

Reoxidation of Bioreduced Uranium Under Reducing Conditions

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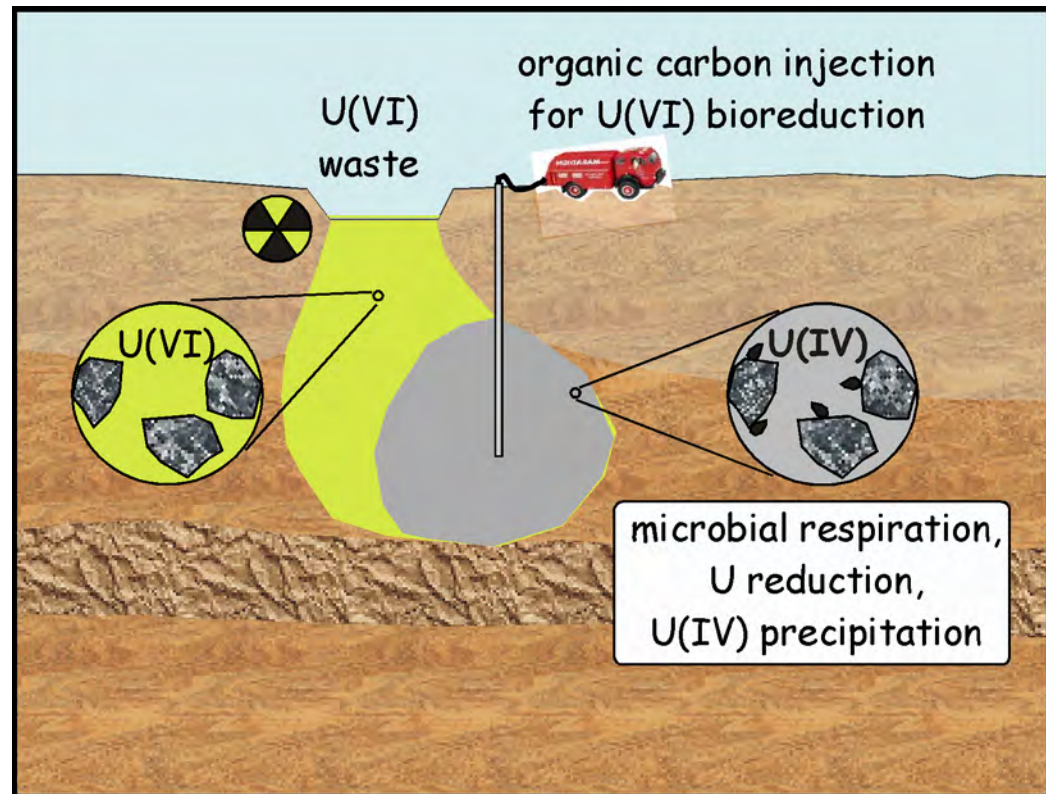
Initial Motivation

Stimulation of U(VI) bioreduction is becoming an attractive in-situ method for stabilizing U in contaminated sediments.

After reduction of U(VI) is completed in sediments, how stable is bioreduced U(IV) upon exposure to oxidizing groundwaters?



S3 Ponds, Oak Ridge



Experimental Approach

Pump organic carbon (lactate) into U-contaminated soil columns and get U bio-reduced to low-solubility U(IV), then permeate these columns with oxidizing waters.

Historically U-contaminated sediment:

NABIR FRC Area 2 soil, 5.6 mm sieved
Total U = 206 mg kg⁻¹ (by XRF and γ activity)
Initial U(IV) fraction = 0.24 (by μ -XANES)
Initial nitrate \approx 0.3 mM
pH 7.5



12 sediment columns:

Length: 20 cm
Porosity: 0.45

Influent solution:

