



Bioremediation of Uranium from Contaminated Sites

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Abstract

Although uranium is naturally scarce, it can accumulate to dangerous levels owing to the requirement of large quantity of ores to generate reactor-grade fuel. Resultant waste, containing uranium radionuclide can cause both chemical and radiological toxicity. The extent of the effect is dependent on the uranium species in the environment as well as the route of exposure. Uranyl ion (UO_2^{2+}) is soluble in water, which can be reduced to uraninite, (U^{+4} species). Microorganisms and plants play a prominent role in reducing uranium. The mechanism of reduction is dependent on the organisms ability to transfer electrons, to which affect several potential uranium reeducates has been predicted. Bioremediation is often more effective in a consortium of microbes isolated from mines or contaminated sites.

Introduction

Uranium is a radioactive element [Atm no. 92] found in natural ores. It occurs principally as three natural isotopes such as uranium-235 (U^{235}) (0.7 percent), U^{238} (99.3 percent) and a minute amount of U^{234} (0.005 percent). With an absorption of neutrons the radioactive nuclides can undergo fission i.e. splitting of the heavy or unstable nuclei of an atom to roughly two equal parts of smaller nuclei with the release of considerable amount of energy. The critical energy required for this fission is crucially provided by the binding energy of absorption of neutrons which can be high energy (or fast) neutrons or low-energy, thermal (slow) neutrons. For example, the binding energy released the by U^{238} absorbing a thermal neutron is less than the critical energy, so the neutron must possess additional energy for fission to be possible. Hence, it can be fashioned only with high-energy neutrons. In contrast, U^{235} is fissile, meaning it is capable of undergoing fission after capturing/absorbing low-energy thermal (slow) neutrons. As a result, in commercial light-water reactors which uses ordinary water as coolant (such as oiling water reactors (BWRs) and pressurized water reactors (PWRs) use U^{235} as fuel. Since this isotope is naturally scarce, various different processes of enrichment is used to increase the U^{235}

content of the fuel to ~ 3 %. In heavy water moderated reactors, unenriched uranium can be used as fuel.

Radioactive waste is defined as material that contains, or is contaminated with, radionuclides at concentrations or activities greater than clearance levels as established by individual countries' regulatory authorities, and for which no use is currently foreseen (Ojovan, 2014). The act of freeing a regulated radioactive material or a material contaminated with radioactive material from regulation is called "clearance". Substances not regulated as radioactive materials and materials contaminated with them are not classified as radioactive wastes, in the first place. Hence, not all waste containing radioactive material is considered radioactive waste. Further, whether a radioactive waste is stored for future use or disposed is dependent on the reprocessing industry of the specific country, leading to requirement of containment of 'storage' or remediation of 'disposal' of the radioactive waste.

Radioactive waste can result from various sources in the production and use of Uranium. The first and foremost source of uranium exposure is from uranium mining which was reported as early as 16th century in the silver mines of central Europe. Mining refers to the act of collecting ore containing a target metal from mines, in this case Uranium. Common uranium containing ores are uraninite, pitchblend and brannerite, and they contain uranium in the form of oxide. Their grade generally ranges from 0.1 to 0.3 % triuraniumoctoxide (U_3O_8) equivalent. Milling, the process of extracting the metal involves dressing i.e. the process of separating valueless rocks based on physical or chemical characteristics by such methods as fire refining and aqueous refining and refining and concentration. The ore is crushed and dissolved in acid or alkali and precipitated with strong alkali to powder form of about 70-80 % U_3O_8 . It is further refined to prepare UF_6 , UO_2 or metallic uranium to be used as fuel in reactor. According to a simplified calculation, to run a nuclear reactor rated electrical output of 1,000 MW is operating at an 80 % loading factor, 40,000 MW/d burn-up and 33 % thermal conversion rate for one year 27 tones of low-enriched uranium fuel would be used, which will be extracted from 130,000 tones, leaving a large amount of residual uranium and almost entire amount of progeny nucleids such as ^{230}Th , ^{222}Rn whose secular equilibrium with uranium is broken after extraction (Okoshi and Nakayama, 2011). In Canada, one of the major uranium producing countries, tailings in storage have reached about 225 million tons, and their confinement and stabilization, as well as closure work at abandoned mines, are recognized as serious problems.

