

Uranium Biomineralization with Phosphate - Biogeochemical Process and Its Application

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Abstract

Rapid development of nuclear energy and science, uranium contamination has been increasing concern worldwide. Various restoration techniques have been developed. At present, *in situ* biomineralization became a promising U remediation technology due to its high stability of mineral products in underground and surface environments. This study reviews the current research status of uranium biomineralization with phosphate developed in recent years. The screening of microorganisms, the organophosphorus sources and biomineralization efficiency were briefly discussed. The objective of this paper is to provide an understanding of biogeochemical processes in relation to relevant microorganisms, biomineralization mechanisms, and biogeochemical conditions. A comprehensive overview of microbial reactions to uranium could facilitate the further development, perfection and optimization of *in situ* bioremediation strategies for uranium remediation in underground water and soils.

Keywords: Biomineralization, Uranium contaminated groundwater. Uranium contaminated soil, Phosphate, microbial processes

1. Introduction

Anthropogenic activities related to nuclear processes such as mining, fuel processing, weapon production or nuclear accidents have resulted in contamination of the environment with uranium (VI).¹⁻² Uranium has biologically dynamic toxicity, metabolism toxicity and chemical toxicity, leading to potential harm to mammalian reproduction and development with reduced biological fertility, abnormal and slow embryonic development.³ Uranium exists primarily as U(IV) or U(VI) depending on the prevailing environmental redox conditions. In oxidizing conditions, U(VI) exists as the aqueous uranyl such as UO_2^{2+} and its hydroxyl complexes.⁴ Uranium hexavalent has a high mobility and chemical toxicity. Because of its chemical toxicity, aqueous uranyl is harmful to aquatic organisms, plants and humans, as well as to all organisms exposed to effluents from uranium mines.⁵ Uranium has a long half-life and will remain in the environment for a long time. Therefore, it possesses long-term potential risks to human health and ecological environment.⁶

Remediation of uranium contaminated groundwater with conventional physical and chemical approaches such as pumping treatment method generated secondary pollution with high operation costs. For small areas of water contaminated by uranium, coagulation, precipitation, evaporation, extraction, and membrane separation technologies were used to remove most U from the water.⁷ Although the traditional treatments effectively removes U(VI) in uranium contaminated environment, it performs poorly in the treatment of U(VI) groundwater with low concentration.⁸

Chicoua N. proposed that zero-valent iron (ZVI) be used to remove U from contaminated water.⁶

Micro-organisms (bacteria and fungi) changed the extracellular binding sites and the pH through biological activity and plants modified the form and bioavailability of U. Therefore both have been used for remediation of groundwater contaminated by uranium.⁹⁻¹⁰ One potential strategy to inhibit the spread of uranium in the groundwater consists of inducing uranium precipitation via bioremediation process. U(VI) biomineralization is U(VI) precipitates with microbe-associated ligands such as phosphate, carbonate, or hydroxide, which provide nucleation foci for precipitation.¹¹ Biomineralization technology has obvious advantages over bioreduction. Uranium biomineralization has been demonstrated in aerobic and anaerobic conditions with both acidic and neutral pH values. U(VI) forms sparingly soluble and stable phosphate minerals over a broad range of pH conditions (pH 4-8). Phosphate minerals are also highly stable under extensive redox conditions compared to other uranium (IV) minerals.¹² In the MIPP biomineralization process, by the addition of a phosphate source, metabolic activity like phosphatase or phytase produced from microbes can increase phosphate availability and then mediate toxic ion mineralization as result of precipitating phosphorus containing minerals on cell surfaces.¹³⁻¹⁴

