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CL:AIRE research bulletins describe specific, practical aspects of research which have direct application to the characterisation, monitoring or remediation of contaminated soil or groundwater. This bulletin describes experimental work to investigate the potential for biostimulation to remove uranium from groundwater.

Investigating the Potential for Biostimulation to Remediate Uranium-contaminated Groundwater

1. INTRODUCTION

Uranium-contaminated groundwater is present at a number of sites across the world, including several nuclear sites in the UK. This potentially could represent an uncontrolled source of radiation exposure and therefore may require remediation. Like any metal, uranium cannot be destroyed, although as it is radioactive its concentrations will decrease over time due to radioactive decay, albeit very slowly as the half-lives of the most common isotopes (^{238}U, ^{235}U) are in the order of hundreds of millions of years. Consequently the fate and transport of uranium in the environment is predominantly determined by its chemical speciation. Under most oxidising groundwater conditions, uranium is present as mobile aqueous U(VI) in the form of the uranyl cation (UO_2^{2+}) or as uranyl-carbonate complexes (Choppin *et al.*, 2002; Newsome *et al.*, 2014a). Under reducing conditions, uranium as U(IV) is poorly soluble and will precipitate from solution. Uranium bioremediation technologies focus on stimulating the removal of uranium from solution as U(IV) phases via microbial reduction processes, or by harnessing other "biomineralisation" processes to precipitate highly insoluble uranium phosphates (Figure 1).

Many sediment microorganisms, particularly iron(III)-reducing bacteria, are able to enzymatically reduce aqueous U(VI) to form insoluble biominerals such as uraninite [UO_2] (Lovley *et al.*, 1991; Newsome *et al.*, 2014a; Williams *et al.*, 2013). This process can be enhanced by increasing the amount of organic electron donor available; an approach known as biostimulation. This has been successfully demonstrated in a number of field trials, where the supply of acetate or other electron donors *in situ* has caused the removal of uranium from groundwater via the reduction of U(VI) and subsequent precipitation of U(IV) biominerals (Anderson *et al.*, 2003; Istok *et al.*, 2004; Watson *et al.*, 2013; Williams *et al.*, 2011). Alternatively, the use of glycerol phosphate has been shown to drive the precipitation of uranium(VI) phosphate minerals such as autunite [$Ca(UO_2)_2(PO_4)_2$] or chernikovite [$H_2(UO_2)_2(PO_4)_2$], due to microbial phosphatase activity (Beazley *et al.*, 2011; Macaskie *et al.*, 1992).

The key advantages of using bioremediation to remove uranium from groundwater are that firstly it can be applied *in situ.* This avoids the need for large-scale ground excavations which could cause unacceptable doses to operators and create large quantities of

