59% and 57% in 40 days at pH 1.7 and 1.9, respectively (Abhilash et al., 2010).
761
762 In general, bioleaching of uranium has been mainly applied to ores that contain accessory Fe-sulfides
763 such as pyrite as they provide Fe^{3+} and acid upon bacterial oxidation. Laboratory studies have also been
764 reported for the bioleaching of uranium from sandstone deposits (Bosecker and Wirth, 1980; Bhatti et al.,
765 1989; Bhatti and Malik, 1997). Because sandstone deposits are deficient in sulfide minerals, supplemental
766 Fe- and S-compounds are required to promote the regeneration of Fe^{3+} and the production of sulfuric
767 acid. Similarly, the bioleaching of a relatively pure uraninite mineral, which was devoid of sulfide minerals,
768 required a supplemental source of iron for lixiviant production and enhancement of uranium solubilization
769 (Bhatti et al., 1998).
770
771 The Elliot Lake area has uranium mineralizations associated with pyritic conglomerates or other Fe-
772 sulfides. Uranium deposits in the southwest U.S. generally have relatively low pyrite contents, and in these
773 cases the addition of pyrite has beneficial effects on the bioleaching of uranium (Muñoz et al., 1995a, b,
774 c). Chen et al. (2019) also showed enhanced uranium bioleaching from granite-type uranium ore with
775 pyrite addition. Pyrite additions for enhancement of uranium bioleaching have usually been tested in the
776 range of 5 kg pyrite/t for Portuguese and Indian uranium ores and 3 kg/t for Spanish uranium ores (Muñoz
777 et al., 1995a, 1995b, 1995c). Pyrite oxidation produces the chemical oxidant but also serves to satisfy acid
778 consumption of the ore caused by the dissolution of alkaline minerals such as carbonates. The pyrite:ore
779 ratio depends on the mineralogical composition of the ore and on specific characteristics of the additional
780 pyrite. In the case of *in situ* bioleaching, pyrite supplementation is not possible to implement unless pyrite

oxidation is conducted above ground to generate the lixiviant. The control or monitoring of the pH is important because it impacts the solubility of ferric iron and dissolution products and thus controls many dissolution reactions. The initial acid consumption must be taken into account in leaching tests until the microbial oxidation of reduced sulfur compounds starts producing acid.

Gangue minerals impact the uranium bioleaching process. Carbonate minerals and K- and Na-containing silicate phases in host rock consume acid and may cause the formation of precipitates and secondary minerals and retard the oxidation of U^{4+} mineral phases and the solubilization of uranium. Secondary solid phases such as gypsum ($CaSO_4 \cdot 2H_2O$) and anhydrite ($CaSO_4$) can hinder solution flow due to compaction and coating of mineral surfaces, resulting in loss of dissolved uranium. Some mica minerals may also cause channeling because they may transform to expanded phases (e.g., vermiculite {ideal formula $(Mg,Fe^{+2},Fe^{+3})_3[(Al,Si)_4O_{10}](OH)_2 \cdot 4H_2O$)} upon contact with acid leach solution (Bigham et al., 2001; Bhatti et al., 2010).

Because of their acid consumption, carbonates are usually neutralized before acid leaching experiments as otherwise they result in excessive precipitation of ferric iron as well as gypsum. Acidic gangue with quartz (SiO_2) and Al-silicates have less of these problems because they usually have low acid consumption (Muñoz et al., 1995a, 1995b, 1995c). Although not documented for uranium bioleaching situations, excessive dissolution of aluminum and silica from silicates (Potysz et al., 2018) may lead to the formation of amorphous gels and blockages in leach columns and heaps (Dopson et al., 2008) and cause channeling of the leach solution. Excessive dissolved silica may also transfer with dissolved uranium to the tertiary amine extraction phase and then precipitate when the pH is adjusted to a higher value for further processing (McDonald et al., 1981).

The rate of acid consumption and the dissolution of target minerals is a function of the specific surface area of the reactive mineral particles. In shake flasks and stirred tank bioreactors the particle size distribution is typically -200 mesh (<74 μm) to allow suspension in agitated solution. The smaller the particle size, the faster is the reaction kinetics because of the increase in the reactive surface area (Wang et al., 2019b). Small particle size is not economically justified in uranium bioleaching, but shake flask and stirred bioreactor studies can be used as a screening tool for assessing the feasibility of using specific microbial cultures and process conditions and for determining acid consumption during bioleaching (Eisapour et al., 2013). The mineral porosity and the diffusion and permeability of the leach solution are less significant factors when ore particles are stirred as finely ground suspensions in leach solutions as opposed to column tests with larger particle size distribution. The mass transfer of O_2 and CO_2 from the air to the solution phase slows down at high pulp densities, but with up to 20% solids this is considered to be insignificant as a rate-limiting factor. Zokaei-Kadijani et al. (2013) evaluated the effects of process parameters on the oxygen mass transfer coefficient in stirred tank reactors during uranium bioleaching by *A. ferrooxidans*. Although the coefficient was dependent on agitation speed, aeration rate and pulp density, the rate limiting factor was the biochemical reaction; i.e., oxygen uptake by *A. ferrooxidans* for ferrous iron oxidation. Wang et al. (2017) concluded that uranium bioleaching was under diffusion control in a 4854-ton pilot-scale heap experiment that resulted in 88% uranium dissolution over 85 days (particle size -8 mm) (Figure 10). Kinetic modeling is complex because of the heterogeneous reactions and the relatively rapid leaching of uranium from fissures and pyritic phases as opposed to the slow dissolution of encapsulated minerals (Wang et al., 2017).

With column tests and pilot and commercial scale heaps as well as underground stopes the reactive surface area (i.e., the particle size distribution) combined with mineral intergrowth and encapsulation can be one of the rate-limiting factors. The permeability must allow the seeping of leach solution to wet all surfaces, but this rarely is the case except in flood leaching. The formation of secondary minerals such as

jarosite-type precipitates, elemental sulfur, gypsum, or mixed interlayer micas can cause changes in the permeability. They may also limit the diffusion of O_2 and CO_2 in the interior layers to reactive sites. The lower zones especially may have poor contact with leach solution due to poor distribution and channeling of the solution and evaporation if the heap is aerated from the bottom. Column leaching in bench-scale can still have probes for monitoring purposes, but this is increasingly difficult in commercial-scale leaching operations. Heap construction, with installation of thermal probes, is limited to particle distribution that allows even solution flow and averts channeling. For example, the Talvivaara (nowadays operated by Terrafame Ltd.) surface heaps are constructed with crushed, agglomerated ore particles with a size of -8 mm (Riekkola-Vanhanen, 2013). The Agnew Lake heap leaching system was targeted for -7.6 cm size (run 1of mine and crushed) and the stope leaching for -20 cm size (rubblized run of mine) range (McCready and Gould, 1990), but neither size promotes good contact and penetration of the leach solution. Comminution with blasting and crushing is a cost factor that must be justified as a compromise against predicted leaching kinetics.

5.2 Physicochemical factors

A number of physicochemical factors, such as temperature, pH, redox potential, and the concentration of dissolved oxygen, CO_2, uranium, Fe^{2+}, Fe^{3+} and other metals affect the efficiency of uranium leaching (Table 2). In open heap bioleaching processes the prevailing temperature is a function of the climatic factors, the intensity of heap aeration and the presence of Fe-sulfide minerals. Depending on the heap design and scale, intense oxidation of Fe-sulfides can heat the inside of the heap up to the range of thermophilic microorganisms (50-70 °C), creating a temperature gradient from outside to inside. High temperatures prohibitive to thermophiles can be controlled by increased aeration, but it may also stimulate bacterial oxidation activity. Additionally, aeration blows off humidity, which is essential to bacterial activity. Liquid flow, aeration rates and the amount and oxidation of Fe-sulfides all affect heat generation and convection and are the most important factors involved in controlling the temperature inside bioleaching heaps (Liu and Granata, 2018).