In soil and groundwater environments, aerobic and anaerobic microorganisms are ubiquitous. They react with uranium through different mechanisms.¹² At present, many researches focus on in situ activation of microorganisms to remediate uranium contaminated environment and screening and identifying efficient strains from

indigenous microorganisms. It is worth noting that not only the indigenous bacteria, but also genetically modified *Deinococcus radiodurans* and *Escherichia coli* expressed phosphatases such as PhoN (a periplasmic acid phosphatase) or PhoK (an extracellular alkaline phosphatase) enhanced the biomineralization of toxic ions in polluted soil.¹⁵ Uranyl phosphate mineral phases are considered to be stable and are not amenable to oxidative remobilization while the other products of uranium reduction may be re-oxidized to mobile U.¹⁶ Martinez's study provided the first evidence of U(VI) precipitation via the phosphatase activity of naturally occurring *Bacillus* and *Rahnella* spp. isolated from the acidic subsurface at the DOE ORFRC.¹⁷ Alternatively, the degradation of polyphosphate (phosphate polymer) resulting in phosphate release and metal bioprecipitation has also been reported in microbes.¹⁸⁻¹⁹ M. Paterson-Beedle confirmed that *E. coli* ATCC 33965 cells entrapped in Hypol polyurethane foam bioaccumulated hydrogen uranyl phosphate(HUP) at pH 4.5. This process relies on the liberation of inorganic phosphate via the phytase-mediated cleavage of phytate.²⁰

Microbial induction of organophosphorus hydrolysis to repair uranium contaminated groundwater/soil is affected by a variety of factors, such as bacterial strain activity and tolerance to uranium, acid and alkaline subsurface environment, organic matter, and other co-existing metal ions. In addition, different types of organophosphorus sources were selected with the different restoration effects. More information on microbe-microbe and microbe-uranium interactions in the subsurface environment is needed to optimize in situ uranium bioremediation.

. The Objective of this study is to review the application and research progress of microbial remediation of uranium contaminated environment in recent decades and discuss the effects of coexisting ions, organic matter, pH, enzyme activity and other factors on the process of biomineralization. Finally 3) the current research gap and the future research will be discussed.

2 Screen of underground microbial strains and potential mechanisms

Uranium in situ remediation process involves a variety of microorganisms such as bacteria, actinomycetes, fungi, and algae.²¹ Many bacteria isolated from uranium-contaminated environments have a strong tolerance to uranium and radiation. Phosphorous solubilizing bacteria release inorganic phosphates from organic phosphates through phosphatase or organic acids in uranium contaminated sites, precipitating uranium as uranyl phosphate and reducing the fluidity and solubility of uranium.²² There were few reports on U resistance by bacterial strains isolated from uranium mine or radioactive waste repositories, which were mostly identified as *Microbacterium* spp., *Arthrobacter* spp., *Rhodococcus globerulus*, *Bacillus* spp. and *Rahnella* sp..²³⁻²⁴ At the same time, some fungi have unique characteristics and bioremediate radioactive contaminated sites through biomineralization of uranium oxide.²⁵ A large number of extracellular enzymes are secreted by fungi and these enzymes are suitable for the treatment of many types of contaminants. Many fungi such as *Pleurotus*, *Aspergillus*, and *Trichoderma* have been proved to be effective in the removal of many metals.²⁶ Fomina et al. showed that fungi had a high tolerance to

uranium oxide. A large number of phosphate-related uranium deposits were found in the mycelium and the mycelium was encased with the well-crystallized uranyl phosphate minerals.²⁷ About 80% of soil microorganisms can convert organophosphorus into inorganic phosphorus through phosphatase activity. U (VI) can transform refractory and stable phosphate minerals under a wide range of pH and redox conditions.^{12,28-29} Therefore, the screening of subterranean strain is important. The resistance level and remediation capability of microorganisms under contaminated conditions should be considered in the remediation of groundwater/soil.³⁰

Heterotrophic Bacteria *Arthrobacter ilicis* and *Deinococcus radiodurans* were isolated by R2A medium from an acidic uranium contaminated site. *Deinococcus radiodurans* and *Arthrobacter ilicis* have a strong tolerance to uranium. *Arthrobacter ilicis* accumulated uranium intracellularly as precipitates closely associated with polyphosphate granules. *D. radiodurans* precipitated nanocrystals of a uranyl phosphate mineral extracellularly, probably as the result of phosphate release during cell lysis.³¹ Yohey et al. have shown that inactive cells precipitate a uranyl phosphate mineral from a phosphorus-free solution within an hour and that microorganisms can induce the precipitation of uranyl phosphate mineral by increasing the local phosphate concentration around the cell.³¹ The surface of microbial cells contains functional groups such as carboxyl group and phosphate group, which adsorb/accumulate uranyl substances and thus produce U-P precipitation.³²

