

# Biogeochemical behaviour and bioremediation of uranium in waters of abandoned mines

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**Abstract** The discharges of uranium and associated radionuclides as well as heavy metals and metalloids from waste and tailing dumps in abandoned uranium mining and processing sites pose contamination risks to surface and groundwater. Although many more are being planned for nuclear energy purposes, most of the abandoned uranium mines are a legacy of uranium production that fuelled arms race during the cold war of the last century. Since the end of cold war, there have been efforts to rehabilitate the mining sites, initially, using classical remediation techniques based on high chemical and civil engineering. Recently, bioremediation technology has been sought as alternatives to the classical approach due to reasons, which include: (a) high demand of sites requiring remediation; (b) the economic implication of running and maintaining the facilities due to high energy and work force demand; and (c) the pattern and characteristics of contaminant discharges in most of the former uranium mining and processing sites prevents the use of classical methods. This review discusses risks of uranium contamination from abandoned uranium mines from the biogeochemical point of view and the potential and limitation of uranium bioremediation technique as alternative to classical approach in abandoned uranium mining and processing sites.

**Keywords** Ecotoxicology · Tailings · Groundwater · Leaching · Bioreduction · Radionuclides · Uranium minerals

## Introduction

Uranium (U) is not only important because of being the heaviest element occurring in nature in weighable amounts and nuclear fuel but also due to its chemical and radioactive implications to human health and environment. Exposures to environmental U are associated with health effects, such as kidney or liver damage, cancer or birth defects (Schuttman 1993; Sram et al. 1993; WHO 2001). Furthermore, U disrupts the endocrine system such that populations exposed to environmental U have increased risk of fertility problems and reproductive cancers. U is one of the most mobile radioactive metals that it are easily washed down through soil together with percolating water to both surface and underlying groundwater. Thus, water pathway is the major exposure route to U, although dust in the air may act as a pathway too. Its geochemical properties especially the chemical form strongly influences the environmental transport. U exists predominantly as hexavalent oxidation state (U(VI)) which forms readily soluble species over a range of pH and redox potential ( $E_H$ ) conditions in the aquatic environments. Furthermore, U(VI) ions are exclusively available as species of uranyl ion ( $UO_2^{2+}$ ), which sometimes behave like divalent metal ions (Mkandawire et al. 2006a).

Small amounts of U are found almost everywhere in soil, rock and water. As a result, it is normal to find dissolved U at very low concentrations in most natural waters, but concern rise only when concentrations increase to levels above  $1 \mu\text{gL}^{-1}$  (Kalin et al. 2005a). Thus, trace contamination occur naturally, but most of alarming U contaminations are anthropogenic associated with activities of the nuclear fuel cycle, phosphate fertilizer production process and the

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improper disposal of U mine tailings (Jerden and Sinha 2006; Chen et al. 2005). The cycle for nuclear fuel, include the mining, milling and processing of U ores or U products. In the production of phosphate fertilizers, the phosphorus is usually extracted from phosphate rocks containing U (Shumilin et al. 2012; Rodgher et al. 2012; Giri et al. 2010; Arogunjo et al. 2009; Vanhoudt et al. 2008). U may also be released from fly ash or stack emissions during coal combustion and from the military use of  $^{235}\text{U}$ -depleted in warheads (WHO 2001; Giannardi and Dominici 2003). However, major contamination concerns currently stems from U waste and tailing dumps in abandoned sites of ore mining and processing (Carvalho et al. 2005; Mkandawire 2005). In most abandoned U mining sites, the discharge of U contamination is also accompanied by other toxic heavy metals and metalloids like Cd, Pb, Ra and As (Al Hashimi et al. 1996; Trontelj and Ponikvar-Zorko 1998).

There are considerable efforts to reclaim most of abandoned and decommissioned U mining sites. Frequently, classical techniques based on civil and chemical engineering strategies are employed in the restoration efforts. The strategies include: covering of tailing and waste dumps with either chemical neutralising or physical stabilising material, or even both, and, treatment of contaminated waters using chemical and electrochemical procedures (Kalin et al. 2005a). These classical remediation techniques are said to be too expensive to meet the remediation demand due to the number of sites requiring restorations, and the technologies are beyond capacity of most poor nations (Benders et al. 2000; Salt et al. 1995; Schnoor et al. 1995; Adler 1996). Additionally, it is claimed that the contamination problems are merely suspended or transferred to elsewhere (Mkandawire et al. 2004b). Furthermore, the discharges of the contaminants from the tailing and waste heaps into the water pathway are mostly non-point source. Moreover, the patterns of discharge of contaminant loads from mine wastes and tailings are usually unpredictable where they are either (a) high volume and concentrated but a short-term, (b) very low concentrated but long-term or (c) low concentrated but short-term discharge. These criticisms have pointed to the need for alternative remediation technology that would be cheap, easy to run, responsive with long-term effect preferably where natural processes are used. Bioremediation fulfils most of these conditions, and it is currently advocated as a promising alternative technology with potential application in abandoned U mining sites. Therefore, the aim of this review is to relate knowledge of U biogeochemistry with opportunities and challenged in application of bioremediation in abandoned U mines. The scope of this review is limited to discussion on toxicology as well as potential and limitation of bioremediation of natural U in abandoned U mines with special consideration to its biogeochemical behaviours in water.

## Abandoned U mining site

Not every decommissioned U mine is an abandoned mine. However, only sites where advanced exploration, mining or mines production ceased without comprehensive rehabilitation or restoration are classified as abandoned mines, also called orphaned mines (Hoffmann 2000; Mkandawire 2005; Mkandawire et al. 2004a). All U abandoned mining sites share identical environmental problems ranging from disturbed physical landscapes to large piles of rocks, mining wastes and tailing to environmental contamination with radionuclides and heavy metals (Diehl 2003; Diehl 1995). Consequently, the overlying characteristics of abandoned U mines are public health and safety, environmental safety and aesthetic concerns. However, the mining and processing procedure determines the characteristics of an abandoned mining site.

### Ore mining and processing

#### *Ore extraction*

There are several techniques used to extract U from its ore deposits but frequently used are open-pit, underground, in situ leaching (ISL)<sup>1</sup> and borehole mining techniques. The open-pit mining of U was predominant until the 1960s (Pettersson and Koperski 1991; Abdelouas 2006). It was used to extract U from ore deposits located near the surface while deposits located deeper are extracted in underground mining. In both open pits and underground mines, traditional mining techniques of drilling and blasting are used to excavating mineral bearing rock. The ISL mining involves injecting either acid or alkaline solutions into the ground to dissolve the mineral ores, and then, pumping the pregnant solution to the treatment plant to recover the U. Alkaline leaching, using a combination of sodium bicarbonate and carbon dioxide, is suitable for ore bodies containing significant calcium (Ca) like limestone or gypsum (Erdem et al. 1995; Beddow et al. 2006; Misaelides et al. 1995). Otherwise, acid leaching using sulphuric acid enriched with oxygen is generally ideal. The ISL technique is ideal in permeable ore bodies especially in secondary mineral deposits (e.g. uraninite or coffinite silicate) that occur in permeable sand or sandstones below the water table (Ulrich et al. 2006). Lastly, the borehole mining of U is the least frequently used technology although it is potentially applicable in both surface and underground mining. The procedure involves injection of water at high pressure

<sup>1</sup> ISL technology was developed independently in both the USSR and USA in the mid-1970s. The Soviets adopted the acid leaching system, while alkaline, primarily carbonate-based leaching system was widely used in the USA. In the USA, the technology is also known as in situ recovery.

through boreholes into the ground. The pressure breaks up the ore-bearing rocks, which results into formation of slurry composed of dirt, rock and minerals. The slurry is pumped to the surface where it is dewatered to recover the U (Salbu 2013); Thompson 2011; Boice et al. 2010; SENES and Alberta Environment 2008).

### U processing

The process of extracting U from ore is summarised in Fig. 1. For open-pit and underground mining, the first step is to crush the ore-bearing rocks into a fine powder. Then, huge amounts of water, leaching agents and thickener are added to the pulverised ore to oxidise and dissolve the U minerals. The U minerals usually available as  $\text{UO}_2$  are oxidised to  $\text{UO}_3$  followed by formation of either a uranyl sulphate ( $\text{UO}_2(\text{SO}_4)_3^{4-}$ ) or a uranyl carbonate (predominantly  $\text{UO}_2(\text{CO}_3)_3^{4-}$ ) in acid leaching or carbonate leaching, respectively. Obviously, there is no crushing in the ISL and borehole mining procedure. After the leaching step, the U is concentrated and purified using either ion exchange or solvent extraction processes. Solvent extraction is the most applied procedure, which uses tertiary amines ( $\text{R}_3\text{N}$ ) in kerosene diluents (El-Nadi et al. 2003). The amines react with sulphuric acid, which removes

impurities from the loaded solvents. Cations are removed using sulphuric acid while anions are removed with gaseous ammonia (Juznic et al. 1989; Levy and Kearney 1999). The solvents are stripped with ammonium sulphate solution. Then, exposure to gaseous ammonia to neutralise the solution leads to the precipitation of ammonium diuranate. The diuranate is subsequently dewatered and dried to produce hydrated U peroxide ( $\text{UO}_4 \cdot 2\text{H}_2\text{O}$ ). Lastly, centrifugation and precipitation of  $\text{UO}_4 \cdot 2\text{H}_2\text{O}$  yields the yellowcake ( $\text{U}_3\text{O}_8$ ) containing at least 75 % U oxides (Abdelouas 2006; Moyes et al. 2000).

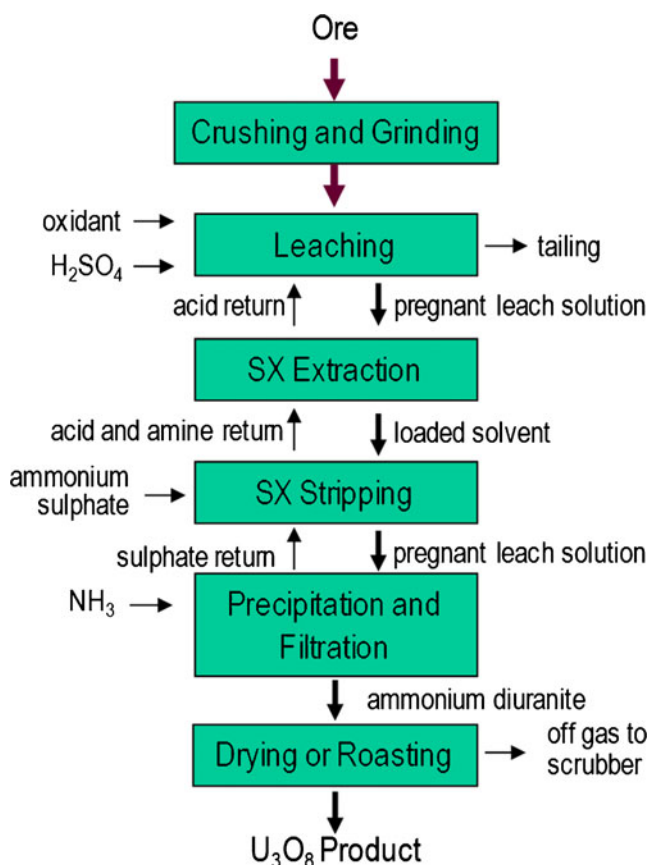
### Characteristics of abandoned U mines

#### Mining wastes and tailing

Huge piles of mine waste and tailing are the first characteristics of abandoned open-pit and underground mines and U content of the ores is often between 0.1 and 0.7 % (Diehl 1995; Diehl 2003; Meinrath et al. 2003). Thus, large amounts of rocks are excavated and large amounts of tailings are generated when extracting the U. A mine can generate up to 40 tonnes of waste rock for every tonne of U ore produced, while underground mines produce about 1 tonne of waste rock/tonne of ore (Morgenstein 2003; Mkandawire 2005; Tomasek et al. 1993; Enderle and Friedrich 1995). Mountains of wastes and debris as well as lakes resulting from flooding of open pits disturb the landscape. Apart from creating undesired physical features, wastes, debris and tailings create massive stockpiles of radioactive and toxic waste rock and sand-like tailings, which become a source of radioactive, toxic metals and radioactive air pollutants. With most of the U removed, the mine wastes and tailings remain very radioactive because almost 85 % of the radioactive elements and U decay progenies contained in the original U ore end up in the tailings and wastes (Mkandawire 2005; Enderle and Friedrich 1995).

