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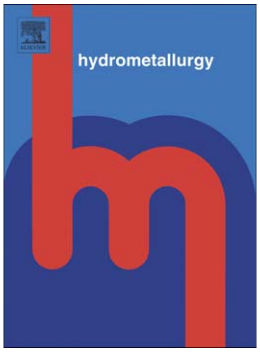

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In situ recovery of uranium – the microbial influence

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ABSTRACT

In situ recovery (ISR) has become an increasingly utilized technology worldwide for the economical extraction of uranium (U). Microorganisms play a significant role in U mobilization/immobilization and have therefore been used for the bioremediation of U contaminated sites. In natural environments a wide range of microorganisms have the ability to oxidize or reduce U compounds as part of their metabolism. Hence, microbiota are very likely to play an important role at all stages of U ISR, however the effect of resident microbial communities subject to ISR has not been investigated. Therefore, this review focuses on the interactions between microorganisms and U and the possible effects this could have on ISR operations. Microorganisms may affect ISR in either a positive or negative way, e.g. assisting in U mobilization via the oxidation of U or immobilizing U by reducing it into an insoluble form. The use of native microbial communities to influence the mobilization/immobilization of U during ISR could help to increase U recovery rates or speed-up post-mining remediation.

Abbreviations

GHG: Greenhouse gas; ISR: *in situ* recovery; XFM: X-ray fluorescence mapping.

Keywords

In situ recovery, ISR, bioleaching, microbiology, geobiology, uranium

Introduction

With the effects of global warming being felt world-wide, there has never been a stronger, more vocal push to protect the stable, yet fragile environment which Earth's creatures inhabit. Governments are being called upon to make urgent, yet dramatic changes to implement legislation, which may help to mitigate global warming. In addition to the environmental impacts of climate change, Stern (1) estimates that global warming could decrease global GDP by as much as 25 % by 2090, while reducing our Greenhouse Gas (GHG) emissions to offset global warming would only cost about 1 % of the current global GDP. We therefore must make changes to the amount of GHG being emitted into the Earth's atmosphere and those changes must be made expeditiously. The major source of GHG emissions is the use of fossil fuels to produce energy (2). To cut down GHG emissions it is imperative that we find energy producing solutions that do not require the use of fossil fuels (which, in itself is a limited, increasingly scarce resource). Such technologies exist and include “renewable” energies, such as wind, solar and geothermal energy, as well as nuclear. Of these solutions nuclear energy is seen as an attractive alternative to fossil fuels in many countries, with 430 nuclear power stations currently in operation and 70 under construction across 31 countries worldwide. Proponents of nuclear technologies argue that it produces more power, with often lower costs than “renewable” energy sources (3, 4). In 2009, Adamantiades and Kessides (3) stated that nuclear power had contributed to a 10 % reduction in CO_2 emissions from energy production. The 4th generation nuclear reactor designs being developed by a US-led association of 13 countries may help to address some of the concerns that traditionally come with the use of nuclear technologies, further promoting the use of nuclear power (3). Hence, it appears that nuclear energy is here to stay and future energy needs will be increasingly met through nuclear energy in some countries.

Driven by the world's ever-increasing need for nuclear power, uranium (U) consumption has been rapidly increasing (Figure 1). Conservative estimates speculate that the annual demand for U in 2030 will reach between 80,000 t and 148,500 t (5), increasing by 50 to 179 % from the 58,000 t having been being produced in 2012 (Figures 2). Kazakhstan (36.5%), Canada (15%) and Australia (12%) currently account for approximately 63.5% of the world's U production (Figure 2). Uranium has been mined using underground mining, open pit mining or *in situ* recovery (ISR) methods from a great diversity of deposits (7). Over the past two decades, the use of ISR has been progressively increasing and now accounts for 45% of the worlds U production (6). The Chinese appear to have been the first to use ISR for the extraction of copper in 907 A.D., with references of solution mining dating back to 177 B.C. (8, 9). This was proceeded with the ISR of elemental sulfur by the French and gold by the Russians (8, 9). During the 1960's the ISR of U was developed by the USA and the Soviet Union (10). By the 1990s ISR accounted for 95% of U mined in the USA, and the technology is being increasingly globally applied (6, 11).

In situ recovery of U involves drilling boreholes into the ore deposit (12); pumping a leaching solution down injection boreholes; flowing the solution through the mineralized horizon so it can dissolve the ore; retrieving the solution from production boreholes; and extracting U from the solution in a plant at the surface (Figure 3). The solution may travel through the ore via natural rock porosity, or via porosity generated by mineral dissolution (acid leach) or artificial fragmentation (hydraulics or explosives). The leaching solution can be alkaline or acidic depending on the mineralogical and geochemical properties of the deposit. In the USA U recovery by ISR uses mainly alkaline chemistry, while in Russia, Kazakhstan, Australia and Asia acid is generally used (10, 13). Acid is used when carbonate content is less than 1.5-2% and is the preferred technique as recovery rates are typically higher than when using alkaline leaching methods (10). However, a good understating of hydrogeology and extensive monitoring are required as the use of acid can lead to heavy metals and radionuclides being mobilized and leached into the environment, contaminating ground water supplies.

Despite these issues, ISR allows for the recovery of U without the need for removing the ore body from the ground (Figure 3). Hence, ISR of U holds many advantages over traditional open pit or underground mining methods, including:

- Reduced environmental impact.