Martinez et al. used full-strength (100%) PTYG, 1% PTYG and R2A medium respectively from Energy (DOE) 's Field Research Center (FRC) located at Oak Ridge,

Tenn. and isolated the heavy metal and radionuclides (U and other actinides) resistant subsurface strains *Arthrobacter* spp. (X34 V45, AA20), *Bacillus* spp. (Y7, X18, Y9-2) and *Rahnella* spp. (Y9602, Y4, Y29).³³ Follow-up studies have shown that *Bacillus* spp. strains Y9-2 and *Rahnella* spp. strains Y9602 exhibiting sufficient acid phosphatase activity to release phosphate in precipitation of 73% and 95% total soluble U. In contrast, an *Arthrobacter* sp. X34 did not liberate phosphate in promoting U(VI) precipitation.¹⁷

The key of the microbial selection process is to established strains' tolerance to a variety of constraints (i.e., high concentration of uranium, acidity/alkalinity, and nutritional limitations) and physiological functions (with phosphatase/phytase enzyme activity) with high enzyme activity to efficiently decompose organophosphorus. Whether happening in extracellular molecules, cell metabolites and cell structure, the specific mechanism of biomineralization is not fully understood.¹⁵ Tables 1 and 2 show several strains of bacteria and fungi involved in biomineralization from the literature.

Han et al. used *Bacillus Subtilis* ATCC-6633 to study uranium biomineralization. The biomineralization process of U(VI) was divided into the following three stages: first amorphous adsorption on the cell surface, and then precipitation crystallization, finally the adsorbed U(VI) in the form of UO_2^{2+} immobilized into uramphite.³⁴ Biomineralization of U(VI) in *Bacillus Sphaericus* JG-7B occurred at pH 3.0 and 4.5 in the absence of organic phosphate substrates. Other studies showed that phosphorus was derived from phosphorylated biomolecules such as nucleic acids released by cells.³⁵

There were three sources of inorganic phosphates, with which microorganisms were capable of precipitating uranium: (a) from supplemented organic phosphate donor through activity of phosphatase enzyme³⁶; (b) from hydrolysis or degradation of intracellular polyphosphate granules³⁷; and (c) Phosphate functional groups on the surface of microbial cells. The formation of uranyl phosphates happened as a result of precipitation of uranium and phosphate within the cells or the surface of the cell. Depending on where the phosphate comes from, two potential mechanisms have been suggested to explain the biomineralization process. The first type of biomineralization occurred due to the interaction between its chemical and compound changes present in the cellular surface as a result of the bacterial metabolic activity, for example extracellular polymeric substances associated with biofilms.³⁸⁻³⁹ Intracellularly synthesized polyphosphate granules, lipopolysaccharide, and phospholipid bilayers contribute to the phosphate storage of cells, which may be used in associating uranium and alleviating the uranium toxicity to cells⁴⁰. The second type of biomineralization was the uranyl precipitation due to the interaction of aqueous U(VI) with ligands PO_4^{3-} released by microorganisms enzymatically digested from additional source of organophosphorus.⁴¹

3 Selection of organic phosphorous source

Phosphate minerals have excellent thermodynamic stability constants and are stable in the groundwater environment.⁴² Compared to organic phosphates, the addition of inorganic phosphates seems to be cost-effective and simple. However, inorganic

phosphates may be not easily dispersed and precipitate rapidly causing clogging in the environment.¹² To immobilize U(VI), phosphate minerals, polyphosphates or organophosphates are introduced to the subsurface to form phosphate-uranium complexation at the contaminated sites.⁴³⁻⁴⁴ Arey et al. (1999) used hydroxyapatite to achieve phosphate-mediated U(VI) immobilization.⁴⁵ Glycerol-2-phosphate(G2P), glycerol-3-phosphate(G3P), tributyl phosphate, phytic acid, and fructose-1,6-phosphate were used as organophosphates with hydrolysis into phosphates by the cells.²⁹

