From nanowires to biofilms: an exploration of novel mechanisms of uranium transformation mediated by *Geobacter* bacteria

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An insufficient knowledge of the biological mechanisms of contaminant transformation often limits the performance of *in situ* subsurface bioremediation and long-term stewardship strategies. The *in situ* stimulation of Fe(III) oxide reduction by *Geobacter* bacteria, for example, leads to the concomitant precipitation of U(VI) from groundwater. However, the biological mechanism behind this reaction has remained elusive for almost two decades. Because Fe(III) oxide reduction requires the expression of conductive pili in *Geobacter*, we evaluated their contribution to uranium reduction in piliated an non-piliated strains of *Geobacter sulfurreducens*. We observed a direct correlation between the levels of piliation and the extent of U removal and reduction. Furthermore, pili expression also prevented the permeation of U in the cell envelope and its periplasmic mineralization. As a result, pili expression preserved the vital respiratory activities of the cell envelope and the cell's viability. Uranium preferentially precipitated along the pili as a mononuclear U(IV) complexed by carbon containing ligands and, to a lesser extent, on outer membrane redox-active foci. These results demonstrate a previously unrecognized role for *Geobacter* pili in the extracellular reduction of uranium and highlight its essential function as a catalytic and protective cellular mechanism that is of interest for the bioremediation of uranium-contaminated groundwater.

The expression of pili by *Geobacter* also promotes cell aggregation and biofilm formation. Thus, we investigated the contribution of biofilms of *G. sulfurreducens* to U transformations. Multilayered biofilms reduced substantially more U than planktonic cells and for prolongued periods of time. They also tolerated higher concentrations of U, making them an attractive option for the development of permeable biobarriers for U bioremediation. While U was immobilized by both monolayered and multilayered biofilms, only pili-expressing multilayered biofilms reduced the U. Similarly, a pili-deficient mutant, which is interrupted at the monolayer stage, immobilized but did not reduce U. Thus, the pili are also the primary U reductase in the biofilms. To gain more insights into how biofilms transform U, we screened a library of 4,000 transposon-insertion mutants and identified mutants with biofilm defects. After identifying the interrupted genes and their functions, we built a genetic model for biofilm formation that links biofilm development to the mechanisms of U transformations. This study confirmed the role of *Geobacter* pili in the biofilm formation and U reduction and identified molecular markers involved in biofilm electron transport and metabolism that can be used to predict and monitor the physiological state of *Geobacter* bacteria during the in situ bioremediation of U.