Bioremediation of metal or radio nuclei principally aims to reduce bio-availability in the environment and confinement. Bioavailability of uranium can be defined as the ability of the nuclide to attack to or traverse an organism. Unlike the toxic organic pollutants which microbes partially or completely assimilate to yield energy, reduction of contaminant/toxic metal mobility for extraction or immobilization the metal through sequestration, complexation, or changes in speciation that reduce solubility is the first step. These repertoire of functions are displayed by microbes which is utilize to this end. In oxic waters and soils, uranium is present primarily as soluble salts of the uranyl ion

(UO_2^{2+}). When reduced from this U(VI) oxidation state to U(IV), the solubility decreases, resulting in immobilization.

In the surface water with dissolved oxygen, U may occur in surface waters in three oxidation states: U^{4+} (U[IV]), UO_2^+ (U[V]), and UO_2^{2+} (U[VI] or uranyl ion). In deep water with anoxic condition (low redox potential), U occurs as U^{4+} and/or UO_2^+ . U(IV) has a strong tendency to precipitate (e.g., uraninite, $\text{UO}_2[\text{s}]$) and to remain immobile, whereas UO_2^+ forms soluble complexes. In oxic waters, uranium occurs as UO_2^{2+} and forms stable, readily soluble ionic and/or neutral complexes that are highly mobile and play the most important role in uranium transport during weathering (Osmond and Ivanovich, 1992). The pH-Eh conditions influence the type of dominant uranium species as well as the concentration and availability of complexing ions (Gascoyne, 1992). The redox and complexation reactions of uranium are strongly influenced by hydrolysis, since hydrolytic reactions may limit the solubility or influence sorption to particle spin (Choppin *et al.*, 1989).

The first report of microbial reduction of U(VI) was reported in *Micrococcus lactilyticus* (reclassified as *Veillonella alcalescens*). In an deoxygenated warbug's apparatus containing the reaction mixture hydrogen was flushed and products were analysed. It was observed that cell-free extracts of *Veillonella alcalescens* was able to reduce a number of inorganic compounds including uranyl compounds, quantitatively yielding uranyl hydroxide ($\text{UO}_2(\text{OH})_2$). Addition of citrate buffer which is known to form a complex with uranyl ions and stabilize, lead to rapid production of uranyl hydroxide (Woolfolk and Whiteley, 1962). However, at that moment the prevailing theory of uranyl reduction was that abiotic processes were responsible for the production of U(IV) in anaerobic or low redox environments, by processes that included reduction by sulfide, Fe(II), or hydrogen. Almost 30 years later, Lovley et established uranium reduction in dissimilatory Fe(III) reducing bacteria (Lovley and Phillips, 1992), (Lovley *et al.*, 1991), (Gorby and Lovley 1992). As of now, wide range of organisms from uranium-enriched sites such as plant, algae, fungi, protozoa have been shown to reduce uranium. Possibly dissimilatory metal-reducing microorganisms gain energy for growth by coupling the oxidation of organic acids or H_2 to the reduction of metals. In temperate circumneutral pH environments, *Geobacter* species are capable of dissimilatory Fe(III) reduction. *Geobacter metallireducens* produced flagella and pili during growth on Fe(III) or Mn(IV) oxides. Further studies suggested that structural pillin protein PiliA was critical for reduction of Fe(III) and the pillin is electrically conductive. The finding that the conductivity could only be measured across the pili in regions where there were no other proteins associated with the pili suggested that the pili filaments themselves were conductive, rather than redox-active, moieties, such as *c*-type cytochromes, associated with the pili conferring conductivity. This suggest that PiliA serves as uranium reductase in *Geobacter* sp. to extracellularly reduce uranium, in addition to Fe(III) oxides. The outer membrane cytochrome *c* function as electron carriers and can also enzymatically reduce uranium.

