Chapter 8

Bioremediation of Radiotoxic Elements under Natural Environmental Conditions

Phalazane Johanna Mtimunye and
Evans M. N. Chirwa

Additional information is available at the end of the chapter

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1. Introduction

1.1. Status of uranium use and pollution

The energy sector around the world is severely affected by the combined impact of population growth, industrialisation and the general movement of people into cities (urbanisation). Controlling carbon emissions from the energy sector lies at the centre of the strategy to curb the problem of global warming and its effects on changing weather patterns – which poses a threat to ecosystems and biodiversity. Several alternatives are under consideration worldwide to reverse the current trend of global warming; almost all strategies are concern in finding cleaner primary energy sources with low or no carbon emissions. The alternative energy sources include hydropower, wind power, nuclear power, solar energy, biomass energy, and geothermal energy. Among the proposed cleaner energy sources, nuclear energy has been demonstrated to be the most stable and concentrated enough to replace fossil fuels such as coal and natural gas on most national grids.

However, in spite of holding the promise of cleaner production in terms of carbon emissions, nuclear technology produces a waste which is highly radioactive and in most instances difficult to treat. The waste compounds originating from nuclear power generation include uranium and its fission products, and transuranic elements. These metallic elements account for over 95% of the total radioactivity of radioactive waste [1].

The lightweight fission products emanating from nuclear fuel processing plants and underground nuclear waste repositories contain high levels of mobile species such as caesium (Cs-137), strontium (Sr-90), and cobalt (Co-60). These elements are characterised by very high radiological decay rates and short half-lives. Due to their high decay rates and high radioac-

tivity, fission products render the waste matrix in which they are detected highly radiotoxic and therefore hazardous [2]. Some of these elements are easily taken up by plants and other animal life forms upon reaching the environment [3]. As an example, the divalent cation strontium-90 ($^{90}Sr^{2+}$) is easily incorporated into bone tissue because its chemical properties resemble calcium (Ca^{2+}) which is a critical component of the mammalian bone structure. Calcium is responsible for the bone's structural integrity and strength and is therefore an essential component of the mammalian diet.

Currently, there are about 438 nuclear power plants in operation in 31 countries around the world providing about 14% of the world's primary energy needs [4]. The slow progression towards wider application of nuclear energy technology worldwide is mainly due to concerns over long term radiation contamination, reactor accidents such as those which occurred in Chernobyl and Fukushima, and the possibility of proliferation of atomic bomb making materials to renegade regimes and terrorists.

Nuclear power generation is not the only source of potential radioactive pollution. Other activities such as nuclear weapon testing, radioisotope manufacturing, and biomedical research have also contributed significant amounts of radioactive waste into the environment [5]. However, most radioactive waste originating from medical and radioisotope manufacturing facilities is predominantly organic and therefore can be easily degraded [6]. Radioactive waste from the power generation industry which is identified as High Level Waste (HLW), on the other hand, consists of a higher proportion of non-degradable metallic elements such as uranium and fission products, and transuranic elements. The waste tends to be "hot" (highly radiotoxic) to living organisms and therefore requires pre-treatment before disposal.

This chapter presents recent findings from research aimed at developing environmentally friendly treatment processes for radioactive waste. Attempts have recently been made to treat components of high level radioactive waste (HLW) prior disposal in specifically engineered facilities by immobilizing and extracting the radioactive elements in the waste using a combination of biological reduction and biosorption of the toxic metallic elements. In the case of U(VI), the reduced element is easily extracted either by precipitation/deposition on cell surfaces or removed by a biologically mitigated ion exchange process using live cells of bacteria.

1.2. Uranium compound toxicity

Uranium is found in the environment in many forms including as an oxide, organic or inorganic complex, and rarely as a free metallic ion. Free elemental uranium primarily exists in higher oxidation states typically bound to oxygen. In the aqueous phase, cationic uranium readily combines with oxygen to form oxy-cations of uranium (uranyl ions) which are highly mobile and highly reactive. For example U(VI) in the form of (UO_2^{2+}) is highly soluble in water. Whereas, the reduced form U(IV), existing as uraninite (UO_2) is less soluble and therefore represents a lower risk in the environment.

The toxicity of uranium compounds is closely related to its mobility. That is, the most soluble of the uranium species are associated with acute toxicity in organisms [7]. The highly soluble

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uranium compounds such as UF_6, $UO_2(NO_3)_2$, UO_2Cl_2, uranyl acetate, uranyl sulphates, and uranyl carbonates exhibit high toxicity to mammalian cells whereas the less soluble uranium compounds including UO_2, U_3O_8, uranium hydrides, and carbides are less reactive and less toxic.

The permissible body level for soluble compounds is based on chemical toxicity, while the permissible body level for insoluble compounds is based on radiotoxicity. Because all uranium isotopes (U^{234}, U^{235}, U^{238}) mainly emit α-particles that have little penetrating ability, the main radiation hazard from soluble uranium compounds occurs when uranium compounds are ingested or inhaled [7]. Although, absorption of some soluble compounds through skin is possible, uptake through the skin is normally superseded by either surface damage due to exposure or accumulation to toxic levels through other routes of entry such as inhalation and ingestion.

2. Treatment and reclamation of uranium

2.1. Physical-chemical treatment

The most common treatment strategy for uranium and radioactive waste involves the extraction of the radioactive component to reduce the volume of radioactive waste followed by treatment of the bulky nonradioactive waste using conventional methods [8]. Various options have been utilised to achieve extraction by employing a combination of physical-chemical and biological methods. For areas that have already been contaminated, further migration of the pollutants is prevented by using *in situ* treatment options [9]. *Ex situ* pump-and-treat processes have also been attempted but these come with very high operational costs and are in most cases unsustainable [10]. *In situ* remediation technologies are generally the most preferred treatment technologies because they are more economical, do not require transportation of toxic materials that may lead to more spillage in transit, and also cause fewer disturbances on site [11, 12].

2.2. Adsorptive/ion exchange processes

Separation processes have been utilised to selectively remove cationic species from wastewater streams. Materials such as activated carbon, saw dust, and peat can remove pollutants from water. However, these materials are not selective and therefore may not be effective in removing metallic elements from nuclear waste. Specially designed resins can be utilised to target specific species by manipulating the composition of functional groups on surface of the resin. Several examples of uranium binding ion exchange systems are reported by several researchers [13-15]. Although proven successful on pilot scale, full implementation of ion exchange uranium separation is hindered by high cost. Additionally, the ion exchange resin surfaces are not self-regenerating, and therefore have limited capacity [13].

2.3. Membrane processes

Conventional membrane systems used in treating uranium includes, nano-pore filtration, ultrafiltration, microfiltration and reverse osmosis [16]. Nano-pore membrane filters have the potential to be used in recovery of radioisotopes from water or gas streams. Membrane technology is now regarded as established technology with predictable and reliable processing capability than most current alternatives. Membranes have become relatively cheap such that there use is no longer regarded uneconomical. In spite of being economically viable membrane processes generate large quantities of used membranes which contribute to the problem of radioactive solid waste from the nuclear industry.

2.4. Chemical extraction

Chemical extraction processes have mostly been used for remediation processes mostly on land. For example, uranium can be extracted from contaminated soil using sodium carbonate/bicarbonate or citric acid [17-19]. Although this process effectively removes uranium from soil, it requires a careful balance during application since overloading the system with the acid agent may further migrate uranium in the environment [18, 20]. Certain chemical agents may oxidise other potentially toxic metals posing further risk to the environment. Furthermore, long-term stability of reaction products is of concern. Changing chemical conditions in future could remobilise the metal to its toxic form.

2.5. Biological treatment processes

Biological methods have been proposed to improve or substitute the conventional physico-chemical methods for the remediation of contaminated environments. Unlike organic compounds, toxic metals cannot be degraded or destroyed but can only be transformed from high oxidation state to lower oxidation state. Microbes can potentially affect the physical and the chemical state of the uranium by altering its speciation, solubility, and sorption properties. Strategies suggested for the removal of metals and radionuclides using appropriate microbes include biosorption, bioaccumulation, bioprecipitation, and bioreduction [21-23].

Biological treatment is based on the prospect of utilising processes already devised by nature in dealing with environmental hazards. During three and a half billion years of evolution, microorganisms have evolved mechanisms to survive in hostile environments and to adapt to changes in the environment [24]. One of the most conserved mechanisms in the living cell is the biochemical pathway for electron-transport through the cytoplasmic membrane to conserve energy through the oxidation of an electron donor and reduction of an electron acceptor such as oxygen. This process has been conserved over billions of years, such that, to this day, all life on earth depends on variants of this pathway [24-26]. Most biochemical processes for degradation and/or detoxification of compounds in the living cell are linked to the above process.

Environmental engineers around the world have undertaken to find ways to tap into the mysteries of nature by diligently studying the action of microorganisms as they adapt to extreme conditions. Lately, microorganisms have been isolated that are capable of reducing

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the toxic forms of heavy metal and transitional metal elements in transuranic waste (TRU) to less mobile precipitable forms [27]. Other researchers have found microbial cultures with the capability to resist high radiation doses.

Most of the microorganisms discovered thus far, utilise the energy derived from change in the redox potential or oxidation states of the compounds for metabolic purposes. No organism has yet been shown to utilise the energy derived from radioactive decay for metabolism. But recent research show promise that this scenario is soon to change. For example, recent studies have shown that microorganism may not only resist radiation, but may to a certain degree utilize the radiation for metabolic advantage. One example was illustrated in a recent study in which cultures of melanising fungi from the cold regions utilized ionizing radiation to derive metabolic energy [28].

2.6. Biosorption

Biosorption is used to describe the metabolism independent sorption (passive process). In this process uranium-bearing water is brought into contact with either living or dead biomass that possesses abandoned functional groups (carboxyl, hydroxyl, amine, and phosphate group) within the surface layer. The charged group within the cell surface layer is able to interact with ions or charged molecules present in the uranium-bearing water. As a result metal cations become electrostatically attracted and bound to the cell surface layer.