#### Mine water quality

Water contamination is a common characteristic of abandoned U mining sites. The tailings and wastes from the U milling process consist of ground rock particles, non-extracted radionuclides, toxic metals, metalloids, remnants of U as well as leach and mill chemicals. As a result, chemically hazardous substances in the wastes and tailings like lead, cadmium, arsenic and mercury are discharged more easily into the environment through leaching and blown dust into the water and air pathway (Al Hashimi et al. 1996; Pressyanov et al. 1995). The excavated and ground rock particles have high surface area that increased their contact with air and water, which naturally favours geochemical process especial redox and acidification. Hence, precipitation leaches out radionuclide (i.e. progeny of U and U debris), heavy metals and metalloids, which may contaminate surface and groundwater. Depending



**Fig. 1** The U extraction chemistry from ore mining to production of yellow cake

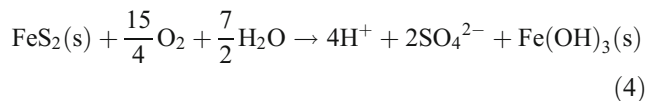
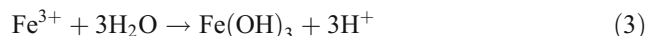
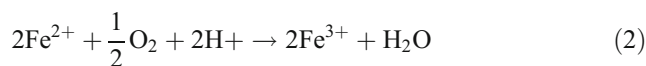
on the type of rock, these wastes may be acid generating. For instance, surface mine water discharged from a few abandoned mining sites in eastern Germany (which the author has studied extensively) have pH values between 2.7 to 3.4. The concentrations of dissolved arsenic ranges from 0.8 to 700 mgL<sup>-1</sup>, iron (Fe) from 750 to 3,000 mgL<sup>-1</sup> and sulphate from 2,000 to 8,000 mgL<sup>-1</sup>. However, 20 to 60 % of the dissolved arsenic is immobilised by co-precipitation with Fe (III) (Clara and Magalhães 2002; Fritzsche et al. 2006; Munoz et al. 2002). Nevertheless, this results in high groundwater concentrations of arsenic and enrichment into the sediments, which can reach as high as 500 % more than the background (Carvalho et al. 2005; Belzile and Tessier 1990).

*Radon gas emanation and air quality*

Another characteristic of abandoned U mining is air pollution, which is manifested either through odours from the waters, dust or both. However, the most dangerous air pollution associated with abandoned U mining is odourless—radon (<sup>222</sup>Rn) emanation. The original source of <sup>222</sup>Rn gas is from the natural radioactive decay of <sup>238</sup>U to its daughter element radium (<sup>226</sup>Ra). U-rich mineral rocks like granite, phosphate, shale and pitchblende store natural high deposits of <sup>222</sup>Rn. Therefore, the piles of rock and tailings in abandoned U mines continuously release Rn to the environment. Apart from being significant sources of Ra gas, winds may also blow dust containing radionuclides, heavy metals and particulate matter from the rock debris and tailing piles. Furthermore, waste rock and tailings dumps of abandoned U mines release nitrogen oxides, volatile organic compounds and carbon dioxide (CO<sub>2</sub>). Acid plants producing acid for milling operations release large amounts of sulphur dioxide—a major contributor to acid rain (Hole et al. 2008; Fowler et al. 2005; Jeffries et al. 2003).

*Acid mine drainage*

The generation of acid mine drainage (AMD) is a common in most abandoned U mining and processing sites because most U ore are associated with pyrite minerals (FeS<sub>2</sub>). Thus, pyrite minerals are abundant in the mine waste rock (Carvalho et al. 2005). Exposure to water and air breaks down the pyrite-rich waste rocks and tailings leading to the formation of sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and FeSO<sub>4</sub>. Then, the FeSO<sub>4</sub> undergoes further reactions, which eventually end up in formation of ferrous hydroxide (Fe(OH)<sub>3</sub>). The Fe(OH)<sub>3</sub> is responsible for reddish or orange colour in the seepage water through mine waste and tailings, which is a symbol that acid drainage is taking place. The whole breakdown process can be summaries in following chemical reactions:



Logically, the AMD affects the acidity as well as pH of the receiving waters due to the high Fe and H ion they contain. However, the pH measurements may not detect heavy AMD in some streams with high dissolved carbonates because pH only indicates the concentration of H ions. AMD depletes the buffering ability of water by neutralizing carbonate and bicarbonate ions to form carbonic acid (H<sub>2</sub>CO<sub>3</sub>), which readily breaks down into water and carbon dioxide (see reactions 5 and 6).



*Aquifer contamination from in situ leaching*

Much as there is also little surface disturbance and no generation of waste rocks and tailing, the abandoned ISL mines have serious groundwater contaminations that are difficult to remediate. During ISL mining, bacterial contamination are common that sometimes facilitate in the leaching process. When the mines are abandoned, the bacterial activities continue to affect the leaching of minerals into the aquifers. Accompanied by prevailing unsaturated conditions, the pyrite oxidise generating significant quantities of sulphuric acid, which further mobilises heavy metals, U and radionuclides trapped in the pore spaces of the rocks thus adding to the contaminant load in the groundwater. The trapped liquids are characterised by elevated amount of heavy metals and metalloids which can reach between 100 and 400 % higher in Cd, As, Ni and U than before the ISL begins (IAEA 2001; Carvalho et al. 2005). In acid ISL mining, an important characteristic of using sulphuric acid in leaching is the deterioration of the ore zone permeability due to chemical and gaseous plugging and rising of salinity levels. In the alkaline ISL, the large quantities of solutions escape beyond the mining zone due to gaseous oxygen plugs. However, the alkaline agents tend to increase the salinity of the groundwater but only slightly. Nevertheless, the contamination potential of surrounding waters with U, Ra, SO<sub>4</sub>, Fe and other heavy metals remains high.

## Geochemistry

### General properties

#### *Physicochemical*

U is the heaviest naturally occurring element, with an atomic number of 92. Despite its high atomic number, it is an ubiquitous element with an average concentration of  $3 \text{ mg kg}^{-1}$  in the Earth's crust (IAEA 1998). The concentrations of U in geological materials are highest in continental-type rocks. Pure U is a silvery white, ductile and malleable heavy metal, which melts at  $1,132.3 \text{ }^\circ\text{C}$  and boils at about  $3,818 \text{ }^\circ\text{C}$ . Its density is about  $19.05 \text{ g cm}^{-3}$  (Kleykamp 1991). However, elemental U hardly exists in nature, because U metal is readily subjected to surface oxidation (Beddow et al. 2006; Whitehead et al. 1971). Consequently, it ignites spontaneously upon contact with air at ambient temperature forming U oxides with six potential oxidation states of U. However, U(IV) and U(VI) are the most common oxidation states of U in nature because U(III) easily oxidises to U(IV) under most redox conditions while U(V) readily disproportionates to U(IV) and U(VI) in nature (Lovley et al. 1993; Benders et al. 2000; Markich 2002). U forms more than 234 mineral species and accounts for only 5 % of all known minerals (Barthelmy 2008). The most common forms of U oxide pitchblende ( $\text{U}_3\text{O}_8$ ) and uraninite ( $\text{UO}_2$ ), have low solubility in water and are relatively stable over a wide range of environmental conditions. The  $\text{U}_3\text{O}_8$  exist mostly as  $\text{U}_2\text{O}_5$   $\text{UO}_3$  (Wersin et al. 1994). Generally, U forms many compounds that tend to have yellowish or greenish colours. In respect to this chemical property, U has been historically used as a colorant for glass and ceramic (i.e. producing orange-red to lemon yellow hues) and for tinting and shading in early photography.

#### *Radioactivity*

U has sixteen radioactive isotopes, but only three isotopes— $^{238}\text{U}$ ,  $^{235}\text{U}$  and  $^{234}\text{U}$ —occur naturally (Jerden and Sinha 2006). Figure 2a, b summarises the decay series of  $^{238}\text{U}$  and  $^{235}\text{U}$  detailing the type of radiation given off at each step and the half-life of each step gone through to ultra-stable and non-radioactive  $^{206}\text{Pb}$  or  $^{207}\text{Pb}$  isotopes. The U isotopes emit mostly alpha and less beta particles accompanied by little emission of gamma radiation especially during the decay of  $^{235}\text{U}$  (Cherdynstsev et al. 1982; Ivanovich and Harmon 1992). The emissions are all ionising radiation, strong enough to break chemical bonds, thereby possessing the ability to damage or destroy living cells (ATSDR 1999; WHO 2001, 2004). In terms of the amount of radioactivity in nature,  $^{235}\text{U}$  contributes about 2.2 %,  $^{238}\text{U}$  about 48.6 % and  $^{234}\text{U}$  about 49.2 %. The total activity level of U depends

on the isotopic composition ratio. The  $^{235}\text{U}$  and  $^{234}\text{U}$  isotopes pose a greater radiological toxicity risk than  $^{238}\text{U}$  because they have much shorter half-lives.

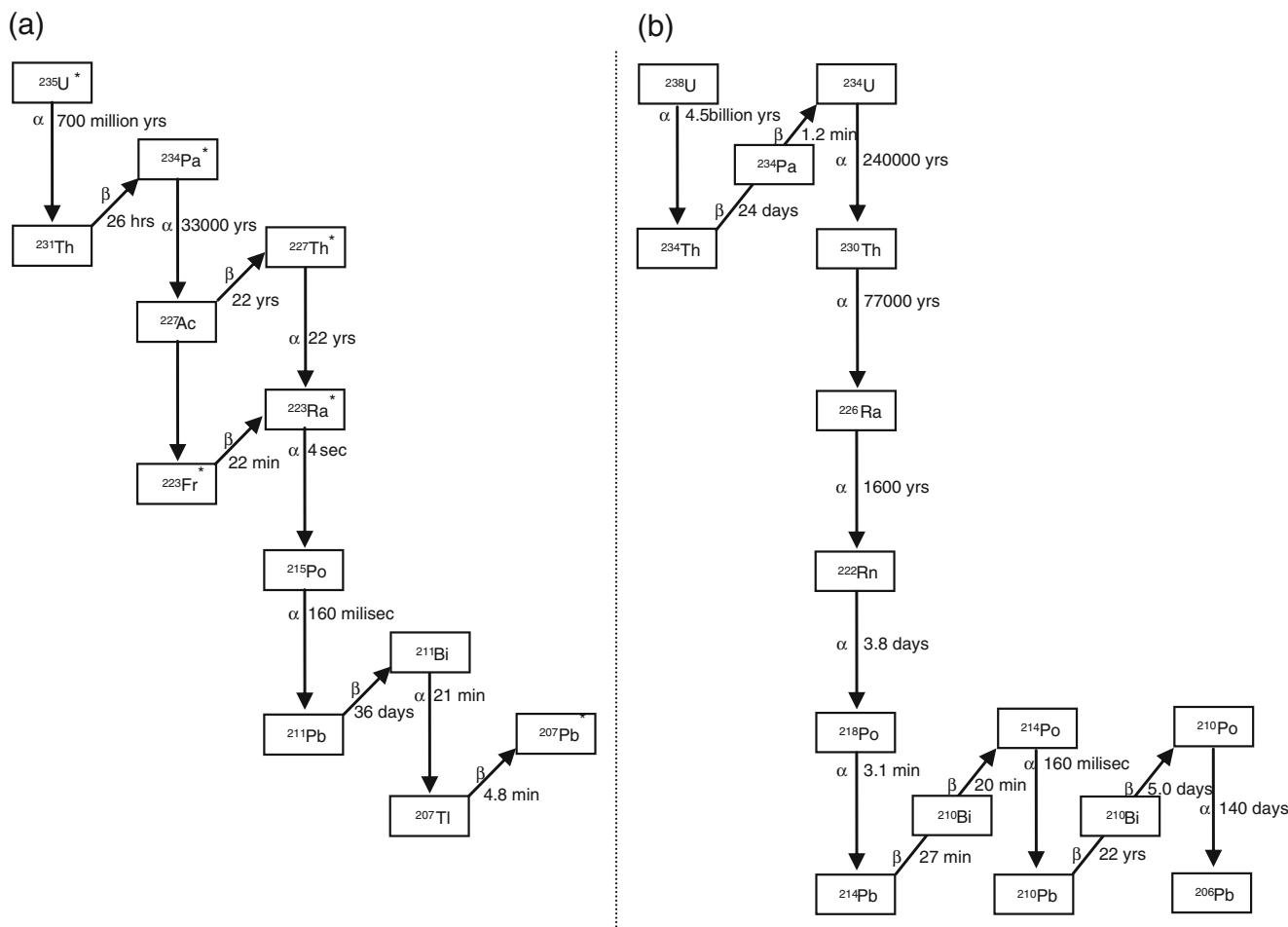
#### *U classifications*

In nature, the U isotopes are distributed as 99.284 % of  $^{238}\text{U}$ , 0.72 % of  $^{235}\text{U}$  and a very small amount ( $\sim 0.0054$  %) of  $^{234}\text{U}$ . Thus, U with this isotope distribution is called natural U. The  $^{235}\text{U}$  has high fission capacity; consequently,  $^{235}\text{U}$  is used as fuel of nuclear reactors. However, a nuclear reactor requires higher than 0.72 % content of  $^{235}\text{U}$  to function. Thus, the natural U is centrifuged to enrich the content of  $^{235}\text{U}$  from 0.72 % to about 1.5–3.0 % (IAEA 1999, 2007). Then, the  $^{234}\text{U}$  and  $^{235}\text{U}$  isotopes are extracted to produce high radioactive enriched U, which is primarily used as fuel in nuclear reactors. Consequently, the remaining U after removal of the enriched fraction is called depleted U and it contains at least three times less  $^{234}\text{U}$  and  $^{235}\text{U}$  by mass than before commencement of enrichment (Table 1). The depleted U is weakly radioactive and a radiation dose from it is about 60 % of natural U with the same mass (WHO 2001; Meinrath et al. 2003). Natural and depleted U differ only in their radioactivity but their chemical properties are the same.