  The surface environmental footprint of ISR is substantially smaller compared to other mining methods. Brierley (14) states that as the world's populations become more urbanized, people will live closer to mining operations and ISR is a technology which markedly reduces the surface impacts of mining. Low grade U deposits, which are produced using open pit or underground mining methods result in large tailings dams contaminated in U and radionuclides; such tailings are not generated by ISR (15).

- Reduced safety hazards.

  The use of ISR has been reported to substantially reduce the radiation dosages experienced by mine-site employees and reduce hazards associated with the movement of large quantities of ore and waste rock (16).

- Reduced production costs.

  Increasing environmental restrictions on U mining coupled with the prevalence of low-grade ore deposits and increasing energy costs has meant that ISR has become an attractive, economically viable extraction method for many U deposits.

In view of the push to apply ISR technology to a wider range of deposits of ever decreasing grades and with increasingly stringent environmental and safety requirements, there are still a number of issues that need to be addressed in order to realize the full potential of U ISR:

- Uranium dissolution as a result of ISR is not well understood.

  Heterogeneous hydrogeological, mineralogical, geochemical and geobiological conditions mean that the recovery rates from ISR vary greatly, and are often lower than using conventional methods (typically 70-90% recovery using acid leaching, and 60-70% recovery from alkaline leaching (10)).

- The consumption of the leaching solution by ‘parasite’ reactions and reduced porosity of materials must be addressed.

  Acid leaching (mainly sulfuric acid) is the predominate form of U ISR, because of its low cost, availability, and relatively high recovery rates

(17). For acidic ISR the ore zone should contain less than 2 % calcium carbonate; at higher concentrations alkaline leaching is required, which is often less effective than acid leaching (10). Reduced porosity, which can be caused by the growth of biofilms or the formation of gypsum can greatly decrease the effectivity of leaching and is another major problem for acid ISR operations (13).

- The activity of leaching solutions must be closely monitored.

  Thorough monitoring and control of leach solutions must be conducted in order to prevent the spread of U, other radionuclides and metals into previously uncontaminated groundwater. For example due to the mismanagement of an acid ISR U mine towards the end of the 1960s in Stráž, Czech Republic, 186 million m^3 of groundwater across 24 km^2 was contaminated with 980,000 t of sulfuric acid, 415 t of dissolved aluminum and 1,000 t of dissolved U (10, 18-20).

- Mine sites must be remediated to pre-mining use category.

  Post-mining remediation of U ISR was overlooked for much of its early history (21). Although acid leach has led to major environmental disasters, by the end of the 1980s the Nuclear Regulatory Commission concluded that there were no significant long-term environmental effects from alkaline ISR (22). Despite this, there will be tighter environmental standards to which mine sites must be remediated and this will continue to be an issue for the mining industry. Additionally, current clean up costs of alkaline leach operations worldwide can run into the billions of dollars (21).

One aspect of U ISR that has remained largely overlooked, and yet could help to mitigate some of the issues listed above as well as improve ISR efficiency, is the use of *in situ* microbial communities to mediate U immobilization/mobilization by promoting dissolution/precipitation of U-bearing minerals. It is well established that microorganisms are key players in elemental cycles on Earth, e.g., the carbon, nitrogen, sulfur and phosphorous cycles, and strongly influence the mobility of a wide range of metals, e.g., iron, manganese, gold, copper and U (23). However, the impact of microorganisms on ISR has not been investigated despite the fact that many studies have shown that microbiota can have a dramatic effect on U mobility (24-31).

Microbial interactions with uranium

The transformation of U by microorganisms through the reduction of U(VI) to U(IV) impacts both the state and distribution of U in the environment (26). Microorganisms are able to use U as an energy source, and a wide range of different microorganisms have the ability to transform U: in general, aerobic, metal-oxidizing microorganisms catalyze the oxidation of reduced metals, hence solubilizing and mobilizing U; while anaerobic microorganisms catalyze metal reduction, hence immobilizing U (32, 33). For example, some bacteria increase U mobility via the production of acid during iron and sulfur oxidation (i.e*.,* bioleaching) (34-37); whereas others limit U mobility by reducing uranyl and forming highly insoluble nano-particles of secondary U(IV) minerals, such as uraninite (26) and coffinite (38). Hence, microbiota are very likely to play an important role at all stages of ISR: they can affect U recovery rates in both positive and negative ways, and play a fundamental role in mine site remediation. Microorganisms may be able to assist in a number of processes that occur during ISR; conversely microorganisms are currently playing a yet-undefined role in ISR operations. Iron and/or sulfur oxidizing microorganisms may assist in U dissolution, when U is found in association with sulfidic ores (36) (for example, Figure 4); dissimilatory metal-reducing microorganisms can solubilize U, assisting in U processing and bioremediation (39, 40); and, microorganisms present in aquifers may affect the ISR of U by 'locking up' intracellular U (41).