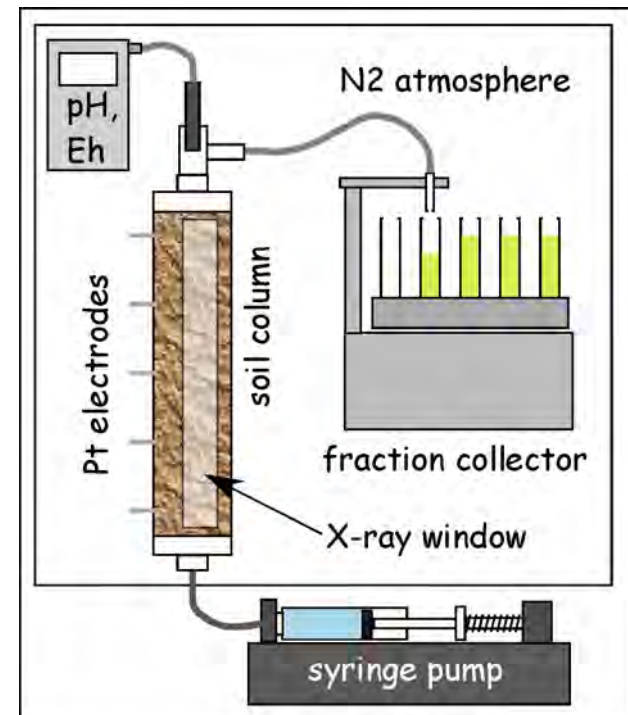
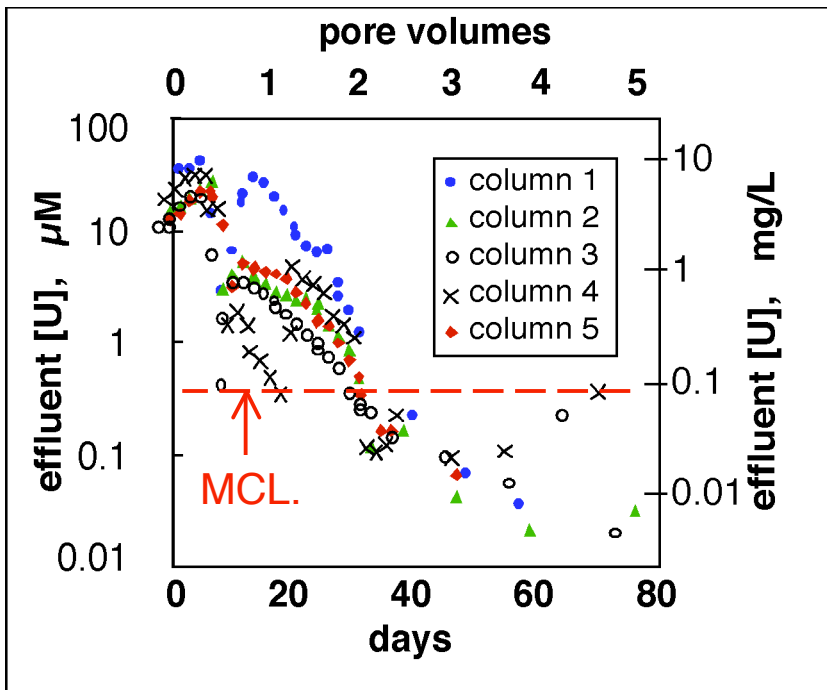
Na-lactate 10.7 mM (32 mM OC)
CaCl₂ 1 mM
MgCl₂ 1 mM
KCl 1 mM
pH 7.2

Pore-water velocity:
1.3 cm day⁻¹ to 1.0 cm day⁻¹



“Short-term” (≈ 80 days) results

- Column redox potentials declined.
- Effluent U(VI) concentrations declined.
initially $> 10 \mu\text{M}$, now $< 0.1 \mu\text{M}$
- Soil U is now primarily U(IV)
U(IV) fraction increased from 0.24 to 0.87.
- U bioreduction appeared successful.



However, later U concentrations in effluents increased by about 30-fold, back to $\approx 1 \mu\text{M}$, under reducing conditions, before we even had a chance to introduce oxidants!

Influent contains no oxidants
(no nitrate, negligible oxygen).

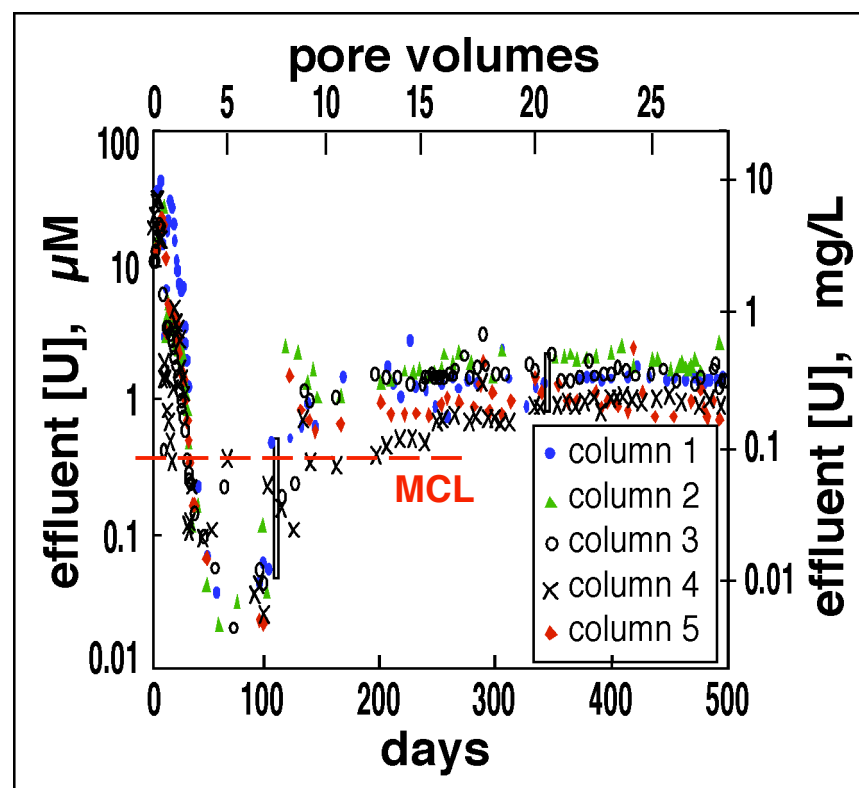
Columns placed in N_2 atmosphere.