Uranium was removed from acidic mine water with enzymatically mediated process of the glycerol phosphate.⁴⁶ A very promising organophosphorus compound is the phosphate-rich natural product inositol (hexaphosphate) or phytic acid, which can be used as a metal chelating agent or precipitator. Seaman et al. conducted batch experiments to assess the ability of different forms of phytic acid $\text{Ca}_n\text{-IP}_6$, $\text{Na}_{12}\text{-IP}_6$, HA (hydroxyapatite) to immobilize U, Ni, and other inorganic contaminants in soil and sediment. Results showed that the use of IP_6 should be cautious since it may increase the solubility of some contaminant metals.⁴⁷ The solubility of the metal- IP_6 complex was largely determined by the ratio of metals to ligands⁴⁸ since most metals with equal molality were highly soluble. In addition, phytic acid to metal concentration ratios was important to prevent abiotic precipitation.⁴⁹

Knox evaluated effects of three phosphates (rock phosphate, calcium phosphate and phytic acid) with two kinds of microbes (*Alcaligenes piechaudii* and *Pseudomonas putida*) on U mobility. Phosphates with three sources decreased U concentration by more than 90%. Thus, soil application of appropriate phosphates

may effectively reduce the metal mobility, bioavailability and toxicity.⁵⁰ Chao remediated uranium contaminated groundwater with glycerophosphate and glycerol and found that sodium glycerophosphate, as a carbon and phosphorus source, could promote the biological reduction and mineralization of U(VI) to immobilize the uranium in situ.⁵¹ Under the condition of aerobic culture at two pHs natural microbial communities could use native occurring organic phosphorus in the soil/sediments such as phytic acid to promote U(VI)-phosphate biomineralization in the underground environment in acidic sediments.⁵² Beazley et al. confirmed that phosphatase activity of three aerobic heterotrophic bacteria isolated from the DOE Field Research Center (FRC) promoted the precipitation of uranium with organophosphate compound glycerol-3-phosphate (G3P).⁵³⁻⁵⁴ Runwei et al. studied the effect of MRS-1 *B.cereus* on UO_2^{2+} transport and immobilization in Savannah River Site (SRS) with column experiments and reported that in the presence of phytate, bacterial-facilitated UO_2^{2+} transport was hindered since phytate promoted UO_2^{2+} - PO_4^{3-} complex and/or $[\text{Ca}(\text{UO}_2)_2(\text{PO}_4)_2]$ formation, leading to enhanced UO_2^{2+} immobilization in the SRS soil.⁴² Further, uranium solubility and its interaction with phosphates in the groundwater was affected by redox conditions, pH value, soil matrix, and the presence of organic ligands, and carbonates.¹⁵

4 Factors affecting U- PO_4^{3-} biomineralization

4.1 Coexisting ions

Wei et al. explored the influence of coexisting cations/anions (such as Ca^{2+} , K^+ , Mg^{2+} , Na^+ , Fe^{2+} , Fe^{3+} , Cl^- , SO_4^{2-} , HCO_3^- , CO_3^{2-} , PO_4^{3-} , NO_3^-) on U(VI) immobilization.

Different cations and anions had different effects on U biomineralization,. The presence of mixed cations and anions promoted the formation of uramphite as a clear crystal.⁵⁵ Carbonate under the alkaline condition had an obvious influence on U-phosphate biomineralization, due to the complexation of carbonate with uranyl as $\text{UO}_2(\text{CO}_3)_2^{2-}$ or $\text{UO}_2(\text{CO}_3)_4^{3-}$. Moreover, the affinity between carbonate and uranyl was higher than that between phosphate and uranyl.^{28,56-57} NH_4^+ could complex with UO_2^{2+} as well as PO_4^{3-} forming $\text{NH}_4\text{UO}_2\text{PO}_4$, which has a lower solubility than $\text{H}_2\text{UO}_2\text{PO}_4$ and $\text{Na}_2\text{UO}_2\text{PO}_4$.^{28,58} The presence of calcium at pH 7 may inhibit uranyl reduction or U (VI)-phosphate precipitation through formation of ternary calcium uranyl carbonate complexes.^{59-60,52}