Nutrient media for culturing acidophilic microorganisms from leach solutions and acid mine water have been formulated and used routinely in leaching tests for many decades (e.g., Silverman and Lundgren, 1959; Tuovinen and Kelly, 1973; McCready et al., 1986; Gonzáles-Toril et al., 2006). Precise quantitative requirements of nutrient ions in liquid media optimized for microbial biomass growth have not been established. Chemical analysis can, of course, detail the elemental content of cell mass but that information cannot be equated to precise solution chemistry. In addition to reduced Fe- and S-compounds as substrates (energy sources), acidithiobacilli need dissolved O_2 and CO_2, N, P, and S sources, Mg and K, and minor and trace requirements for transition metals (at least Fe and Cu, and possibly Ni, Zn, and Co) for catalytic functions and redox properties of several enzymes. Trace nutrient requirements for acidophiles are not substantiated although some media formulations include trace metals. Some iron-oxidizing acidophiles are nitrogen fixers (Norris et al., 1995; Parro and Moreno-Paz, 2004; Tyson et al., 2005; Valdés et al., 2009) but their diazotrophic lifestyle in bioleaching processes is not clear. The addition of ammonium has been shown to enhance iron oxidation in Terrafame bioleach solutions in the laboratory experiments (Ahoranta et al., 2017), suggesting possible N-limitation of iron-oxidizers in the heap. In other studies the concentration of ammonium in bioleach solution has been shown to decrease with contact time, indicating precipitation (Niemelä et al., 1994). In acid sulfate-rich leaching solutions NH_4^+ can be incorporated into ammoniojarosite precipitate $[(NH_4)Fe_3(SO_4)_2(OH)_6]$ and this is not a reversible reaction in oxidative leaching systems. The additions of mineral salts in laboratory experiments have proven to increase iron oxidation rates or leaching yields, whereas similar trials in commercial mines have not produced distinct enhancement. For example, Duncan and Bruynesteyn (1971a) reported on nutrient

supplementation of stope leaching using bacteria-containing solutions that were supplemented with fertilizer N and P to provide inorganic nutrients, but beneficial effects of nutrients on uranium bioleaching were not established unequivocally. Lee et al. (2005) reported that nutrient addition did not significantly increase uranium bioleaching over a two week contact time in batch experiments with a black shale, but such findings do not pertain to large scale bioleaching systems. Trials of nutrient additions in pilot scale leaching of low-grade uranium ores have also been reported by others (e.g., Jayaram et al., 1976; McCready et al., 1986; Dwivedy and Mathur, 1995) but specific, detailed qualitative and quantitative analyses of nutrient effect are absent.

Some nutrient requirements may be satisfied by the dissolution of metals, S, Fe, P, N, organic substances, and micro and trace elements from the uranium-bearing phases and associated sulfide and gangue minerals during the contact with the leach solution, which further defies efforts of defining quantitative nutrient chemistry relevant in bioleaching processes. Organic compounds are not required in media formulations for acidithiobacilli and leptospirilli, but all heterotrophic microbes associated with leach operations require organic substrates. Heterotrophs have been found in acid bioleaching environments and they grow in close association with autotrophic iron- and sulfur-oxidizers, likely by scavenging organic compounds excreted by the autotrophic acidophiles (Johnson and Roberto, 1997). Organic compounds may also originate from surrounding soil as well as residuals from solvent extraction. Whether heterotrophs should be stimulated with readily biodegradable organic carbon amendments is debatable because their respiration competes for oxygen consumption. A beneficial role of heterotrophs in acid bioleaching of uranium has not been established, although mixed culture studies suggest that heterotrophs can consume organic compounds (organic acids especially) that are otherwise or potentially toxic to *A. ferrooxidans* in culture media (Shuttleworth and Unz, 1987; Fournier et al., 1998; Marchand and Silverstein, 2003). Under extreme circumstances heterotrophs may produce organic acids that are detrimental to autotrophic bacteria. The effect of organic acids has only been demonstrated in pure culture studies of iron- and sulfur-oxidizers (Fang and Zhou, 2006; Ren et al., 2009) and their relevance to uranium bioleaching systems is questionable. It is conceivable that, over time, heterotrophic biomass growth in interstitial and intraparticle pore spaces in ore heaps and piles may occupy physical space and interfere with solution flow and chemical and bacterial leaching reactions. Excessive organic compounds may also promote undesired biofouling effects typically caused by the proliferation of heterotrophic bacteria with large amounts of extracellular polymeric substances (such as polysaccharides) as well as fungal biomass, as demonstrated for example in connection of *in situ* bioremediation of uranium contaminated groundwater (Williams et al., 2011). However, biofouling in uranium bioleaching operations has not been documented.

5.3 Microbiological factors

Laboratory experiments of uranium bioleaching have used pure cultures of Fe- and S-oxidizing acidophiles or their mixtures. Enrichment cultures obtained from mine site samples have also been tested successfully. Microbial consortia have been generally considered to be superior to pure cultures in laboratory bioleaching experiments, but this is difficult to document unequivocally. The source inocula have always been variable in bioleaching studies conducted by different research groups. Standard protocols have not been established that would make it possible to compare the bioleaching rates and efficiencies by cultures of different origin and growth history under otherwise identical conditions. Pilot-scale trials at mines may still involve inoculum transfer from the laboratory in an effort to minimize the lag period preceding the active bioleaching stage (Halinen et al., 2012). Acidophilic Fe- and S-oxidizers are indigenous in acid mine drainage and exposed mineral surfaces at mine sites (Riekkola-Vanhanen, 2013), and they may be enriched for in the microbial population during pilot trials (Halinen et al., 2012). In pilot-

and commercial scale operations there is only a limited extent of control of the microbial population, most notably by the supply of Fe and S, and via adjustment of pH and aeration.

Microorganisms vary in their tolerance to uranium, other nuclides and non-radioactive metals that may be present in uranium leach liquors. UO_2^{2+} acts as an uncoupler of CO_2 fixation from the substrate (Fe or S-compounds) oxidation (Tuovinen and Kelly, 1974). Uranyl ion inhibits *A. ferrooxidans* and other bacteria involved in iron and sulfur oxidation at >5 mM concentrations (Tuovinen and DiSpirito, 1984). In general, as with other potentially toxic metals, the resistance to uranium can be increased by successive subculturing of *A. ferrooxidans* in the presence of increasing concentrations of UO_2^{2+}. Growth in the presence of 10 mM UO_2^{2+} has been reported (DiSpirito and Tuovinen, 1982b). Plasmid borne resistance is implicated but the underlying mechanism remains elusive. Culture resistance has been increased to as high as 20 mM (4.76 g U l^{-1}) at least with one strain of *A. ferrooxidans* (Tuovinen and DiSpirito, 1984). Merroun and Selenska-Pobell (2001) reported that uranium resistance varied among *A. ferrooxidans* strains; some were resistant to 8-9 mM U and some did not tolerate more than 2 mM U. Several isolates were sourced from the Agnew Lake Mine leach solutions that contained 0.07-0.92 mM U and 3.28-5.99 mM Th (Tuovinen et al., 1981). Resistance to 2 mM U was developed in one isolate by increasing stepwise the uranyl sulfate concentration in subcultures. *A. thiooxidans*, an isolate from Vulcano (Aeolian Islands) has been reported to have resistance in the range of 5.0-7.5 g U/l (31.5 mM UO_2^{2+}) (Ebner and Schwartz, 1973, 1974). A strain of *A. ferrooxidans* isolated from the same location had a 7 to 10 fold lower resistance to uranium (Ebner and Schwartz, 1973, 1974).

Iron-and sulfur-oxidizing microorganisms have the propensity to develop resistance to dissolved uranium at concentrations relevant to uranium mine leach solutions. Duncan and Bruynesteyn (1971a) observed bacteria in uranium mine leach solutions that contained as much as 12 g L^{-1} U_3O_8 (43 mM U). However, the presence of bacterial cells is not synonymous with their activity. The presence of bacteria in mine water and leach solutions is to be expected in leaching processes. Bacteria may also slough off from surfaces of ore particles where their growth as biofilms may confer higher tolerance to uranium as compared to planktonic cells in the solution.

A. ferrooxidans usually has a broad spectrum of tolerance to metals, but the toxic threshold levels vary with the metal, with Ag^+ and Hg^{2+} being among the most toxic (Figure 11). Fluoride toxicity (HF p*Ka* = 3.2) has also been a concern in bioleaching practices (Brierley and Kuhn, 2010; Peng et al., 2013; Ma et al., 2016; Rodrigues et al., 2016; Mo et al., 2017; Zhou et al., 2019a). Chen et al. (2016) tested column bioleaching of a uranium ore sample (1.8% F) that contained pitchblende, coffinite and a uraniferous silicate phase as well as Ca-fluorite (CaF_2). No toxicity of fluoride was evident in the results that showed 89% uranium dissolution from the ore (-8 mm) in 120 days (Supplementary Table 2). Wang and Qiu (2011) concluded that 1 g/l of F was a threshold concentration for viability of *A. ferrooxidans*. Rodrigues et al. (2016) reported active bioleaching of copper with concurrent dissolution of fluoride of up to 2.5 g/l from biotite and fluorite phases. Peng et al. (2013) studied fluoride toxicity at 20-50 mg F/l in Fe-oxidizing cultures of *A. ferrooxidans*. The toxicity increased with acidity of the medium (pH 2.5-1.5), which may reflect the opposite effect of pH on jarosite formation in the leach solution. Fluoride ions have been shown to partially replace the structural hydroxyl group in jarosite (Gunneriusson et al., 2009). The reason for the 15 to 20-fold difference in the toxic concertation of fluoride between iron oxidation and bioleaching studies is not clear. Fluoride is also an inhibitor of enolase (2-phospho-D-glycerate hydrolyase), which converts 2-P-glycerate to P-enolpyruvate. The inhibition of the enolase activity has multiple effects downstream in the central metabolic pathways. Information to date indicates that the reported toxicity of fluoride to bacteria in bioleaching systems is variable and is influenced by leach solution chemistry and properties of the F-containing minerals and probably also the microbial composition. For example, the

complexation of fluoride with aluminum has been suggested to decrease fluoride toxicity (Brierley and Kuhn, 2010).