Polymers secreted by many metabolizing microbes can also immobilize metals. Different studies on biosorption demonstrated that uranium biosorption is reversible, species-specific, and depends upon the chemistry and pH of the solution, physiological state of cells as well as the presence of the extracellular soluble polymers (EPS) [29, 30]. In this process desorption and recovery of heavy metals and radionuclides for further reuse is easy [31]. Biosorption of radionuclides to the cell surface and polymer substance is a promising technology for remediating contaminated waters. However, the effectiveness of this process is highly affected by pH of the solution and saturation of the biosorbent when metal interactive sites are occupied. Studies were done [32, 33] to investigate the biosorption of uranium under acid conditions. It was observed in these studies that biosorption under acidic conditions is not favoured in several species as at low pH the protons (H^+) compete with UO_2^{2+} for sorption sites (surface hydroxyl groups–SOH), thus indicating poor selectivity of the biosorbent surface against competing ions.

In order to understand the interaction between the cell and metallic species in wastewater, the cell surface can be thoroughly characterised using Fourier Transform Infrared spectroscopy (FTIR) or Energy-Dispersive X-ray spectroscopy (EDX). In our research group at the University of Pretoria, we were able to demonstrate that different functional groups on the cell wall of sulphate reducing bacteria acted as ion exchange sites in different pH ranges.

Using autotitration date in the software MINTEQ, four equilibrium states were identified which were associated with carboxylic functional groups (pK_a 4-5), phosphates (pK_a 6-7), phenolics (pK_a 8-9), and hydroxyl/amines (pK_a 10-12). The most abundant reaction sites in sulphate reducing bacteria (SRB) were associated with the hydroxyl functional groups.

Adsorption of the divalent fission product (Sr^{2+}) was inhibited at higher pH, supposedly as a result of increased hydroxylation at the high OH- concentration in solution. Additionally, increased pH could increase the formation of SrII-OH precipitates which is counterproductive to the processes of adsorption to the cells.

Biosorption offers a unique advantage in that the biosorbent media (bacteria) is self-regenerating and can be safely disposed after expiry. Apart from the uranium species, the biosorbent can remove a range of other toxic heavy metals from the wastewater without creating hazardous sludge at costs much lower than conventionally used ion exchange systems. Regeneration of the biosorbent and concentration of the metal solution for eventual recovery further increase the cost effectiveness of the process.

2.7. Bioaccumulation

Bioaccumulation is an active process wherein metals are taken up into living cells and sequestrated intracellularly by complexing with specific metal-binding components or by precipitation. Intracellular accumulation of metals occurs among all classes of microorganisms by an energy-dependent transport system. Unlike metabolically essential metals such as Fe, Cu, Zn, Co, and Mn, which accumulates intracellularly via energy transport system, intracellular uranium sequestration is attributed to non-specific transport system mainly due to increased membrane permeability resulting from uranium toxicity in the living cell [34]. Therefore, intracellular accumulation of uranium is considered as metabolism-independent process. The major drawbacks associated with the use of active uptake systems is the requirement of metabolically active cells and also the challenge in metal desorption and recovery [35]. Specifically, the cells will need to be destroyed to release the metal either by lysis or by incineration. Therefore, in this case, the medium for the uptake of metals cannot be reused.

2.8. Bioprecepitation

Bioprecipitation also known as biocrystallization or biomineralization is the process by which metals and radionuclides can be precipitated with microbially generated ligands such as phosphate, sulphide or oxalate [35]. In these processes bacteria interact strongly with metal ions and concentrate them, eventually generating carbonates and hydroxide minerals at the surface of the cell. Macaskie et al. [35] investigated *Citrobacter sp*. accumulation of heavy deposits of UO_2^{2+}-phosphate, derived from enzymatically liberated phosphate ligand. Cells showed no saturation constrains and it could accumulate several times their own weight of precipitated metal. The above method showed that metal deposition occurs via an initial nucleation pathway involving phosphate groups localized within certain cell-surface lipopolysaccharides (LPS). Accumulation of metal-phosphate complex within the LPS was suggested to prevent fouling of the cell surface by the accumulated precipitate. The limitations of method during application in an industrial process could be similar to those encountered in biosorption. Firstly, the process is hindered by the formation of negatively charged uranyl carbonate complexes, U(VI)–CO_3, arising from microbial metabolism of the carbon source under anaerobic conditions and over time the U(VI) carbonate complex formed stimulate U(VI) oxidation

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over time [36]. Additionally, these processes precipitate metals other than uranium and forms insoluble uranyl-phosphate complex on the cell surface.

2.9. Bioreduction

Reduction of highly toxic and mobile U(VI) to sparingly soluble U(IV) using appropriate microbes has been proposed as a mechanism for preventing the migration of U(VI) with groundwater [37, 38]. The strategy is based on injecting physiological electron donors such as acetate, lactate, ethanol, or glucose to stimulate U(VI) reduction by microbial community native to contaminated aquifers [39]. Microorganisms are known to have evolved biochemical pathway for degradation or transformation of toxic compounds from their immediate environment either for survival or to derive energy by using toxic compounds as electron donors or acceptors [40, 41]. The overall transfer of electrons from the carbon sources to active uranium species can be visualised by the figure below (Figure 1).

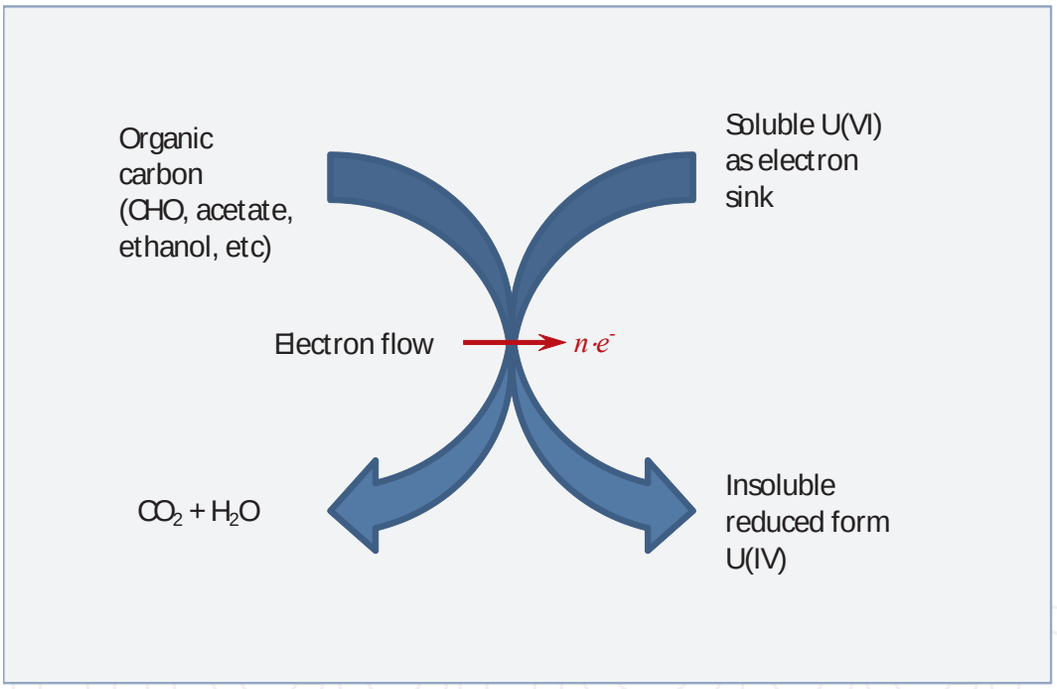

Figure 1. Electron flow during biological reduction of uranium (VI) to U(IV)

An example of a balanced stoichiometric relationship during U(VI) reduction using propanoate as an electron donor is represented by Equation 1 (below):

$$UO_2^{2+} + 1/2CH_3CH_2COO^- + 1/2H_2O \longrightarrow UO_2 + 1/2CH_3COO^- + 1/2CO_2 + 2H^+ \quad (1)$$

Microbial reduction of U(VI) was first reported in crude extracts from *Micrococcus lactilyticus* by assaying the consumption of hydrogen which was dependent on the presence of U(VI) [42].

To date, U(VI) reduction capability has been identified in more than 25 species of phylogenetically diverse prokaryotes. Examples of these are the mesophilic sulphate-reducing bacteria (*Desulfovibro sp*.) [43], Fe(III)-reducing bacteria (*Geobacter* and *Shawanella sp*.) [44], fermentative bacteria from *Clostridium sp*., [45], *Acidotolerant bacteria* [46], as well as *Myxobacteria sp*. [47]. Some of these organisms have been reported to conserve energy for growth from U(VI) reduction [43, 47], while others reduce U(VI) without energy gain [45, 48, 49]. Researchers such as Lloyd [50], and Wade and Di Christina [51], have demonstrated the importance of dissimilatory metal-reducing bacteria (DMRB) in reducing toxic form of uranium (U), iron (Fe), manganese (Mn), and other toxic metals. The unique physiological property of DMRD is that they are obligate anaerobes that are capable of utilising (Fe(III) and Mn(IV) oxide) as terminal electron acceptors. In the presence of the radionuclides (U(VI) and Tc(VII)), these could be mostly bioavailable since Fe(III) and Mn(IV) exist as insoluble hydroxides in the natural pH range.

