Uranium reduction by *Geobacter* spp. as a bioremediation strategy in the subsurface contaminated environments.

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INTRODUCTION:

Anthropogenic use of uranium. → Widespread contamination in the subsurface. → Need to devise cheap and efficient methods of remediation. → BIOREMEDIATION STRATEGY. → Decreased uranium bioavailability.

Stimulating the microbial reduction of U(VI) could stop the spread of uranium contamination in the subsurface and might concentrate it into a discrete zone for the subsequent recovery. At *in field* trials this stimulation results in the concomitant removal of U(VI) and domination of *Geobacter* spp., known to be Fe(III) reducing microorganisms. It is assumed then, that these bacteria are the major responsible of the U(VI) reduction. Apart from *Geobacter* spp. other phylogenetically distinct microorganisms have been found to be capable of this reduction, although in any of these bacteria the pathway is completely understood. The aim of this review is to give a synopsis of the uranium reduction process by *Geobacter* spp. as a bioremediation strategy in contaminated groundwater and associated sediments.

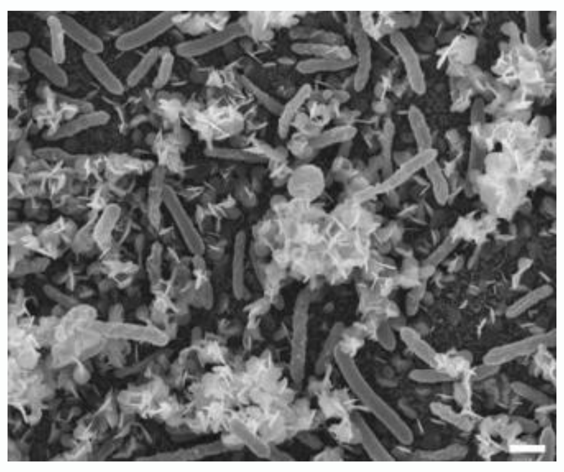

Figure1: extracellular needle-like uranium precipitates in *G. Sulfurreducens*. Scale bar, 1μm. [2].

U(VI) Soluble Mobile → Stimulated MICROBIAL REDUCTION → U(IV) Precipitates Immobile

1. URANIUM REDUCTION *in field* by *Geobacter* spp.

The addition of acetate in a contaminated aquifer results in the concomitant reduction of Fe(III) and U(VI) and it also correlates with the greatest enrichment of *Geobacter* spp. However, other bacterial genus are usually found in uranium contaminated sites so it is necessary to analyze the integrated biological, hydrological and geochemical factors to design a strategy to stimulate an U(VI) reducing microbial community.

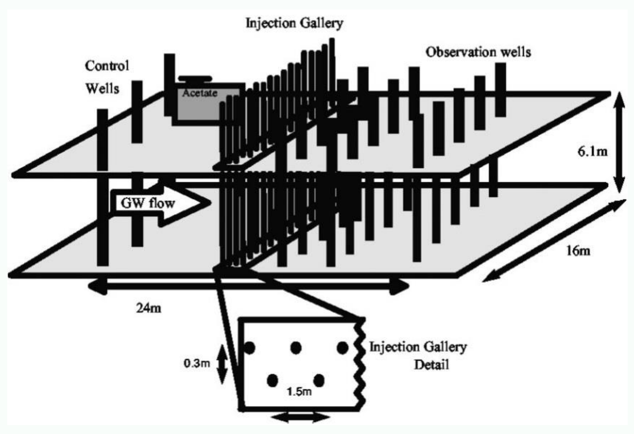

Figure2: Layout of the *in field* test plot installed at the Old Rifle UMTRA site in Colorado [1].