In general, the tolerance to toxic ions involves the selection of resistant cells over time that have the genetic traits, possibly involving plasmids or other transmissible genetic elements, to protect them from the toxicity. Stress proteins are likely to be involved as well as uncharacterized proteins, which are upregulated when *A. ferrooxidans* is challenged with uranium (Dekker et al., 2016). Some physical protection is conferred by cellular exopolysaccharides of *A. ferrooxidans* that accumulate uranium from the solution phase (Merroun et al., 2003). As with other microbes, phosphate groups are ligands for uranium sequestration, another detoxification mechanism (Suzuki and Banfield, 2004; Hennig et al., 2009).

A. ferrooxidans and related acidophiles have multiple stress proteins that act as molecular chaperones that mediate correct assembly and folding of proteins in response to adverse conditions such as nutrient starvation and toxic shocks due to heat, extreme pH, toxic metals, and oxyradicals. Some stress proteins act as proteases to degrade unfolded proteins. For example, Hubert et al. (1995) demonstrated the presence of multiple heat and cold shock proteins in *A. ferrooxidans*. Ribeiro et al. (2011) characterized several small (12-43 kDa) heat shock proteins in *A. ferrooxidans* and suggested that some of the genes of heat shock proteins may be inherited via horizontal gene transfer. Seeger et al. (1996) demonstrated that two molecular chaperones responded to phosphate starvation. Responses to oxidative stress in *A. ferrooxidans* were characterized by Bellenberg et al. (2019); this involved multiple barriers to reactive oxygen species such as metal homeostasis, proteins quenching oxyradicals, various repair mechanisms and production of capsular polysaccharides to protect the cells. Various molecular repair mechanisms and stress responses have also been examined for example in *A. caldus* (Guo et al., 2014) and the thermoacidophile *Metallosphaera sedula* (Peeples and Kelly, 1995). As a side note, bioinformatics and synthetic biology have promise as tools for understanding and increasing the tolerance of bioleaching microorganisms to various stress factors such as elevated concentration of metals, salinity, low pH and temperature extremes (Valdés et al., 2008; Campodonico et al., 2016; Gumulya et al., 2017; Osorio et al., 2019; Wang et al., 2019a; Johnson and Quatrini, 2020).

Uranium has relatively high affinity for cellular molecules and biopolymers. Prokaryotic and eukaryotic microorganisms including acidithiobacilli have been shown to biosorb and accumulate uranium from the solution (DiSpirito et al., 1983; Tsezos et al., 1989; Nakajima and Sakaguchi, 1993; Panak et al., 1998; Suzuki and Banfield, 2004; Choudhary et al., 2012). Biosorption refers to a metabolism independent process that occurs through adsorption (accumulation of uranium at the surface) or absorption (penetration of atoms or molecules into the cells), whereas bioaccumulation refers to metabolism-dependent accumulation (Lloyd and Renshaw, 2005). Both living and dead cells are capable of biosorption and ligands involved in binding include amine, carboxyl, hydroxyl, phosphoryl and sulfhydryl groups, while bioaccumulation occurs only when the cells are living and active (Lloyd and Renshaw, 2005; Wang and Chen, 2009). The capacity of uranium sorption onto cell surfaces depends on the number of available binding sites (phosphoryl groups especially), uranium speciation and the properties of the solution (Suzuki and Banfield, 2004; Hennig et al., 2009; Mkandawire, 2013; Acharya 2015). Cationic uranyl species are more easily adsorbed than anionic species as there are generally more negatively charged ligands on cell surfaces. However, in acidic solutions cations such as UO_2^{2+} compete with protons for negatively charged binding sites on the biomass and hence sorption is lower than at higher pH values (Mkandawire, 2013). *A. ferrooxidans* can take up uranium from solution and in the cells uranium has been found to be associated primarily with the cell wall and membrane fractions (DiSpirito et al., 1983). Tuovinen and DiSpirito (1984) reported that the accumulation was influenced by the external uranium concentration and was greater at

pH 1-1.5 compared to pH values 2-4. By contrast, Panak et al. (1998) observed increased accumulation of uranium in two *A. ferrooxidans* strains when pH was increased from 1.5 to 4.0.

In addition to biosorption and bioaccumulation, microorganisms can decrease the solubility of uranium by reduction and precipitation (Lloyd and Renshaw, 2005; Merroun and Selenska-Pobell, 2008). Microbial reduction of soluble U^{6+} to insoluble U^{4+} can decrease the mobility of uranium and help in uranium recovery or remediation. To date, the number of species that are known to be able to reduce uranium are distributed among phylogenetically diverse prokaryotes (Suzuki and Suko, 2006; Choudhary and Sar, 2015). They also include also some acid-tolerant bacteria (Shelobolina et al., 2004). Some of the microbes are able to reduce uranyl carbonate and form U^{4+}-oxide minerals such as uraninite (Suzuki and Suko, 2006). Some of these organisms have been reported to conserve energy for growth when using UO_2^{2+} as an alternative electron acceptor, while others reduce uranium without an energy gain (Lovley et al., 1991; Merroun and Selenska-Pobell, 2008; Wufuer et al., 2017). Moreover, the hydrogen sulfide generated by sulfate reducing microorganisms can also indirectly reduce U^{6+} to U^{4+} in conjunction with precipitation of U^{4+} species (Mohagheghi et al., 1985; Spear et al., 1999).

Extracellular precipitation of uranium can be caused by biogenic phosphate, carbonate, or hydroxide or the consumption of uranium complexing organic compounds (Lloyd and Renshaw, 2005; Merroun and Selenska-Pobell, 2008). Biogenic phosphate can precipitate uranium as H-autunite (HUO_2PO_4) (Macaskie et al., 2000, Merroun and Selenska-Pobell, 2008), autunite/meta-autunite (e.g., calcium autunite $Ca(UO_2)_2(PO_4)_2$ and meta-autunite $Ca(UO_2PO_4)_2$) as well as other mineral phases (Jroundi et al., 2007; Martinez et al., 2007; Nedelkova et al., 2007; Merroun and Selenska-Pobell, 2008) at pH values ranging from 4.5 to 7. This process is based on passive uranium sorption by the negatively charged cell wall extracellular polymers and active secretion of phosphate groups due to a phosphatase activity (Macaskie et al., 1992; Jeong et al., 1997; Renninger et al., 2004; Martinez et al., 2007; Merroun and Selenska-Pobell, 2008). Microbial production of CO_2 and NH_3 increases carbonate alkalinity and solution pH, contributing to the precipitation of uranium with vaterite, a polymorph of calcite in the microenvironment around the cells, and directly onto the surface of bacterial cells (Rodriguez-Navarro et al., 2003, 2007; Merroun and Selenska-Pobell, 2008; González-Muñoz et al., 2010). Alkalinity generating microorganisms can decrease the solubility of uranium as $[UO_2]^{2+}$ forms insoluble precipitates with hydroxide at neutral pH values (Lloyd and Renshaw, 2005). Although biosorption, bioaccumulation and bioprecipitation of uranium may be unwanted during ferric sulfate bioleaching of uranium, these bioprocesses may hold potential for treatment of uranium contaminated waters. In fact, a number of biological approaches have been tested for the recovery of uranium from dilute solutions both as a means of recovery of U and pollution abatement (Tuovinen and DiSpirito, 1984; Li et al., 2004; Wall and Krumholz, 2006; Newsome et al., 2014; Jain et al., 2018b; Zhao et al., 2019; Ohnuki et al., 2020). The cost-efficiency of these bioprocesses must be competitive with traditional methods of uranium separation and removal. New approaches such as functionalised and ligand-based composite adsorbents (Shahat et al., 2018; Awual, 2019a; Awual, 2019b; Jiang et al. 2020) have promise for large-scale implementation. The rehabilitation of mine sites always requires complementary approaches of ecological restoration and revegetation (Hernandez-Santin et al., 2020).