2.10. Enzymatic U(VI) reduction

Members of genera *Shewanella* [43], *Desulfovibro* [52], *Clostridium* [45], *Geobacter* [50], *Thermus* [53], *Pyrobaculum* and *Desulfosporosinus* [54], display U(VI) reduction activity. The two approaches (ie. biochemical and genetic) are responsible for identifying U(VI) reductases in DMRB [46, 51, 52, 54, 55]. The mechanism by which Fe(III)-reducing bacteria (FeRB) transfer electrons to insoluble Fe(III) oxides during anaerobic growth have been extensively studied in *Shewanella* and *Geobacter* species [50]. In these organisms, an electron transfer chain containing c-type cytochromes is thought to pass through the periplasmic and terminate at the outer membrane facilitating electron transfer to the extracellular solid phase [50]. U(IV) is reported to precipitate in the periplasm and outside of both gram-negative and gram-positive cells suggesting that U(VI) complexes do not generally have access to intracellular enzyme. Thus, imply that the best candidates for the reductases would be electron carrier proteins or enzymes exposed to the outside of the cytoplasmic membrane, within the periplasm, and/or in the outer membrane. Several purified c-type cytochromes display U(VI) reductase activity in vitro. *Shewanella* and *Geobacter* has been reported to enzymatically reduce U(VI) to U(IV) via a respiratory process that conserve energy to supports their anaerobic growth [52].

2.10.1. Desulfovibrio reductase

The enzymatic system responsible for U(VI) reduction, tetraheme or periplasmic cytochrome-*c3* was characterize in the cell-free extract of the sulphate-reducing bacterium *Desulfovibrio vulgaris* in the presence of hydrogenase using H_2 gas as a physiological electron donor [43, 52]. The involvement of tetraheme cytochrome-*c3* was confirmed in the whole-cell and results on whole-cell showed that cytochrome-*c3* was oxidized during U(VI) reduction, but not during sulphate reduction [56]. Further evidence that cytochrome-*c3* was biologically important for *Desulfovibrio* U(VI) reduction came from the impairment of this process when a mutation was constructed in a related strain that eliminated the homologous cytochrome [54]. U(VI) reduction by the mutant *D. desulfuricans* strain was inhibited by at least 90% in the presence of H_2 (gas) as an electron donor and partially impaired (inhibition between 50-70%) in the

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presence of acetate or pyruvate as electron donors [57]. Other researchers observed a partial increase in the U(VI) reduction activity in the presence of either lactate or pyruvate, suggesting the presence of additional proteins capable of metal reduction [54].

2.10.2. Geobacter reductase

Genome sequence of *Geobacter sulfurreducens* revealed putative open reading frames (ORFs) for 73 multiheme c-type cytochromes [58]. The genes responsible for U(VI) reduction include, triheme periplasmic cytochrome-*c7*, diheme periplasmic cytochrome, and others [59]. Mutations have been constructed in the number of genes for these proteins and it was observed that each of the mutant constructed from a number of genes negatively affected Fe(III) reduction rates with acetate as electron donor. However, analysis over 15 cytochromes mutants of *G. sulfurreducens* showed that there was no good correlation between effects on reduction rates of Fe(III) and U(VI) [46]. Interestingly, although this organism is proficient at reducing a broad range of extracellular Fe(III) and Mn(IV) minerals, and UO_2^{2+} it was observed to be inefficient in reducing, NpO_2^{+}, the reduced species of neptunyl (NpO_2^{2+}) exiting in the spent fuel nuclear waste [60]. The authors suggested that the enzyme system responsible for uranium reduction in *G. sulfurreducens* is specific for hexavalent actinides and is capable of transferring one electron to an actinyl ion, and the instability of the resulting U(V) then generates U(IV) via disproportionation.

2.10.3. Shawanella reductase

Among the species that conserve metabolic energy from dissimilatory respiration utilising U(VI) an electron sink is *Shewanella oneidensis* MR-1 [61]. *S. putrefaciens* cells cultures under Fe(II) lost their orange colour indicating a major decrease in c-type cytochrome content [61]. The interpretation of these observations was that cytochromes were involved in the transfer of electrons to the terminal electron acceptor or were the terminal reductases. Mutant analysis implicated the nitrate reductase in U(VI) reduction because of the simultaneous loss of U(VI) and NO_3^- reduction observed in the absence of these reductase [51]. Further mutant studies have implicated other proteins and cytochromes to be involved in metal reduction and a model for electron transfer was proposed. The function of these electron carriers for U(VI) reduction was only recently evaluated as a part of the analysis of global transcriptional responses to U(VI) [55].

By assay of mutants, several proteins including the one involved in menaquinone biosynthesis, decaheme outer membrane cytochrome, a periplasmic decaheme cytochrome, outer membrane protein, and a tetraheme cytochrome were all shown to be needed for optimal U(VI) reduction [55]. The multiple pathways for electron delivery to U(VI) available in *Shawanella* are associated with the capability of U(VI) reduction with lactate as an electron donor in mutants lacking one or more of the above electron transfer components [55]. Comparison of uraninite (UO_2) deposition by mutants lacking outer membrane decaheme *c*-type cytochromes showed accumulation predominantly in the periplasm versus the deposition of UO_2 external to wild-type cells [61]. This result indicate that U(VI) reduction is not eliminated by any of the single mutants analysed and also supports the hypothesis that uranium reductase are likely

non-specific, and that low potential electron donors are present in both the periplasm and outer membrane. It remains to be determined whether the mutants altered for U(VI) reduction are similarly affected in their ability to use U(VI) as terminal electron acceptor for growth.

2.11. Permeable reactive barriers

Waste from power generation and fuel process facilities contains high levels of uranium and transuranic elements. This type of waste, classified as high level waste (HLW) or transuranic waste (TU), is usually solidified in a concrete or bitumen before it is stored in specially engineered facilities above the ground. The chances of environmental contamination from such facilities are slight. However, most of the voluminous intermediate level and low level waste (ILW and LLW) can be packed and stored underground. The underground storage facilities pose a high risk of groundwater contamination. Where contamination has actually occurred, pump-and-treat processes are utilised to intercept the polluted groundwater for treatment above ground. The water can be treated using chemicals or using biological reactors and the clean effluent is returned to the aquifer. For toxic metals, chemical agents may be added followed by precipitation to reclaim the metals [62]. The chemical reduction process utilizes toxic reducing agents that produce toxic sludge requiring further treatment before disposal into natural waters. Biological processes have been proposed for the pump-and-treat process, but this does not eliminate the problem of disposal of the product of the precipitation stage. Several techniques for installing a biological barrier have been attempted such as construction of semi-porous walls which require a fair amount of excavation (Figure 2), injection of nutrients to encourage the growth of certain types or native species in the environment (a form of bioaugmentation), and inoculation of a region down gradient of a pollutant with specialized cultures of bacteria. Molecular *in situ* bioremediation, the process of introducing new genetic material in native species, has not been put to practice anywhere apart from small scale experimental projects on petrochemical pollution [63].

Insitu bioremediation techniques using permeable reactive barriers (PRBs) have been used to treat organic pollutants that can be completely mineralized to safe products, carbon dioxide (CO_2) and water (H_2O), by microorganisms in the environment. A wide range of toxic recalcitrant organic compounds have been treated this way. For example, case studies have been reported on the treatment of petrochemical pollutants [i.e., benzene, toluene, ethylbenzene, xylene, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs)], and agricultural pollutants: methyl-tert-butyl-ether (MTBE) and its congeners [7, 63].

The decreasing concentration of U(VI) across the barrier is envisioned if barrier is inoculated with U(VI) reducing bacterial species. In the case of U(VI) reduction across a barrier system, we hope to utilise U(VI) as an electron sink in a dissimilatory respiration process in which the organisms introduced in the barrier (X_a) will require U(VI) to optimise their growth. If the organisms require U(VI) as a growth limiting electron sink, their survival away from the barrier zone will be limited. This will prevent increased microbial counts in the aquifer water if the aquifer downstream of the direction of flow is utilised as a drinking water supply source.

The main limitation of *in situ* bioremediation for treatment of metals such as uranium is that the element is not destroyed but rather trapped in the aquifer matrix in a reduced state. Should

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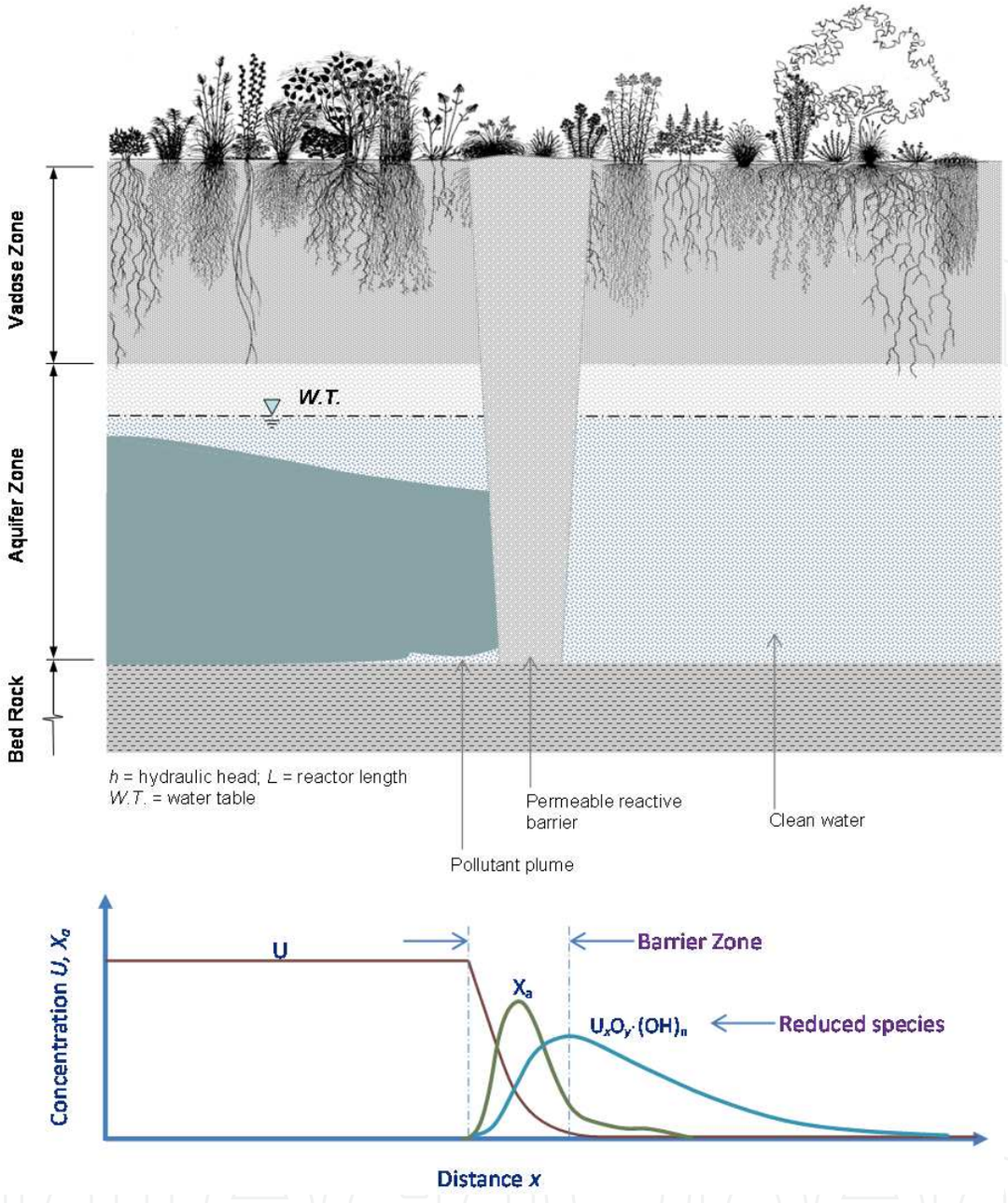