Anaerobic culture from uranium mine (with uranium conc. 168mg/kg) was able to completely reduce upto 200 mg/L in soil isolates such as *Pantoea* sp., *Pseudomonas* sp.

and *Enterobacter* sp. These organisms were able to reduce U(VI) to U(IV) (Chabalala and Chirwa, 2010). In this study from Limpopo, South Africa, the soil consortium was a mixture of uranium reducing and oxidizing microorganisms. However, several microbial consortium showed ability to reduce uranium with the highest efficiency shown by non-contaminated soil collected in Monchique thermal place. TGGE and phylogenetic analysis of 16S rRNA gene showed that the uranium (VI) removing bacterial consortia are mainly composed by members of Rhodocyclaceae family and *Clostridium* genus (Martins, 2010). In another study, Anaerobic granular biomass from upward-flow anaerobic sludge blanket (UASB) reactors used for the high-rate treatment of agro-industrial wastewater were used to reduce uranium with high efficiency. This methanogenic granular sludge have endogenous electron donors which could be stimulated with exogenous H₂ addition (Tapia-Rodriguez *et al.*, 2010). *In-situ* reduction of U(VI) was studied in the highly contaminated aquifer (U.S. DOE Integrated Field Research Challenge site, Oak Ridge, TN), using ethanol as an electron donor to reduce the uranium concentration below EPA drinking water standard (0.03mg/L). GeoChip-based analysis revealed that Fe (III)-reducing bacterial (FeRB), nitrate-reducing bacterial (NRB), and sulfate-reducing bacterial (SRB) functional populations reached their highest levels during the active U(VI) reduction phase (days 137 to 370), in which denitrification and Fe(III) and sulfate reduction occurred sequentially. The results suggest that, exogenous addition of electron donors stimulate uranium reduction *in situ* and change in microbial community composition help maintain the stability of U(IV) produced (Van Nostrand *et al.*, 2011). The composition of microbial community varied with the type of electron donor even though the exogenous donor to reduce uranium could stimulate each community (Barlett *et al.*, 2012). Stimulation with acetate in aquifer in Rifle, Colorado, known to enhance uranium reduction caused enrichment of the Fe(III)- and sulfate-reducing lineages, Desulfuromonadales and Desulfobacterales (Handley *et al.*, 2012). Initially a bloom of *Geobacter* followed by an increase in the sulfate-reducing bacteria. Decrease in *Geobacter* population is a challenge in uranium bioremediation.

A geo-microbial analysis of the Uranium ore using DGGE of 16S rRNA genes identified hitherto uncultured aerobic heterotrophic bacteria consisting of Proteobacteria, with the predominance of γ - over β - and α -subdivisions, along with Actinobacteria and Firmicutes. More than 50% of the bacterial isolates affiliated to *Stenotrophomonas*, *Microbacterium*, *Acinetobacter*, *Pseudomonas* and *Enterobacter* showed resistance to uranium and other heavy metals (Islam and Sar, 2011). A group of aerobic, chemoheterotrophic bacteria, *Serratia marcescens* were isolated from the the subsurface soils of India's largest sandstone-type uranium deposit, Domiasiat. All these isolates were able to remove nearly 90-92% (21-22 mg/L) and 60-70% (285-335 mg/L) of U(VI) on being challenged with 100 μ M (23.8 mg/L) and 2 mM (476 mg/L) uranyl nitrate solutions, respectively, at pH 3.5 within 10 min of exposure (Kumar *et al.*, 2011).

Uranium removal from groundwater is reported with 90% efficiency using rhizofiltration. Sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L. var. *vulgaris*) were able to clean-up system was about 25 mg/kg of wet plant mass (Lee and Yang, 2010). Uptake in hydroponically grown plants was demonstrated (upto EC₅₀ = 0.71mM) in *Cucumis sativa* and accumulated in the plant upto 0.16mg/g dry weight. In

both cases, acidic medium increased uranium uptake and uranium was mainly localized in the root system (Soudek *et al.*, 2011). In the soil of three black sand habitats in the Mediterranean coast of Egypt, namely, sand mounds and coastal sand planes and dunes, uranium transfer from soil-to-plant was found to be negatively correlated with clay and organic matter content of soil suggesting the improving phytoremediation of contaminated site using black sand species (Hegazy and Emam, 2011).

Bioremediation of uranium depends on both science and policy. Research in the last decade has resulted in important factors accelerating uranium immobilization. Insight into uranium reductase activity of microorganisms and biochemical pathways supporting them opens new avenues for biotechnological approaches to aid to remediation. However, being a mineral that cannot be completely assimilated, storage and disposal of radioactive waste and treated immobilized uranium is highly dependent on the policies of different regulatory agencies.

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