Effluents remained reducing.

| | |
|---------------|--------------------------|
| Eh | $-200 \pm 50 \text{ mV}$ |
| CH_4 | 10 mM |
| Fe | $45 \mu\text{M}$ |
| Mn | $35 \mu\text{M}$ |

pH remained stable, ≈ 7.4

(acid dissolution of UO_2 did not occur)



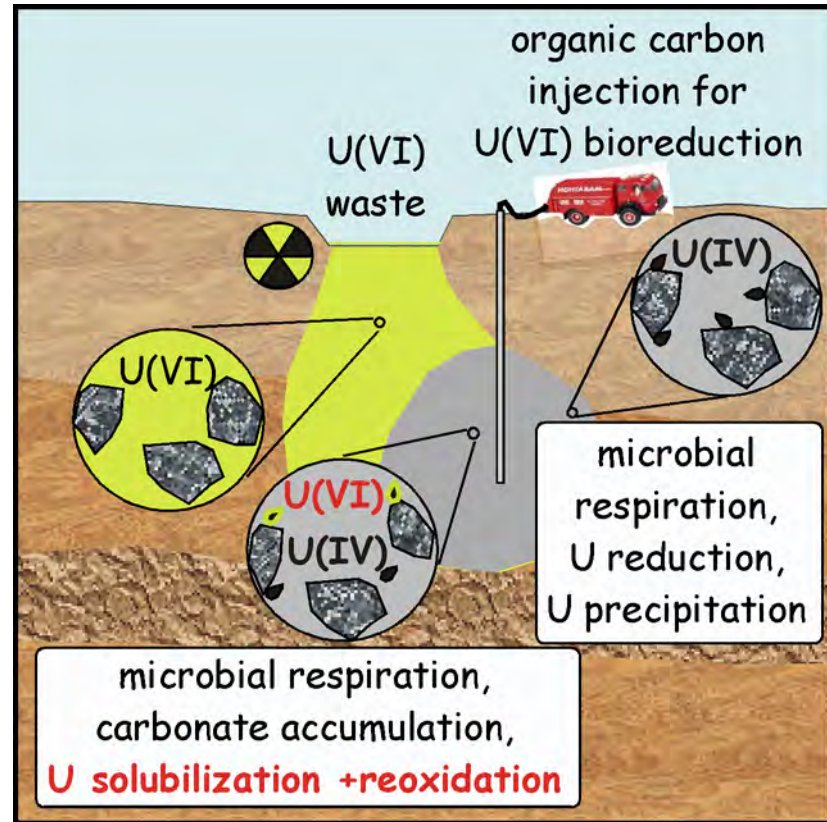
Overview of general solution chemistry entering and exiting these sediment columns

| | | Influent | Steady-state effluent |
|--|----|----------|---|
| pH | | 7.2 | 7.4 ±0.14 ^a |
| redox, pe | | > 0 | -3.4 ±1.0 ^b ; -4.2 ±0.3 ^c |
| log ₁₀ (P _{CO2} , atm) | | < -3.5 | -1.5 ^d |
| OC (lactate) | mM | 32.0 | 1.01 ±0.25 |
| bicarbonate | mM | 0 | 12.7 ±1.4 |
| CH ₄ | mM | 0 | 10.4 ±1.9 |
| C mass recovery | | | 75% |
| Ca | mM | 1.0 | 1.0 ±0.4 |
| Fe | mM | 0 | 0.045 ±0.005 |
| Mn | mM | 0 | 0.035 ±0.005 |

- **All measurements are consistent with sustained reducing conditions.**
- Bicarbonate concentrations in columns are elevated from OC oxidation.