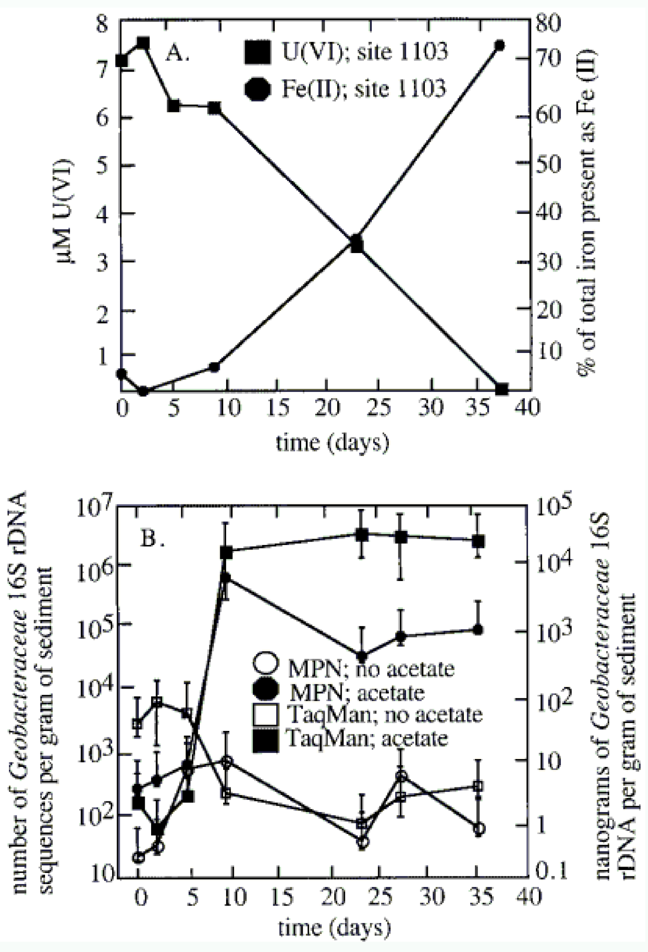

Figure3: U(VI) and Fe(II) concentrations in an acetate amended site over time [3].

ELECTRON ACCEPTORS AND DONORS:

Diverse organic electron donors, such as acetate stimulate the uranium reduction because it promotes the anaerobic respiration of *Geobacter* spp. On the other hand, the presence of various electron acceptors in the environment such as nitrate or sulfate, apart from U(VI) and Fe(III), influences the microbial community and the uranium reduction efficiency. For example:

NITRATE REDUCING BACTERIA. → Reduce nitrate. → *Geobacter* spp. won't oxidize U(IV).

SULFATE REDUCING BACTERIA. → Outcompete *Geobacter* spp. for electron donor. → Decreased efficiency in U(VI) reduction.

STABILITY OF THE REDUCED URANIUM:

In oxic waters U(IV) and U(VI) are able to form complexes with carbonate, calcium, phosphate, and humic substances, affecting the susceptibility of both U(VI) reduction and U(IV) oxidation. If U(IV) is oxidized, it will remain at the subsurface, and this is the main LIMITATION of the microbial U(VI) reduction as a bioremediation strategy. Recently, an alternative has been proposed, it consists on emplacing electrodes that serves as the electron donor and acceptor, then the U(IV) would precipitate on the electrode facilitating the uranium removal [5].

3. COMPUTATIONAL MODELS

There is an increasing interest in developing computational models to accurately predict the outcome of bioremediation before field implementation and consequently enabling a better optimization of the strategy. These computational simulations couple genome-scale metabolic models of key microorganisms to hydrogeochemical models to predict the microbial impact in the environment [7].

2. THE URANIUM REDUCTASE in *Geobacter* spp.

The ability of *Geobacter* spp. to transfer electrons onto insoluble electron acceptors outside the cell is essential to their function as an uranium reducing bacteria. They completely oxidize organic compounds to carbon dioxide through the reduction of these extracellular electron acceptors including Fe(III) oxides and U(VI).

ELECTRON TRANSFER → DIRECT CONTACT / PILI / ELECTRON SHUTTLES

Pili enhance the cell's reactive surface by accepting electrons from electron transfer proteins. The observation of uranium precipitates along pili suggests that catalyze the extracellular reduction of U(VI) to U(IV) helped by associated c-cytochromes that would preserve the cell's viability [9].

It has been seen in some *Geobacter* spp. that abundant c-cytochromes decorate uniformly the cell surface [10]. Periplasmic and outer-surface reduced c-type cytochromes can transfer electrons to oxidized metal ions. This ability would permit electrons to diffuse through the membranes.

However, the specific location of the uranium reductase remains unclear. The detection of uranium precipitates in the periplasm and the surface of the cell suggest that the cytochromes exposed at this sites are the best candidates to enable the U(VI) reduction. The Fe(III) reduction by *Geobacter* spp. is an anaerobic respiratory process, electrons derived from NADH oxidation are probably transferred to Fe(III) via cytochromes [6], but there is a lack of correspondence between Fe(III) and U(VI) reductases suggesting that they are reduced by different pathways and different reductase structures. Even though, it is predictable that both pathways might share some cytochromes.