6. Postlude: control of microbial processes and uranium contamination after mine closure

Upon closure of uranium bioleaching operations, decommissioning procedures are required to ensure minimal environmental impacts and risks to human health. Without efficient containment, release of uranium and daughter nuclides from the abandoned mine site and tailings piles can continue long after the mining and milling operations have ceased (IAEA, 1997, 2004; Bernhard et al., 1998). Transport of

radionuclides involves both surface waterways and aquifers as well as airborne particulate matter (IAEA, 1997, 2004; Bernhard et al., 1998). Any post operation procedures at the mine site need to be designed with due consideration of the site-specific biogeochemical, hydrological and climatic conditions.

Many processes and techniques have been reported that can potentially restrict the dissemination of uranium and/or remove uranium from contaminated soil, sediments and water. Biological processes can be useful in some mitigation efforts. For example, the anaerobic reduction of water-soluble hexavalent U^{6+} to poorly soluble tetravalent U^{4+} helps limit uranium mobility in subsurface sediments and aquifers (Williams et al., 2013; Selvakumara et al., 2018; Lakaniemi et al., 2019). Best known in these bioprocesses are iron- and sulfate-reducing bacteria as they are native in soils and sediments. Other U-reducers include for example *Geobacter*, *Shewanella* and *Clostridium* spp. (Gao and Francis, 2008) and *Anaeromyxobacter dehalogenans* (Wu et al., 2006). The anaerobic reduction of uranium must be coupled with anaerobic oxidation of electron donors (e.g., acetate). Many microbes can utilize organophosphates that are hydrolyzed by phosphatases, leading to precipitation of uranium as poorly soluble uranyl phosphates (Figure 1) (Newsome et al., 2014). In general, other approaches involve soil washing with uranium-complexing acids or bases such as citric acid or (bi)carbonates (Francis et al., 1999), phytoremediation with uranium accumulating terrestrial plants (Dushenkov et al., 1997), and use of sorbents such as plant or microbial biomass, biochar, zero-valent iron and activated carbon (Fiedor et al., 1998; Mellah et al., 2006; Jain et al., 2018a). Although not within the scope of this review, large-scale adoption of uranium bioreduction and phytoremediation have shown positive outcomes in environmental trials but control and prediction of uranium plumes is difficult and sometimes not even feasible. Conjugated methods (chemical, physical and biological) are usually the most successful approaches for implementation and remediation of uranium contamination (Li and Zhang, 2012; Malaviya and Singh, 2012; Rosenberg et al., 2016; Selvakumar et al., 2018). Monitoring is an important component in the reclamation. Geochemical modelling can also provide ways to investigate and predict the potential effectiveness of reclamation activities (Williams et al., 2013).

Microorganisms in bioleaching operations are suspended in the solution phase and attached on solid phases and are also associated with tailings and low-grade waste and overburden piles. Mine closure does not the stop the microbial action. The inactivation of microbes is a challenge because iron- and sulfur-oxidizing bacteria continue to produce sulfuric acid and release metals from exposed sulfide minerals in tailings, overburden, and leftovers in the mine. Acidophiles in bioleaching systems are sensitive to high chloride (Shiers et al., 2005; Gahan et al., 2009; Zammit et al., 2012; Bomberg et al., 2018) and nitrate concentrations as well as various organic compounds (e.g., low molecular weight fatty acids, benzoic acid, and C1 to C8 alcohols) (Ballerstedt et al., 2017). Laboratory tests have also identified toxic surfactants (Ballerstedt et al., 2017). Although some potential inhibitors can be embedded in slow-release matrices to ensure extended contact time, their usefulness on-site is debatable because the toxic organic and inorganic compounds tested against the bacteria are water-soluble or at least water miscible and can run off with storm water. The excessively high concentrations also make these compounds problematic to the environment. Silicate and phosphate coatings of pyrite have been developed (Evangelou, 1995; Kargbo and Chatterjee, 2005; Bessho et al., 2011; Kang et al., 2016) that provide a barrier against bacterial oxidation, but, again, their field applications may be rather limited. Preventing access of water and surface layering of exposed spoil or tailings with soil or waste sludge have proven by and large successful, although often impracticable, mitigation approaches in some closed uranium mine sites. Most success has been achieved by increasing the impermeability of affected areas and vegetation of the surface cover by capping the tailings with clay, geo textile membrane, gravel and soil.

7. Concluding remarks

Since the historic times when ore bodies were excavated and exposed to air, humidity and rain, iron- and sulfur-oxidizing bacteria have always been spread in mine water and mineral surfaces. Following about ten years of the first discovery of acidophilic iron- and sulfur-oxidizers (*A. ferrooxidans*), commercial uranium bioleaching using heap, dump and stope technology was practiced already at the turn of the 1960's. Acidophiles in the bioleaching of uranium produce sulfuric acid by pyrite and sulfur oxidation and maintain high redox potential in the ferric iron-based lixiviant by ferrous iron oxidation. Past practices in uranium leaching operations confirm that microbes contributed to the oxidation and dissolution of uranium minerals even when no special effort was made to augment the role.

In the 1960's, the bacteria in uranium and sulfide leaching processes were more or less synonymous with iron- and sulfur-oxidizers as modern methods for analysis of genotypes and phylotypes were not available and archaea were not known to exist. Information on prokaryotic diversity in the environment rapidly expanded in the ensuing decades and microbial life in uranium and sulfide mine environments was understood to involve complex biological, chemical, and physical interactions with cells, solutes and mineral surfaces. Molecular and biochemical aspects of acidophiles have since received a great deal of attention. Their genome sequences, gene regulation and expression, and bioinformatics can be exploited as resource to select for traits for improving bioprocess conditions. Transmissible metabolic traits and gene regulation in free-swimming and biofilm-associated acidophiles in environmental situations are some key areas for advancing research that may benefit bioleaching technology. Strain improvement through genetic modification is also possible but safeguards require containment in closed environment.

Research in uranium bioleaching parallels many problems also relevant in the bioleaching of copper, nickel and zinc from sulfide ores. Optimization of bioleaching processes is specific for the ore type, requiring interdisciplinary approaches and expertise at each stage of research. Studies in the bioleaching of sulfide minerals have progressively led to multiple commercial scale bioprocesses. Investigations on potential bioleaching applications are underway for extracting metals from electronic and other metal-containing waste streams. New applications of uranium bioleaching are expected in conjunction with extraction of other commodities, such as rare earths, base metals and phosphate. The long-term environmental mitigation and monitoring and public pressure and opposition to uranium mining are some of the prohibitive factors in this regard. Global uranium demand and national self-sufficiency of uranium supply are some of the mitigating factors that may justify opening new mine sites. The role of microorganisms is now so well understood that mining operations can be designed to include bioleaching steps with specifications that take into account their metabolic, physiological and environmental requirements.

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2130 Table 1. Some uranium minerals of economic importance mentioned in the text.
2131

U-mineral	Ideal formula
Autunite	$Ca(UO_2)_2(PO_4)_2 \cdot 10H_2O$
Betafite	$U_2(Ti,Cb)_2O_6(OH)$
Brannerite	$(U,Ca,Fe,Th,Y)(Ti,Fe)_2O_6$
Carnotite	$K_2(UO_2)_2(VO_4)_2 \cdot 3H_2O$
Coffinite	$U(SiO_4)_{1-x}(OH)_{4x}$
Pitchblende	UO_2 [UO_2 to $UO_{2.25}$]
Uraninite	UO_2 [$(U^{4+}_{1-x},U^{6+}_x)O_{2+x}$]
Uranophane	$Ca(UO_2)_2Si_2O_7 \cdot 6H_2O$
Uranothorianite	$(Th,U)O_2$
Uranothorite	$(U,Th)SiO_4$

2132

2133 Table 2. Mineralogical, physicochemical, and microbial factors affecting uranium bioleaching

Factor	Variable	Notes
Mineralogy	Uranium mineralogy	Oxides, phosphates, sulfates and carbonates solubilized more easily while dissolution of silicates is relatively slower
	Pyrite	Oxidation generates acid, dissolved Fe and heat
	Gangue minerals	May consume acid and result in precipitate formation
	Particle size	Affects leaching rate and yield
	Porosity and permeability	Affects recovery rates of *in situ* leaching
	Secondary precipitates	Precipitates remove Fe^{3+} from solution; passivate mineral surfaces and decrease uranium dissolution
Physical and chemical factors	Particle size distribution	Reactive surface area increases with ore comminution and grinding
	Temperature	Temperature affects reaction rates, solubility of gasses, microbial diversity and activity, and acid consumption
	pH	Solution pH affects microbial activity and solubility of uranium and other elements; low pH enhances uranium leaching
	Redox	High redox increases uranium leaching
	Dissolved O_2	Dissolved O_2 is required for the oxidation of Fe^{2+} and reduced sulfur compounds; aerobic growth not limited at >1.5 mg/L O_2
	Dissolved CO_2	CO_2 is required by autotrophic microorganisms; 3-7 mg/L CO_2 optimal for *A. ferrooxidans*
	Nutrients	Nutrients (e.g., N, P, Mg) and some trace metals (e.g., Cu^{2+}) are required by microorganisms
	Fe^{2+}	Fe^{2+} addition increases the bioleaching of U^{4+} provided that Fe^{2+} is oxidized to Fe^{3+}
	Fe^{3+}	Fe^{3+} increases uranium leaching and redox potential, oxidizes U^{4+}; may precipitate as jarosite; excessive concentration may inhibit microbial activity; reduces acid consumption due to ferric iron hydrolysis