Nitrate does not complex with uranium (VI) and has no role in the presence of other inorganic ligands in waters. However, in a nitric acid medium, uranium (VI) coordinates with two nitrate groups to produce $\text{UO}_2(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$ ($x = 2, 3, 6$) where x value depends on acid concentration.⁶¹ Chloride, which is a common ion present in all natural waters, forms very weak complexes with uranium (VI) such as UO_2Cl^+ and $\text{UO}_2\text{Cl}_2(\text{aq})$.⁶² The higher concentration of chloride, the higher U complexes were observed. In general, the relative strength of the uranyl ion (hard acid) complexation towards inorganic ligands (hard bases) decreases in the following order: $\text{CO}_3^{2-} > \text{OH}^- > \text{F}^- > \text{HPO}_4^{2-} > \text{SO}_4^{2-} > \text{Cl}^-$ and NO_3^- .⁶³ The removal of uranyl ions from solution by a *Citrobacter* sp. was improved substantially by adding ammonium acetate (NH_4Ac) to the solution and the end product $\text{NH}_4\text{UO}_2\text{PO}_4$ had a lower solubility than $\text{H}_2\text{UO}_2\text{PO}_4$ and $\text{Na}_2\text{UO}_2\text{PO}_4$.⁶⁴ Due to the complexity of the subsurface environment, the effects of co-existing heavy metals or radioactive substances on uranyl biomineralization need to be further studied.

4.2 pH values

pH is another important factor affecting U(VI) biomineralization because it not only controls protonation and deprotonation in the interaction with cells, but also affects the adsorption capacity and the structure of uranium precipitation.⁸ Uranyl mobility was mainly controlled by adsorption, precipitation and complexation in natural systems below the neutral pH. However, above the neutral pH, uranyl mobility was controlled by carbonates found in most groundwater systems.⁶⁵ The pH value of uranium contaminated groundwater was different with areas. Thus it is necessary to explore the influence of pH values on the process of biomineralization locally. The pH value strongly affected the microbial population and enzyme activity. The optimal pH value for different microorganisms and biomineralization performance may be different and the. Some areas in FRC site with low pH, high nitrate and oxidation conditions inhibited the biological reduction process, but the impact on the biological mineralization process was not significant. Underground microbials hydrolyzed phytic acid releasing inorganic phosphate to precipitate uranium⁵². However, under the condition of pH 7, the hydrolyzation of phytic acid was not observed with no precipitation of uranium.⁵² Perhaps there may be no phytase production at this pH, which affected the biomineralization process. Beazley et al. studied the biomineralization of uranyl in two types of uranium-contaminated soils in the department of Energy's Oak Ridge Field Research Center (ORFRC) and reported that changes in pH may affect the rate of phosphorus production by soil bacteria.⁵³ Due to the low isoelectric points on the

surface of bacteria, functional groups such as carboxyl group, phosphoryl group and hydroxyl group as well as proteins on the surface of bacteria combined with UO_2^{2+} over a large pH range.⁶⁶

Uranyl can be precipitated with phosphate to $\text{UO}_2\text{HPO}_4 \cdot 4\text{H}_2\text{O}$ or $(\text{UO}_2)_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$, depending on the distribution of uranyl and phosphate at different pH values. ; The precipitate was amorphous in acidic environments and remained crystalline in neutral and alkaline systems. This indicates that the stability of uranium phosphate minerals decreases with increasing acidity.⁶⁷ Different pH values of the solution significantly affect the electrical properties of the cell surface and the form of uranium hydrolysis, thus affecting the binding of the cell surface to uranium. In addition, Kelly et al. showed that at extremely low pH values (pH 1.67), UO_2^{2+} bound exclusively with phosphoryl functional groups and with the increase in pH (3.22 and 4.80), UO_2^{2+} tended to bind with carboxyl functional groups.⁶⁶

Differential localization of uranyl phosphate precipitates at different pHs. Analysis of cells at pH 5 revealed the presence of electron-dense uranyl deposits first on the cell surface, and then when the deposits were located in the cell at pH 7 and 9, uranyl deposits were found to be essentially extracellular.⁶⁸ When pH value is changed, whether precipitation is directly related to pH, uranium could be biomineralized by *S. maltophilia* JG-2 and *Microbacterium oxydans* SW-3 at pH 4.5 as a uranium phosphate mineral, while no precipitation was observed at pH 2.⁶⁹ Therefore, the influence of subsurface environment or soil acidity and alkalinity on uranium biomineralization is complex.