Figure 2. Theoretical representation of the microbial permeable reactive barrier system as an intervention for U(VI) pollution in an unconfined aquifer system. The graph shows the U(VI) concentration and biomass propagation under optimum operation conditions. U = uranium (VI) concentration, X_a = concentration of active biomass, and $U_xO_y(OH)_n$ = hydroxide precipitates of reduction products. The number of complexed hydroxyl ions, *n*, will depend on the charge on the uraninite group $U_xO_y^{ne}$.

the conditions in the soil change one day in future, the metal may be remobilized to its chemically toxic and mobile state and migrate down gradient to further contaminate groundwater and surface water resources. The alternative is to let the reduced form migrate without precipitating in the aquifer medium. This can then be removed by a pump-and-treat method as described above.

3. Determination of U(VI)

U(VI) reductase activity was determined by measuring the decrease in U(VI) in the solution using UV/vis spectrophotometer (WPA, Light Wave II, and Labotech, South Africa). Arsenazo III (Sigma-Aldrich, St. Louis, Missouri, USA) (1, 8-dihydroxynaphthalene-3, 6 disulphonic acid-2, 7-bis [(azo-2)-phenylarsonic acid]), a non-specific chromogenic reagent, was selected as the complexing agent for facilitating U(VI) detection. The accuracy and the precision of the method on the UV/vis spectrophotometer was determined by measuring the concentration of standard U(VI) solution in the range of (0-80 mg/L). A linearized U(VI) standard curve was generated by plotting the absorbance at 651 nm versus the known U(VI) standard concentration. Standard curve for U(VI) measurement demonstrated high degree of accuracy with R^2 = 99.7% and was used to estimate unknown U(VI) concentration.

Measurement of U(VI) was carried out by withdrawing 2 mL of homogenous solution from a 100 mL serum bottle using a disposable syringe. The sample was then centrifuged for about 10 minutes at 6000 rpm (2820 *g*) using a Minispin® Microcentrifuge (Eppendorf, Hamburg, Germany). The sample (0.5-1 mL) was then diluted with 0.4 mL of 2.5% diethylene-triamine-penta acetic acid (DTPA) and then diluted up to mark with basal mineral medium (BMM) (1:10) dilution. The homogenous solution was then mixed with 2 mL of complexing reagent (Arsenazo III) and then allowed to stand for full development of the pink-violet color prior analyses for U(VI) at a wavelength of 651 nm (10 mm light path) against a reagent blank. Total uranium in the unfiltered sample was measured using inductively-coupled plasma mass spectrometry (ICP-MS).

4. Uranium (VI) reduction capability in pure cultures

In order to achieve *ex situ* and *in situ* biological treatment of water and soil contaminated by U(VI) and transuranic elements, it is necessary to search for microorganisms capable of reducing U(VI) under natural conditions. In a current investigation at the University of Pretoria, cultures isolated from a uranium mine dump were tested for uranium (VI) reduction under anaerobic and micro-aerobic conditions. The following section presents results from detailed batch experiments conducted with non-purified and purified cultures isolated from soil samples.

Microorganisms were isolated from the soil samples collected from tailings dumps of an abandoned uranium mine. Background uranium concentration in the original sample was detected at levels as high as 29 mg/kg, much higher than values observed in natural soils (0.3-11.7 mg/kg). To select U(VI) tolerant species, microorganisms from soil were cultured overnight into a 100 mL of sterile basal mineral medium (BMM) amended with glucose as sole carbon source and a dose of U(VI) (75 mg/L uranium (VI) as uranyl nitrate). The inoculum was grown under anaerobic conditions for 24 hours at 30±2°C in 100 mL serum bottles purged with pure (nitrogen) N_2 gas (99.9% pure grade) for about 5-10 minutes to expel residual oxygen before sealing the bottle with rubber stoppers and aluminium seal. After 24 hours enriched

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bacterial strains were isolated by serial dilution of the cultivated culture. U(VI) reduction activity was evaluated for the isolates starting with evaluation for abiotic processes to make sure that physical-chemical processes are taken into consideration when analysing the biological process.

5. Abiotic U(VI) reduction

Heat-killed cultures and sodium azide exposed cultures were used to determine the extent of abiotic U(VI) reduction in batch experiments. For U(VI) reduction experiments cultures were grown over night in a sterile nutrient or Luria-Bettani (LB) broth under anaerobic conditions. Overnight grown cells were heat killed by autoclaving at 121°C for 20 minutes and another set of overnight grown cells were incubated with 0.1% of sodium azide (NaN_3). Cells were then harvested by centrifuging at 6000 rpm (2820 *g*) for 10 minutes. The supernatant was then decanted and the remaining pellet was washed three times with sterile 0.85% NaCl solution. The washed pellet was then re-suspended in 100 mL serum bottle containing U(VI) stock solution and sterile basal mineral medium (BMM) amended with D-glucose as sole carbon source. The serum bottles were then purged with 99.9% (N_2) for about 5-10 minutes to expel residual oxygen before sealing the bottle with rubber stoppers and aluminium seal. The experiments were all conducted at 30±2°C with continuous shaking on lateral shaker (Labotec, Gauteng, South Africa). The experimental conditions in the abiotic controls and the live cells experiments were kept the same (100 mL serum bottles containing BMM amended with D-glucose, 100 mg/L U(VI) solution, and incubated at 30±2°C under anaerobic conditions). A sample was withdrawn at regular time interval using a disposable syringe for U(VI) reduction analysis as described above.

The results showed insignificant difference U(VI) reduction between live and heat-killed cells (Figure 3). The instantaneous U(VI) reduction in heat-killed cells may be due to the presence of the cells that escaped destruction by heat. The reduction of U(VI) observed during the first 2 hours in all treatment containing biomass presented an anomaly. It was clear from these results that another mechanism rather than the direct metabolic process was involved in the U(VI) removal from solution. On the other hand abiotic control (without bacteria) showed that U(VI) reduction process is biologically mediated.

Reverting back to the biosorption studies, it is suggested that functional groups on the cell wall surfaces (-OH, -NH_2, and –COOH) may serve as ligands for U(VI)-U(IV) complexation with the cell surface. U(VI) reduction may serve as a step towards this complexation step. To evaluate these effects we conducted experiments where the pH was varied and the oxidation reduction potential (ORP) was measured with time. Results presented in Figure 4 show that that the rate of U(VI) reduction was pH dependent (Figure 4a). Electronegative conditions under anaerobic conditions created a strongly reducing environment as expected, after which the ORP increased to electropositive values (Figure 4b). As a result insignificant change over time in ORP indicated poor oxidation-reduction, while significant change in ORP over time indicated that the oxidation-reduction process approaches completeness.

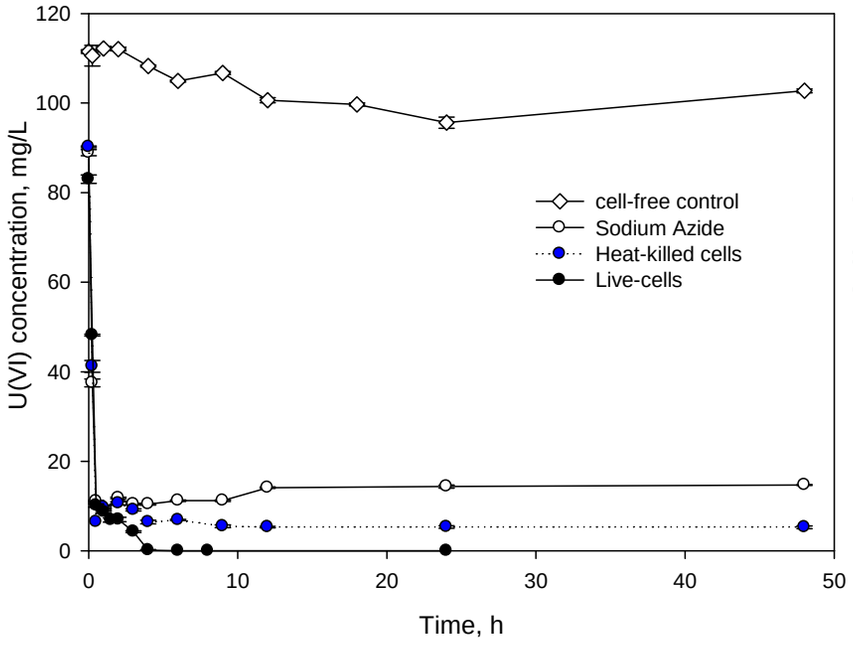

Figure 3. Evaluation of abiotic U(VI) reduction in heat-killed and azide inhibited cells

6. U(VI) Reduction by non-purified cultures (Consortium)

Preliminary experiments were conducted over a wide range of U(VI) concentration (30-400 mg/L) under similar experimental conditions (100 mL serum bottles containing BMM amended with D-glucose, U(VI) solution, and then incubated at 30±2°C under anaerobic conditions) using a reconstituted consortium culture of several identified U(VI) tolerant species. Results showed complete U(VI) reduction in batch cultures at initial U(VI) concentration up to 300 mg/L within 24 hours. In all batch studies with U(VI) concentration up to 400 mg/L (80-100%) U(VI) removal was achieved within the first 5 hours of incubation. However, inhibition of the reduction process was observed at the initial U(VI) concentration of 400 mg/L over time (Figure 5a).