#### *Aquatic chemistry of U*

##### *Behaviour in surface mine waters*

The aquatic chemistry of U is governed by a linear dioxocation  $\text{UO}_2^{2+}$  ( $[\text{O}=\text{U}=\text{O}]^{2+}$ ) (Meinrath 1996; Meinrath 1999), which is mainly controlled by the pH, the  $E_H$  and the type of the available complexing ligand such as carbonates, phosphates, vanadates, fluorides, sulphates and silicates (Bernhard et al. 1998; Duff et al. 2002; Martinez Aguirre et al. 1995). The U(III) is very easily oxidised whilst the U(V) is prone to disproportionation (Steele and Taylor 2007; Shilov et al. 2007). In oxidised subsurface waters with  $E_H > 200 \text{ mV}$ , dissolved  $\text{UO}_2^{2+}$  form carbonate complexes of varying stoichiometry as a function of the pH and the partial pressure of  $\text{CO}_2$  (g) (Meinrath 1996). The uranyl carbonate species prevail in the neutral to alkaline range, while the  $\text{UO}_2^{2+}$  hydrolyses in low pH values. Most of the hydrolysis species are oligomeric nature. Thus, oligomeric hydrolysis  $(\text{UO}_2)_2(\text{OH})_2^{2+}$  species are usually the most dominants. These are followed by other oligomeric and monomeric hydrolysis species, which include  $[(\text{UO}_2)_3(\text{OH})_5]^+$ ,  $[(\text{UO}_2)_3(\text{OH})_4]^{2+}$ ,  $[(\text{UO}_2)_2(\text{OH})_3]^+$ ,  $[(\text{UO}_2)_3(\text{OH})_7]^-$ ,  $(\text{UO}_2)_4(\text{OH})_7^+$ ,  $\text{UO}_2\text{OH}^+$ ,  $\text{UO}_2(\text{OH})_2$ ,  $\text{UO}_2(\text{OH})_3^-$  and  $\text{UO}_2(\text{OH})_4^{2-}$  (Bernhard et al. 1998; Brendler et al. 1998; Markich 2002; Meinrath 1997; Ulrich et al. 2006; Zanonato et al. 2004). The monomeric carbonate



**Fig. 2** The decay series of **a**  $^{235}\text{U}$  and **b**  $^{238}\text{U}$ . The  $\alpha$  and  $\beta$  symbols indicate the alphas and beta decay, while the time indicated the half-life. The asterisk next to the isotope indicates the element that emit  $\gamma$  particles too. Modified from Peterson et al. (2007)

species include  $\text{UO}_2\text{CO}_3^0$ ,  $\text{UO}_2(\text{CO}_3)_2^{2-}$  and  $\text{UO}_2(\text{CO}_3)_3^{4-}$  while oligomeric carbonate compound include  $(\text{UO}_2)_3(\text{CO}_3)_6^{6-}$  (Bernhard et al. 1998; Meinrath 1998; Meinrath 1996). The distribution and occurrence of the uranyl species in water is summarised in Fig. 3.

Under the reduced conditions with  $E_H$  below zero volts, the dissolved U(VI) species are reduced to the U(IV) and precipitate as  $\text{UO}_2 \cdot 2\text{H}_2\text{O}$  (Meinrath 1999; Luo et al. 2007; Liu and Xu 2007). This occurs mostly in sediments and very deep reservoirs. The U(IV) has a strong tendency to bind to organic material (Meinrath 1999). U in the mine water is derived

mainly from oxidation of uraniferous bitumen or dissolution of carbonates (Meinrath 1996; Gavshin et al. 2005; Milodowski et al. 1990). Consequently, the dissolved U is associated with the concentrations of  $\text{NO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$  and not silica (Petrescu and Bilal 2006).

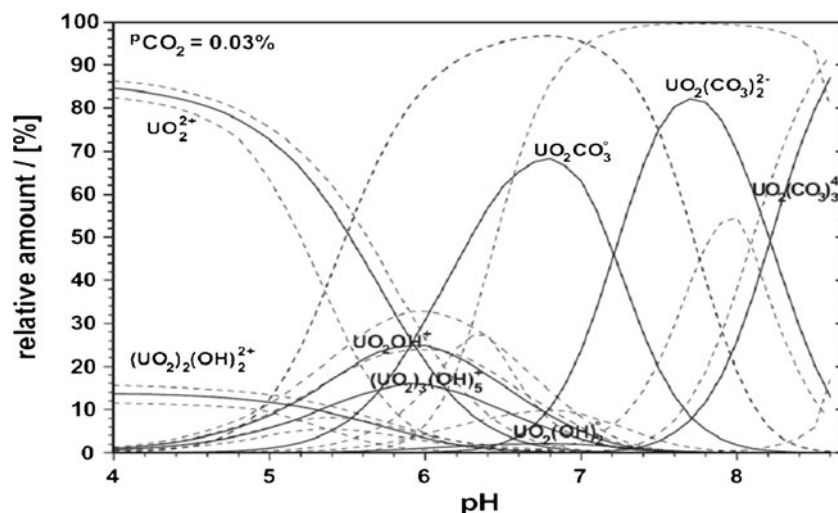
*U behaviour in oxidising aquifers*

The chemistry of U in oxic aquifers is very much similar to the chemistry in the surface waters. Under oxidising conditions, U(VI) is the most stable form of U and it mostly exists as uranyl ion ( $\text{UO}_2^{2+}$ ). It forms complexes readily with carbonate, phosphate or sulphate ions. In low carbonate-free systems, the important species include  $\text{UO}_2^{2+}$ ,  $\text{UO}_2(\text{OH})_2^0(\text{aq})$ ,  $\text{UO}_2(\text{OH})_3^-$ ,  $(\text{UO}_2)_3(\text{OH})_5^+$  and  $(\text{UO}_2)_2(\text{OH})_2^{2+}$  (Meinrath 1999; Meinrath 1997). In predominately carbonate-containing water, major uranyl species are carbonate anions with  $\text{UO}_2(\text{CO}_3)_3^{4-}$  accounting for almost 60 %,  $\text{UO}_2(\text{CO}_3)_2^{2-}$  for 30 % and  $(\text{UO}_2)_3(\text{OH})_5^+$  (Meinrath 1999). However, the real distributions of the species in relation to other U(VI) species are a function of

**Table 1** Distribution of isotopes in natural and depleted uranium

Isotope	Fractions of the isotopes	
	Natural uranium (%)	Depleted uranium (%)
$^{234}\text{U}$	0.006	0.001
$^{235}\text{U}$	0.72	0.20
$^{238}\text{U}$	99.28	99.80

**Fig. 3** Distribution of U(VI) aqueous species at 25 °C and ionic strength  $\mu=0.01$  M NaCl as a function of pH at atmospheric  $\text{CO}_2$  partial pressure. Dashed lines give 90 % confidence limits. The speciation diagram has been modified and used with courtesy from Meinrath (1999)



pH and the total concentration of dissolved U(VI). Concentrations of dissolved U are controlled by the aqueous speciation of U(VI) and adsorption of U(VI) species onto mineral and organic material (Murakami et al. 1997; Davis et al. 2004; Ohnuki et al. 2004). The adsorption of U(VI) onto specific mineral phases primarily controls U mobility in oxic aquifers. The minerals prevalent in oxic conditions include carnotite  $[(\text{K}_2(\text{UO}_2)_2(\text{VO}_4)_2)]$ , schoepite  $(\text{UO}_3 \cdot 2\text{H}_2\text{O})$ , rutherfordine  $(\text{UO}_2\text{CO}_3)$ , tyuyamunite  $[\text{Ca}(\text{UO}_2)_2(\text{VO}_4)_2]$ , autunite  $[\text{Ca}(\text{UO}_2)_2(\text{PO}_4)_2]$ , potassium autunite  $[\text{K}_2(\text{UO}_2)_2(\text{PO}_4)_2]$  and uranophane  $[\text{Ca}(\text{UO}_2)_2(\text{SiO}_3\text{OH})_2]$  (Allard et al. 1999; Jerden and Sinha 2006; Hiess et al. 2012; Luo and Gu 2011; Stefaniak et al. 2009). However, adsorption onto colloidal organic matter facilitates U transport and dispersal in the oxic aquifers. Furthermore, the solubility of U(VI)-containing minerals controls the maximum concentrations of dissolved U. In most abandoned U mines, the dissolution of U is dependent on the equilibrium between partially oxidised uraninite and the precipitation of coffinite (Cai et al. 2007). U coprecipitates with Fe oxyhydroxides, which leads to the Fe oxyhydroxides taking up nearly all the U dissolved in the mine waters before they reach the surface (Gomez et al. 2006).

#### *U behaviour in reducing aquifer*

Under the reducing conditions of negative  $E_{\text{H}}$  values, the dissolved U(VI) species are reduced to the less soluble U(IV) (Duff et al. 1999; Bots and Behrends 2008). Consequently, there are sparingly soluble U(IV) species or mixed U(IV)–U(VI) solids (e.g.  $\text{U}_4\text{O}_9$ ) in reducing and anoxic conditions like in sediments and groundwater. The speciation of these sparingly dissolved U(IV) is dominated by hydrolytic species  $\text{U}(\text{OH})_3^+$  and  $\text{U}(\text{OH})_4^0(\text{aq})$  in extremely low pH, especially of values below pH. The solid phases that control the U concentration in the groundwater

are partially oxidised pitchblende. Other important U(IV) minerals associated with reducing conditions include coffinite  $(\text{USiO}_4)$  and ningyosite  $(\text{CaU}(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O})$  (Jerden and Sinha 2006). There are usually accumulations of U(IV) precipitates in the reducing aquifers because the dissolved U(VI) species are in most cases, transported through the permeable where they get into reduced state. This is how the U roll-front deposits in sandstone rocks come about. These ore bodies occur when U(VI) carried by oxic groundwater contacts a zone containing a naturally occurring reductant such as natural organic matter and  $\text{H}_2\text{S}(\text{g})$  (Duff et al. 1999; Gadelle et al. 2001). The resulting U(IV)-containing minerals include uraninite and coffinite.

#### *U in waste-rocks and tailings heaps*

Generally, primary U ore in the waste rocks and tailing weathers into saprolites, which retain U as uranyl phosphates of the meta-autunite group mainly as meta-uranocircite in saturated zones (Jerden and Sinha 2006; Vinay Kumar Reddy et al. 2012; Rodgher et al. 2012). The U is retained in the abandoned mine wastes and tailings containing primary ore by two processes:

- 1 Incorporation into barium–strontium–Ca aluminium phosphate minerals of the crandallite group (mainly gorceixite); and
- 2 Sorption of U with phosphorous onto Fe oxides that coat the surfaces of other soil minerals.

Thermodynamically, the meta-autunite group minerals are present in the saprolites in pH values below 4.5. Once exposed to the unsaturated environment, the meta-autunite react to form U(VI)-bearing aluminium phosphates and U(VI) surface complexes or nano-precipitates associated with ferric oxides (Jerden and Sinha 2006; Shvareva and Albrecht-Schmitt 2006; Raicevic et al. 2006).

## Hazard transfer from abandoned U mines

The prime environmental concerns in abandoned U mining and processing sites are the dissolution of minerals containing radioactive elements and heavy metals from waste-rock piles and tailings (Landa 2004). All chemical elements exist in conditions close to secular equilibrium in ores deposits, but the state of secular equilibrium is altered during the mining and processing of the ore material. The ore mining and processing lead to both physical and chemical equilibrium changes, which enhance the entry of radionuclides and the heavy metals hazards into human and environmental pollution pathways. The physical changes are responsible for the following major hazards transfers: (1) atmospheric dispersion of radioactive aerosol particles from mining waste and tailings heaps to adjacent sites by blowing winds; (2) increased erosion and seepage of water through loose waste-rock and tailing heaps, which may result in transport of contaminants by surface water; and (3) emanation of radon gas into the atmosphere.

The physical stability of the tailings and waste-rock heaps decreases over time due to internal natural processes, including physical and chemical diagenesis and effects of root penetration from a vegetative cover (Riley 1995; Roussel et al. 2000). Thus, the abandoned U mining and processing sites become prone to erosion, leaching as well as emanation of radioactive gases. Erosion occurrences can expose the underlying waste materials to either air or water dispersion. Seepage of water through the tailings pile may transport the radionuclides and other substances into groundwater. Seepage at near-surface groundwater can lead the contamination to re-enter the surface water bodies or air pathways (Biswas and Wu 1998; Sims et al. 1996). The seepage to deeper groundwater may act as a long-term source of contamination. Extreme events such as earthquakes, floods and dam failures, though rare, may lead to a short-term but large discharge. Therefore, the physical changes facilitate alteration of chemical stability that is responsible for the transfer of both radionuclide and heavy metal hazards to environmental and human exposure pathways.