Microbial uranium solubilization

Aerobically, microorganisms can directly (enzymatically) or indirectly (chemically) oxidize U. Indirect, microbially assisted oxidation of U(IV) to U(VI) is used for the recovery of U from low-grade ores worldwide (34, 35, 42). During this process iron and/or sulfur oxidizing microorganisms metabolize the iron and/or sulfur in the ore, producing Fe(III) and sulfuric acid, which act as effective oxidants for the insoluble U(IV) within the ore (36, 42-45);

$$4\ Fe^{2+} + O_2(aq) + 4H^+ \rightarrow\ 4\ Fe^{3+} + 2\ H_2O \qquad (1)$$

[microbial, aerobic]

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

[inorganic - with Fe^{3+} from equation 1]

$$UO_2^{2+} + SO_4^{2-} \rightarrow UO_2SO_4(aq) \qquad (3)$$

[complexation with sulphate, enhances uranyl mobility]

$$UO_2(s) + 2H^+ + ½O_2 \rightarrow UO_2^{2+} + H_2O \qquad (4)$$

[inorganic and microbial]

$$5\ UO_2(s) + 2\ NO^{3-} + 12\ H^+ \rightarrow 5\ UO_2^{2+} + 2\ N_2 + 6\ H_2O \qquad (5)$$

[microbial, anaerobic]

In some instances pyrites have been added to leaching solutions, providing microorganisms with the fuel to assist in the dissolution of U (44, 46). According to free energy calculations, it was hypothesized that some metal-oxidizing microorganisms maybe able to directly oxidize U(IV) to U(VI) (Equation 4) (37, 45, 47-49). However, at the time of these calculations, there was no direct evidence for the existence of microorganisms with such capabilities (45). In 1982, DiSpirito and Tuovinen demonstrated that the bioleaching microorganism, *Acidithiobacillus ferrooxidans* (formally *Thiobacillus ferrooxidans*), oxidized U(IV) at pH 1.5, conserving the energy during oxidation and then using it for carbon dioxide fixation. Bioleaching with iron and/or sulfur oxidizing microorganisms can only occur aerobically, presenting a major hindrance for ISR, which takes place in the subsurface (37, 48). However, during long-term studies of contaminated Oak Ridge sediments high levels of dissolved oxygen have been shown to solubilize U(IV), despite the presences of U(VI) reducing microorganisms (50-55). In these samples the iron(II) oxidizing bacterium, *Thiobacillus*, which is able to oxidize U(IV) anaerobically in the presence of nitrate, was detected (Equation 5) (55-57).

The anaerobic oxidation of U(IV) can also occur via direct and indirect mechanisms. Heterotrophic and autotrophic, dissimilatory nitrate-reducing bacteria, have been shown to oxidize Fe(II) and U(IV) when nitrate serves as electron acceptor under anaerobic, near neutral conditions (57-59). The energy gained from U(IV) oxidation is not used for cellular growth, rather it is coupled to nitrate reduction (57-60). Microorganisms may employ extracellular proteins to anaerobically oxidize U(IV) (49, 61-63). Kalinowski *et al.* (49) showed that microorganisms can leach U anaerobically, possibly due to the production of chelators. Using three facultative anaerobes, Kalinowski *et al.* found that after five days 0.005% of U was leached from the ore at anaerobic, neutral to alkaline conditions.

Studies of bioremediation sites have shown on multiple occasions that the anaerobic oxidation of U(IV) occurs in the environment (50-55). The closure of the mine at Ranstad, in the north of Sweden, resulted in the leaching of U into surrounding areas (49, 64). To stop this process, the mine was covered, rendering it anaerobic; despite this measure metals were still being leached from the mine. Studies of a U.S. Department of Energy site also found that U(IV) was being reoxidized, and attributed this to the presence of *Anaeromyxobacter* and *Geobacter* spp. which can oxidize U(IV) in the presence of nitrate (29, 53, 58, 65, 66). In addition, methanogens, which require anerobic conditions, produce methane which has been correlated to the remobilization of U(VI) (50, 67). During the bioremediation of the Rife site in Colorado, U(IV) oxidation was reported to occur following the addition of acetate to groundwater; the authors suggest that certain *Dechloromonas* spp. may have actually inhibited U(IV) reduction (68).

This suggests that microorganisms can increase the solubility of U ore amenable to ISR, and hence further investigations of these processes could lead to improvements in ISR technologies. Based on studies of microbial composition and activities in U-bearing sediments and deep aquifers (28, 53, 65), it is highly likely that geobiological reactions leading to U mobilization occur naturally in ISR operations, and supporting the growth of these beneficial microorganisms would increase leaching rates.

Microbial reduction of uranium

Microorganisms can also reduce U(VI), into the sparingly soluble U(IV), resulting in the formation of the minerals uraninite and coffinite, or the biosorption, bioaccumulation and biotransformation of U (24, 25, 40, 58, 69-71). Some microorganisms can use U(VI) as an electron acceptor and a few of these microorganisms have been shown to conserve energy for growth from U(VI) reduction (39, 72-76). Microbial U(VI) reduction is also catalyzed via direct and indirect mechanisms, the metabolic byproduct of iron(III) and sulfate reduction can chemically reduce U(VI) (77, 78). The ability to reduce U(VI) is widespread amongst microorganisms (39, 73, 79-81) including sulfate- and iron(III)-reducing microorganisms (72, 74, 75), as well as other microorganisms such as *Deinococcus radiodurans* (82), *Clostridium* spp. (83) and *Lactobacillus* spp. (84). The ability to reduce U has been reviewed elsewhere (32, 85), hence this review will focus on the microbial reduction of U(VI) solely in the context of utilization by the mining industry.

The growth of U(VI) reducing (immobilizing) microorganisms during ISR could adversely impact dissolution rates, and the conditions which encourage the growth of U(VI) reducing microorganisms should be avoided. One practical solution to the presence of U(VI) reducing microorganisms could be the addition of nitrate, which is a thermodynamically more favorable electron acceptor for microorganisms than U(VI) (58, 86, 87). During processing, the ability of microorganisms to reduce U(VI) could be used to remove U(VI) from solution. Takehiko (84) found that *Lactobacillus* cells could remove ~2,370 µM of U per gram of dry weight cells from U-contaminated seawater. It has been proposed that such microorganisms could be immobilized on a polyacrylamide gel and repeatedly used as an absorbing agent for the recovery of U from nuclear fuel effluents, mine tailings, seawater etc. (81, 84).