	Other metals	Metals may inhibit microbial activity; the toxic threshold concentrations are highly variable
	Uranium	Uranium can inhibit microbial activity; complete inhibition of *A. ferrooxidans* occurs usually at approximately 250-500 mg U/L
Microbiology	Microbial density	The rate of microbial activity is a function of active cell numbers if no other limitation exists
	Microbial activity	Can increase or decrease U solubility due to oxidation, reduction, or sequestration with complexing agents
	Microbial diversity	Consortia generally superior to pure cultures in uranium bioleaching
	Tolerance to U and toxic elements	Tolerance varies among species and strains as well as with growth conditions
	Adaptation to toxic metals	Resistance to uranium and other metals can be increased by successive subculturing in the presence of increasing concentrations of the metal
	Biofilms	Biofilms are more resilient to toxic elements than suspended cells
	Sorption	Uranium sorbs on fungal and bacterial biomass

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2136 Table S1. Examples of shake flask bioleaching studies published in the literature. PD = pulp density.
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Pulp density, uranium mineral, U_3O_8 content	**Experimental conditions**	**U solubilization (%)**	**Reference**
0.356% U_3O_8, brannerite and uraninite,	-48 mesh, 20-40% PD, 28 °C, *A. ferrooxidans*, 9 d	98%	Zajic and Ng (1971)
<0.2% U_3O_8, 10%, ningyoite and autunite	-17 mesh, 10% PD, 25 °C, *A. ferrooxidans*, 12 d	95%	Tomizuka and Takahara (1972); Tomizuka and Yagisawa (1978)
0.11% U, brannerite	<37 μm, 2-40% PD, 30 °C, *A. ferrooxidans*, 10 d	80-100%	Guay et al. (1975)
0.11%, brannerite	-64 μm, 10% PD, 32 °C, *A. ferrooxidans*, 10 d	96%	Guay et al. (1976)
0.03-0.15% U, pitchblende, pyrite, chalcopyrite	5% PD, 30 °C, *A. ferrooxidans* ± *A. thiooxidans,* 40 d	90%	Bosecker and Wirth (1980)
0.047, 0.051, and 0.116% U, three apatite-bearing rock samples; U in uraninite and apatite	-200 mesh, 10% PD, 28 °C, *A. ferrooxidans* and culture filtrates, 24 h	57-94%	Tuovinen et al. (1983)
0.205% U associated mostly with apatite quartzite-muscovite-cericite rock	-325 mesh, 10-20% PD, 28 °C, dilute sulfuric acid, no added iron, 24 h	72-88%	Tuovinen and Hsu (1984)
	-325 mesh, 10-20% PD, acidic ferric sulfate (chemical and biogenic), 24 h	99-100%	
0.047, 0.051, and 0.116% U, three apatite-bearing rock samples; U in uraninite and apatite	-200 mesh, 30% PD, 28 °C, acidic biogenic ferric sulfate filtrates, 7 h; about 15% dissolved U co-precipitated with biologically produced ferric iron	60-99%	Vuorinen et al. (1986)
0.097% U_3O_8, pitchblende and UO_3, pyrite	< 180 μm, 5% PD, 35 °C, mixed culture from Río Tinto mine water, 24 h	100%	Muñoz et al. (1995a)
0.28% U, museum grade carnotite, calcite, quartz	-200 mesh, 5% PD, 22 °C, *A. ferrooxidans*, 15 d		Bhatti et al. (1997)

0.38% U, museum-grade uranophane, plagioclase, quartz, K-feldspar, mica		100% 100%	
1.30% U, museum-grade uraninite, ferroan rhodochrosite, quartz, microcline, kaolinite	-200 mesh, 5% PD, 28 °C, *A. ferrooxidans* 7-15 d	80-100%	Bhatti et al. (1998)
0.04% U_3O_8, quartz-chlorite-cericite-apatite	<150 μm, 10-20% PD, 30 °C, *A. ferrooxidans*, 14 d	49%	Pal et al. (2010)
0.018% U_3O_8, uraninite, pyrite, quartz, Al-silicates	<76 μm, 10% PD, 35 °C, mixed culture of *A. ferrooxidans*, 40 d		Abhilash and Pandey (2011), Abhilash et al. (2009)
0.0465% U, uraninite magnetite, pyrite, quartz	<106 μm, 2.5% PD, 35 °C, mixed culture of *A. ferrooxidans*, *A. thiooxidans*, *L. ferrooxidans*, 8 d	100%	Abdollahy et al. (2011)
0.047% U_3O_8, uraninite, apatite	<45 μm, 10% PD, 35 °C, *A. ferrooxidans*, 40 d	96%	Abhilash et al. (2012)
0.052% U, coffinite	10% PD, 25 °C, *A. ferrooxidans* ± *A. thiooxidans*, 90 d	72-85%	Umanskii and Klyushnikov (2013)
0.48% U in uraninite, weeksite, boltwoodite, uranophane, U-thorianite	-100 μm, 5-15% PD, 35 °C, *A. ferrooxidans*, 2 d	84-88%	Rashidi et al. (2014)

2138 ND, data not given.
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2140

2141 Table S2. Examples of column bioleaching studies published in the literature. Inoculation has varied between *A. ferrooxidans* and enrichment
2142 culture from the mine site, but in view of the long time courses it is likely that the final microbial populations have been mixed cultures in each
2143 case.
2144

Pulp density, uranium mineral, U_3O_8 content	**Experimental conditions**	**U solubilization (%)**	**Reference**
0.0278-0.0373% U	-5 mesh, 8 kg ore, 109 d	63%	Barbič et al. (1976)
0.024% U, uranothorite, brannerite, monazite (Agnew Lake ore)	+1" – -50 mesh, 6 kg ore, mixed culture, 120	34-55%	Bruynesteyn et al. (1981)
0.13-0.56% U_3O_8 , coffinite, uraninite and organo-uranium complexes	-16 – +35 mesh, 100 g ore, mixed culture, 54 d	95%	Brierley (1978)
0.146% U_3O_8, pitchblende in black slate rock	-40 – 0.5 mm, ambient temperature, 20 t ore, 477 d	57-83%	Floeter et al. (1983)
0.145% U_3O_8, pitchblende in granite rock	-40 – 0.5 mm, ambient temperature, 20 t ore, 429 d	31-86%	
0.097% U_3O_8, pitcblende	-6 mm, amount of ore ND, mixed culture from mine water, 24 d	~90%	Muñoz et al. (1995b)
0.11% U, brannerite	-4.75 mm, 2 °C, *A. ferrooxidans*,; 16 kg ore, 39 d	67%	Guay et al. (1977)
0.027% U_3O_8, U-rich sandstone uranophase, carnotite, tyuamunite, uraninite, pitchblende, coffinite	+30 to -300 mesh, *Acidithiobacillus* spp., 100 kg, 90 d	66-70%	Bhatti et al. (1989)
0.113% U, uraninite ore,	-10 mm, 20 kg ore, *A. ferrooxidans*, 38 d	74-79%	Ding et al. (2010)
0.0308% U_3O_8, uraninite	2.5-0.5 cm, 6 kg, 60 d	60%	Abhilash et al. (2010)
0.164% U, uraninite, brannerite, uranothorite	-2-5 mm, 24.7 kg, pooled enrichment culture from uranium mines, 64 d	97%	Qiu et al. (2011)

0.208% U, pitchblende, coffinite, fluorite silicate uranium	-8 mm, ambient temperature, 120 d	89%	Chen et al. (2016)
0.058% U, multiple U-bearing phosphates and silicates	<0.2 mm – 0.63 mm, 3 kg, 25 °C, mixed culture from a mine site, 55 d	75%	Szolucha and Chmielewski (2017)
0.0230% U, uraninite, talc, magnetite, hematite and pyrite	d_{80} = 5 mm, 3 kg, 25 °C, *A. ferrooxidans*, 19 d	50-62%	Zare Tavakoli et al. (2017b)
0.0218% U, uraninite, talc, magnetite, hematite and pyrite	d_{80} = 10 mm, 3 kg, 25 °C, *A. ferrooxidans*, 19 d	48-59%	Zare Tavakoli et al. (2017b)
0.0211% U, uraninite, talc, magnetite, hematite and pyrite	d_{80} = 15 mm, 3 kg, 25 °C, *A. ferrooxidans*, 19 d	46-54%	Zare Tavakoli et al. (2017b)
0.025% U_3O_8, uraninite, talc, magnetite, hematite and pyrite	d_{80} = mm, 3 kg, 25 °C, *A. ferrooxidans*, 21 d		Zare Tavakoli et al. (2017c)

ND, data not given.