4.3 Enzyme activity

Enzyme activity is affected by many factors, such as temperature, pH and heavy metal concentration. The higher the enzyme activity, the higher the concentration of organic phosphate hydrolyzed, and the higher the biomineralization efficiency. The removal of uranium under acidic, neutral or alkaline pH conditions with aerobic and anaerobic conditions is related to phosphatase activity of microorganisms. Heavy metals can also deactivate enzymes and disturb the vital chemical reactions inside cells. They may change the configuration of enzymes through competitive and noncompetitive interactions.⁷⁰ The important chemical groups of the enzyme may be affected by uranium because of its enhanced toxicity.

Uranium removal by microbes under oxic conditions has generally focused on enzymatic precipitation (via phosphatases/phytase) forming stable uranyl phosphate minerals. On the other hand, reduced uranium minerals, such as uraninite resulted from reductive bioprecipitation which are susceptible to oxidation in the environment. Phosphatases are broadly categorized as acid or alkaline phosphatases, based on the pH required for their optimum activity.⁶⁸ Wei et al. studied the effect of *Bacillus Thuringiensis* 016 on the fixation of UO_2^{2+} , by enzymatic denaturation and showed that due to the absence of enzymes in cell fragments, intact cells had better biomineralization ability than cell fragments.⁵⁵

4.4 Organic matter

Humic substance is mainly composed of humic acid and fulvic acid. It is the main form of natural organic matter and an important nutrient for microorganisms. It constitutes an important pool of ligands for complexing metals.⁷¹⁻⁷² Humic and fulvic acids can strongly bind with uranyl,⁷³ and affect uranyl's chemical properties. Tu et al. showed that HA and FA did not significantly inhibit or promote the precipitation of uranyl phosphate minerals, but HA/FA had a significant impact on the formed precipitation morphology. With the addition of HA or FA, the mineral microstructure changed from small particles to layered accumulation structure.²⁸

In most water systems, organic matter is an important ligand library for complexing metals, especially uranyl ions, which show a strong affinity for organic ligands.⁷² Tu et al. showed that the effects of several organic ligands (acetic acid, lactic acid, salicylic acid, citric acid and oxalic acid) on biomineralization were significantly correlated with their ability to complexate uranyl.²⁸ In the presence of low molecular weight organic ligands such as citric acid and oxalic acid, uranium formed highly soluble complexes with them.⁷⁴ Excretion of organic acids not only resulted in U-ore dissolution but also U-complex formation.⁷² \, Therefore it is necessary to further investigate the influence of organic matter on microbial and uranium biomineralization in groundwater and soils.

4.5 Others

Indigenous microorganisms could be activated by adding electron donors to the underground environment.. Microorganisms in the families Burkholderiaceae, Comamonadaceae, Oxalobacteraceae, and Rhodocyclaceae predominated when

microbial activity was stimulated with ethanol and methanol in sediment incubations.⁷⁵ U (IV) products are easy to be reactivated and oxidized under aerobic conditions. Thus the remediation of uranium contaminated groundwater or soil by adding electron donors is not an ideal remediation method.⁷⁶ In addition, temperature is also one of the factors affecting the process of biomineralization. Phosphate mineralization bacteria (PMB) grew most rapidly at 30°C compared to 20°C and 40°C.⁷⁷ Different microorganisms have different activities at different temperatures.

