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Biogeochemical Mechanisms Controlling Reduced Radionuclide Particle Properties and Stability

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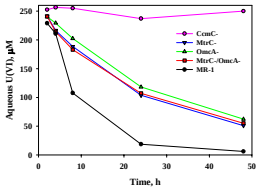
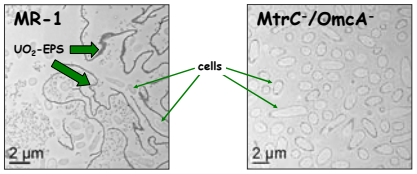
Uranium(VI) reduction

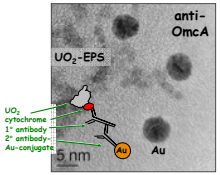
A redundant network of outer membrane (OM) and periplasmic *c*-type cytochromes are required for bioreduction of U(VI).



The reduction kinetics of U(VI) by wild type MR-1, a disruption of the heme maturation system (CcmC$^-$) or deletions of genes encoding OM *c*-type cytochromes (OMCs) (MtrC$^-$ and/or OmcA$^-$) cells demonstrate that while the *c*-type cytochromes are required for U(VI) reduction, deletions of OMCs only partially inhibit the U(VI) reduction.

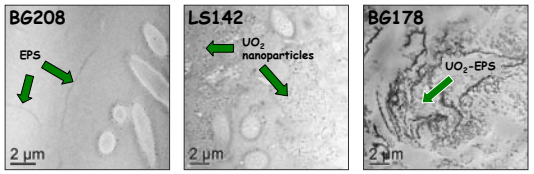
OMCs influence the sub-cellular localization of UO_2 and are components of organized UO_2-exopolymeric substance (UO_2-EPS).





While the MtrC$^-$/OmcA$^-$ mutant cells localized UO_2 nanoparticles predominantly in the cell periplasm, wild type MR-1 localized extracellular UO_2 at high densities in association with EPS. Immune-electron microscopy localized the OMCs (OmcA or MtrC [not shown]) with UO_2-EPS, confirming their co-localization with UO_2 nanoparticles.

S. oneidensis MR-1 mutants producing altered polysaccharide reveal differences in UO_2 nanoparticles associated with EPS.



A comparison of MR-1 and MR-1 strains containing mutations in putative polysaccharide biosynthesis genes (BG208, LS142, and BG178) revealed that altered polysaccharide (exo- and/or lipopolysaccharide) significantly influences the association of UO_2 nanoparticles with EPS. The BG208 and LS142 mutants did not form UO_2-EPS, but revealed only extracellular EPS without nanoparticles or UO_2 nanoparticles not associated with EPS, respectively. In contrast, the BG178 mutant produced copious amounts UO_2-EPS relative to wild type MR-1.

The BG208 and BG178 were kindly provided by Daad Saffarini, University of Wisconsin, Milwaukee, Wisconsin

***Shewanella oneidensis* MR-1 can effectively transform soluble U(VI) and Tc(VII) to U(IV)O_2 and Tc(IV)O_2 nanoparticles; however, the biomolecular mechanisms of reduction and controls on nanoparticle formation are not well understood.**

The physical and chemical properties of biogenic radionuclide solids have important implications for their long-term fate and transport in subsurface environments.

Key findings from this research:

- Multiple *c*-type cytochromes serve as U(VI) and Tc(VII) reductases in *S. oneidensis* MR-1.
- OMCs directly transfer e$^-$ to U(VI) and Tc(VII).
- Polysaccharide biosynthesis influences the fate of UO_2 nanoparticles association with EPS.
- Columbia River *Shewanella* can reduce Hanford subsurface contaminants, U(VI) and Tc(VII).
- UO_2-EPS production is conserved among Columbia River 300 Area *Shewanella* strains.

300 Area *Shewanella* spp.

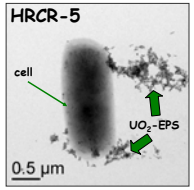
Hanford subsurface contaminants are reduced by *Shewanella* isolated from the Columbia River 300 Area U seeps.