## Human health concerns

### Exposure routes

There are several pathways through which humans and the entire environment can be exposed to U toxicity hazard. These include inhalation of aerosol particles, dermal exposures and ingestion through contaminated water and food-stuffs. Inhalation, ingestion and external radiation are the most considered in human exposure risk. Although

absorption of some soluble compounds through the skin is possible, such dermal exposures generally are not significant. The inhalation of insoluble compounds such as U dioxide ( $\text{UO}_2$ ) and triuranium octaoxide ( $\text{U}_3\text{O}_8$ ) lead to deposition in the lungs that can remain for long periods. In such conditions, inhaling aerosols with short-lived U progeny may lead to radiation toxicity as well as chemical toxicity of progeny heavy metals like Pb. For ingested U, insoluble compounds are poorly absorbed from the gastrointestinal tract and they are only retained in the body for a short time, thus generally having a low toxicity. However, ingested soluble U compounds enter the bloodstream and eventually reach the kidneys and other internal organs where chemical toxicity is of primary importance. About 98 % of U entering the body via ingestion is not absorbed (Hoffmann et al. 1993; Kusiak et al. 1993; Simon and Garnier-Laplace 2005). Typical gut absorption rates for U in food and water are about 2 % for soluble and about 0.2 % for insoluble U compounds (Veiga et al. 1998). The fraction of U absorbed into the blood is generally greater following inhalation than following ingestion of the same chemical form. The fraction will also depend on the particle size distribution. For some soluble forms, more than 20 % of the inhaled material could be absorbed into blood. Of the U absorbed into the blood, approximately 70 % is filtered by the kidney and excreted in the urine within 24 h. This amount may increase to 90 % within 48 to 96 h. On average, approximately 90  $\mu\text{g}$  of U exists in the human body from normal intakes of water, food and air (Piao and Hei 1993; Morris and Meinhold 1995). The distribution of U in the human body is estimated to 66 % in the skeleton, 16 % in the liver, 8 % in the kidneys and 10 % in other tissues (Henge Napoli et al. 1995; Russell et al. 1996).

### Chemical toxicity

U has been reported to cause kidney malfunction, respiratory disorders, DNA damage, mutagenicity, cancer and neurological defects (Stearns et al. 2005; Mitrovic et al. 2005; Yazzie et al. 2003). Insoluble forms of U exhibit the least chemical toxicity risks because they are readily excreted via the digestive tract, while soluble forms of U interact with biological ligands and lead to toxicological effects. Once in the bloodstream, the kidneys filter the U compounds out of the body system. U ions, specifically uranyl cations are verified classic nephrotoxins (Dang et al. 1995; Russell et al. 1996; Periyakaruppan et al. 2007). They bind to at least ten different proteins in the serum; its tissue distribution is affected by bicarbonate. Among the identified proteins are ceruloplasmin and hemopexin as binding uranyl with a 1:1 stoichiometry (Rybicka 1996; Miller et al. 1998; Van Horn and Huang 2006). Generally, uranyl cation is partitioned in the serum into the uranyl bis- and tris-carbonate complexes



and  $\text{UO}_2$ –protein–carbonate complexes with human serum albumin, transferrin and other protein. The uranyl–carbonate complexes are more diffusible into tissue especially the liver, kidneys and bones, while the portion bound to protein is removed from the serum in the kidneys (Dang et al. 1995; Russell et al. 1996). The uranyl compounds damage the proximal tubules, which are the main filtering component of the kidney. The renal injury is characterised by cellular injury and tubular necrosis (Cooley et al. 2000; Bellés et al. 2007). One mechanism of U toxicity may relate to the disruption of the first step in glycolysis by uranyl displacing magnesium (Mg) in the enzyme, hexokinase (Van Horn and Huang 2006). Uranyl may also disrupt the para-aminohippurate transporter system and damage to peritubular cell membrane (Miller et al. 1995). Thus, U intakes of above 50 mg is fatal in humans (Van Horn and Huang 2006; Tsuruta 2006).

Furthermore, a few studies have shown that exposure to chemical U lead to genomic instability in lymphocytes (Müller et al. 2004; Kryscio et al. 2001). This is attributed to breaks and cross-linkages of DNA strands because hydrolysis in the backbone of the DNA molecule caused by uranyl ions, acting as a hard Lewis acid (Periyakaruppan et al. 2007; Geissler et al. 2009; Stearns et al. 2005; Yazzie et al. 2003). U also alters the proteome in lung cells (Periyakaruppan et al. 2007). U induces significant oxidative stress in lung epithelial cells followed by concomitant decrease in the antioxidant potential of the cells due to loss of total glutathione and superoxide dismutase induced by the presence of U (Periyakaruppan et al. 2007; Pressyanov et al. 1995; Piao and Hei 1993).

### Radiological toxicity

Radiological toxicity of U is primarily manifest in bone sarcomas, leukaemia, lung cancer and general increase of cancer risk, whereas combined chemical and radiological effects are responsible for nephrotoxicity (Bellés et al. 2007; Nagarkatti et al. 1996; Veiga et al. 1998). The main radiation hazard from U occurs through injection of insoluble U compounds and inhalation of U containing aerosols because all U isotopes mainly emit alpha particles that have little penetrating ability (Lloyd et al. 1996). However, communities near abandoned U mines are exposed radiation from U decay products, which are sometimes stronger and more dangerous than the radiation from U. For instance, an increased risk of lung cancer is attributed to exposure from radon decay products.

The cancer cases induced by radiation are generally indistinguishable from other naturally occurring cancers. The development of cancer occurs years after the exposure takes place. The probability of developing a radiation-induced cancer rises with increasing U intakes. A high radiation dose

increases lung tissue damage, which may lead to lung cancer. Risks for other radiation-induced cancers, including leukaemia, are lower than for lung cancer. Generally, the WHO and ILO recommends that exposure to U radiation should not exceed a dose of more than 1 mSv in a year (WHO 2001; Simpson et al. 2004). In special circumstances, an effective dose of up to 5 mSv in a single year is permitted if the average dose over five consecutive years does not exceed 1 mSv/year. An equivalent dose to the skin should not exceed 50 mSv in a year.

### Ecotoxicity

The chemical ecotoxicity risks are greater than radiological because U has high solubility in various ecosystems. For instance, the Predicted No Effect Concentration (PNEC) is listed in Table 2 showing the toxicity in different compartments and organisms. Generally, the detrimental effects of U on soil bacteria are less than the effects upon plant cells (Chmielowski et al. 1994). Based on information from studies in plant communities that colonise natural U ore bodies, toxicity to U may occur at concentrations of 50 ppm in the rhizosphere, and acute toxicity symptoms occurs in higher plants at about ten times this concentration. A study in a U anomaly site in Kayerekera, Malawi showed that visible poisoning symptoms appear at concentrations above 50-ppm U, while acute toxicity symptoms appear at ten times the concentrations (Zeman et al. 2008; Vanhoudt et al. 2008). High U content may lead to special vegetation formations in some sites. For instance, nitrogen fixing (leguminous) plant species dominated the hotspots where

**Table 2** A selection of predicted no effect concentration (PNEC) for the types of non-human biota showing interesting difference in ecosystem compartments

Compartment	PNEC
Terrestrial plants	250 mg Ukg <sup>-1</sup> dry soil
Soil biota (from microbes to animals)	100 mgkg <sup>-1</sup> dry soil
Freshwater plants	0.005 mgL <sup>-1</sup>
Freshwater invertebrates	0.005 mgL <sup>-1</sup>
Freshwater benthos	100 mgkg <sup>-1</sup> dry sediment
Freshwater fish at water	0.4 mgL <sup>-1</sup> in very soft water (<10 mg CaCO <sub>3</sub> L <sup>-1</sup> ) 2.8 mgL <sup>-1</sup> in soft water (10–100 mg CaCO <sub>3</sub> L <sup>-1</sup> ) 23 mgL <sup>-1</sup> in hard water (>100 mg CaCO <sub>3</sub> L <sup>-1</sup> )
Mammals	0.1 mgkg <sup>-1</sup> body weightday <sup>-1</sup>

Sheppard et al. (2005), data source, are the only research group in literatures (up to now, October 2012) that have approached uranium ecotoxicity from this perspective; therefore, there is no any other information and data to compare with

surface mineralisation of U occurred within 0.5 m at Kayerekerera U anomaly site in Malawi. This is probably a long-term response of plant communities to an environmental gradient, the most important aspect being the chemical properties of U.

However, the ecotoxicity of U cannot be generalised. The potential for development of critical ecotoxicological conditions is different from one ecosystem compartment to another as well as the type of organism. In all, the most important is the endpoint used to assess the U ecotoxicity because effective assessment depends on the endpoint used at a specific ecological complexity level (Sheppard et al. 2005; Franklin et al. 2000; Charles et al. 2002b; Hogan et al. 2005). Consequently, information of U ecotoxicity is scarce and controversial. Assessment of U ecotoxicity is controversial because most organisms exhibit hermetic responses to low concentration and chronic exposure to U while as toxicity is observed only at acute exposure (Mkandawire et al. 2006b). Some organisms relocate their resources (like energy or multiply rapidly) to withstand the U toxicity in low concentration (Mkandawire and Dudel 2005). Some invertebrates as well as some macrophytes secrete or exude organic compounds that protect them from U uptake as well as toxicity (Schaller et al. 2008). As a result actual effect of U is debatable—is it the lethal or inhibition effect or just presence of stress? The hermetic response can itself be an endpoint depending on the level of complexity at which U ecotoxicity is assessed. For instance, the effect of U at the organism metabolic level can be assessed effectively using molecular biomarker, inhibition of specific and important metabolic process, absence of some proteins and energy chemical molecules like ATPs, etc. Other endpoints are oxidative stress at cell molecular level and growth rate inhibition at population level. At community level, U ecotoxicity seems to be invisible. Thus, several areas of information need further definition to describe adequately the ecological consequences of U. Long-term observations are fundamental to accurate evaluation of the environmental impact of U because of the gradual modified response of ecosystems to U toxicity that follows acute effects (Yang and Volesky 1999a; Mkandawire et al. 2006a; Mkandawire et al. 2007; Charles et al. 2006).

Furthermore, biomagnifications of U via the trophic contamination route is also scarce. The clay and organic matter content, acidity, and the presence of cations of Ca and Mg as well as Fe in the soil (Saric et al. 1995; Fresquez et al. 1998) influence the U uptake by terrestrial plants. Similarly, carbonate content and presence of cation (e.g.  $H^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$ ) mostly determines U uptake in aquatic ecosystem because of the formation of soluble U carbonate complexes and the competitive interaction between cations with the uranyl ion ( $UO_2^{2+}$ ), respectively (Charles et al. 2002a; Fox et al. 2006; Zanonato et al. 2012; Kim et al. 2012; Doudou

et al. 2012; Kerisit and Liu 2012; Doudou et al. 2011). Most of the U penetrate the roots surface through the epidermis, and then precipitates within the root, which explains the localisation of U in plant roots (Dushenkov et al. 1997; Dushenkov 2003; Rodriguez et al. 2006). The highest concentrations are usually found in seeds and roots of most plant species, rather than leaves (Saric et al. 1995; Dushenkov et al. 1997; Yong and Macaskie 1998).

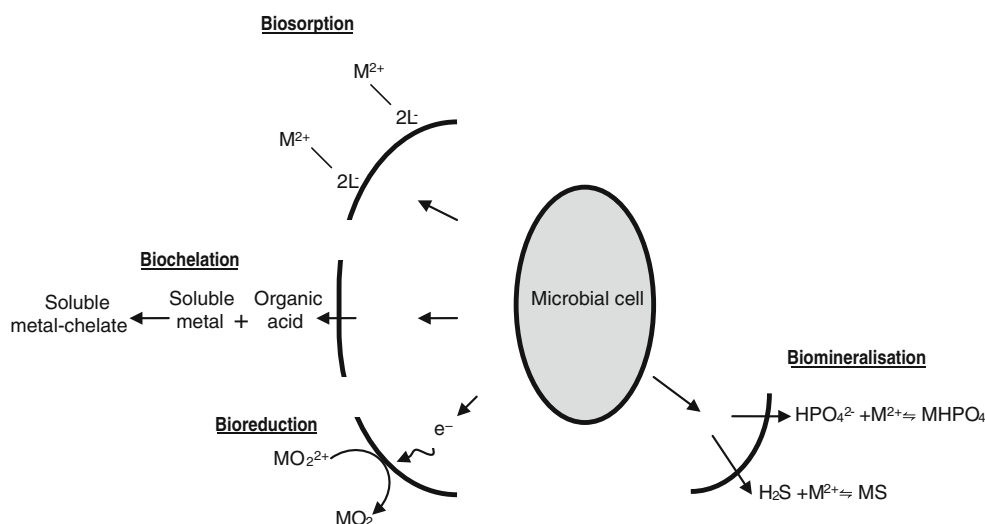
## U bioremediation<sup>2</sup>

Unlike many organic compounds, U and all other metals do not disintegrate into small components that are non-toxic. Logically, biological activity can (1) only transform the metal from toxic to non-toxic species or (2) immobilise the metals from the pathway. Figure 4 demonstrates the interactions of U with biotic component and summarise the mechanisms involved metal bioremediation. Uptake, sorption and precipitation by purified biopolymers derived from microbial cells provide alternative methods and additive processes for conventional physicochemical bioremediation (Malekzadeh et al. 2002). Some plants and algae species can sequester U and other metals into their biomass, while other species exude bioligands that trap U into biometallic colloids. A few bacterial species are capable of reducing mobile U(VI) to immobile U(IV) species (Smeaton et al. 2008). Furthermore, the algae-microbial bio-films have the ability to sequester U and other metals (Allan et al. 2002; Kalin et al. 2005b; Hsieh et al. 1994). The bioreduction, sequestration and trapping of U in biocolloids can nucleate biomineralisation processes of U. However, practical applications of bioremediation techniques under natural conditions are still far from reality.