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2151 Table S3. Examples of stirred tank bioleaching of uranium ores. PD = pulp density.
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Sample	Parameters	Contact time	U solubilisation (%)	Reference
0.12% U, pitchblende	<0.6 mm, 10% PD, *A. ferrooxidans*, 30 °C	3 d	90	Bosecker and Wirth (1980)
0.047% U_3O_8, uraninite	-45 and -100 μm, 10% PD, 35-40 °C, *A. ferrooxidans* and *L. ferrooxidans*	10 h	57-63	Abhilash and Pandey (2013a)
0.065% U, brannerite (Saghand anomaly II)	80% -80 μm, 5.8% PD, 25 °C	6 d	95	Eisapour et al. (2013)
0.024% U, uraninite	<76 μm, 20% 35 °C, *A. ferrooxidans*	14 d	98	Abhilash and Pandey (2013b)
1.3% U, ningyoite	10%, 30 °C, *A. ferrooxidans*	Continuous culture	92 excluded	Tomizuka et al. (1976)
0.03% U, uraninite	d_{80} = 100 μm, *A. ferrooxidans*	7 d	99	Zare Tavakoli et al. (2017a)
0.036% U_3O_8, carbonaceous-siliceous-argillaceous type uranium	< 1 mm, 10% PD, 30 °C, *A. ferrooxidans*	10 d	85	Wang et al. (2018)

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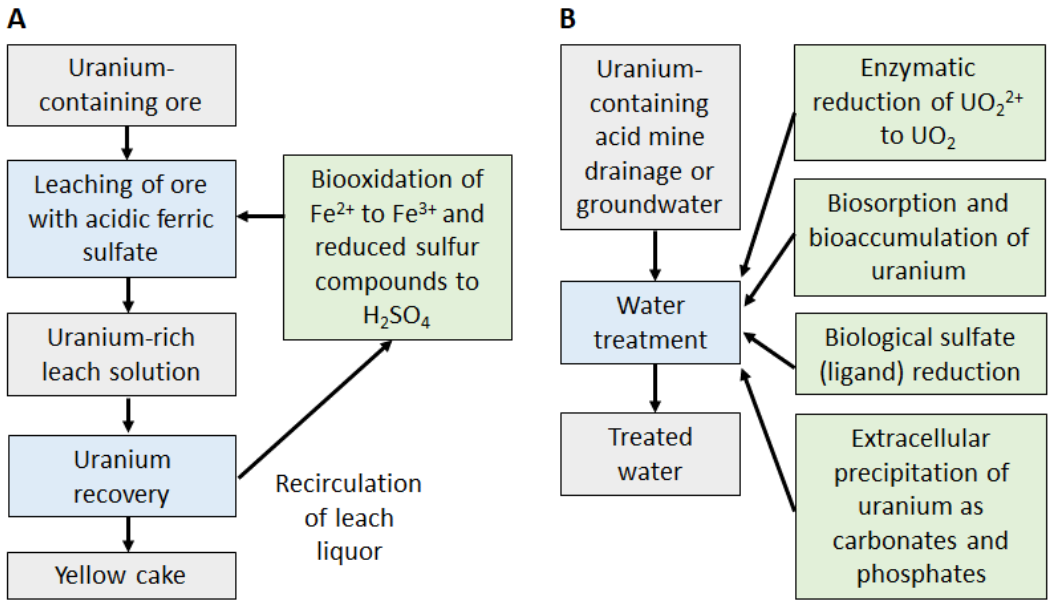

Figure 1. Potential roles of microorganisms in A) acid and ferric sulfate leaching of uranium ores and B) treatment of uranium-containing acid mine drainage and groundwater.

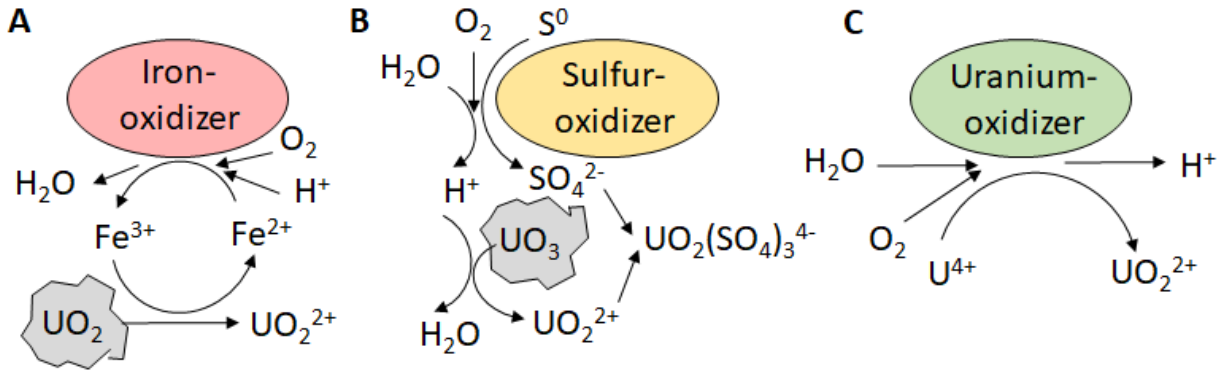

Figure 2. A schematic diagram of the roles of A) iron-oxidizing, B) sulfur-oxidizing and C) uranium-oxidizing microorganisms in the bioleaching or uranium ores.

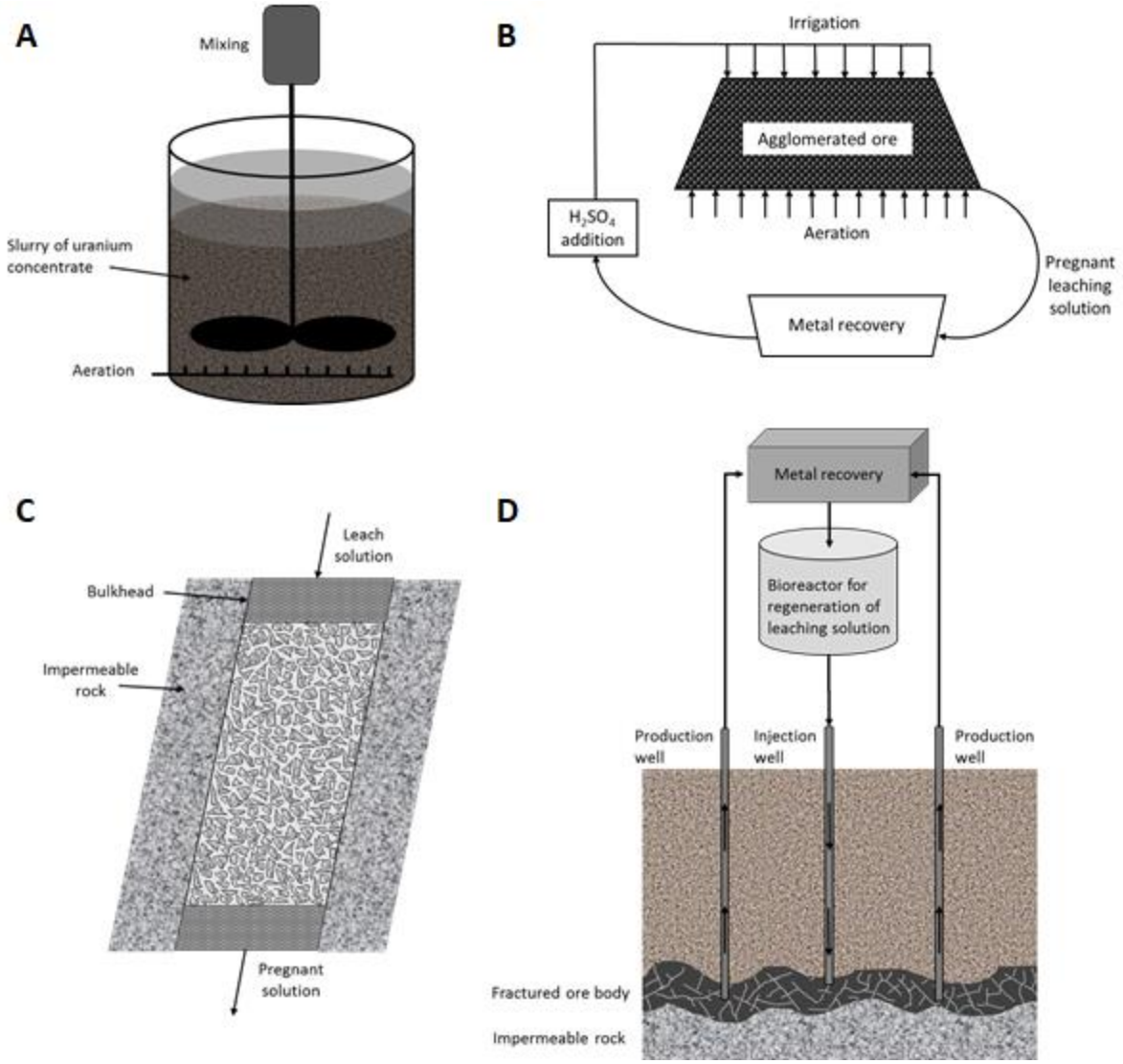

Figure 3. Schematic diagrams showing the basic principles of (A) tank bioleaching, (B) heap bioleaching, (C) stope leaching (adapted from McCready and Gould, 1990), (D) in-place bioleaching and. The geometries of stope and in-place leaching can vary depending on the geometry of the stope and uranium ore body, respectively.