U(VI) reduction trends in batches using purified cultures with single species per batch showed similar trends of U(VI) reduction. Figure 5b shows the summary of results from the best performing cultures labeled Y1, Y5, and Y6. The species characterisation results for these pure cultures are presented later in the chapter. The results in Figure 5b show that the microorganisms existing as a community possess significant stability and metabolic capabilities which can be linked to the effectiveness of synergistic interactions among members of bacterial communities [64].

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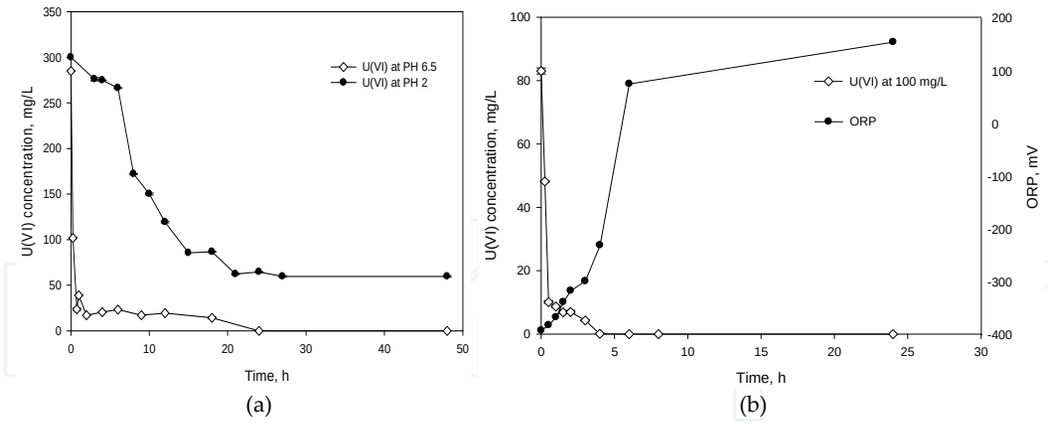

Figure 4. (a) U(VI) reduction at different pH values – U(VI) reduction rate increased with increasing pH, and (b) data showing loss of U(VI) reduction capacity as ORP increases.

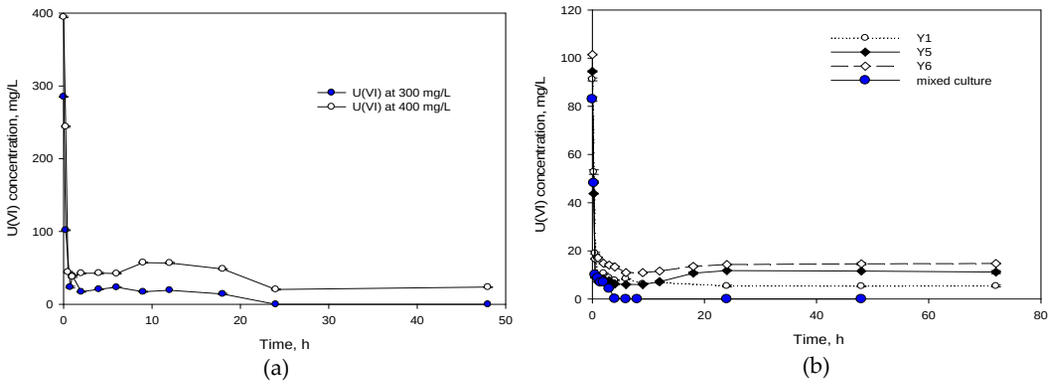

Figure 5. (a) U(VI) reduction in reconstituted consortium culture from mine soil under the initial concentration of 300 and 400 mg/L, and (b) comparative performance of three pure isolates against the reconstituted consortium culture. The reconstituted consortium culture shows the best performance possibly due to symbiotic interactions within the culture system.

7. Fate of reduced uranium species in a cell

Proportional distribution of uranium precipitates in the medium and cells can be used to determine the location of U(VI) reductase activity in the culture system. This is because most the precipitates are formed from reduced uranium species. Transmission electron microscopy (TEM) was used to establish the distribution and localization of uranium deposits in the cells. The energy dispersive X-ray (EDX) spectrometer coupled to the TEM was also used for elemental characterization of the metal deposits in the medium. TEM result images show crystal structures in the medium and very little crystallisation inside the cells (Figure 6a). EDX analysis of the crystals deposited on the cell surfaces confirmed the accumulation of uranium elements in the crystal matrix. Extracellular depositions of uranium also indicate that bacteria are excellent nucleation sites for mineral formations. EDX spectra derived from the uranium

deposits show that they are composed of the following elements U>Cu>P>Os>Ca>Co>Fe (according to their descending order of their weight %). The higher copper (Cu) peak results from the specimen to support grid used. Phosphorous observed in the spectrum could either be from the added phosphorous or could microbially produced. On the other hand no uranium was observed in the metal free biomass (Results not shown).

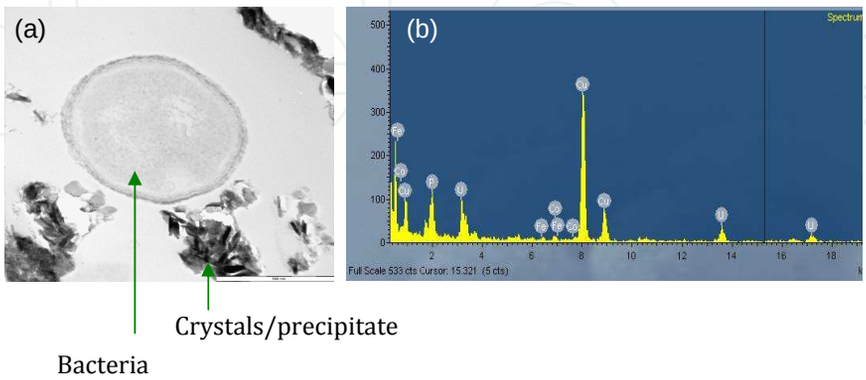

Figure 6. (a) TEM Scan of bacterial cells indicating deposition of uranium species on cell surface and (b) EDX spectrum of precipitate.

8. Microbial characterisation and activity

8.1. Correlation of U(VI) reduction to enzyme activity

Proteins make up a large fraction of the biomass of actively grown microbes. To determine microbial activity over time, protein concentration was determined using a UV/Vis Spectrophotometer (WPA, Light Wave II, and Labotech, South Africa) at the wavelength of 595 nm using Coomassie Dye as a complexing agent to facilitate protein detection. Samples required pre-treatment to reduce interferences during the spectrophotometric analyses. Cell lysis was achieved by ultrasonification of acid treated cells. Results showed that microbial activity decreased with increasing U(VI) reduction (Figure 7). These results served as a confirmation of enzymatic activity as responsible agent for U(VI) reduction.

8.2. Culture composition analysis

The phylogenetic characterization of cells from the mine dump soil was conducted after subculturing the cells in nutrient or Luria-Bettani broth. Individual colonies from a serially diluted preparation were carefully examined for colony morphology and cell morphology by Gram-staining. This process, we recognize, could eliminate a wide range of potential U(VI) reducers especially anaerobic species in the samples. But at this stage, we were targeting the species that can survive under facultative anaerobic conditions.

The purified colonies were streaked on nutrient agar followed by incubating at 30°C for 18 hours in preparation for 16S rRNA gene sequence analysis. Microbial pure cultures were

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grown from loop-fulls from individual colonies, transferred to fresh media containing low amounts (30-75 mg/L) of uranyl nitrate. The process was repeated at least three times for each colony type to achieve close to a pure culture of each identified species.

Genomic DNA was extracted from purified colonies according to the protocol described for the Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI, USA). 16S rRNA genes were amplified by a reverse transcriptase-polymerase chain reaction (RT-PCR) using primers pA and pH1 (Primer pA corresponds to position 8-27; Primer pH to position 1541-1522 of the 16S gene under the following reaction conditions: 1 min at 94°C, 30 cycles of 30s at 94°C, 1 min at 50°C and 2 min at 72°C, and a final extension step of 10 min at 72°C). PCR fragments were then cloned into pGEM-T-easy (Promega) [Promega Wizard® Genomic DNA Purification Kit (Version 12/2010)]. The 16S rRNA gene sequences of the strains were aligned with reference sequences from *Desulfovibrio sp.*, *Geobacter sp.*, *Acinetobacter sp.*, *Anthrobacter sp.*, and *Shewanella putrefaciens* using Ribosomal Database Project II programs. Sequence alignment was verified manually using the program BIOEDIT. Pairwise evolutionary distances based on an unambiguous stretch of 1274 bp were computed by using the Jukes and Cantor method [65].

U(VI) reducing colonies were identified from the genera *Bacilli*, *Acinobacter*, *Actinomycetes* and *Chrysebactreium*. Sections of phylogenetic tree diagrams with closet associations to know species are shown in Figures 8d. The associations shown Figure 8 have been reported among U(VI) reducing groups in literature. Fowle et al. [66] has shown that *Bacillus* species are effective biosorbents for uranium. Additionally, the capability of *Anthrobacter* species isolated from a uranium-contaminated site in accumulating uranium intracellularly as uranium precipitates closely associate with polyphosphate granules was also reported [21].