For instance, U can be immobilised through adsorption onto cell surfaces, extracellular polysaccharides and low molecular weight organic acids including biogenic colloids (Lloyd and Renshaw 2005; Malekzadeh et al. 2002). Furthermore, U can be internalised into cells through metabolic active procedures. In the cells, U may be sequestered as inert compounds in cell organelles such as vacuoles, cell membrane and cell wall. Some species excrete the U compounds back into the water as precipitates or organometallic colloids. Under conducive conditions, the interaction may result into U biomineralisation. For instance, some marine and freshwater algae are able to incorporate U into aragonite ( $CaCO_3$ ) mineral structures.

<sup>2</sup> Bioremediation is defined in this chapter as any process that uses microorganisms, fungi, green plants or their enzymes to remove contaminants from the contamination pathway. Under this definition, phytoremediation belongs to bioremediation.

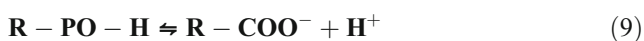
**Fig. 4** The interaction of microorganism with metals emphasising the important processes that lead to U immobilisation in aquatic environments. The structure is modified from Bruins et al. (2000)



## Processes of U bioremediation

### Biosorption

U biosorption involves passive deposition of the metal ions into the biosorbent through various sorption processes, such as ion exchange, complexation, chelation and micro-precipitation (Akhtar et al. 2007; Bhainsa and D'Souza 1999; Bustard and McHale 1997). The cell walls of most plant and algal or microbial materials are composed mainly of polysaccharides and carbohydrates, e.g. cellulose, xylan and mannans (Chmielowski et al. 1994). The backbone of polysaccharide include ligands, such as amino, carboxyl or hydroxyl, sulphide groups, which eventually has an overall negative charge influence on the cell wall (Gonzalez-Munoz et al. 1997; Malekzadeh et al. 2002). The negatively charged groups attract and bind U cations (O'Flaherty 1995; Sar and D'Souza 2001). The chemistry involved on the cell surface can be simplified as follows:



Deprotonation leads to negatively charged sites, which are convenient for uranyl adsorption:



The cell wall structure and the characteristics of the U ion determine the capacity and efficiency of bioremediation of U water using sorption techniques. The capacity of U adsorption onto cell surface depends on the number of available ligand binding sites, U speciation and the physico-

chemical properties of the media. The chemical composition and cell size are reasonably constant for a given species, implying that the number of ligand groups on a surface is also fixed. Furthermore, the complexation is limited to a single layer (Ariff et al. 1999; Prikryl et al. 2001; Kalin et al. 2005a). Therefore, the surface of the cells carries a constant concentration of U with respect to the cell composition, even if the U ions are in excess of the concentration of affinity sites (Kalin et al. 2005a). The number of available surface binding sites can only increase with cell growth, thus the rate at which U ions are removed from water by biosorption procedure is a function of growth rate. A few studies have estimated the plausible number of high affinity surface binding sites on algae and bacteria cells. For instance, phytoplankter have about 108 U binding sites per cell (Kalin et al. 2005a; Charbonneau 2009), while on the bacterium *Shewanella putrefaciens* has approximately  $2.0 \times 10^{19}$  carboxyl sites,  $5.5 \times 10^{18}$  phosphoryl sites and  $2.3 \times 10^{19}$  amine sites per gram of bacteria, which translates to a biosorption capacity of up to 100 mg of U (Kalin et al. 2005a; Daković et al. 2008; Yang and Volesky 1999a).

There are many ligand types on a cell surface, which result in selective binding of U cation species. Some ligand groups may carry a positive charge and are thus able to absorb and complex anionic metal species (Bonfada et al. 2005; Haas et al. 1998). The mixture of positively and negatively charged groups on a cell surface determines the number of cations and anions complexation. The U cation adsorbed onto most cell surfaces, while binding anionic, complexed species of U are more difficult, as there are fewer positively charged ligands on cell surface (Kalin et al. 2005b; Markich 2002; Worms et al. 2006). In acidic aqueous solutions of low pH value, U is present mainly as free  $\text{UO}_2^{2+}$  and adsorbs poorly to cell surface due to competition with protons for binding sites on the biomass (Yang and Volesky 1999a; Yang and Volesky 1999b). At pH values

higher than 6, sorption is higher because of ion exchange between hydrolysed uranyl ions,  $\text{UO}_2\text{OH}^+$ ,  $(\text{UO}_2)^3(\text{OH})_5^+$ ,  $(\text{UO}_2)_2(\text{OH})_2^{2+}$  and protons (Kalin et al. 2005a; Meinrath 1998).

Living and dead cells behave in a similar fashion with respect to adsorption of U, provided the cell wall structure remains intact (Donnellan et al. 1995; Mkandawire et al. 2003; Volesky and May Phillips 1995). Thus, the dead algae, microbes and plants biomass still contain negatively charged ligands, capable to adsorb positively charged U. With the help of invertebrate shredders, the biomass is reduced to particulate organic matter to which more U is adsorbed because of the increase in the surface to volume ratio (Kalin et al. 2005a). Furthermore, the dead biomass decays into humic and fulvic substances through microbial activities. The humic and fulvic substances are rich in dissolved ligands probably because of the carboxyl and hydroxyl groups associated with the breakdown of lignin. The carboxyl group has a dissociation constant ( $\text{p}K_s$ ) of about five, which facilitate the complexation with U at wide range of pH values (Dodge and Francis 1997; Pollmann et al. 2006; Shanbhag and Chopping 1981).

#### Bioprecipitation

Bioprecipitation occurs when U species interact with biogenic compounds. The processes can take place either internal or external of the cell. Bioprecipitation of U is involved in biosorption as well as in active or metabolic internalisation. Most plant and microbe populations release extra-cellular polysaccharides as a by-product of metabolism (Francis and Dodge 1998; Macaskie and Basnakova 1998; Macaskie et al. 2000). Additionally, when imbalance in resource stoichiometry occurs, homeostatic regulation causes the affected organisms to exude chelates, low molecular weight organic substance and polyphenolic substances (Charbonneau 2009; Kalin et al. 2005a; Schmeide et al. 2003). These excreted by-products or exudates interact with U resulting in precipitate formation but not necessarily on the cell surface. Furthermore, their introduction into the water alters the chemical speciation, which also results in immobilisation of U. For instance, microbes such as *Pseudomonas* (Gamma Proteobacteria) and *Citrobacter* (Omnibacteria) also produce large quantities of extra-cellular polysaccharides (Allan et al. 2002; Malekzadeh et al. 2002), while some macrophytes like *Lemna* sp., and *Myriophyllum aquaticum* exude oxalates and polyphenolic substance that complex U ions (Mkandawire 2005; Schmeide et al. 2003; Mazen et al. 2003; Ma 2000). There is also evidence that interaction of phosphate with U leads to formation of very stable crystalline structures (Duff et al. 2002; Murakami et al. 1997; Mkandawire et al. 2005). For instance, a homeostatic regulation of sudden uptake of U by

*Peltigera* sp. lichen) leads to cellular formation of uranyl phosphate crystals, which are stored in the cellular mycobiont (Haas et al. 1998; Branquinho et al. 1997). This formation of U(VI) phosphate crystals is widespread and occurs mostly on the surface of cells like in bacteria *Citrobacter* sp. (Thomas and Macaskie 1998). The mechanism involves production of alkaline phosphatase, which causes the cells to excrete phosphate to the cell surface. The phosphorus acts as the nucleus for the precipitation of U(VI) as uranyl phosphate species (Macaskie et al. 2000; Jerden et al. 2003; Geissler et al. 2009). Another homeostatic regulation of stoichiometry imbalance induced by U uptake is apparent in *Lemna gibba*. In *L. gibba*, uranyl oxalate crystals develop to counteract presence of U in the cells (Mkandawire et al. 2005).

#### Chelation

Chelates are organic molecules that trap or encapsulate highly reactive trace metal cations, and consequently prevent them from involvement in unwanted chemical reactions. Sometimes, the binding between metals and chelates may lead to precipitation (i.e. form insoluble compounds). Thus, most plants and microorganism use chelates to aid the uptake and transportation of essential metal nutrients. Due to unique binding properties, chelates are often used to stabilise metals, which makes them critical compounds in bioremediation (Kalin et al. 2005a). Chelating agents produced by microorganism are mostly siderophores, which enhance the dissolution of Fe and increase its mobility and bioavailability. Siderophores are low molecular weight, and function primarily as Fe transport compounds. Since there are some similarities in the biological behaviour of ferric and uranyl ions, chelates can bind U in a manner similar to those produced for Fe (Premuzic et al. 1985). Plants first release special chelates known as phytosiderophores that bind to metals in the rhizosphere and allow them to be absorbed by the plant (Chiang et al. 2006; Jin et al. 2006; Wasaki et al. 2005). Functioning of chelates is stereoselective, depending on recognition of the metal ion coordination geometry. For example, some chelates like ethylenediaminetetraacetic acid function hardly on U because of the linear structure of uranyl cation (Meinrath et al. 1999). Nonetheless, benefits of chelating agent on U remediation are enormous. Low molecular weight organic acids like citric acids, oxalic acids exuded by most aquatic plants, facilitate the dissolution of U and uptake by macrophytes and microorganism (Ebbs et al. 1998b). Other chelates include metallothionens and phytochelatins, which facilitate both uptake and transportation of metal in organisms. Phytochelatins are heavy metal complexing peptides composed of different amino acids (*r*-glutamic acid cysteine) *n*-glycine  $n=3-7$ , which are involved in detoxication and

homeostatic balance of heavy metals in plant cells (Kaplan et al. 1998; Maksymiec 1997; Klapheck et al. 1995). Excess heavy metals are bound to cell wall in a process called metathiolate formation through mercaptide complexes (Shah and Dubey 1998). Consequently, presence of chelating agents facilitates bioreduction of U because of the influence on U bioavailability.

#### Active intracellular uptake

Probably the most widely recognized biological processes for metal removal in wetlands is plant uptake. The main route of U uptake in aquatic plants is through roots in emergent and surface floating. In plants with either completely submerged leaves or both floating and submerged leaves or free-floating, metals are taken up through both leaves and roots. In the case of foliar uptake of U, a passive movement in aqueous phase through cracks in the cuticle or through the stomata to the cell wall and then the plasmalemma is involved. Submerged rooted plants potentially extract water or metals from sediments, while rootless plants like *Wolffia* sp. or floating macrophytes like *Lemna* sp. extract metals only from water.

Previously, it was thought that a few aquatic macrophytes and microorganisms including algae and plants actively pump metals across their cell membranes (Curie and Briat 2003; White and Broadley 2003; Van Assche and Clijsters 1990). The energy source is usually coupled to photosynthesis and temperature. However, emerging reports indicate clearly that a majority of macrophytes actively avoid uptake of U ions into their system (Duquène et al. 2009; Daković et al. 2008; Bhat et al. 2008). U uptake takes place when the external U concentrations far exceed an arbitrary threshold point of cell resistance. U may be taken up by some cells when the U species mimic an essential metal or interact closely with nutrient resources like  $\text{PO}_4^{3-}$ . Once inside the cell, U may be concentrated and sometimes be precipitated into vacuoles like in some plants and algae, or sequestered in the cell wall in some U resistant microphytes (Martinoia et al. 2007; Samardakiewicz and Wozny 2000; Neumann et al. 1995). In several plant and algae species, U may also interact with internal inorganic phosphate, to create U-phosphate crystals, which are stored in the cellular mycobiont (e.g. in *Lemna* sp.) (Samardakiewicz and Wozny 2000). Some organisms react to potential U toxicity, by changing the U redox status, which induces their binding and subsequent excretion as non-mobile organometallic compounds. Furthermore, some plants species through toxicity-avoidance mechanism take up and sequester U in the roots like *Phragmites australis*, while other species transfer the U into the above ground parts vis-à-vis shoots. The realisation of the different allocations of bioaccumulated U is important in designing the remediation procedures.

The rate of U uptake by aquatic macrophytes and algae varies widely depending on plant species, growth rate, U

speciation and concentration of the U in plant tissue. The pH, organic matter context and plant genotype have marked effects on U uptake. The  $\text{UO}_2^{2+}$ , uranyl carbonate complexes and  $\text{UO}_2\text{PO}_4^-$  are preferentially taken up by roots and transferred to the shoots (Vandenhove et al. 2007). Furthermore, when in mixtures, U(VI) competes for uptake with other metallic ions in terms of molecular size and shape and the configuration of the ligand. For instance, Li, Na, K and  $\text{NH}_4$  do not affect U accumulation. However, metals of the transition group as well as Al, Ca, Fe and Zn ions compete with U(VI) for uptake by biotic system especially macrophytes and algae (Kalin et al. 2005a). U uptake is hindered in the presence of phosphate and carbonate ions but not affected by cations (Na, K, Mg, Ca, manganese (Mn), Co, Ni and Zn), nitrates, sulphates and thiosulphates. Nevertheless, the overall contribution of bioaccumulation procedure in the overall immobilisation of U in an aquatic system is quite small.