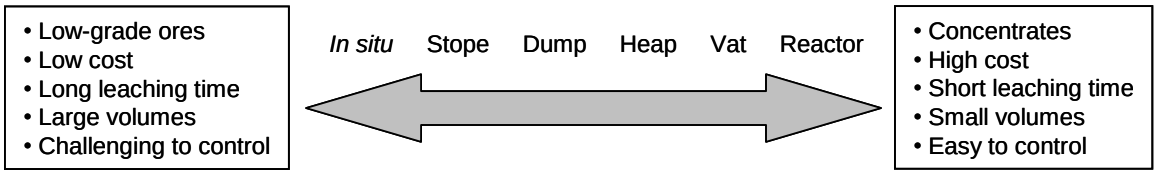

Figure 4. Characteristics of various biooxidation and bioleaching methods (adapted from Kinnunen, 2004).

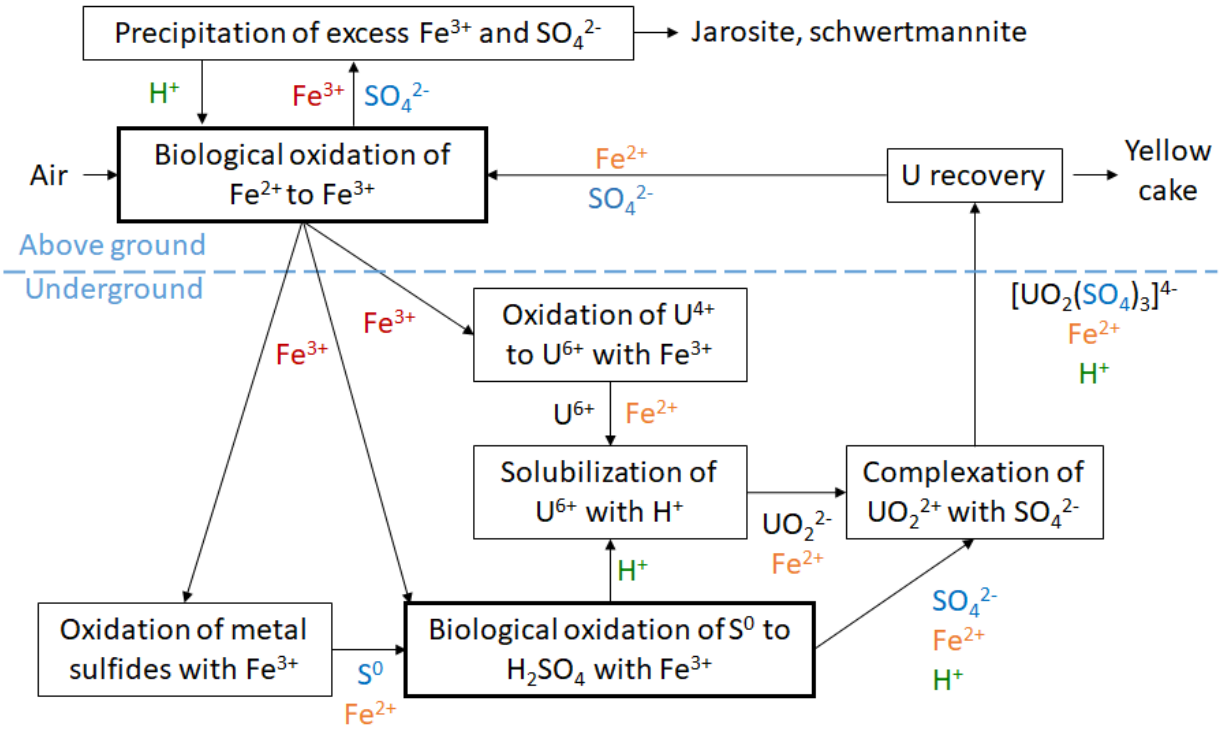

Figure 5. A generalized flow sheet for *in situ* leaching of uranium with biologically generated ferric iron and acid, and excess iron and sulfate removal through jarosite precipitation. Due to the lower solubility of oxygen as compared to ferric iron, biological oxidation of reduced sulfur compounds in the subsurface has been expected to be facilitated mainly by ferric iron rather than oxygen as the electron acceptor.

Figure 6. Agnew Lake mine operation in 1978 (photos: O.H. Tuovinen).

Figure 7. Terrafame heap bioleaching in 2008 (photos: O.H. Tuovinen).

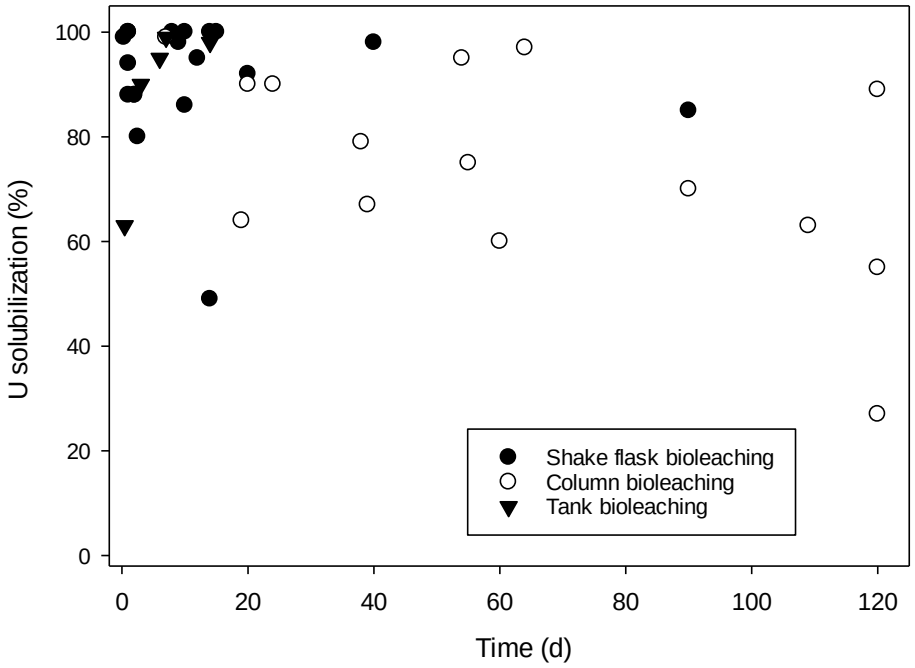

Figure 8. Examples of uranium bioleaching yields in shake flask (●), column (O), and stirred tank (▼) studies. Data pooled from the literature (see Tables S1, S2, and S3). Shake flasks: pulp density 5-40%, U-content 0.035% – 1.03% U; Columns: 100 g – 100 kg ore, U-content 0.024% -- 1.42% U; stirred tanks: pulp density 5.8-20%, U-content 0.024% – 0.12% U.