The ability of microorganisms to reduce and immobilize U has been successfully utilized in the bioremediation of many sites globally (88). Bioremediation offers a cost-effective strategy for dealing with the contaminated relics of the cold war area, with over 7,200 km^2 contaminated with radioactive wastes (30, 89, 90). However, the bioremediation of U remains a significant challenge as natural settings offer a diverse range of variables that can influence the resident microbial populations. Bioremediation of U contaminated sites have been successfully conducted but they require constant monitoring and environmental customization (29, 65). Some sites, such as Oak Ridge (discussed above) have gone through U reduction, reoxidation followed by a final reduction phase until U contamination was contained to accepted environmental standards (50-55). Additionally, U mineralogy is complex, the U.S. geological survey recognizes 185 U (and thorium) minerals (17, 91). This demonstrates the need for continual research in the field of U bioremediation.

Microorganisms can create U(IV) particles that are locked in cells, and as the live cells are attached onto mineral grains they cannot move with the leaching solution (92-94). Ray *et al.* (41) recently showed that reduction and formation of nano-particulate U(IV) minerals is not the only mode by which microbiota affect U mobility, presenting evidence for the biota-mediated formation of U(VI) phosphates (Equation 6 and 7). Additionally, *Thermoterrabacterium ferrireducens* has been shown to couple organotrophic growth to the reduction of sparingly soluble U(VI) phosphate, precipitating U(IV) in the form of ningyoite ($CaU(PO_4)_2.H_2O$) (95). In terms of ISR, the microbial uptake of U from solution could inhibit leaching, thus leaching conditions would be optimal when microbial uptake and complexation of U can be suppressed.

$$Ca_5(PO_4)_3F + 6\ H^+ \rightarrow 5\ Ca^{2+} + 3\ H_2PO_4^- + F^- \qquad (6)$$

[microbial phosphate formation (96)]

$$2\ UO_2^{2+} + 2\ H_2PO_4^{-} + Ca^{2+} + xH_2O \rightarrow Ca(UO_2)_2(PO_4)_2.xH_2O + 4\ H^{+} \quad (7)$$

[inorganic uranium-phosphate complexation]

Acidic versus alkaline ISR

The influence of microorganisms on U ISR would be substantially different for acidic or alkaline leaching. Acidic leaching occurs around a pH of 2.0-3.0, whereas alkaline leaching occurs at pH 7.5-8.5, the microbial populations at these two pH ranges would vary significantly, as would their ability to mobilize U. At the acidic end of the spectrum, acidophilic iron and/or sulfur oxidizing microorganisms would assist in the leaching of U (37, 48). Under near neutral conditions, anaerobic U(IV) oxidation has been demonstrated, however under alkaline conditions microbial oxidation of U(IV) has not been demonstrated (97). In terms of ISR this means that microbial oxidation of U(IV) could especially occur during acidic ISR. However, results from bioremediation studies have shown that at neutral to alkaline pH, U(IV) oxidation continues to occur (50). At this site they found that the elevated levels of carbonate resulted in reducing conditions, making the oxidation of U(IV) thermodynamically favorable, however whether or not this was microbially mediated has yet to be determined. This demonstrates the complex interaction between U, the surrounding material and microorganisms.

The microbial formation of phosphate, leading to the complexation and immobilization of U, has been demonstrated to occur at pH 3.5-8.2 (98), hence this process would impact both acidic and alkaline leaching. The reduction and immobilization of U(VI) by microorganisms occurs across a range of pH. At near-neutral pH, dissimilatory Fe(III)-reducing and/or sulfate reducing microorganisms reduce U(VI) while *Clostridium* spp. can reduce U(VI) at pH 4.0 (99). Site-specific conditions could also exacerbate the effects of pH, for example varying concentrations of ferric hydroxides, manganese oxides or nitrates (85). The effect of pH on the net mobilization of U is complex and multifaceted with much basic research still required in the field.