#### Biological-induced redox reactions

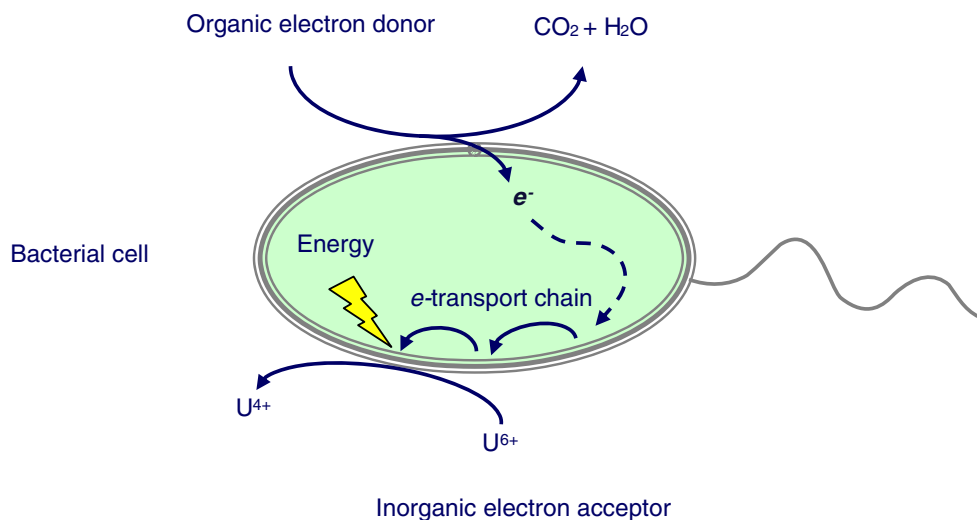
**General mechanism** Numerous natural-occurring metal dissimilatory and SRB are capable of obtaining energy through coupling the oxidation of organic matter and  $\text{H}_2$  to the reduction of soluble U(VI) to insoluble U(IV) (Fig. 5). Hydrocarbons are electron donors that provide a source of electrons to reduce metals in a redox reaction. For example, the half reaction for U(VI) to U(IV) is:



When metabolised by the bacteria, the hydrocarbons provide the necessary electrons to carry out the reaction 11. Remediation benefits from the process because once reduced, the metals are less soluble and easily precipitate out of solution. For instance, the reduced product, U(IV), is a non-toxic precipitate and a mineral, uraninite ( $\text{UO}_2$ ) (Jerden and Sinha 2006; Min et al. 2005). However, many bacteria are not capable of using U(VI) as a sole terminal electron acceptor. Nevertheless, microbial reduction of soluble U(VI) to insoluble U(IV) is one potential strategy of immobilising U from water pathways in abandoned U mines because it promotes U mineralisation under in situ environmental conditions (Mkandawire et al. 2005; Virk et al. 1999; Ginder-Vogel et al. 2006).

**Enzymatic reduction mechanism** The pathway of enzymatic reduction of U(VI) involves c-type cytochromes as a reductase in presence of an electron donor, such as hydrogen gas (Behrends and Van Cappellen 2005; Liger et al. 1999). In the  $\text{H}_2$ -utilising bacteria, the electron is transferred by the tetraheme cytochromes c3, while the triheme periplasmic cytochrome c7 play a role in the transfer of electrons from

**Fig. 5** Principle of bioreduction of U(VI) to immobile U(IV), which may result into uraninite minerals. Modified from Ginder-Vogel et al. (2006)

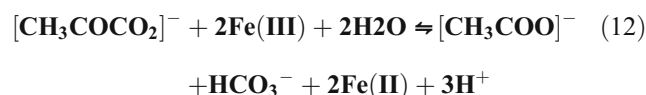


acetate to U(VI). There are suggestions that periplasmic and outer membrane cytochromes also play an important role (DiSpirito et al. 1983; Macaskie et al. 2000). The reduction of U(VI) to U(IV) requires two electrons. However, the microorganism can transfer only one electron. The one-electron reduction process initially generates U(V), for instance, the U reduction (Luo et al. 2007; Wall and Krumholz 2006). Due to the instability of U(V) complexes, it disproportionates to form U(IV). The enzymatic reduction of U can sometimes be hindered by the presence of some natural occurring chemicals. For instance, bicarbonate ion concentrations reduce enzymatic reduction of U(VI) by lowering the half-cell potential of the U (IV)–U(VI) couple (Ortiz-Bernad et al. 2004; Rawlings and Silver 1995). Thus, there is formation of ternary Ca–U(VI)–CO complexes that make it a less energetically favourable electron acceptor than U(VI)–carbonato complexes because of direct interaction of Ca with the cells or electron donor. The U (VI) reduction is sometimes hindered by the presence of ferrihydrite (Raicevic et al. 2006).

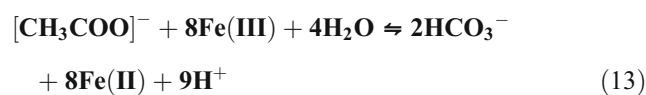
**Bioreduction through Fe-reducing bacteria** The majority of Fe(III)-reducing microorganisms can conserve energy by coupling the oxidation of hydrogen or organic compounds to the reduction of Fe(III). Similarly, they also have the ability to reduce soluble U(VI) to insoluble U(IV) in the same manner (Luo et al. 2007; Madden et al. 2007; Wall and Krumholz 2006). For example, the *Geobacter* sp., which normally gains energy by reducing Fe(III) to Fe(II), can reduce U(VI) to U(IV) instead. They can use U(VI) as an electron acceptor, instead of Fe(III) (Min et al. 2005). Further, bacteria from both the delta and gamma subdivision of the Proteobacteria like *Geobacter metallireducens*, *Geobacter sulfurreducens*, *Desulfuromonas palmitatis* and *Desulfuromonas acetoxidans* couple complete oxidation of acetate to CO<sub>2</sub> via the citric acid cycle with Fe(III) as the electron acceptor (Fredrickson et al. 2000b; Wall and Krumholz 2006; Wilkins et al. 2006). In

addition to acetate, *Geobacter* sp. can also oxidise ethanol, propionate, butyrate, valerate, pyruvate, propanol and toluene amongst others to carbon dioxide with Fe(III) as the electron acceptor (Fredrickson et al. 2000a; Wall and Krumholz 2006; Davis et al. 2006). The bacterial *Shewanella oneidensis*, *Shewanella alga* and *Pseudomonas* sp. do not use organic electron donors like acetate (Luo et al. 2007) but mostly couple the oxidation of hydrogen to the reduction of Fe(III) (Luo et al. 2007; Choppin 2006; Wilkins et al. 2006; Takashima et al. 2002). Given the wide range of Fe(III)-reducing bacteria, it is therefore not surprising that they exhibit a high degree of metabolic diversity, with various species able to reduce U(VI). Despite the apparent diversity of Fe(III)-reducing bacteria, *Geobacter* species are the dominant Fe(III)-reducing microorganisms (Anderson et al. 2003; Neal et al. 2004; Lovley 1995; Lovley 2003).

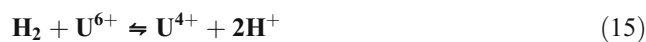
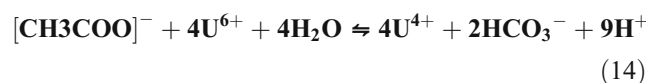
Therefore, the capability to couple the oxidation of organic matter to the reduction of Fe(III) and alternatively U (VI) is through the following typical reactions:



In addition, then:



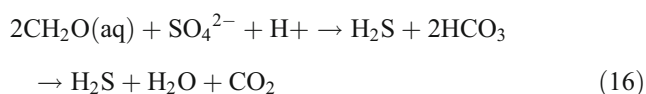
Concurrently or alternatively existence of Fe(III) with U (VI), the reactions include the following:



The Fe(III) dissimilatory procedure involves the ferric Fe reductase, probably located on the outer membrane in Gram-negative bacteria (Creanga et al. 2004; Lovley 2008; Ortiz-Bernad et al. 2004). The ferric Fe reductase acts as the terminal reductase of an electron transport chain, which is linked to the cytoplasmic membrane (Lovley 2003; Anderson and Lovley 2002). Electrons are transferred down the transport chain to the ferric Fe reductase, which then transfers them onto insoluble extra-cellular Fe(III) oxides. The transfer of electrons is a mode of conserving energy through the generation of ATP (Vauclaire et al. 2002; Cohen et al. 1998). The Fe(III) is reduced by the ferric reductase either before or after uptake into the cell, thus forming a weak Fe(II)-chelate complex from which the Fe can be easily dissociated for use by the cell. Three possible mechanisms employed through which the Fe(III)-reducing bacteria reduce Fe(III) oxides as well as U(VI) have been identified as follows:

1. Direct contact, where the microorganism contact the insoluble Fe oxide and transfer an electron from the cell onto the Fe(III) oxide surface with outer membrane bound c-type cytochromes playing some role in this process (Lovley 2008; Anderson et al. 2003; Lloyd and Lovley 2001; Lovley 1995). Some bacteria utilise pili in electron transfer from the cell to the mineral surface as highly conductive ‘nanowires’ (a pilus is typically 6 to 7 nm in diameter);
2. Fe(III) chelators, where the bacteria produce siderophore to dissolve Fe(III) oxides which become more accessible to Fe(III) reductase than insoluble Fe(III) oxides (Lovley 2008; Anderson et al. 2003; Lloyd and Lovley 2001; Lovley 1995); and,
3. Extra cellular electron-shuttling molecules, such as quinone-containing compounds or c-type cytochromes, to transfer electrons from the cellular electron transport chain to the metal terminal electron acceptors (Yang et al. 2001; Fredrickson et al. 2000a; Koike et al. 1996). The extracellular electron shuttles may either be already present in the environment due to decomposition of organic material or produced by cellular processes and excreted into the environment (Lovley 2008). Proteins never act as electrons-shuttling compound while humic substances are very good electron-shuttling compounds.

**Bioreduction through sulphur-reducing bacteria** Sulphur-reducing bacteria (SRB) equally reduce U(VI) to U(IV) like it sulphur that it is salvaged for bioremediation in abandoned U mines. Just like the Fe(III)-reducing bacteria, the chemical basis of SRB remediation involves microbial-mediated sulphate reduction coupled with organic matter oxidation. The reaction occurs as follows:

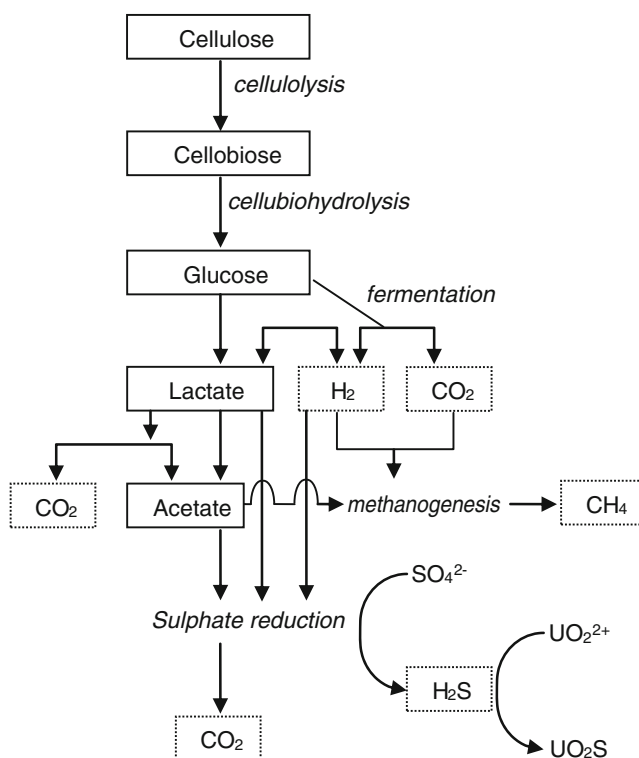


where  $\text{CH}_2\text{O}$  can be replaced by other organic substance. Hydrogen sulphide ( $\text{H}_2\text{S}$ ) then reacts with U, to form a sulphide that precipitates out of the effluent:



As indicated in reaction 17, some bicarbonate ( $\text{HCO}_3^-$ ) may be accompanied by  $\text{CO}_2$  production in the sulphur redox reaction. This consumes acidity, raising the pH, which facilitates the precipitation of U and related heavy metals. SRB generally rely on simple carbon compounds such as organic acids or alcohols to serve as electron donors for sulphate reduction, though some are capable of using hydrogen (De Schampelaere et al. 2010; Lovley 2008; Spear et al. 1999; Basnakova et al. 1998; Charbonneau 2009). When organic matter is used as a carbon source, other heterotrophic bacteria degrade it into simple carbon compounds (Fig. 6). Similar to mechanism in Fe(III)-reducing bacteria, the SRB transfers one electron to U(VI), reducing it to U(V). As mentioned earlier, U(V) is unstable and prone to disproportionate, forming U(VI) and U(IV).

**Pre-conditions for bioreduction processes** Generally, bacteria couple the oxidation of organic matter to the reduction of oxygen as the terminal electron acceptor in aerobic environments. Under



**Fig. 6** Microbial processes of involving oxidation of organic carbon substance coupled to sulphatereduction, which further reduces U(VI) to U(IV). Modified from Doshi (2006)

anaerobic conditions, microbes can utilise  $\text{NO}_3^-$ , Mn(IV), Fe(III),  $\text{SO}_4^{2-}$  and  $\text{CO}_2$  as terminal electron acceptors in the oxidation of organic matter (Wilkins et al. 2006). Thus, amount of  $\text{NO}_3^-$ , Mn, Ca (because of its relationship to  $\text{CO}_2$  in water) influence the U(VI) reduction because of competition. Fortunately, Fe(III) reduction accounts for the oxidation of up to 65 % of organic matter in some anaerobic sediment (Seidel et al. 2002; Finneran et al. 2002; Rittle et al. 1995; Charbonneau 2009). However, the reduction of Fe(III) to Fe(II) lead to other significant chemical changes, such as the release of trace metals and radionuclides into the water pathway and the inhibition of methane production (Mishra et al. 1999; Zayed and Winter 2000), as well as Fe(II) functioning as a catalyst in reduction on U(VI) to U(IV). Nevertheless, the bioreduction of U occurs much faster than abiotic reduction. For SRB, anaerobic conditions where the  $E_H$  is less than  $-200$  mV are required because oxygen, nitrate, Mn and Fe reduction all yield more energy per equivalent than sulphate (Doshi 2006). The redox conditions are also suitable for Fe reduction to the ferrous ( $\text{Fe}^{2+}$ ) state, which co-precipitate with sulphide. When electron donors are in sufficient to drive the reduction of U, some bioremediation strategies like addition of nutrients or organic compounds, especially alcohol, are done to be electron source. In some situations, there may be a more suitable electron acceptor than the metal itself, such as oxygen. In such case, bioremediation strategies can focus on creating an anaerobic environment such as a constructed wetland.