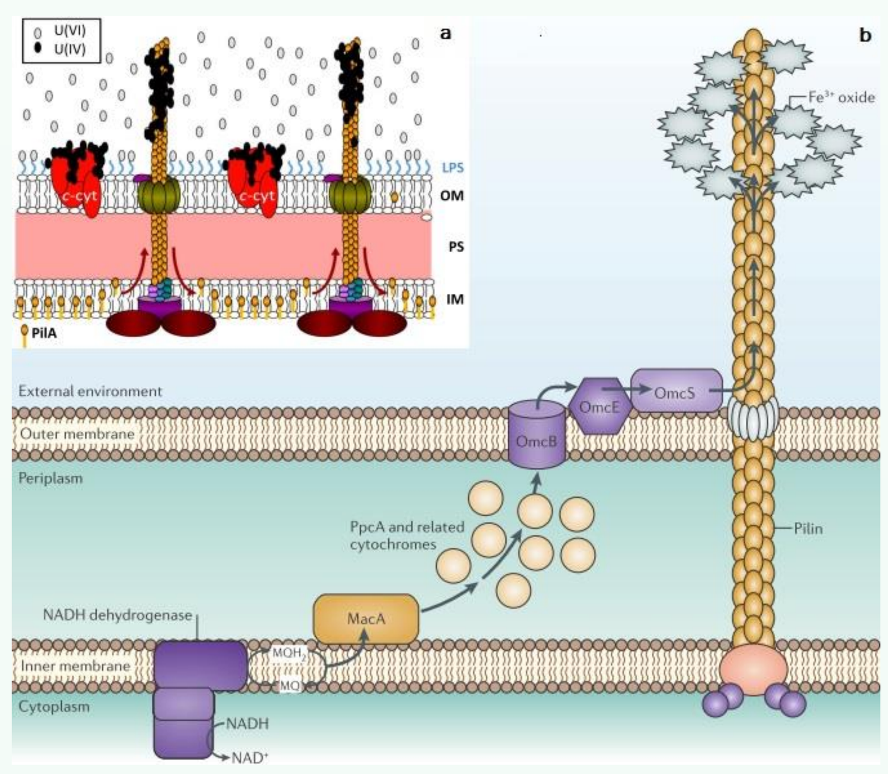

Figure5: predicted pathway for Fe(III) and U(VI) reduction. **a**: Extracellular reduction of U(VI) to U(IV). IM: inner membrane, PS: periplasmic space, OM: outer membrane, LPS: lipopolysaccharide, PilA: pilin subunit [2]. **b**: Electron transfer to Fe(III) oxides by *G. sulfurreducens* [4].

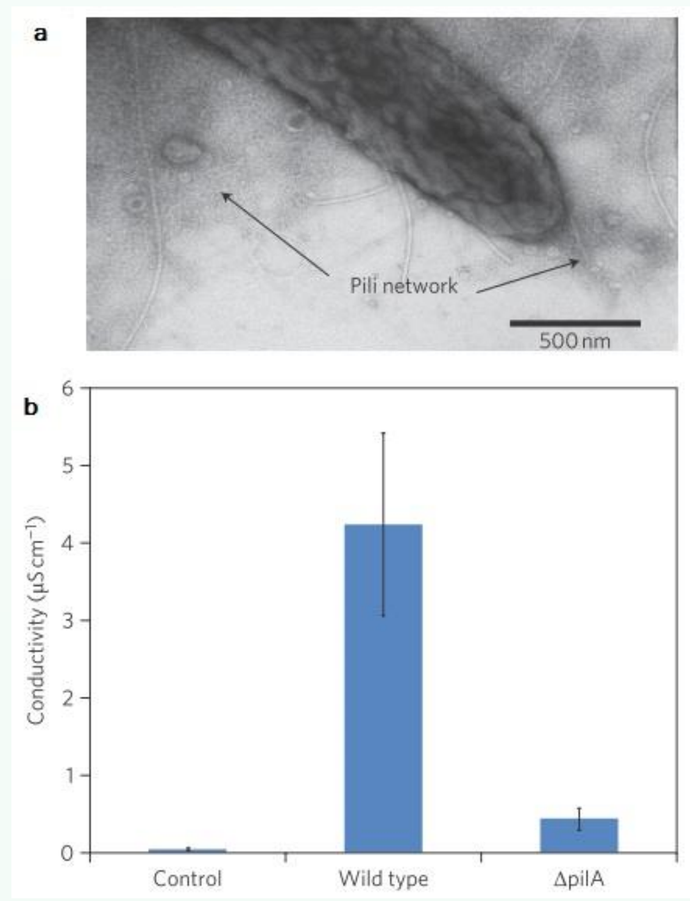

Figure4: Evidence for pili being associated with biofilm conductivity [8]. **a**: *G. sulfurreducens* biofilm. Scale bar, 500nm. **b:** Conductivity of filaments of wild-type and PilA mutant in comparison with control buffer.

CONCLUSIONS:

To efficiently remove uranium from contaminated groundwater and associated sediments, U(VI) reduction must be achieved under controlled hydrogeochemical conditions to provide a suitable microbial community in which *Geobacter* spp. predominates. However, there's still a great uncertainty about the long-term sustainability of the approach. Further investigation is necessary to address the impact of bioreduction products and secondary minerals on uranium immobilization to eventually optimize the bioremediation strategy.

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