Uranium containing (palaeo) roll-fronts – A microbial perspective

Uranium is mined from a wide range of deposits, including magmatic, hydrothermal, sedimentary and supergene deposits. Based on the ubiquity of microbial communities down to depths of several kilometers below the Earth's surface (100), it is likely that microbiota play a role in the cycling of U at many of these deposits post formation. The formation of some deposit styles may, however, occur as a result of direct and indirect microbial influences on the mobility of U. These types of deposits include calcrete-hosted and (palaeo)-roll-front deposits (Figure 5). Calcrete formation in many environments is largely mediated by microbial processes and U-enrichment is likely the result of microbial co-precipitation of U with calcium carbonates and biomineralization of secondary U minerals, for example, carnotite and coffinite (100). Roll-front deposits typically are some of the largest deposit and are hence of high economic importance, especially as they have the advantage of being highly amenable to low-cost ISR. Roll-front deposits are generally hosted within permeable and porous sediments, most commonly sandstones and conglomerates (Figure 5). To form a roll-front deposit primary U is mobilized from a nearby source, and transported as soluble U(VI) complexes in the ground waters to a suitable host unit (Figure 5). The U transporting groundwater is oxidizing, whereas the host unit is reducing, with high contents of organic carbon and biogenic sulfides, commonly pyrite (Figure 5). This results into deposition of U and other redox-sensitive elements (e.g., Se, V) at a migrating (i.e., 'rolling') redox boundary. Tabular-style sandstone hosted deposits form via similar processes in similar environments, but display a different geometry. Roll-front deposits are currently mined in the United States, Kazakhstan and Australia. For example, the Frome Embayment in South Australia contains a number of sandstone-hosted deposits, including Honeymoon Well, Oban, Beverley and Four-Mile, the latter being one of the largest deposits of this kind worldwide. These deposits are hosted in palaeochannels filled with Cainozoic sediments and/or in Cretaceous and Cainozoic sediments; the U is sourced from U-rich Mesoproterozoic rocks of the Mount Painter Inlier and the Olary Domain of the Curnamona Province (101, 102). By directly or indirectly mediating the solubilization of U (discussed above) from the source materials microbial processes play a critical role for the transport of the U (Figure 5). Upon contact of oxidized U-bearing groundwaters, microbial communities metabolically reduce U(VI) to U(IV), Fe(III) to Fe(II) and sulfate to sulfide, leading to the immobilization of U (Figure 5). At Berverley, the reductive environment is also demonstrated by the presence of native metals such as Cu and Pb (103, 104). Microbially mediated biomineralization then leads to the formation of U-bearing sulfide minerals and secondary U minerals, such as uraninite and coffinite (Figure 5). For example Min *et al.* (2005) showed that uraninite and coffinite in high-grade ore samples (U_3O_8>0.3%) from the Wuyiyi, Wuyier, and Wuyisan sandstone-hosted roll-front U deposits, Xinjiang, northwestern China were biogenically precipitated and pseudomorphically replace fungi and bacteria (105). The authors suggested that U(VI), which was the sole electron acceptor, was likely to have been enzymatically reduced. They also showed that post-mortem accumulation of U may have also occurred through physio-chemical interaction between U and negatively-charged cellular sites, and inorganic adsorption or precipitation reactions. The nano-particulate nature and geochemical composition (phosphorous-rich) of coffinite at the Berverly U-deposit, together with the absence of reductant preserved in many ore samples, led Wulser *et al.* (103) to suggest that bacterial reduction may have been the main ore-forming process for this particular orebody. A recent study by Ingham (2013) from a typical roll-front system in the Lake Frome embayment (abundant organic matter as reductant) showed the presence of U-bearing framboidal pyrite indicative of a microbial formation history (104). The stable isotope composition of pyrite (δ^{34}S as low as -43.9) indicated a strong biogenic influence on sulfide formation, with abiogenically formed U-bearing pyrites also present at the site. This is further evidence that biogenic and abiogenic processes are strongly interconnected during the formation of rollfront deposits.

Conclusion

The use of ISR is increasing worldwide and it is a technology that will progress into the future. ISR is a mature technology for U mining, and may serve as a platform for the development of this method to other commodities. Not only does the potential for using microorganisms in ISR exist, microorganisms undoubtedly play a yet-undetermined role in current U ISR operations. In the case of sandstone-hosted deposits, the ISR process essentially involves reverse engineering the processes that led to the formation of these deposits. Acidophilic iron, sulfur and U oxidizing microorganisms contributed to the mobilization of U and other metals (36), a process common in many environments (27, 49, 78, 106, 107). A detailed understanding of microbial community structures and their functions will lead not only to improved understanding of ore formation processes and controls on ore grade and mineralogy, but also have direct implications for the optimization of the ISR process. In terms of microorganism communities, ISR provides new opportunities and challenges, which will increase the rate and diversity of microbial activity, as has been observed in the case of U-contaminated aquifers (31, 53, 54, 65). Microorganisms can interact with U mobility in via a wide variety of pathways (e.g. direct oxidation and reduction of U; controlling oxido-reduction of species that interact with U (e.g., Fe^{2+}/Fe^{3+}); controlling release and speciation of solubility-controlling ligands such as PO_4^{2-}), either assisting or hindering U extraction. Encouraging or retarding the growth of these microorganisms could improve U extraction rates. The opportunity exists here for researchers to frontier the development of microbially assisted ISR operations as well as further developing bioremediation strategies.

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Figure One. World nuclear energy consumption.

Data extrapolated from U.S. Energy Information Administration (108).

Figure Two. World uranium production.

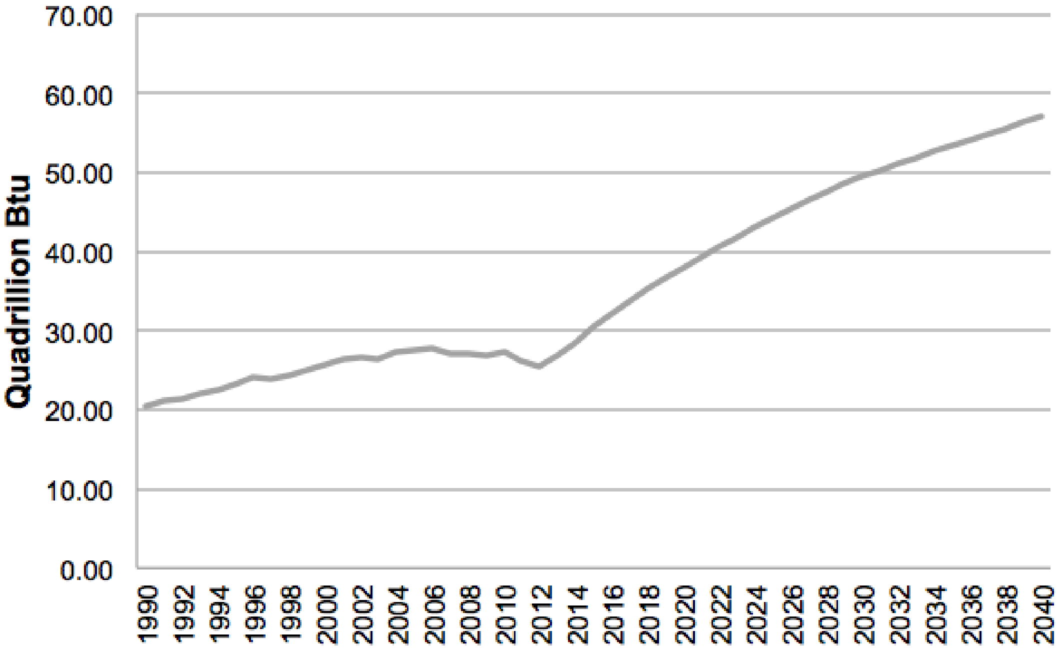
Data extrapolated from (6).