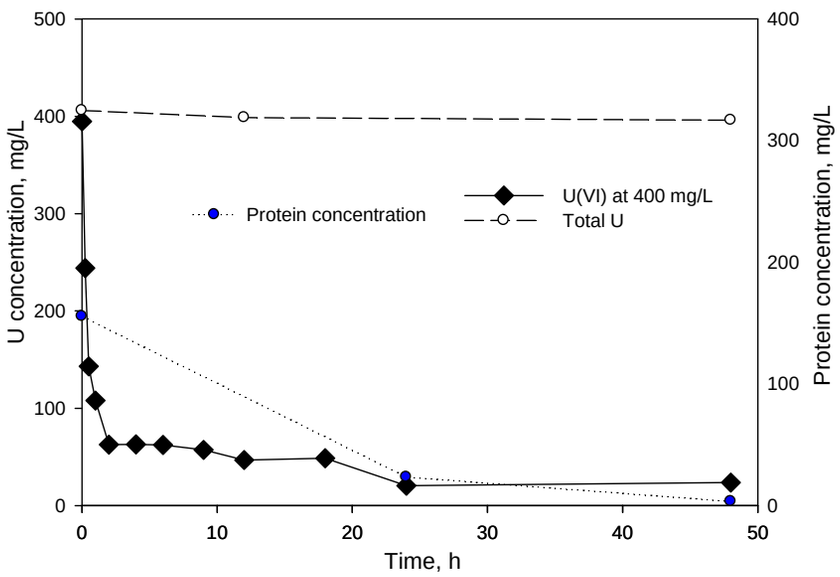

Figure 7. Evaluation of U(VI) reduction, protein concentration and total uranium under an initial concentration of 400 mg/L.

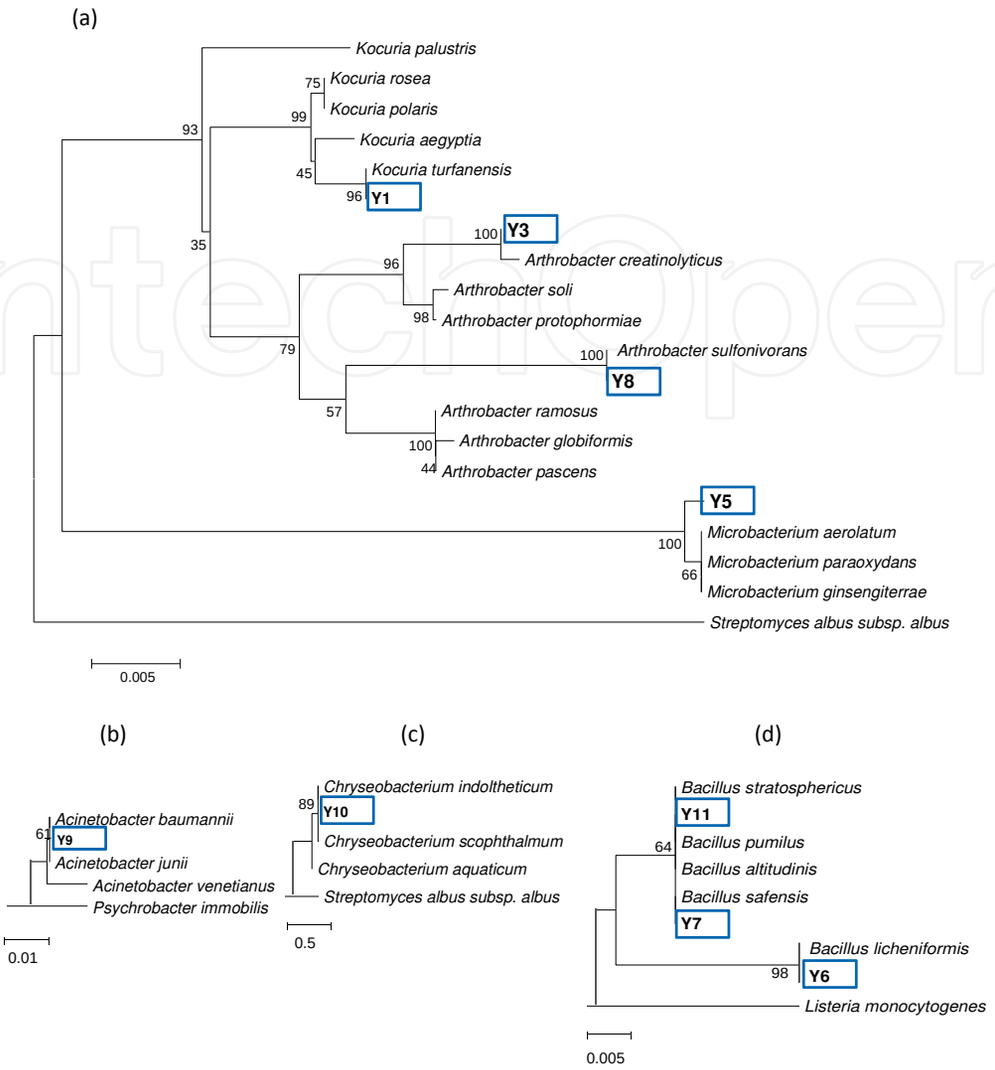

Figure 8. Phylogenetic analysis results showing the predominance of the Gram-positive (a) *Microbacterieceae* and *Anthrobacteriae*, and Gram-negative (b) *Acinetobater* under low U(VI) exposure. Colonies Y10 in figure 8c did not reduce uranium. Another uranium (VI) reducing species, Y6, was also identified among the Bacilli shown in Figure 8d.

In the phylogenetic analysis, the scale indicated at the bottom of the plots, e.g., 0.005 for Figure 8a represents the genetic distance, while the percentage numbers at the nodes indicate the level of bootstrap based on neighbour-joining analysis of 1000 replicates. The three species related to *Actinomycetes* were tolerant at 75 mg/L of U(VI) suggesting the capability of the species in reducing U(VI) in a basal mineral medium (BMM) amended with D-glucose (Figure 8a). The species related to *Acinobacter* as indicated in Figure 8b were also tolerant to U(VI) at concentration levels around 75 mg/L with D-glucose as a sole added carbon source. The group presented in Figure 8c, although tolerant to U(VI), did not reduce U(VI) under the conditions tested. Three species of 16S rRNA gene of *Bacilli* were tolerant at 75 mg/L and at least one of these, Colony Y6, was able to reduce U(VI) in a basal mineral medium (BMM) amended with D-glucose as a sole added carbon source.

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9. U(VI) removal kinetics

9.1. Kinetic model adaptation

To model a biological U(VI) reducing system, the reaction scheme, rate equations and kinetic constants for the processes taking place in the batch reactor are chosen from published models on enzymatic reduction hexavalent toxic metals such as U(VI). Shen and Wang [67] demonstrated that the rate of U(VI) reduction by enzymes can be expressed as the Monod equation if viable cell concentration X is correlated to enzymes produced E:

$$-\frac{dU}{dt} = \frac{k_u \cdot U}{K_u + U} \cdot X \tag{2}$$

where: U = U(VI) concentration at time, t (mg/L); X = density of active bacterial cells at time, t (mg cells/L); k_u = specific rate of U(VI) reduction(mg U(VI)/mg cells/h); and K_u = half-velocity constant (mg/L). However, the active cell concentration, X, may be assumed to decrease in proportion to the amount of U(VI) reduced due to the toxicity of U(VI):

$$X = X_0 - \frac{U_0 - U}{T_u} \tag{3}$$

where: U_0 = initial U(VI) concentration (mg/L); X_0 = initial cells density of U(VI)-reducing strains (mg cells/L); and T_u = maximum U(VI) reduction capacity of cells (mg U(VI)/mg cell). Substituting Equation 3 into Equation 2 yields the following equation:

$$-\frac{dU}{dt} = \frac{k_u \cdot U}{K_u + U}\left(X_0 - \frac{U_0 - U}{T_c}\right) \tag{4}$$

U(VI) reduction data obtained with the pure cultures and the mixed culture were analyzed using Equation 4. Parameters in Equation 4 can be analyzed using simulation software such as AQUASIM or SigmaPlot. The model is calibrated using batch data over the incubation period. The values collected under non-inhibiting conditions are suitable for estimating the kinetic parameters k_u and K_u since these respond to cell growth dynamics. The parameter T_u is estimated under overloaded conditions since this parameter is related to the U(VI) reduction capacity of the cells.

9.2. Uranium (VI) reduction under inhibiting conditions

The inhibition model is suitable for application where the U(VI) loading per cell is very high. This is expected during startup (inoculation) of a systems with U(VI) already present. Such would be the case during the initial operation *in situ* bioremediation system. To account for

toxic inhibition in such situations, a simple Monods non-competitive inhibition kinetic model incorparating inhibition term, K is suggested:

$$-r_u = \frac{k_u U}{\left(K_u + U\right)\left(K^{\left(1-\frac{U_T}{U_0}\right)}\right)}\left(X_0 - \frac{U_0 - U}{T_u}\right) \tag{5}$$

where: k_u = maximum specific rate of U(VI) reduction (1/h); K_u = half-velocity constant (mg/L); U_T = U(VI) toxicity threshold concentration (mg/L); X_o = initial biomass concentration (mg/L); K = limiting constant (mg/L); and T_u = U(VI) reduction capacity (mg U(VI) reduced/mg cells).