Having remobilized U even without introducing oxidants, we needed to revise our goals

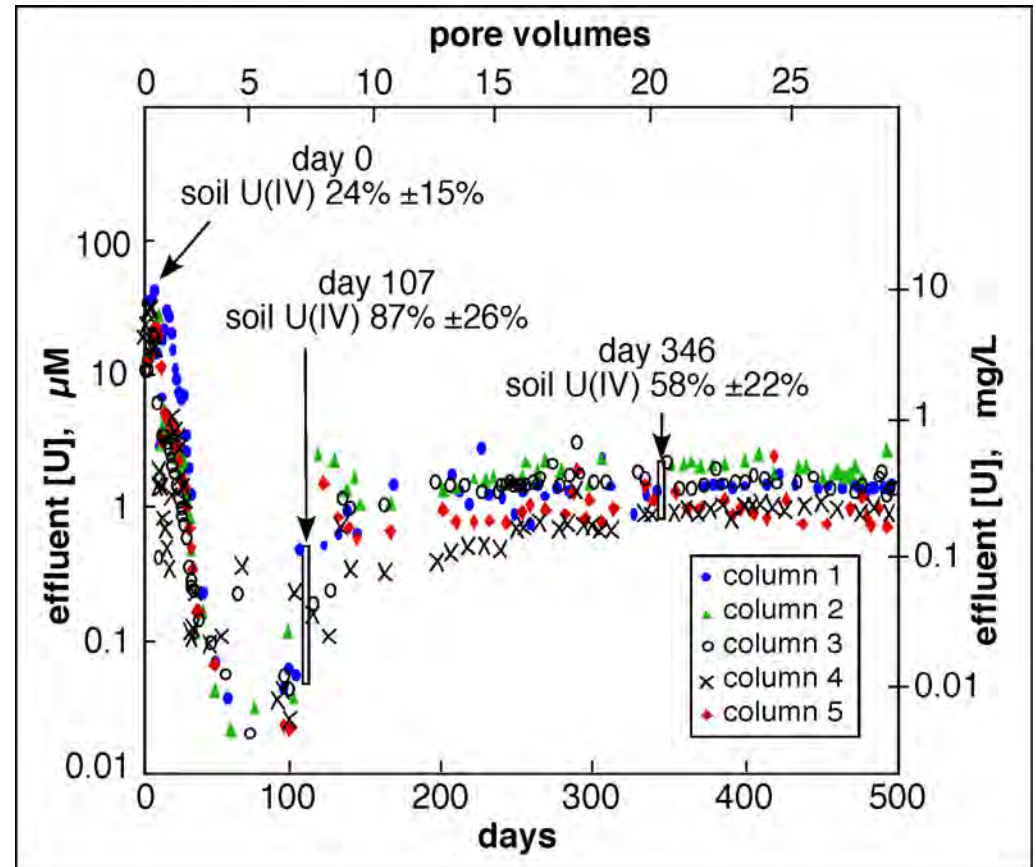
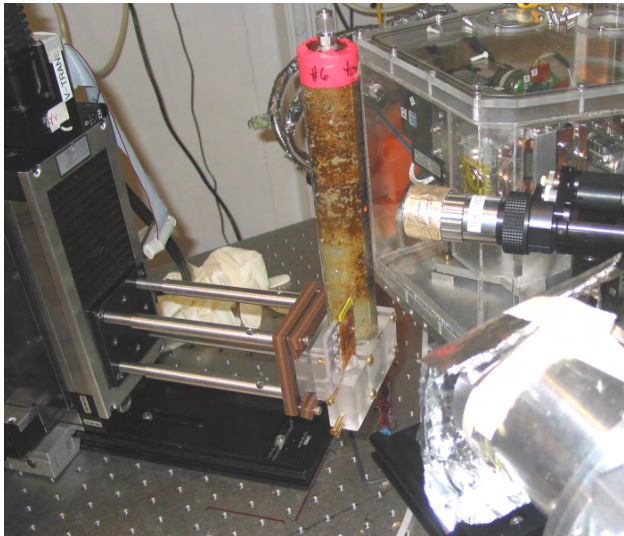
- Why do effluent U concentrations return to high levels under reducing conditions?
- What conditions are necessary for long-term stability of U bioreduction?



Was U being reduced initially, then reoxidized?