#### Phytoremediation

Phytoremediation is the use of plants to clean-up contaminated sites (Pilon-Smits 2005). Metals can be phytoremediated by several different processes including phytoextraction, rhizofiltration and phytostabilization. Plants also have the ability to remove trace metals from the water through biological uptake and surface adsorption (Slaveykova and Wilkinson 2003; Slaveykova and Wilkinson 2002). In phytoextraction, plants take up and concentrate the U and other metals in their tissues. Certain plants species like *Lemna* sp. and *Phragmatis* sp. have evolved the capacity to extract particularly high levels of U. Once U and other metals taken up, they are either stored in the root or transported to the shoots through the xylem (Overall and Parry 2004; Ebbs et al. 1998a). Normally, U has difficulty migrating through the high cation exchange capacity of the xylem. Most metals are chelated to facilitate translocation process, but the transport process for U is not yet clear. However, U is probably immobilised and stored in the cell vacuoles as well as cell wall. Inside the vacuole, U is bound to organic compounds, probably chelates to prevent further damage to the plant. Furthermore, the U may form crystals after uptake in the cell, for instance U oxalates observed in *L. gibba* (Mkandawire et al. 2005). Some plant species use their roots to filter U and other toxic

metals from the water. With this technique, U is deposited in the rhizosphere of the macrophytes either via precipitation, ion exchange or chelation (Kalin et al. 2005a). The processes can be direct when the plant exudes organic substances that facilitate symbiotic, allelopathic associations with microorganisms. For some plant species, the filtering of U is through the rhizospheric microflora, which associates with the plants.

#### Biogenic facilitated abiogenic reduction of U

Based on strict thermodynamics, Fe(II) has high potential of reducing U from U(VI) to U(IV). Many subsurface environments in abandoned U mines, especially sediments, are saturated with biogenic Fe(II) due to long-term microbial reduction of Fe(III) (Behrends and Van Cappellen 2005; Lack et al. 2002). Logically, the Fe(II)-rich condition should facilitate the immobilisation of U and put abiotic reduction above direct microbial reduction process in U immobilisation in wetland ponds of abandoned U mines (Antunes et al. 2007; Zänker et al. 2007; Mkandawire et al. 2004b). Additionally, Fe(II) species adsorbed onto ferrihydrite, magnetite and hematite surfaces are able to reduce U(VI) to U(IV). The reduced U(VI) vis-à-vis U(IV) is then present as a  $\text{UO}_2(\text{s})/\text{Fe}(\text{OH})_3(\text{S})$  precipitate in the water. Therefore, abiotic reductions of U(VI) by biogenic Fe(II) are likely the dominant reductive pathway. However, the Fe(II) does not significantly contribute to U(VI) reduction in the sediments because, the reduction of U(VI) by Fe(II) is only a minor process due to the complexation of U(VI) with carbonate.

#### Applicable bioremediation strategies in abandoned U mines

There are varieties of bioremediation strategies that can be explored to reduce U and other contamination associated with abandoned U mining and processing sites. Here, three commonly applied strategies in abandoned U mining sites that offer some of the most promising bioremediation treatments are discussed. These strategies provide cheap and efficient alternatives to traditional methods of metal decontamination such as ‘pump and treat’ soil washing systems, excavation and reburial of contaminated areas.

#### Constructed wetlands

One strategy for bioremediation metals in abandoned U mines is the creation of artificial wetlands (Vymazal 2005; Batty and Younger 2004; Lüderitz and Gerlach 2002; Zayed et al. 1998), which is applicable for treatment of contaminated surface and groundwater as well as acid drainage. Groundwater is pumped into constructed wetland for passive treatment. The wetland strategy relies on using several basic physical, chemical and biological processes to remove or immobilise U from the aquatic system. Thus, an effective strategy can involve all



the bioremediation processes once well designed. The U remediation occurs in the three main compartments of a wetland vis-à-vis the substrate (sediment), hydrology and vegetation.

The substrate harbours consortia of detritus microbial, which are responsible for uranyl reduction, and transformation, which may nucleate U biomineralisation. Wetlands with appropriate substrate promote the growth of sulphate-reducing bacteria in anaerobic conditions. Thus in acid mine water which is rich in sulphates, these bacteria will generate hydrogen sulphide, which reacts with metals that leads to formation of insoluble metals sulphides (Hallberg and Johnson 2003; Seidel et al. 2002). This is very important in controlling generation of AMD from abandoned U mines.

The hydrology compartment contains heterogeneous poly-ligands like fulvic, humic and tannic acids, amorphous metal oxyhydroxides, clay, bacterial surfaces and associated exopolymers, suspended particles and macromolecules (e.g. polysaccharides and proteins) (Gomez et al. 2006; Müller et al. 2002; Martinez Aguirre et al. 1995). Anaerobic conditions also develop easily in the upper portion of the sediment because of hydric condition that supports hydrophytic vegetation. Uranyl ion can coordinate with most biological material, living or dead. It is also transported through the water column to the sediments. This is slow in well-oxygenated water and fast in anoxic water.

While sediments of wetlands form primary sinks for U, macrophytes absorb U through roots and shoots. The wetland plants are also autotrophic organisms, creating a biomass of reduced carbon compounds that provide nutrients to a variety of both micro- and macroscopic (Lüderitz and Gerlach 2002). The expansive rhizosphere of wetland plant species provides an enriched culture zone for microbes involved in U species biotransformation (Sheoran and Sheoran 2006). Physical processes in constructed wetlands through which U is removal from water pathway include settling and sedimentation following adsorption on particulate matter (Schaller et al. 2008; Selig et al. 2002; Markich 2002). To some extent, mats of floating plants serve as sediment traps while rooted and floating plants regulate the flow rate of the water by providing resistance. For particles, which are light or less dense than water, sedimentation occurs after flocculation. Flocculates generally settle more rapidly in a wetland than do individual particles. The flocculation is enhanced by high pH, concentration of suspended matters, ionic strength and high algal concentration (Kalin et al. 2005a). Before sedimentation, other chemical processes like precipitation, adsorption and coprecipitation should occur first. More than 50 % of the U is easily adsorbed onto particulate matter in the wetland (Dudel et al. 2004; Fritzsche et al. 2006; Dienemann et al. 2003). In sediments, U adsorbed to the particles by either cation exchange or chemisorptions. The U speciation changes with time as the sediment conditions change.

### *Injection wells*

Well injection is another promising bioremediation strategy applicable in abandoned U mine (Anderson et al. 2003). This well injection strategy is illustrated in Fig. 7, and it is one of the major procedures for U bioremediation in groundwater especial in abandoned ISL mining procedure as well as leaching contaminated water and AMD from the tailing and waste heaps. Mostly, bioremediation in the groundwater occurs through the bioreduction of U by Fe and sulphur-reducing microorganism. In most cases, the underground environments are rich with microorganisms, but the environmental conditions are usually not conducive for their growth and reduction of U. The environments usually lack carbon sources and contain high nitrates. Thus, biostimulation of microorganism activities is necessary for the U bioremediation to occur. A common method to deliver carbon sources are injection wells. For such constructions, a series of wells are installed to control groundwater flow and allow the injection of solutes, such as ethanol, required to create a geochemical environment conducive for microbial growth and subsequent U(VI) reduction. For instance, ethanol is added to stimulate in situ denitrification of residual nitrate and reduction of U(VI) (Wu et al. 2006). The addition of acetate to groundwater promotes the growth and activity of dissimilatory Fe(III)-reducing microorganisms and results in the removal of soluble U(VI) from contaminated groundwater (Vrionis et al. 2005).

### *Bioreactive barrier*

This is a bioremediation strategy targeting leaching and ground water. The strategies capitalises on the process of bioaugmentation, where microorganisms are introduced in a form of a barrier to clean the leaching water (Ellen 2006; Fuller et al. 2002). Figure 8 illustrates how the barriers are constructed so that the contaminated waters passes through the barrier and is treated through the same process of U reduction using dissimilating Fe-bacteria or SRB. To stimulate bioremediation, a SRB source such as cow manure or organic matter is added to passive treatment systems.

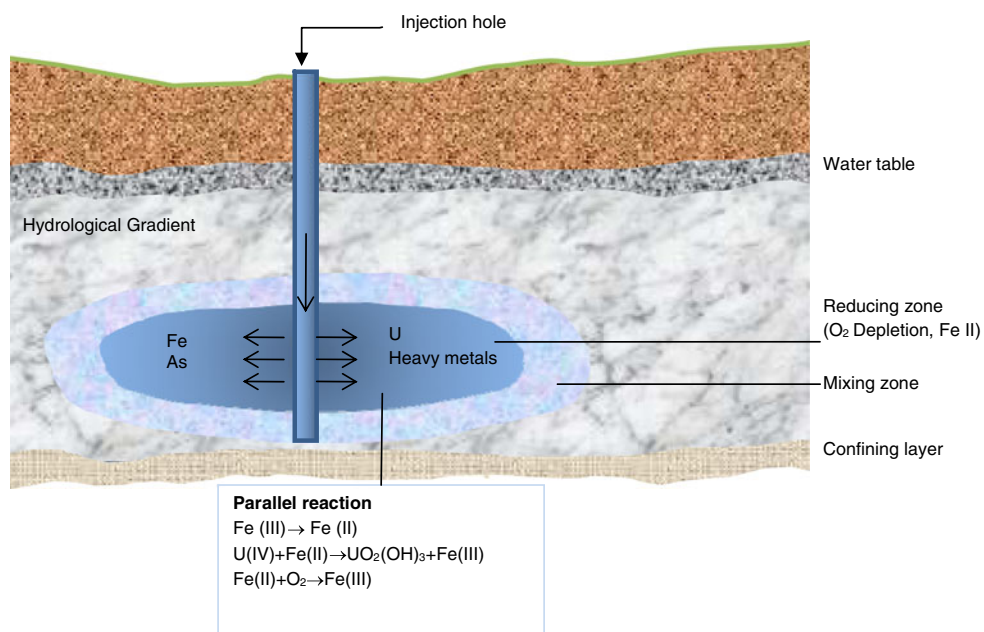
### Challenges in application of bioremediation in AUM

The challenge to apply bioremediation effectively in abandoned U mines lies in the ability to overcome several limitations, uncertainties and failures related to bioremediation systems.

### *Stoichiometry and homeostasis limits*

There are several limitations to application of bioremediation in contaminated waters of abandoned U mining sites.

**Fig. 7** Schematic illustration of well injection strategy of bioremediation where biostimulants like ethanol are injected into underground to stimulate U reduction by bacteria. The well can also be used for bioaugmentation where U-reducing bacteria are injected into the wells (Vrionis et al. 2005)

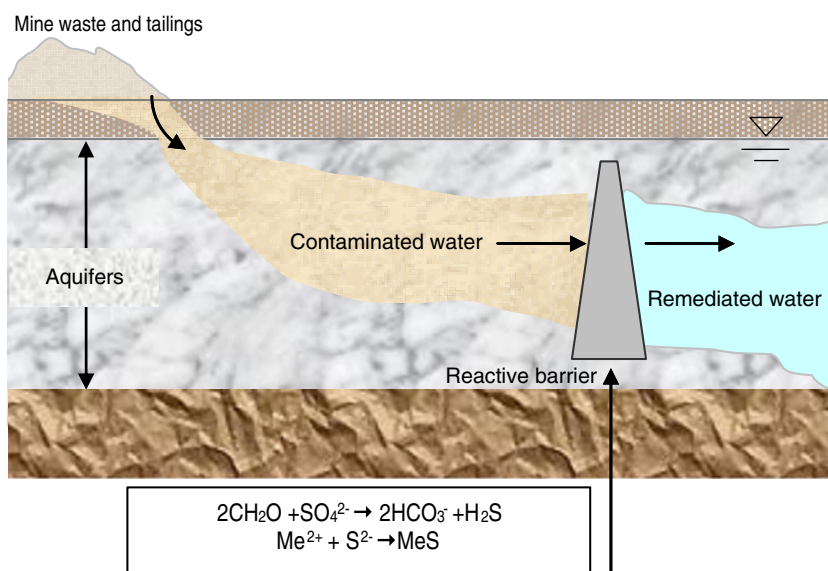


One major limitation is with the nature of the organisms, whose immobilisation of U is not a benevolent gesture but a strategy for survival. Most bioremediation agents only work under environmental conditions that suit the organisms' needs. Thus, unless conducive bioremediation conditions are set, organisms use alternative substrates as source of energy than U. Consequently, some type of environmental modification is needed to encourage the organisms to take up or bio-reduce the U at an acceptable rate. Furthermore, the organism must be presented with low levels of the pollutant over a period to induce the organism to develop metabolic pathways needed to reduce the U. As a result, bioremediation can also become very costly due to modifications, labour intensive and can take several months for the remediation to achieve acceptable levels.