Microbes also have different tolerances to various heavy metals and radionuclides. Low concentration of heavy metal ions had little effect on the growth of bacteria, while high concentration of heavy metal ions significantly inhibited the growth of bacteria.⁷⁷ Therefore, when considering using microbes to repair groundwater polluted by uranium, the microbial activity in the presence of uranium and other metals should be considered. The concentration of U (VI) in natural water such as groundwater plays an important role. High concentration of uranium may inhibit the production and growth rate of bacterial cells used for biological reconstruction.⁶¹ Toxic metals often increased the bioremediation lag period or the growth rate, thus affecting the bioremediation process in the site. Uranium inhibited growth of various organisms such as *Deinococcus radiodurans*, *Escherichia coli*, *Pseudomonas putida*, *Caulobacter crescentus* and *Shewanella putrefaciens* at the concentrations ranged from 500 μM to 3 mM.⁷⁸⁻⁷⁹⁻⁸⁰ Similar consideration for bioremediation at uranium contaminated sites was the tolerance of microorganisms to high concentrations of other metals.⁶¹

5 Biomineralization Applications

Some limitations occurred with application of biomineralization technology. . The metabolic process of microorganisms is usually slower and more complex than the chemical process. Therefore, it is necessary to optimize all the factors involved, such as temperature, pH value, organophosphate concentration, and the presence of salt. The type of bacteria and the environmental conditions are necessary to facilitate their metabolism.⁸¹ The restoration of uranium contaminated groundwater with biomineralization technology requires multidisciplinary expertise in mineralogy, microbiology, chemistry, etc., as well as consideration of economic limitations and effectiveness.

Uranium biomineralization was first observed with *Citrobacter* sp. in 1992.⁸² Fomina et al. analyzed the mycogenic mineralization of uranium. This was the first experimental evidence for fungal transformations of uranium solids and the production of secondary mycogenic uranium minerals.²⁷ Choudhary et al. used the strain metal-resistant *Pseudomonas Aeruginosa* isolated from uranium mine waste for biomineralization of uranium. Experimental results showed that *P. aeruginosa* J007 cells survived well within the contaminated mine water while removing aqueous uranium efficiently as cell bound uranium biominerals.²⁴ Hu et al. used isolated *Penicillium funiculosum* from uranium mine tailings mud to precipitate uranium as chernikovite, reducing the concentration of uranium solution in water below $50\mu\text{g L}^{-1}$, a standard set by the Environmental Protection Agency of China.⁴¹ Lianget al. studied uranium bioprecipitation mediated by yeasts. *Candida Sake* et al. utilized

organic phosphorus substrates glycerol 2-phosphate and phytic acid sodium and demonstrated phosphatase-mediated uranium biomineralization with yeasts.⁸³ Zhao et al. reported that biomineralization process induced by *Rahnella* sp. LRP3 could immobilize DTPA-Cu in the contaminated dark brown soil, producing phosphate crystal of $\text{Cu}_3(\text{OH})_3\text{PO}_4$.¹⁴ Most recently Microbial Fuel Cell coupled with biomineralization technology to remove U(VI) and nitrate from wastewater⁸⁴. The denitrifying bacteria consortia at Microbial Fuel Cell cathode produced phosphatase enzyme, which catalyzed the controlled release of phosphate from glycerol-3-phosphate, the inorganic phosphate combined with U(VI) resulting in insoluble uranyl phosphate.⁸⁴

6 Future research needs

At present, many studies on uranium biomineralization have been based on artificial groundwater or using chemical reagents to simulate uranyl solutions, which might not reflect the real field underground environment. Based on laboratory experiments, a small model was successfully built to reflect the real underground field environment. Therefore, further in situ microbial bioremediation studies are required. The focus should be on field pilot scale experiments with contaminated groundwater/soil to explore the limiting concentration of U(VI) which could be achieved through in-situ bioremediation in the presence and absence of dissolved oxygen. Second, U(VI)-phosphate mineral has demonstrated its thermodynamic stability, more recalcitrant to disturbance from environmental factors and oxidizing conditions.⁸⁵ However, the size