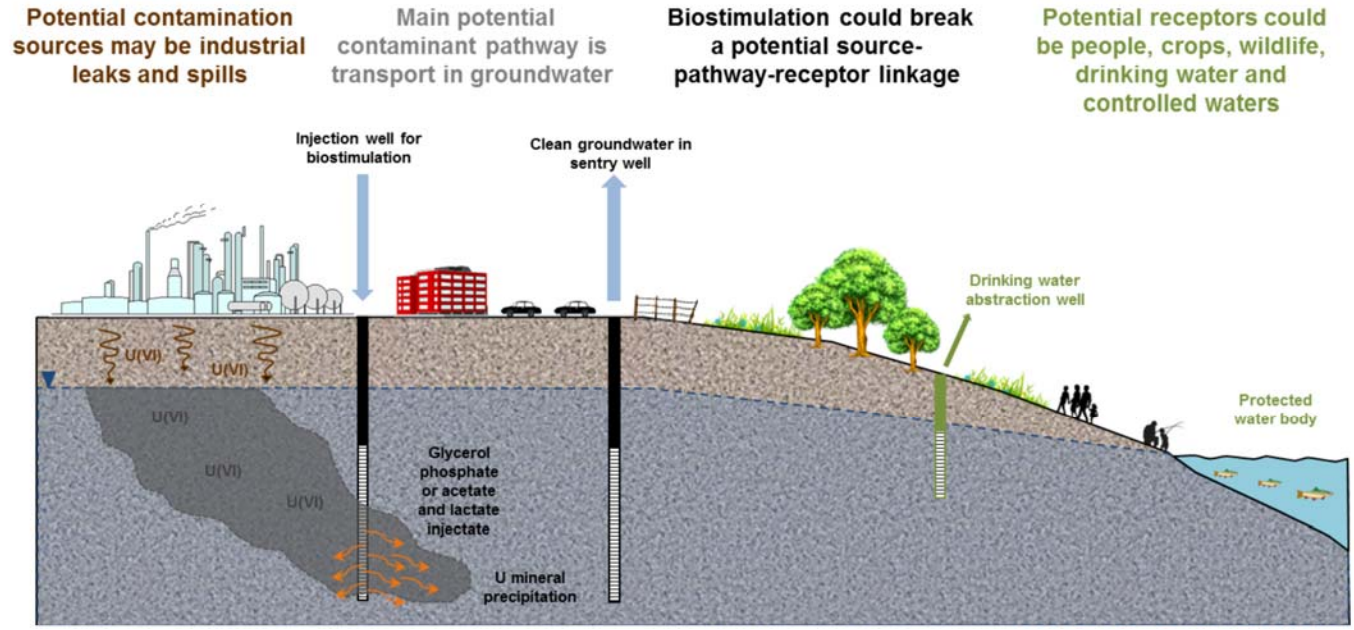

Figure 1: Conceptual model of *in situ* uranium bioremediation at a nuclear site (after Newsome, 2015). Biostimulation of sediment microbial communities with acetate/lactate or glycerol phosphate can lead to the removal of uranium(VI) from groundwater by the precipitation of U(IV) biominerals.

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radioactive waste, which is expensive to dispose of. Secondly it avoids the problem of clogging at injection locations, such as those encountered during chemical remediation of uranium with phosphates in column experiments and field trials (Vermeul *et al.*, 2009; Wellman *et al.*, 2006). However, some questions do remain regarding the long-term stability of uranium biominerals in the subsurface (and hence the prolonged effectiveness of bioremediation), particularly in response to oxidising conditions (Senko *et al.*, 2007).

This bulletin describes the results of a series of experiments to investigate the potential for biostimulation to remove U(VI) from solution under conditions relevant to UK nuclear sites. These also included exploring the long-term fate of uranium biominerals in response to oxidising conditions, including after periods of simulated ageing.

2. METHODS

Full details of the materials and methods are provided in a series of recently published manuscripts, which are available with full Open Access (Newsome *et al.*, 2015a, 2015b, 2015c, 2014b). In brief, microcosms were set up containing sediments, an artificial groundwater representative of the Sellafield site and an electron donor to stimulate sediment microorganisms. This comprised either a mixture of acetate and lactate as a simple electron donor system; or glycerol phosphate as a source of orthophosphate and energy. An artificial spike of uranium as uranyl [UO_2^{2+}] was added to the microcosms at an environmentally-relevant concentration. The sediment samples were collected from the Sellafield nuclear licensed site, and are representative of a range of lithologies that might be also found at nuclear sites elsewhere in the UK. Changes in geochemistry were monitored for up to 100 days, and uranium speciation was analysed using X-ray absorption spectroscopy. To investigate changes in the microbial community during biostimulation, DNA was extracted from the sediments and analysed via a pyrosequencing methodology. Pure and enrichment bacterial cultures were also used, including with elevated uranium concentrations, to elucidate the mechanisms of uranium removal and to generate mineral endpoints that could be studied using electron microscopy and X-ray absorption spectroscopy. Additional experiments were performed to expose the uranium biominerals to air or nitrate in order to assess their long-term fate under oxidising conditions. These included after periods of ageing for up to 15 months, in order to investigate whether the uranium biominerals aged to become more crystalline, and consequently whether this increased their recalcitrance to oxidative remobilisation.

3. REMOVAL OF URANIUM CONTAMINATION FROM GROUNDWATER

The results show that the biostimulation of a variety of different Sellafield sediments with acetate and lactate as the electron donors led to the removal of 12 ppm uranium(VI) from solution (Newsome *et al.*, 2014b). This also occurred at 10°C, representative of UK groundwater conditions. X-ray absorption spectroscopy was used to confirm the formation of microbially-reduced uranium(IV) associated with the solid phase. Initially it was precipitated as a non-crystalline "monomeric" uranium(IV) phase, which after ageing for 15 months had partially crystallised to nano-scale uraninite (Newsome *et al.*, 2015b). DNA analysis revealed increases in bacteria closely related to known U(VI)- and Fe(III)-reducing bacteria such as *Geobacter, Shewanella* and *Rhodoferax* species, as well as bacteria involved in the nitrogen cycle and the degradation of organics (Newsome *et al.*, 2014b). Uranium was not removed from solution, however, in two of the seven sediments that were stimulated with acetate and lactate. Additional investigations suggested that this might be due to these sediments containing low concentrations of bioavailable iron(III) and hence iron(III)-reducing bacteria. This highlights the requirement for sediment-specific investigations to be performed in order to assess the feasibility of *in situ* uranium bioremediation. Augmentation of extant microbial populations with known metal-reducing bacteria may be required in some circumstances.