9.3. Continuous flow systems

Continuous flow systems better simulate actual systems especially where *in situ* bioremediation is planned. Batch systems cannot simulated the effects of diffusion, clogging of pores, advection rates. Packed columns have been used in simulating the operational conditions of a barrier system [68]. The non-steady state dynamic process of uranium removal in packed column is represented by Equation 6. The mass balance of uranium (VI) across the packed column includes U(VI) reduction rate (r_u), mass transport rate (j_u), adsorption rate (q_u). Due to the inclusion of the interstitual term u and the mass transport term j_u, the predominant process affecting performance during the transient-state in the advection and diffussion:

$$\frac{d(U \cdot V)}{dt} = A\sum_{l=0}^{l=L_i} u(U_{in} - U) - r_u \cdot \Delta V - j_u \cdot A_f - q_u \cdot \Delta V \tag{6}$$

where: U = effluent U(VI) concentration (mg/L); V = volume of the reactor (L); U_{in} = influent U(VI) concentration (mg/L); Q = influent flow rate (L/h); r_u = U(VI) reduction rate coefficient (mg/L/h); t = time (h); A_f = biomass surface area (m^2); J_u = flux of dissolved species into the biofilm ($mg/m^2/h$); and q_u = rate of U(VI) removal by adsorption (1/h). The interstitial velocity u (m/h) is assumed to be constant throughout the entire column. The diffusional flux (J_u) can be expresed using Fick's law:

$$\frac{\partial \mathbf{c}_i}{\partial t} = D_w \frac{\partial^2 \mathbf{c}_i}{\partial x^2} \tag{7}$$

Where: $\mathbf{c}_i$ = concentration of dissolved species (mg/L); D_w = diffusivity of dissolved species in water (m^2/h); t = time (h); and x = spatial coordinate (m).

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The reaction term r_u is dependent on the amount of biomass accumulated in the void space of the column. However, due to space limitations, cells may only grow to a certain maximum concentration. The time at which the cells reach the maximum allowable concentration is dependent on initial cells, U(VI) toxicity, and hydraulic loading rate. These conditions cause the cells to follow a logistic curve defined by Equation 8:

$$X = X_0 + \frac{X_{\max}}{1 + \left(\frac{t}{t_0}\right)^b} \tag{8}$$

where: X = viable cell density (mg/L) at any time t (h); X_{max} = maximum attainable viable cell concentration (mg/L) in the barrier column, t_0 = logistic interval (h); and b = pitch (dimensionless). The impact of the adsorptive process was determined to be minimal during continuous flow operation for an extended period of time. This is because reaction sites tend to become saturated as the system approaches equilibrium.

10. Conclusion

The chapter addresses the main feature of various U(VI) remediation techniques involving the *in situ* bioremediation using permeable reactive barrier. The technique is well known for its effectiveness for remediating organic pollutants. However, its effectiveness for removal of metallic species is hindered by possible accumulation of precipitates. In our preliminary batch studies it was observed that isolated organisms are capable of immobilizing U(VI) by means of more than one mechanism, i.e., biosorption and enzymatic reduction. These results open a new research field for understanding which of these mechanism is predominant and in what sequence does the U(VI) reduction take place under anaerobic conditions. Modelling *in situ* U(VI) bioreduction involve many uncertain parameters, including those of aqueous U(VI) speciation, surface complexation, and bioreduction kinetics. Therefore, for efficient application, sensitivity analysis is needed to simplify the models such as presented. Furthermore, re-oxidation of the biologically reduced uranium needs to be included in future models to evaluate long-term stability of bioreduction techniques.

Nomenclature

A - effective cross sectional area (m^2)

A_{f-} biomass surface area (m^2)

b - pitch factor (dimensionless)

D_{w} - diffusivity of dissolved species in water (m^2/h)

J_{u} - U(VI) flux rate (mg/m^2/h)

k_{u} - reaction rate coefficient (1/h)

$K_{}$ - inhibition coefficient (mg/L)

K_{u} - half-velocity constant (mg/L)

Q - inflow rate (L/h)

q_{u} - rate of U(VI) by adsorption (mg/L/h)

q_{m} - maximum specific uptake of metal corresponding to site saturation (mg/g)

r_{u} - U(VI) reduction rate (mg/L/h)

t - time (h)

t_{0} - logistic time interval (h)

T_{u} - U(VI) reduction capacity (mg U(VI) reduced/mg cells)

U - effluent U(VI) concentration at time, t (mg/L)

U_{in} - influent U(VI) concentration (mg/L)

U_{0} - initial value, U(VI) concentration at time zero (mg/L)

U_{T} - U(VI) toxicity threshold concentration (mg/L)

V - volume of the reactor (L)

ΔV - differential volume (L)

x - spatial coordinate (m)

X - biomass concentration at time t (mg/L)

X_{max} - maximum attainable viable cell concentration (mg/L)

X_{0} – initial biomass concentration (mg cells/L)

Author details

Phalazane Johanna Mtimunye and Evans M. N. Chirwa

*Address all correspondence to: mtimunyepj@gmail.com

Water Utilization Division, Department of Chemical Engineering, University of Pretoria, Pretoria, South Africa

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References

[1] IAEA. Nuclear Technology Review. International Atomic Energy Agency Scientific and Technical Publication Series, Vienna, Austria; 2009.

[2] Greve, K., Niesen, E., Ladefogen, O. Evaluation of health hazards by exposure to strontium in drinking water. Toxicology Letters 2007; 172(1) S210.

[3] Ajlouni, AMS. Health consequences of nuclear fission products. Journal of Applied Science and Environmental Management 2007; 11(3) 11-14.

[4] World Nuclear Association (WNA); 2008. http://www.world-nuclear.org/info/inf88.html (accessed 7 November 2012).

[5] Tikilili PV, Chirwa EMN. Characterization and Biodegradation of Polycyclic Aromatic Hydrocarbons in Radioactive Wastewater. Journal of Hazardous Materials 2011; 192(3) 1589-1596.

[6] Bouwer EJ, Zehnder AJB. Bioremediation of organic compounds-putting microbial metabolism to work. Trends Biotechnology 1993; 11(8) 360-367.

[7] Craig DK. Chemical and Radiological Toxicity of Uranium and its Compounds, Report WSRC-TR-2001-00331, Contract No. DE-AC09-96SR18500, Westinghouse Savannah River Company, Aiken/U.S. Department of Energy; 2001.

[8] Chirwa EMN. Developments in bioremediation for separation/recovery. K.L. Nash and G.J. Lumetta (Eds.), in Advanced Separation Techniques for Nuclear Fuel Reprocessing and Radioactive Waste Treatment, Woodhead Publishing, Ltd, Cambridge, UK; 2011. p436-472.

[9] Doherty R, Phillips DH, McGeough KL, Walsh KP, Kalin RM. Development of modified flyash as a permeable reactive barrier medium for a former manufactured gas plant site. Northern Ireland. Environmental Geology 2006; 50(1) 37-46.

[10] IAEA. Treatment of Liquid Effluent from Uranium Mines and Miles. Report of a Co-ordinated Research Project 1996-2000. International Atomic Energy Agency (IAEA), 2004; Vienna, Austria.

[11] Gavrilescu M. Overview of in situ remediation technologies for sites and groundwater. Environmental. Engineering and Management Journal 2006; 5(1) 79-114.

[12] Olexsey RA, Parker RA. Current and future *in situ* treatment techniques for the remediation of hazardous substances in soil, sediments, and groundwater I. Twardowska, H.E. Allen, M.M. Häggblom, S. Stefaniak (Eds.), Soil and Water Pollution Monitoring, Protection and Remediation, NATO Science Series, IV. Earth and Environmental Science, Springer, Netherlands 2006; 69, 211-219

[13] Zaganiaris EJ. Ion Exchange Resins in Uranium Hydrometallurgy, Books on Demand GmbH, Paris, France; 2009.

[14] Traut DE, Nichols IL, Seidel DC. Design requirements for uranium ion exchange from ammonium bicarbonate solutions in a fluidized system. Department of the Interior, Bureau of Mines, USA; 1978.

[15] Ross JR, George DR. Recovery of uranium from natural mine waters by countercurrent ion exchange.US Department of Interior, Bureau of Mines, University of Michigan; 1971. p7471-7480.

[16] Pabby AK, Rizvi SSH, Sastre AM. Membrane techniques for treatment in nuclear waste processing, a global experience. Membrane Technology 2008; 11: 9-13.

[17] Zhou P, Gu P. Extraction of oxidized and reduced forms of uranium from contaminated soils: effects of carbonate concentration and pH. Environmental Science and Technology 2005; 39(12) 4435-4440.

[18] Phillips EJP, Landa ER, Lovley DR. Remediation of uranium contaminated soils with bicarbonate extraction and microbial U(VI) reduction. Journal of Industrial Microbiology 1995; 14(3-4) 203-207.

[19] Gramss G, Voight KD, Bergmann H. Plant availability and leaching of heavy metals from ammonium-calcium, carbohydrate, and citric acid-treated uranium-mine-dump soil. Journal of Plant Nutrition and Soil Science 2004; 167, 417-427.

[20] Kantar B, Honeyman BD. Citric acid enhanced remediation of soil contaminated with uranium by soil flushing and soil washing. Journal of Environmental Engineering 2006; 132(2) 247-255.

[21] Suzuki Y, Banfield J. Resistance to, and accumulation of, uranium by bacteria from a uranium-contaminated site. Geomicrobiology Journal 2004; 21(2) 113-121.

[22] Nancharaiah YV, Joshi HM, Mohan TVK, Venugopalan VP, Narasimhan SV. Aerobic granular biomass: a novel biomaterial for efficient uranium removal. Current Science 2006; 91(4) 503-509.

[23] Nedelkova M, Merroun ML, Rossberg A, Hennig C, Selenska-Pobell S. Microbacterium isolates from the vicinity of a radioactive waste depository and their interactions with uranium. FEMS Microbiology Ecology 2007; 59(3) 694-705.

[24] Bush MB. Ecology of a Changing Planet, 3rd Edition, Prentice Hall, New Jersey, USA, 2003.

[25] Nealson KH. Post-Viking microbiology: new approaches, new data, new insights. Origins of life and evolution of the biosphere. Journal of the International Society for the Study of the Origin of Life 1999; 29(1), 73-93.