Figure Three. *In situ* leaching operation. Adapted from (109).

Figure Four. Synchrotron X-ray fluorescence map of the distribution of U, Fe and Ti in a sandstone-hosted sedimentary ore from the Frome Embayment, South Australia. **A.** full image, covering a 2 x 0.6 cm^2 area at 2 µm resolution. **B.** detail of a U-rich band. The red spheres correspond to biogenic pyrite.

The images are modified from (110); they were obtained at the XFM beamline at the Australian Synchrotron (111) using the 384-element Maia detector (112, 113). The X-ray images were processes and quantified using the GeoPIXE software (112).

Figure Five. Model of uranium roll-front deposit


Quadrillion Btu
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
2010
2012
2014
2016
2018
2020
2022
2024
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2032
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2036
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2040

Figure 1

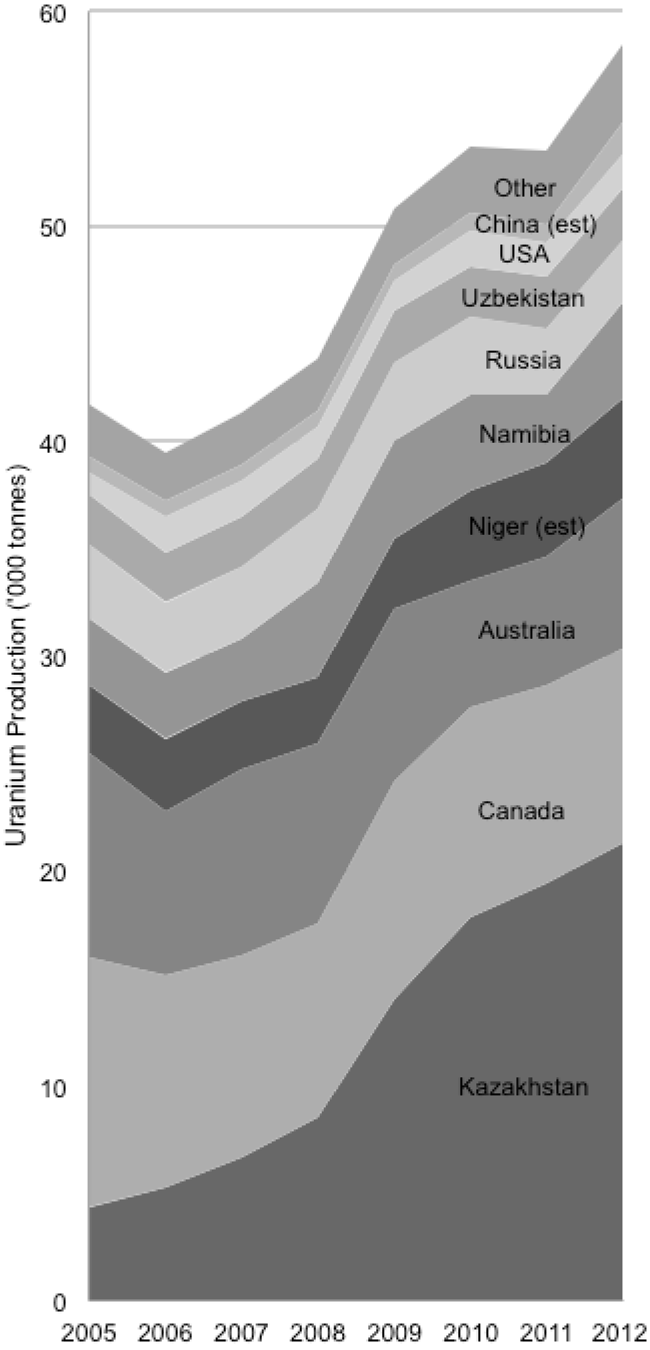

60
50
40
30
20
10
0
Uranium Production ('000 tonnes)
2005
2006
2007
2008
2009
2010
2011
2012
Other
China (est)
USA
Uzbekistan
Russia
Namibia
Niger (est)
Australia
Canada
Kazakhstan