Biostimulation with glycerol phosphate was shown to induce the removal of uranium from solution. A *Serratia* species previously isolated from Sellafield sediments (Thorpe *et al.*, 2012) was able to metabolise glycerol phosphate, leading to the removal of 238 ppm uranium(VI) from a test solution as autunite (Figure 2), including under anaerobic conditions (Newsome *et al.*, 2015a). In addition this bacterium was able to reduce uranium(VI) to nano-scale uraninite (Figure 2). Stimulation of a Sellafield sediment with glycerol phosphate also led to the removal of 12 ppm uranium(VI) from solution, but under these conditions it was precipitated as a crystalline uranium(IV) phosphate phase (Newsome *et al.*, 2015c). DNA analysis showed that the microbial community was dominated by bacteria closely related to *Pseudomonas* species which are known to denitrify and also bacteria closely related to *Pelosinus* species that and can fix uranium by multiple mechanisms (Ray *et al.*, 2011).

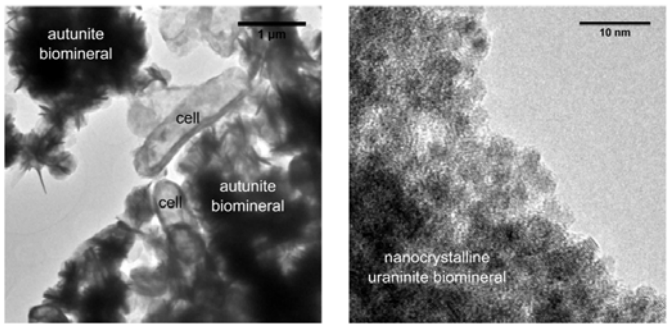

Figure 2: Uranium biominerals precipitated by a *Serratia* environmental isolate. Different experimental set ups were used to produce the autunite and uraninite mineral phases. Note the difference in image scales; the scale bar on the left image is 1 µm, on the right image it is 10 nm.

4. LONG-TERM STABILITY OF BIOGENIC URANIUM PHASES

Experiments were performed to investigate the stability of poorly crystalline monomeric uranium(IV)/nano-scale uraninite produced by acetate and lactate biostimulation, and the uranium(IV) phosphate mineral phase produced by glycerol phosphate biostimulation, under oxidising conditions. These included exposure of the biominerals to air to represent a fall in groundwater level or the influx of oxidising groundwater, and exposure to elevated concentrations of nitrate, which is a strong oxidant and common contaminant at nuclear sites (Figure 3).

Following exposure to air, the monomeric U(IV)/uraninite was fully reoxidised after 60 days and the observed partial transformation of monomeric U(IV) to crystalline uraninite during ageing did not increase its recalcitrance to oxidative remobilisation (Newsome *et al.*, 2015b). In comparison, just 40% of the uranium(IV) phosphate was reoxidised after 90 days under the same conditions (Figure 3) (Newsome *et al.*, 2015c). It should be noted that these experiments were performed under highly oxidising worst-case conditions and were not designed to replicate *in situ* conditions that might occur in the natural environment. However, they do suggest that producing a uranium(IV) phosphate phase could be a more successful long-term bioremediation strategy, and further experiments such as column studies and field trials should be conducted in order to fully assess its potential.

Following exposure to elevated concentrations of nitrate, monomeric U(IV)/uraninite was partially reoxidised (70%), while in contrast very little of the uranium (IV) phosphate was reoxidised (3%) (Newsome *et al.*, 2015b, 2015c). A sterile control confirmed that the microbial production of denitrification intermediates was required for nitrate induced uranium(IV) reoxidation to occur. It was observed that the rate of reoxidation of monomeric U(IV)/uraninite was controlled by the amount of residual electron donor present, which acted as a buffer to protect the monomeric U(IV)/uraninite from reoxidation (Newsome *et al.*, 2015b). Maintenance of reducing conditions via the continual slow supply of electron donor could therefore be a means of addressing this susceptibility to oxidative remobilisation, although again, the products of biostimulation via an organic phosphate donor such as glycerol phosphate are likely to outperform those from the use of simple electron donors such as acetate and lactate.

