Evaluating the role of Spanish bentonite indigenous microorganisms in the speciation of uranium over time

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BACKGROUND

It is estimated that high-level radioactive waste (HLW) will continue to emit radiation for hundreds of thousands of years. Therefore, containment within deep geological storage (DGR) systems is considered the most effective strategy for managing this waste and protecting the biosphere. These DGR systems will employ multiple barriers, including a metal canister (e.g., copper, carbon steel, or titanium) and a secondary bentonite barrier, used for backfilling and sealing. Bentonite would act as a containment barrier in the event of potential system failure (Tondel & Lindahl, 2019; WNA, 2023). Due to its excellent physicochemical properties, bentonite has been selected for various DGR concepts in countries such as Finland, Sweden, Spain, Switzerland, and Canada. Additionally, previous research has highlighted the interaction capacity of microorganisms with heavy metals (e.g., uranium (U)) through mechanisms such as bioaccumulation, bioprecipitation and biomineralization (Martinez-Rodriguez et al., 2023; Lopez-Fernandez et al., 2018; Merroun & Selenska-Pobell, 2008). In the event of canister failure and waste leakage, metal-tolerant microorganisms could serve as an additional barrier, preventing waste migration into surrounding environments. One of the most abundant and hazardous radionuclides is ^{235}U, which exists primarily in two oxidation states: oxidized U(VI) and reduced U(IV). The U(VI) form is more soluble and, consequently, more toxic to living organisms due to its higher bioavailability. In contrast, U(IV) is less soluble and therefore less bioavailable (Lakaniemi et al., 2019).

METHODOLOGY

In this context, the present study investigates the behavior of native microbial communities in Spanish bentonite under a hypothetical worst-case scenario involving waste leakage (e.g., uranium), high microbial activity, and groundwater infiltration. To achieve this, Spanish bentonite were saturated with equilibrium water and amended with 1.26 mM of uranyl acetate. Additionally, these microcosms were supplemented with sodium acetate and glycerol-2-phosphate (G2P) as electron donors. A bacterial consortium consisting of four bacterial genera previously identified in Spanish bentonite (*Pseudomonas, Stenotrophomonas, Bacillus*, and *Amycolatopsis*) was inoculated to stimulate microbial activity. All microcosms were incubated under oxygen-free conditions at 28 °C for a period of three years. The study was conducted using a combination of multidisciplinary analyses, incorporating microbiological, microscopic, and spectroscopic techniques.

RESULTS & DISCUSSION

After a three-year anoxic incubation, Illumina sequencing results revealed a bacterial diversity dominated by anaerobic and spore-forming microorganisms. These findings are consistent with anticipated conditions in future DGRs, where oxic conditions will initially be present following repository closure. Over time, a shift toward reducing conditions is expected, characterized by the dominance of anaerobic bacterial communities. In our study, certain strains with significant roles in uranium interactions, such as *Pseudomonas* and *Stenotrophomonas*, showed increased abundance in the uranium-treated condition. Furthermore, the enrichment in the Postgate culture medium (specific for SRB) of uranium-treated bentonite confirmed the presence of viable sulfate-reducing bacteria after 3 years. Many SRB are known for their ability to reduce uranium and have even been identified in uranium mines.

All these microbiological findings were corroborated and complemented by microscopic and spectroscopic characterizations of the uranium species. X-ray photoelectron spectroscopy (XPS) and X-ray diffraction analysis (XRD) analyses showed uranium in both U(VI) and U(IV) species. For U(VI), biogenic phosphates of U(VI), $U(UO_2)\cdot(PO_4)_2$, were identified within the inner bacterial cell membranes, alongside U(VI) adsorbed on montmorillonite clays. These U-phosphates may result from a biomineralization process, likely driven by microbial phosphatase activity, which releases inorganic phosphates from G2P. Lastly, biogenic U(IV) species, such as uraninite, may result from bacterial enzymatic reduction of U(VI).

CONCLUSION AND ENVIROMENTAL IMPLICATION

Here, we provide evidence of the enrichment of anaerobic, spore-forming microbes and viable SRB in uranium-amended bentonite after a 3-year incubation. Additionally, uranium speciation was influenced by biological processes, resulting in its immobilization as U(VI) phosphates, uraninite, and sorption onto bentonite minerals. These findings suggest that, under water-saturated conditions rich in electron donors, the bentonite microbial community could influence uranium speciation, immobilizing it and thereby enhancing the long-term safety of DGRs in the event of waste leakage.

REFERENCES

Tondel, M. & Lindahl, L. (2019): Intergenerational Ethical Issues and Communication Related to High-Level Nuclear Waste Repositories. Curr. Environ. Health Rep., **6(4)**, 338-343. DOI: 10.1007/s40572-019-00257-1

WNA. World Nuclear Association. (2021): Storage and disposal of radioactive waste. https://www.world-nuclear.org/

Martinez-Rodriguez, P., Sánchez-Castro, I., Ojeda, J. J., Abad, M. M., Descostes, M., Merroun, M. L. (2023): Effect of different phosphate sources on uranium biomineralization by the *Microbacterium* sp. Be9 strain: A multidisciplinary approach study. Front. Microbiol., **13**, 1092184. DOI: 10.3389/fmicb.2022.1092184

Lopez-Fernandez, M., Romero-González, M., Günther, A., Solari, P. L., Merroun, M. L. (2018): Effect of U(VI) aqueous speciation on the binding of uranium by the cell surface of *Rhodotorula mucilaginosa*, a natural yeast isolate from bentonites. Chemosphere, **199**, 351-360. DOI: 10.1016/j.chemosphere.2018.02.055

Merroun, M. L., & Selenska-Pobell, S. (2008): Bacterial interactions with uranium: an environmental perspective. J. Contam. Hydrol., **102(3-4)**, 285-295. DOI: 10.1016/j.jconhyd.2008.09.019

Lakaniemi, A.-M., Douglas, G.B., Kaksonen, A.H. (2019): Engineering and kinetic aspects of bacterial uranium reduction for the remediation of uranium contaminated environments. J. Hazard. Mater., **371**, 198–212. DOI: 10.1016/j.jhazmat.2019.02.074