[26] Kalckar HM. Origins of the concept oxidative phosphorylation. Molecular and Cellular Biochemistry 1974; 5(1-2), 55-63.

[27] Lloyd JR. Microbial reduction of metals and radionuclides. FEMS Microbiology Reviews 2003; 27, 411-425.

[28] Dadachova E, Bryan RA, Huang X, Moadel T, Schweitzer AD, Aisen P, Nosanchuk JD, Casadevall A. Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. PLoS One, May 23, 2007.

[29] Francis AJ, Gillow JB, Dodge CJ, Harris R, Beveridge TJ, Papenguth HW. Uranium association with halophilic and non-halophilic bacteria and archaea. Radiochimica Acta 2004; 92(8) 481-488.

[30] Nakajima A, Tsuruta T. Competitive biosorption of thorium and uranium by Micrococcus luteus. Journal of Radioanalytical and Nuclear Chemistry 2004; 260(1) 13-18.

[31] Kratochvil D, Volesky B. Advances in the biosorption of heavy metals, Trends in Biotechnology 1998; 16(17) 291-300.

[32] Sar P, D'Souza SF. Biosorption of thorium (IV) by a Pseudomonas strain. Biotechnology Letters 2002; 24(3) 239-243.

[33] Jroundi F, Merroun ML, Arias JM, Rossberg A, Selenska-Pobell S, González-Muñoz MT. Spectroscopic and microscopic characterization of uranium biomineralization in*Myxococcus xanthus*. Geomicrobiology Journal 2007; 24(5) 441-449.

[34] Suzuki Y, Banfield JF. Geomicrobiology of uranium. Reviews in Mineralogy Geochemistry 1999; 38(1) 393-432

[35] Macaskie LE, Bonthrone KM, Yong P, Goddard DT. Enzymically mediated bioprecipitation of uranium by a Citrobacter sp. a concerted role for exocellular lipopolysaccharide and associated phosphatase in biomineral formation. Microbiology 2000; 146(8) 1855-1867.

[36] Reeder RJ, Nugent M, Tait CD, Morris DE, Heald SM, Beck KM, Hess WP. A Lanzirotti Coprecipitation of uranium(VI) with calcite: XAFS, micro-XAS, and luminescence characterization. Geochimica et Cosmoschimica Acta 2001; 65(20) 3491–3503.

[37] Lovley DR, Widman PK, Woodwar, JC, Phillips EJP. Reduction of uranium by cytochrome *c*3 of *Desulfovibrio vulgaris*. Applied Environmental Microbiology 1991; 59(11) 3572-3576.

[38] Gorby YA, Lovley DR. Enzymatic uranium precipitation. Environmental Science and technology 1992; 26(1) 205-207.

[39] Anderson C, Pedersen K. In situ growth of *Gallionella* biofilms and partitioning of lanthanides and actinides between biological material and ferric oxyhydroxides. Geobiology 2003; 1(2) 169-178.

[40] Rothschild LJ, Mancinelli RL. Life in extreme environments. Nature 2001; 409(6823) 1092-1101.

[41] Istok JD, Senko JM, Krumholz LR, Watson D, Bogle MA, Peacock A, Chang YJ, White DC. In situ bioreduction of Technetium and Uranium in a nitrate-contaminated Aquifer. Environmental Science and Technology 2004; 38(2) 468-475.

[42] Woolfolk CA, Whiteley HR. Reduction of inorganic compounds with molecular hydrogen by *Micrococcus lactilyticus*. I. Stoichiometry with compounds of arsenic, selenium, tellurium, transition and other elements. Journal of Bacteriology 1962; 84(4) 647-658.

[43] Lovley DR, Phillips EJP. Reduction of uranium by *Desulfovibrio desulfuricans*. Applied and Environmental Microbiology 1992; 58(3) 850-856.

[44] Coates JD, Bhupathiraju VK, Achenbach LA, McInerney MJ, Lovley DR.*Geobacter hydrogenophilus* and *Geobacter chapellei* and *Geobacter grbiciae*, three new, strictly anaerobic, dissimilatory Fe(III)-reducers. International Journal of Systematic and Evolutionary Microbiology 2001; 51(2) 581-588.

[45] Francis AJ, Dodge CJ, Lu F, Halada GP, Clayton CR. XPS and XANES studies of uranium reduction by *Clostridium sp*. Environmental Science and Technology 1994; 28(4) 636-639.

[46] Shelobolina ES, Sullivan SA, O'Neill KR, Nevin KP, Lovley DR. Isolation, characterization, and U(VI)-reducing potential of a facultatively anaerobic, acid-resistant bacterium from low-pH, nitrate- and U(VI)-contaminated subsurface sediment and description of *Salmonella subterranea* sp. nov. Applied and Environmental Microbiology 2004; 70(5) 2959-2965.

[47] Wu Q, Sanford RA, Löffler FE. Uranium reduction by *Anaeromyxobacter dehalogenans* strain 2CP-C. Applied and Environmental Microbiology 2006; 72(5) 3608-3614.

[48] Lloyd JR, Chesnes J, Glasauer S, Bunker DJ, Livens FR, Lovley DR. Reduction of actinides and fission products by Fe(III)-reducing bacteria. Geomicrobiology Journal 2002; 19(1) 103-120.

[49] Kashefi K, Lovely DR. Reduction of Fe(III), Mn(IV), and toxic metals at 100°C by *Pyrobaculum islandicum*. Applied and Environnemental Microbiology 2000; 66(3) 1050-1056.

[50] Lloyd JR, Leang C, Hodges-Myerson AL, Coppi MV, Cuifo S. Biochemical and genetic characterization of PpcA, a periplasmic *c*-type cytochrome in *Geobacter sulfurreducens*. Biochemical Journal 2003; 369(1) 153-161.

[51] Wade R, DiChristina TJ. Isolation of U(VI) reduction-deficient mutants of *Shewanella putrefaciens*. FEMS Microbiology Letters 2000; 184(2) 143-148.

[52] Lovley DR, Roden EE, Phillips EJ, Woodward JC. Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Marine Geology 1993; 113(1-2) 41-53.

[53] Payne RB, Gentry DM, Rapp-Giles BJ, Casalot L, Wall JD. Uranium reduction by Desulfovibrio desulfuricans strain G20 and a cytochrome c3 mutant. Applied and Environmental Microbiology 2002; 68(6) 3129-3132.

[54] Kieft TL, Fredrickson JK, Onstott TC, Gorby YA, Kostandarithes HM. Dissimilatory reduction of Fe(III) and other electron acceptors by a *Thermus* isolate. Applied and Environmental Microbiology 1999; 65(3) 1214-1221.

[55] Bencheikh-Latmani R, Williams SM, Haucke L, Criddle CS, Wu L. Global transcriptional profiling of *Shewanella oneidensis* MR-1 during Cr(VI) and U(VI) reduction. Applied and Environmental Microbiology 2005; 71(11) 7453-7460.

[56] Elias DA, Suflita JM, McInerney MJ, Krumholz LR. Periplasmic cytochrome c3 of Desulfovibrio vulgaris is directly involved in H_2-mediated metal but not sulfate reduction. Applied and Environmental Microbiology 2004; 70(1) 413-420.

[57] Caccavo FJ, Blakemore R, Lovley D. A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay Estuary, New Hampshire. Applied and Environmental Microbiology 1992; 58(10) 3211-3216.

[58] Methe BA, Nelson KE, Eisen JA, Paulsen IT, Nelson W. Genome of Geobacter sulfurreducens: metal reduction in subsurface environments. Science 2003; 302(5652) 1967-1969.

[59] Wu Q, Sanford RA, Löffler FE. Uranium reduction by Anaeromyxobacter dehalogenans strain 2CP-C. Applied and Environmental Microbiology 2006; 72(5) 3608-3614.

[60] Renshaw JC, Butchins LJ, Livens FR, May I, Charnock JM, Lloyed JR. Bioreduction of uranium: environmental implications of a pentavalent intermediate. Environmental Science and Technology 2005; 39 5657-5660.

[61] Kennedy DW, Marshall MJ, Dohnalkova AC, Saffarini DA, Culley DE. Role of *Shewanella oneidensis c*-type cytochromes in uranium reduction and localization. ASM 105th General Meet (Abstr.) Q-389; 2004.

[62] Mukhopadhyay B, Sundquist J, Schmitz JR. Removal of Cr(VI) from Cr-contaminated groundwater through electrochemical addition of Fe(II). Journal of Environmental Management 2007; 82(1) 66-76.

[63] Liu S-J, Jiang B, Huang G-Q, Li XG. Laboratory column study for remediation of MTBE-contaminated groundwater using a biological two-layer permeable barrier. Water Research 2006; 40(18) 3401-3408.

[64] Mukred AM, Hamid AA, Hamzah A, Yusoff WMW. Development of Three Bacterial Consoritum for the Bioremediation of Crude Petroleum-oil in Contaminated Water. Journal of Bio sciences 2008; 8(4) 73-79.

[65] Jukes TH, Cantor CR. Evolution of protein molecules, In Munro, H.N. (Ed.), Mammalian Protein Metabolism. Academic Press, New York, 1969, p21-123.

[66] Fowle DA, Fein JB, Martin AM. Experimental study of uranyl adsorption onto *Bacillus subtilis*, Environmental Science and Technology 2000; 34(17) 3737-3741.

[67] Shen H, Wang Y T. Modeling hexavalent chromium reduction in Escherichia coli ATCC 33456. Biotechnology and Bioengineering 1994; 43(4) 293-300.

[68] Mtimunye PJ. Steady-state model for hexavalent chromium reduction in simulated biological reactive barrier: microcosm analysis. Master's Thesis. University of Pretoria, Pretoria, South Africa; 2011. http://upetd.up.ac.za/thesis/available/etd-09222011-104550/ (accessed on 30/10/2012).

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