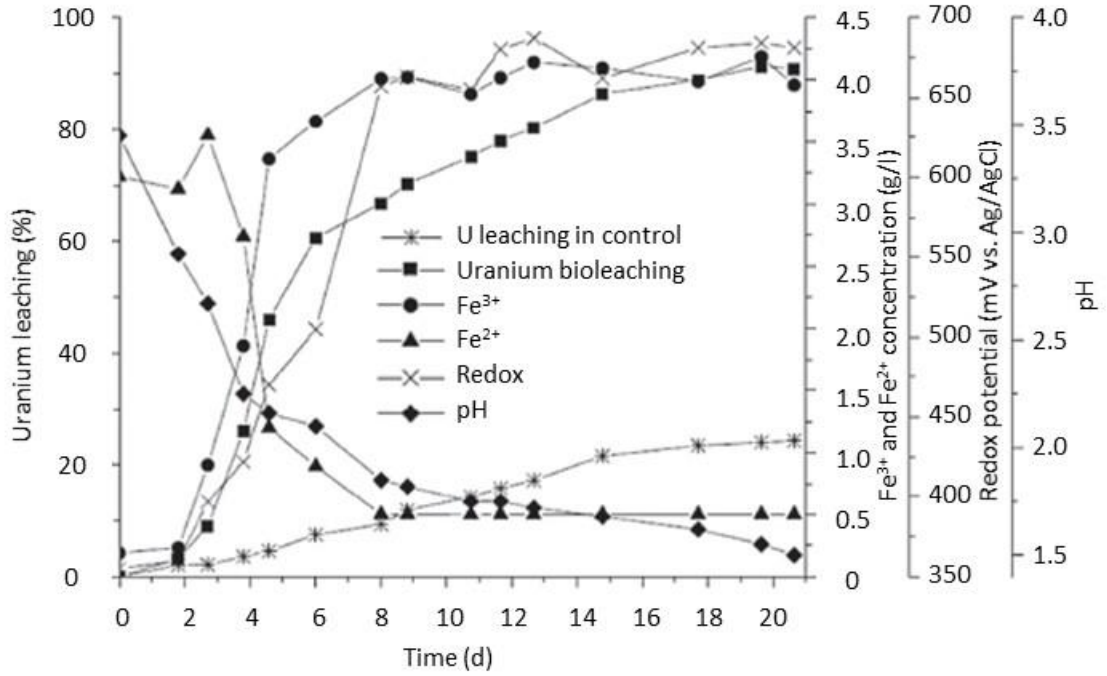

Figure 9. An example of column bioleaching of a uraninite-containing ore (adapted from Zare Tavakoli et al., 2017a with permission from Elsevier).

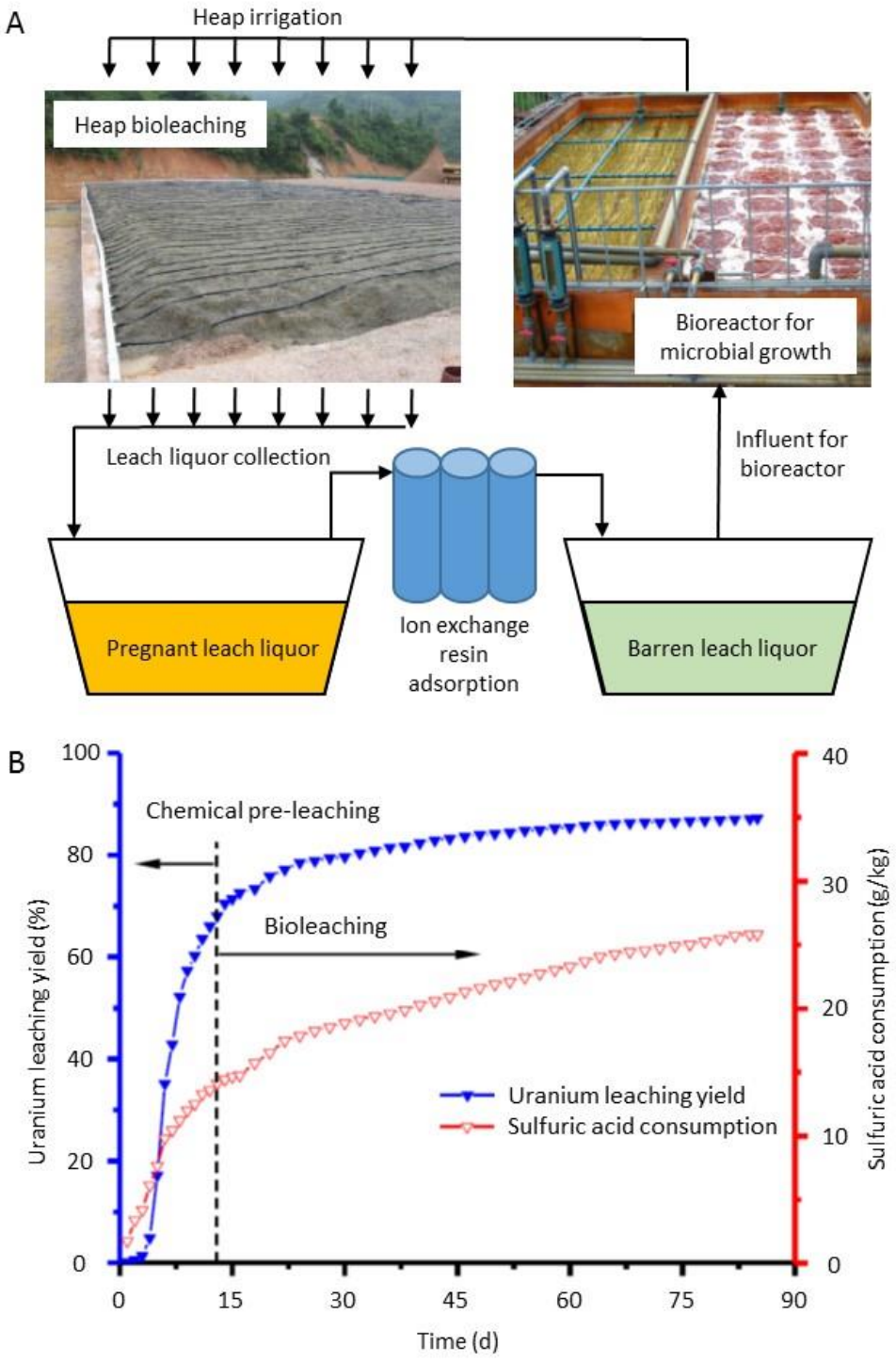

Figure 10. A) Schematic of a 4854-ton bioheap leaching system (U-content 0.082%), B) uranium leaching yield and sulfuric acid consumption during chemical pre-leaching and bioleaching. Acid consumption started immediately and continued throughout the time course of 85 days (adapted from Wang et al., 2017 with permission from Springer Nature).

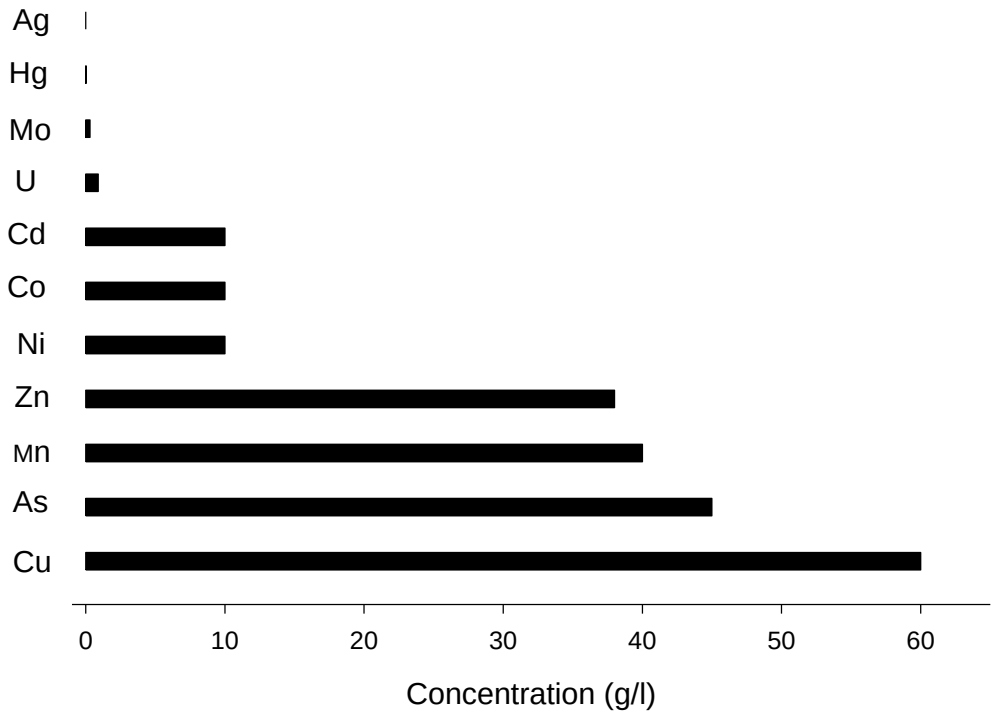

Figure 11. Schematic comparison of toxic threshold concentrations of metals inhibitory to iron oxidation by *A. ferrooxidans*. Data pooled and averaged from various literature sources. There are numerous differences in the threshold concentrations of each metal due to adaptation, strain variation and growth conditions.