5. CONCLUSIONS

In situ biostimulation is a promising technology to remediate current uranium and radionuclide groundwater contamination at nuclear sites, and to potentially deal with any future contamination that might arise during decommissioning. These results show that stimulating a variety of different sediment microbial communities with electron donor led to the removal of soluble uranium from groundwater, under conditions relevant to the UK subsurface. Dynamic shifts in the microbial communities were observed in response to biostimulation, including increases in bacteria closely related to species known to be able to remove uranium from solution. The use of glycerol phosphate generated biogenic uranium (IV) phosphate, which was much more recalcitrant to oxidative remobilisation compared to the uranium(IV) phases formed from the use of a simple electron donor, and may therefore represent a better targeted long-term remediation strategy for the treatment of uranium -contaminated groundwater.

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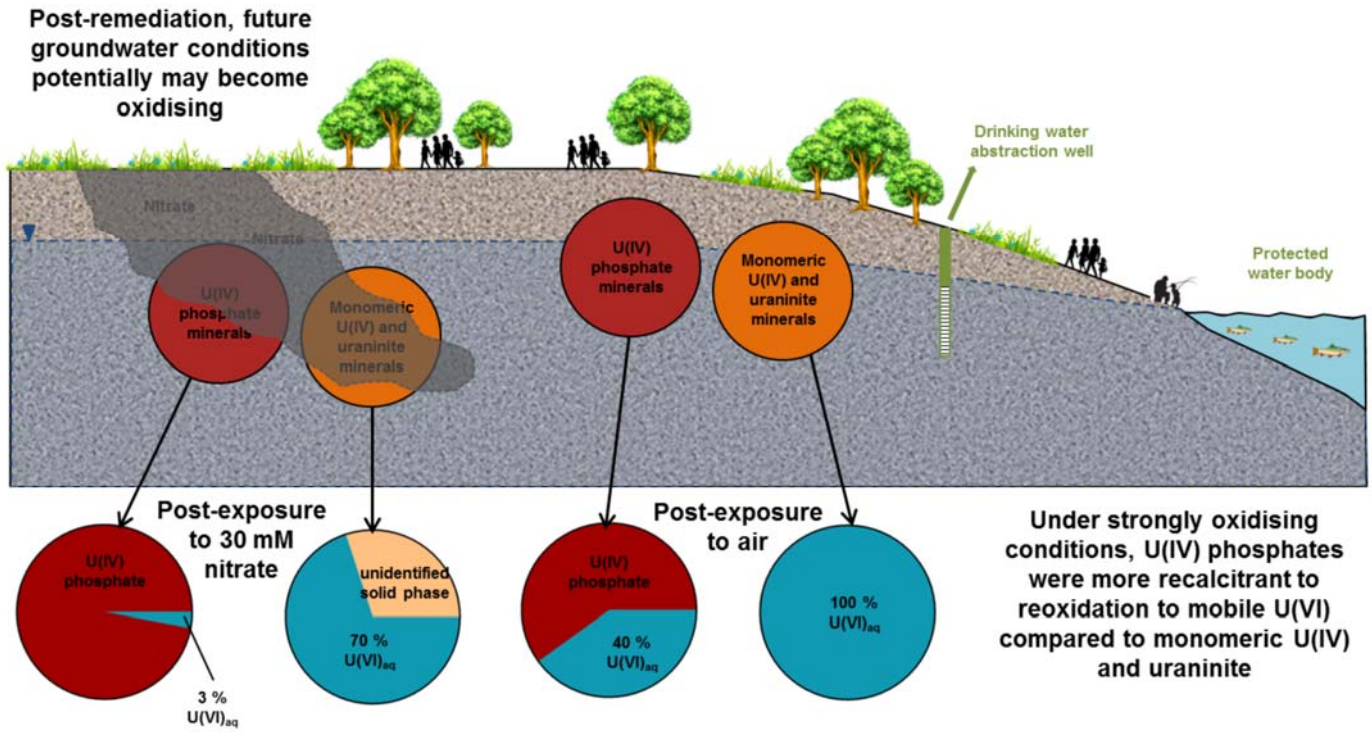

Figure 3: Conceptual model of future oxidising conditions that may potentially occur at a site after *in situ* uranium bioremediation (after Newsome, 2015), with results from reoxidation experiments illustrated (Newsome *et al.*, 2015b, 2015c).

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