of the uranium minerals formed through biomineralization under various biogeochemical conditions is far from fully understood, especially during various reaction times.⁸ Third, extracellular polymeric substances (EPS) are the main structures of biofilm secreted by microorganisms. The main components are proteins, saccharides, uronic acids and humic substances, which influenced the migration and adhesion of bacterial cells by altering the surface chemistry of underlying substrate.⁸⁶⁻⁸⁷⁻⁸⁸ Recent studies have shown that EPS had a significant impact on biomineralization. EPS in the culture solution produced by *Rahnella* sp. LRP3 reduced the concentration of the copper through biomineralization.⁸⁹ An acidic fungal strain *Purpureocillium lilacinum* Y3 secreted extracellular polymeric substances (EPS) specifically synthesized jarosite through biomineralization in laboratory acidic conditions.⁹⁰ Therefore, the role of the key components of EPS in the restoration of uranium contaminated subsurface environment through biomineralization could be explored in the future experiments.

7 Conclusions

This paper briefly reviews the selection of uranium biomineralization strains, the source of phosphates, the biogeochemical conditions affecting the mineralization process, and the its application in reducing uranyl.. The screening of effective strains, the source supply of phosphates, the pHs of subsurface environment, organic matter, and the characteristics of microorganisms were the important consideration controlling in situ bioremediation. Many studies have shown that microbial induced biomineralization was feasible to restore uranium contaminated groundwater/soil. In

order to efficiently remediate uranium contaminated underground environment with in-situ biomineralization, more studies should be performed at field pilot scale experiments. The success of in-situ applications depends on the long-term stability of the final mineral phase, thereby reducing the potential of U (IV) reoxidation processes in the environment.

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Table 1. The bacteria used in uranium biomineralization

Designation	Screened site	Exhibit	P source	U removal rate	Uranyl phosphate species	References
Rahnella sp. Strain Y9602	Acidic subsurface at the DOE ORFRC	phosphatase positive phenotypes	glycerol-3-phosphate	precipitated 95% of soluble uranium	autunite/meta-autunite group mineral	Beazley, M. J.,(2007) ⁵³
Caulobacter crescentus NA1000	U-contaminated sites	alkaline phosphatase activity	glycerol-2-phosphate	remove soluble uranium 85.7%	meta-autunite	Yung,et al.(2014) ⁹¹
Pseudomonas Aeruginosa J007	uranium mine waste	/	Cellular ionizable group; beta-glycerol phosphate	remove soluble uranium (99%)	crystalline U phosphate [UO ₂ (PO ₃) ₂ , (UO ₂) ₃ (PO ₄) ₂ ·H ₂ O and U ₂ O(PO ₄) ₂] compounds	Choudhary, S., & Sar, P. (2011) ²⁴
Strain Rhodanobacter A2-61	U-contaminated wastewater	phosphatase activity	intracellular phosphate	removal of ~70%U(VI)	meta-autunite-like	Sousa, T., et al. (2013) ⁹²
Serratia sp. strain OT II7	Acidic sub-surface soil of a uranium ore deposit	Acid and alkaline phosphatase enzymes	The sodium salt of β-glycerophosphate	Precipitated 91% uranium at pH 5; removed 93–94% of Uranium at pH 7 and 9	calcium uranyl phosphate hydrate and uranyl phosphate hydrate at pH 5; calcium uranyl phosphate hydrate at pH 7 and 9	Chandwadkar, P., et al. (2018) ⁶⁸

Table 2. The fungi/yeast used in uranium biomineralization

Designation	Screened site	Exhibit	Phosphorus source	Uranium removal	Uranyl P species	References
Candida sake	a lead polluted area in Wales	phosphatase activity	glycerol-2-phosphate	/	Meta-ankoleite, Chernikovite, Uramphite	Liang, X.,et al.(2016) ⁸³
Saccharomyces cerevisiae	purchased	/	S.cerevisiae cell wall functional group phosphoryl	89.94 ±0.39% of U(VI) removal (100 mg L ⁻¹) .	tetragonal-chernikovite	Shen, Y.,et al.(2018) ⁹¹
Penicillium funiculosum	uranium mine tailings mud	/	phosphate rock	25 mg L ⁻¹ to 47.3 µg L ⁻¹	chernikovite	Hu, N.,et al.(2018) ⁴¹