Reduction of U(VI)

	HRCR-1	HRCR-2	HRCR-3	HRCR-4	HRCR-5	HRCR-6	MR-1
Specific rate (nmole/h^{-1}/mg^{-1}) (0-6 hours)	14.7	13.2	8.40	2.90	8.51	7.43	8.82
% U(VI) reduced (24h)	98.6	97.7	91.9	39.4	73.8	82.3	96.4

Reduction of Tc(VII)

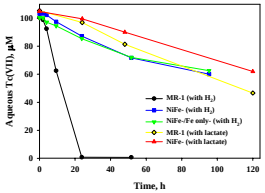
	HRCR-1	HRCR-2	HRCR-3	HRCR-4	HRCR-5	HRCR-6	MR-1
Specific rate (nmole/h^{-1}/mg^{-1}) (0-6 hours)	3.31	3.95	5.84	2.23	2.47	3.30	2.98
% Tc(VII) reduced (24h)	99.6	98.4	99.7	99.4	99.5	97.7	94.9



The *Shewanella* Federation has isolated multiple *Shewanella* spp. from locations along the Hanford Reach of the Columbia River (HRCR), including the 300 Area U seeps. Putative isolates were confirmed by 16S rDNA sequencing (Tiedje lab, MSU). All HRCR isolates from seep 7 (HRCR 1-3) and seep 9 (HRCR 4-6) reduce both U(VI) and Tc(VII) in resting cell reduction assays. Several strains reduce these radionuclides at rates different than wild type MR-1. **Significantly, these Columbia River *Shewanella* isolates also localized extracellular UO_2 nanoparticles with EPS.**

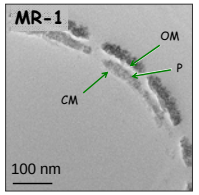
Technetium(VII) reduction

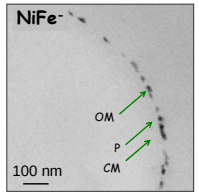
NiFe hydrogenase-catalyzed Tc(VII) reduction is coupled to H_2 oxidation.



Tc(VII) reduction using wild type MR-1 or deletions of genes encoding the NiFe hydrogenase (NiFe$^-$) or both hydrogenases (NiFe$^-$/Fe only$^-$). Assays demonstrate that hydrogenase has a role in Tc(VII) reduction with H_2. In contrast, hydrogenase does not play a role in Tc(VII) reduction coupled to lactate oxidation. This suggests that an alternative mechanism of Tc(VII) reduction exists in MR-1 cells.

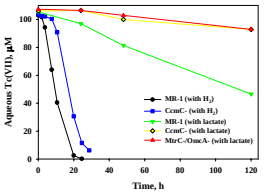
Hydrogenase influences the sub-cellular localization of TcO_2 nanoparticles.





A comparison of MR-1 and the NiFe$^-$ mutant revealed that the NiFe hydrogenase significantly influences the sub-cellular localization of TcO_2 nanoparticles during H_2-driven Tc(VII) reduction. Wild type MR-1 localized TcO_2 in both the periplasmic space and at the outside face of the outer membrane. Analysis of the NiFe$^-$ mutant revealed that TcO_2 was only localized at the outside face of the outer membrane. Locations of the cell membrane (CM), periplasmic space (P), and outer membrane (OM) are shown.

OMCs function as pertechnetate reductases during lactate-driven Tc(VII) reduction.



Specific rate (nmole/mg^{-1}/min^{-1})	MtrC	OmcA
	56.9	4.20

The reduction kinetics of Tc(VII) using wild type MR-1, CcmC$^-$ and MtrC$^-$/OmcA$^-$) mutant cells using either H_2 or lactate as the sole e$^-$ donor. Results demonstrate that *c*-type cytochromes have a role in Tc(VII) reduction with H_2 and that the OMCs (MtrC and OmcA) are pertechnetate reductases when lactate is the e$^-$ donor.

To confirm this novel finding, purified OMC were shown to directly transfer e$^-$ to pertechnetate in a system that was independent of an e$^-$ donor source.

Publications

Marshall, M.J. *et al.* (2006) *c*-Type cytochrome-dependent formation of U(IV) nanoparticles by *Shewanella oneidensis*. *PLoS Biol* **4**: e268.

Marshall, M.J. *et al.* (2007) Biomolecular mechanisms of Tc(IV)O_2 nanoparticle formation by *Shewanella oneidensis* MR-1: A novel technetium(VII) reductase activity by *c*-type cytochromes. *submitted.*