*Environmental condition limits*

Some environmental factors may also limit the applicability and effectiveness of the U bioremediation process. There are occasions where sediment or soil matrices prohibit contaminant microorganisms contact. The matrices form a barrier that the contaminated water may not reach. A worse situation occurs when there is preferential colonisation by microbes, which may cause clogging of nutrient, and water flow in the pores of the sediments. Furthermore, preferential flow paths may severely decrease contact between the biostimulants and U as well as the bioremediation agents. Similarly, bioremediation in clay and highly layered or heterogeneous subsurface environments limit the application because such environments interfere with oxygen or other electron acceptor transfer processes (Luo et al.

**Fig. 8** Schematic illustration of how passive treatment using a permeable reactive barrier functions in treatment of tailing heap leachates especially AMD. For AMD, the reactive barrier can be filled with immobilised microorganisms



2007; Ginder-Vogel et al. 2006). Therefore, the rate of U immobilisation from the aqueous phase depends on the rates of mass transfer of contaminants from the low permeability matrix blocks to higher permeability regions within fractures.

#### *Discharge pattern limits*

Application of bioremediation is limited to the type of contaminant discharge in abandoned U mining sites because the procedure is not responsive to sudden changes. High concentrations of heavy metals are likely to be toxic to microorganisms. Low contaminant concentrations may be below the survival threshold for the organism. Consequently, bioremediation attempts fail and become costly because the microorganisms are sometimes site specific. Each new site represents a different set of conditions, therefore microorganisms used for bioaugmentation need to be adapted to each site. Bioremediation of air in particular is very inefficient, and therefore radon emanated from abandoned U mines is hardly tackled.

#### *Ecological implication fears*

Another problem is that the applicable bioremediation technologies can cause environmental disruption beyond the damage done by the pollution. In most cases, biostimulation and bioaugmentation is unavoidable. These induce environmental conditions can be disruptive to other organisms thereby disrupting the ecological balance. For instance during biostimulation procedures, continued addition of acetate may result in a shift in the dominant terminal electron accepting process from Fe(III) reduction to sulphate reduction, as well as complete degradation of the acetate under sulphate-reducing conditions and an apparent decrease in the rate of removal of soluble U(VI) from groundwater (Vrionis et al. 2005). Therefore, the maintenance of Fe(III)-reducing conditions is critical for sustaining reductive precipitation of U(IV), and highlights the need to correlate U(VI) removal with alterations in subsurface microbial community. Furthermore, the modification of the environment can equally be expensive as classical treatments due to the cost, for example of acetate or ethanol in biostimulation. In bioaugmentation, the long-term effects of introducing naturally occurring non-native bioremediation organisms into an area can alter the ecological balance when they become invasive. Worse, most of high and fast acting are genetically altered bioremediation organisms, long term environmental effects are not yet clear. As a result, there are always uncertainty of horizontal transfer of the modified gene, creation of new pathogens and possibility of mutations that allow the organism to become invasive.

#### *Physicochemical characteristics limits*

In many abandoned U mining and processing sites, ambient groundwater has a very low pH and high levels of

aluminium, Ca and nitrate. The pH values lower than 3.4 are not favourable for the growth of most organisms, which requires manipulation of subsurface pH to a value better suited for microbial activity. However, increasing the pH in situ is not desirable because aluminium hydroxide precipitates form at approximate pH values above 4.5 and Ca solids precipitate at near-neutral pH. Ca is present in the groundwater at levels that inhibit U(VI) reduction especially at neutral pH conditions. The formations of U-carbonate-Ca complexes also retard bioreduction (Wilkins et al. 2006). The aqueous species,  $\text{CaUO}_2(\text{CO}_3)_3^{2-}$  and  $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$  also have an important role on inhibition of microbial reduction and aqueous speciation of U(VI) in contaminated groundwater.  $\text{Ca}^{2+}$  have a significant impact on the aqueous speciation of U(VI), and consequently, on the sorption and mobility of U(VI) in aquifers (Fox et al. 2006).

Nitrate also inhibits U(VI) reduction and is present as a co-contaminant with U in groundwater (Franklin et al. 2000). Prior to the reduction of U, persistent nitrate must first and continuously be removed or transformed prior to U because nitrate is a preferred electron acceptor to U, and the stimulation of metal-reducing bacteria are hindered by low-pH environments (Madden et al. 2007). In situ denitrification of bulk nitrate has high probability of incomplete denitrification because of nitrate levels are usually very high in abandoned U mining sites. Any nitrite formed can oxidise U(IV) back to the more soluble U(VI). In addition, complete denitrification of nitrate generates  $\text{N}_2$  in excess of its solubility limit. The presence of a gas phase can alter flow direction and decrease solid surface area accessible for sorption as well as desorption and redox reactions.

#### *Processes and counteraction limits*

The performance of wetland treatment system depends upon the input water quality and the specific mechanism of metal removal processes. The wetland plants translocation oxygen from the shoots to the root rhizomes through their internal gas space parenchyma. The roots and rhizomes in turn leak the oxygen to the reduced environment. Much as this is advantageous, it also limits U bioremediation. If the U immobilisation is aerobic, these oxidised conditions promote precipitation of oxyhydroxides of  $\text{Fe}^{3+}$  and  $\text{Mn}^{2+}$  that may adsorb or co-precipitate with the U ions (Davis et al. 2006). However, the volume available to collect and store chemical precipitates or the number of available removal sites governs elimination rate in aerobic systems. The primary removal processes, which include sedimentation, adsorption, ion exchange and complexation, are finite. Thus, U removal or immobilisation ceases unless new removal sites are generated. Hence, periodic removal of precipitated U from wetlands is necessary. Only systems that are well vegetated can generate sufficient new removal sites to offer

the possibility of long term, low maintenance treatment. Therefore, only site conditions may determine whether it is more economical to simply let the wetland system develop into a new ecosystem or to excavate away the accumulated solids for proper disposal. Since the generation of new removal sites is a slow process, long-term treatment require a large wetland with a relatively small input load. Further, the wetland concentrates the U in one place, which increases the radiation dangers and radon emanation.

If the primary U immobilisation mechanism uses anaerobic systems, oxygenating macrophytes affect the performance of U bioremediation agents in the sediments and sub-surfaces. The system also works efficiently as long as sulphate-reducing bacteria reduce sulphate to sulphide thus precipitating U sulphides complexes and generating alkalinity. The mechanism is limited by the amount of available carbon for sulphate-reducing bacteria, but the oxygen availability provides a more preferable electron acceptor than U. Therefore, the bioreduction of U ceases. Furthermore, in the absence of organic-reducing sediments, U-bearing particles are released back into the water column when the macrophytes decompose. In reality, both aerobic and anaerobic processes take place simultaneously in the wetlands. This is a big challenge to designing of a constructed wetlands where these processes do not counteract once another.

#### New perspectives—a paradigm of the eco-remediation

In most cases, the laboratory success stories have failed to be applied in the real situation (Bennett 1995). The main reason is that laboratory procedures do not completely apply the multiple interactions that take place in nature, especially the roles of different ecological interactions and hierarchy. A natural system is self-organised in an ecological hierarch where the interaction determines the role of different organism in the system. Hence, use of bioremediation procedure should be used at higher ecological interaction, where the multiple interactions are part of the processes. Thus, U remediation in abandoned U mines can be effective when of ecological engineering is employed to enhance natural ecological processes (Kalin et al. 2005a) vis:

1. Association of the U with organic particles in the water column;
2. Provision of conditions whereby organic particles sink onto and into organic sediments; and,
3. Provision of low  $E_H$  conditions in the sediments for metal-reducing microbial populations.

Consequently, oxidised U associates with particulates, settles, is reduced and is bio-mineralised in the sediments—effectively creating biogenic ores (Ketterer et al. 2000; Martinez Aguirre et al. 1995; Kalin et al. 2005a). The advantage of using living material in the initial step is that it

continually grows, providing new organic material for sequestration. This is especially useful in flow-through systems, where the supply of U to be removed is continuous. The removal of U using ecological approach follows a three-step process: (1) first is the sequestration of U on, in and around aquatic plants and periphyton biofilms. Periphytons are mixed communities, which are composed of algae, fungi and bacteria that grow on macrophytic-vascular plants and on sediment surfaces (Kalin et al. 2005a). Biogenic ligands produced by plants and microorganism facilitate the removal U(VI) from the mine water. The biofilms continuously renew the cellular surface area; (2) the next is the removal of the biological sequestered U units from the water column. The macrophytes, phytoplankton and bacteria become aggregated into larger particulates, which sink rapidly to the bottom. Invertebrate shredders (e.g. zooplankton, diatoms etc.) and small aquatic vertebrates (e.g. herbaceous fish) play an important role in formulation of the U rich organic particulates. The faecal pellets are part of biogenic organic particles that bind U and are together transferred into the sediments (Kalin et al. 2005a). However, the production of biogenic organic particles is attributed to high primary productivity. This organic particulate matter adsorbs the U and other metals from the water pathway. Thus, U(VI) is reduced to U(IV) which forma stable precipitates in the sediments. Once the biomass of U-biofilms reaches the sediment interface, it continues to decompose, releasing nutrients and colloidal-bound U. The dead organic material provides organic carbon and other nutrients to heterotrophic microbial consortia to maintain the low  $E_H$ , within which the U is transformed and consequently bio-mineralised (Kalin et al. 2005a). The nutrients that sustain microbial populations in the anaerobic sediments come from natural organic matter or algal particles. The biomass of the plant and biofilms encounters sediment primarily as decomposing material. The decomposition of the biomass in the sediments provides nutrients to sustain heterotrophic microbial communities which further degrade organic matter, and provide dissolved organic material for anaerobic metal-reducing microbial consortia (Kalin et al. 2005a; Wielinga et al. 2000).

Dissolved oxygen is reduced first, followed by denitrification, followed by  $UO_2CO_3$  reduction to uraninite and sulphate reduction to sulphide. At each step in the redox ladder, different microbial enzymes may catalyze reactions. There is a succession of microbial activity ranging from denitrifies, to metal reducers, to sulphate reducers. Denitrification process is complete before sulphate reducers are activated. Thus, the U(VI) reduction does not occur until the nitrate has been utilised by nitrate-reducing microorganisms (Finneran et al. 2002). When nitrate enter a reduced zone where U(IV) is present, the subsequent reduction of nitrate may remobilise the U though facilitation of oxidation of U(IV) to U(VI). Therefore, the anaerobic pond should be planted with high nitrogen demanding macrophytes like *Lemna* sp. and *Eichonia* sp. and

denitrifying bacteria. Further, the intermediates of dissimilatory nitrate reduction (nitrite, nitrous oxide and nitric oxide) can also oxidise and mobilise U(IV). Therefore, the accumulation of carbonate arising from microbial respiration may promote the formation of highly stable carbonato-U(VI) complexes, thereby increasing the thermodynamic favourability of U(IV) oxidation (Gavshin et al. 2005).

### Concluding remarks

Bioremediation of U shows high potential as alternative to classic remediation under laboratory conditions. However, the crossing from pure academic research to actual marketable technology applicable in abandoned U mines is slow to come. Like many technologies with good scientific foundations, its merits are marred by over-optimistic speculations and fraudulent claims. Furthermore, U bioremediation is usually considered in the absence of other influential ecological interactions. This undermines its potential application in situ because natural aquatic environment including abandoned U mining sites are heterogeneous; and complex interactions involving water column, bed sediments, air and biotic compartment continuously take place. It is characterised with presence of multiple stressors, ligand heterogeneity and polydispersity and non-equilibrium conditions. Consequently, metals and metalloids undergo an array of dynamic physical, chemical and biological processes. For instance, the sulphides minerals, acid-buffering capacity, presence of organic matter, colloids, Fe and Mn oxide minerals affect the solubility, mobility and bioavailability of U and other metal ions. The toxicity of U is a function of its ability to cross biological barriers (e.g. plasma membrane) which is partially regulated by the concentration or flux of internalised metals and the physicochemical conditions of the medium. When the homeostasis is disrupted due to the interactions chemical interactions, the resulting stoichiometry imbalance is reversed by homeostatic regulation of the biotic compartment. Thus, the homeostatic regulation of resource stoichiometry in an aquatic system is one of the prime regulatory processes that can be salvaged for enhancing bioremediation technology as well as toxicity alleviation. The idea of stimulating these biological processes for the purposes of stabilising U in the subsurface is therefore promising as a basis for U remediation technologies. Therefore, a realistic functioning is only feasible if the bioremediation procedures are encapsulated in ecological engineering processes where multiple interactions are manipulated but left to emulate the rule of nature. For instance, cascade wetland procedure would be more effective than just monoculture constructed wetlands in treating U. The long-term stability of biologically reduced U will be determined by the complex interplay of sediment mineralogy, aqueous geochemistry, microbial and invertebrate activity, stabilisation by aquatic plants

and potential U(IV) oxidants. Many of these factors have been studied under laboratory conditions; however, the impact of these factors on U cycling in natural, subsurface environments is yet to be understood.

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