Figure 2

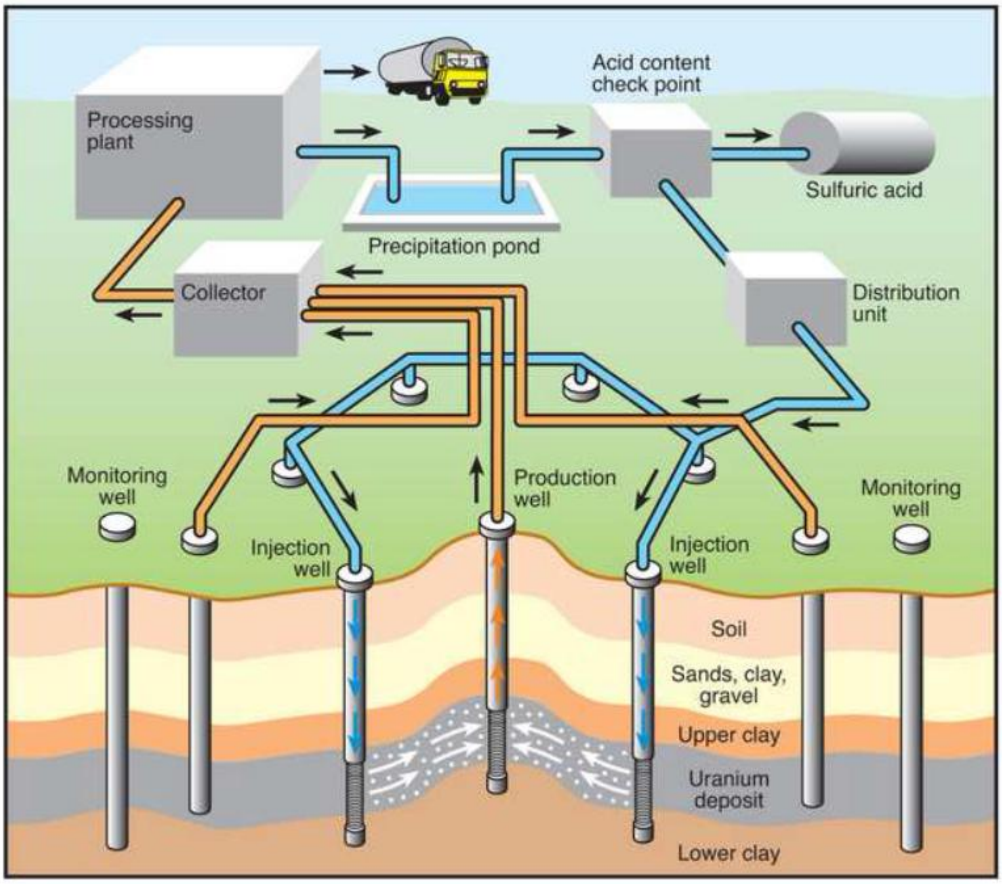

Processing plant
Acid content check point
Sulfuric acid
Precipitation pond
Collector
Distribution unit
Monitoring well
Injection well
Production well
Injection well
Monitoring well
Soil
Sands, clay, gravel
Upper clay
Uranium deposit
Lower clay

Figure 3

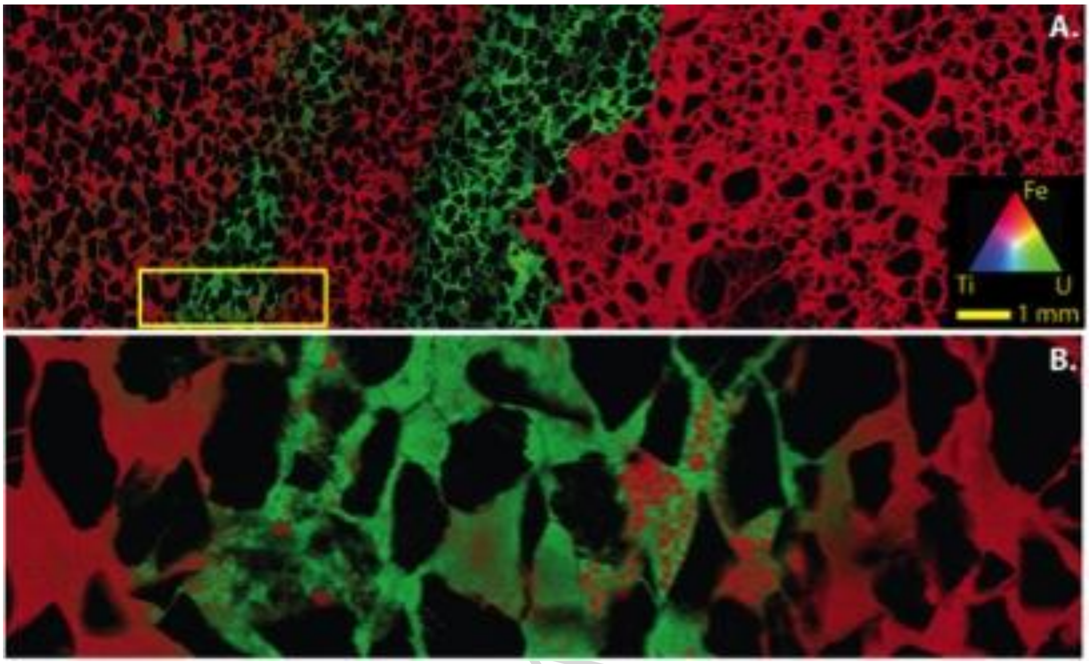


Figure 4

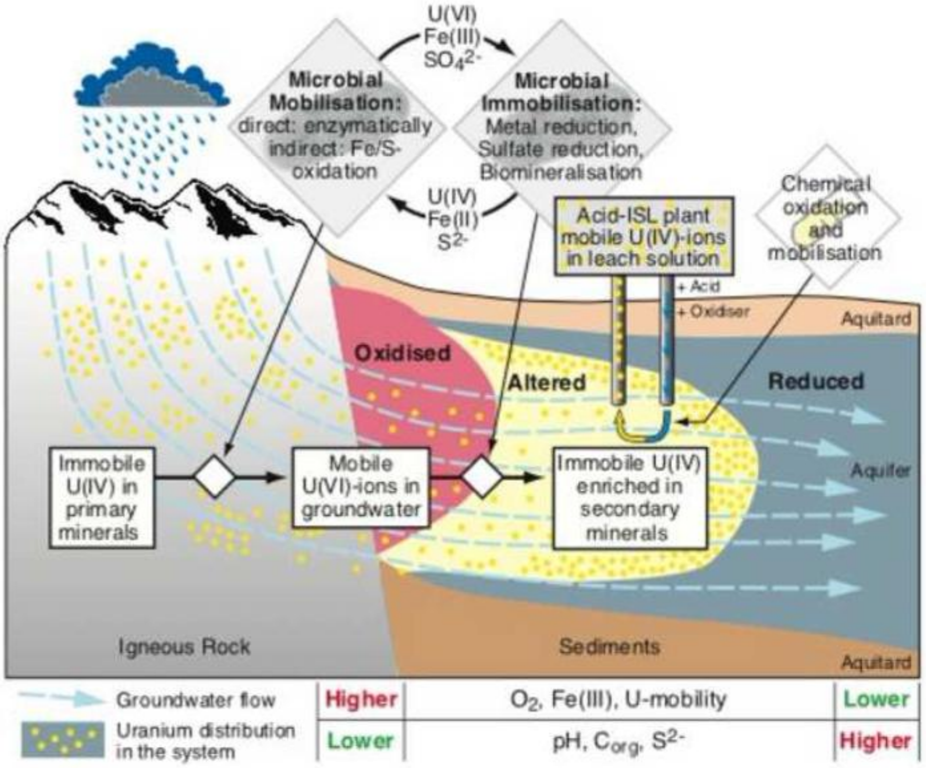
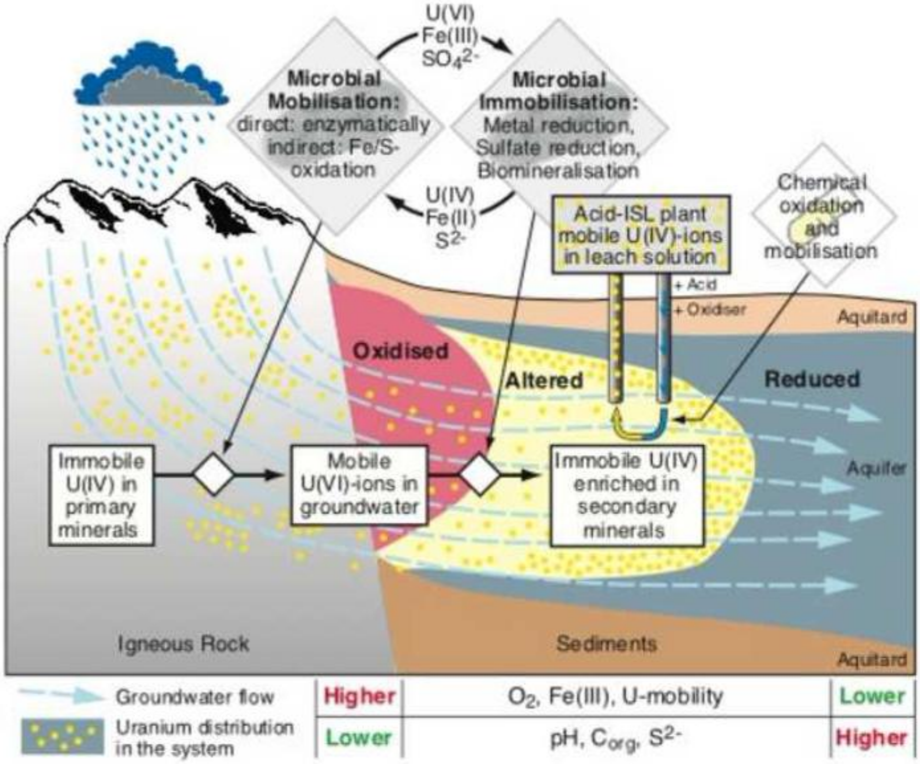

U(VI)
Fe(III)
SO_4^{2-}
Microbial Mobilisation:
direct: enzymatically
indirect: Fe/S-oxidation
Microbial Immobilisation:
Metal reduction,
Sulfate reduction,
Biomineralisation
U(IV)
Fe(II)
S^{2-}
Acid-ISL plant
mobile U(IV)-ions in leach solution
Chemical oxidation and mobilisation
+ Acid
+ Oxidiser
Aquitard
Oxidised
Altered
Reduced
Immobile U(IV) in primary minerals
Mobile U(VI)-ions in groundwater
Immobile U(IV) enriched in secondary minerals
Aquifer
Igneous Rock
Sediments
Aquitard
Groundwater flow
Uranium distribution in the system
Higher
O_2, Fe(III), U-mobility
Lower
Lower
pH, C_{org}, S^{2-}
Higher

Figure 5



Graphical abstract

HIGHLIGHTS

- 45% of uranium is mined by *in situ* recovery worldwide
- Microorganisms play a significant role in the mobilization/immobilization of uranium
- Microorganisms could be used to increase uranium *in situ* leaching and recovery rates, as well as be part of remediation strategies