X-ray absorption near edge structure spectroscopy permits direct determination of U(IV) and U(VI) fractions within undisturbed sediments.

- U oxidation states determined in-situ, by scanning sealed sediment columns with monochromatic X-rays.
- 20 to 36 locations per soil column
- Measurement volumes $\approx 1 \text{ mm}^3$.



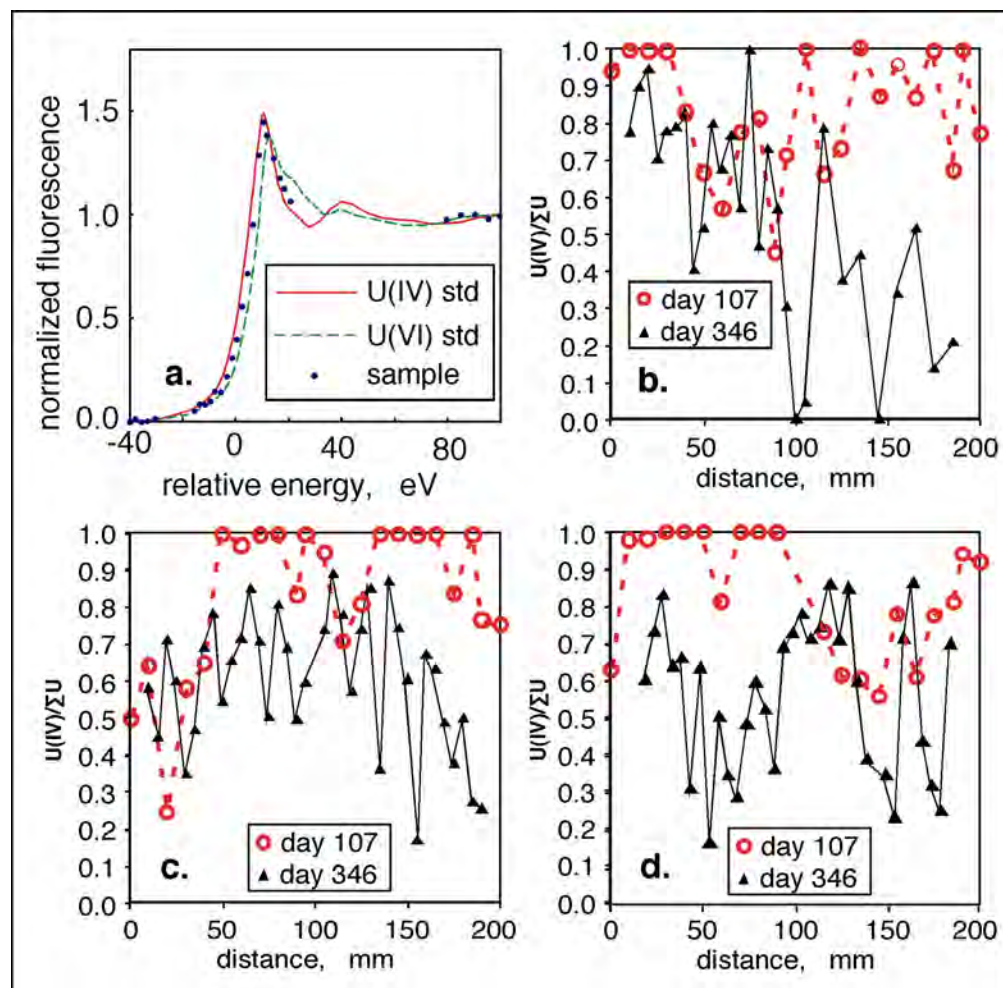
GSECARS beamline 13-IDC, Advanced Photon Source, Argonne National Laboratory

μ -XANES profiles demonstrate net U oxidation

Net increases in U oxidation state (decreases in the U(IV) fraction) were observed upon comparing data from triplicate columns (days 107 and 346):

| day | U(IV) fraction |
|-----|-----------------------|
| 0 | 24 \pm 15% |
| 107 | 87 \pm 26% (80 pts) |
| 346 | 58 \pm 22% (80 pts) |

differences significant at $p = 0.005$.



a. Example comparison of μ -XANES spectra from sediment and U(IV) and U(VI) reference standards. **b,c,d.** Comparisons of profiles of U(IV) fractions along sediment columns on day 107 versus day 346.

Has the microbial community changed significantly between the U reduction and U oxidation stages?

- Stable microbial community throughout the U reduction and reoxidation phases of this experiment.
- 16S rDNA amplicon abundance of most *Geobacteraceae* detected remained consistent over time, and along the lengths of sampled columns.
- Real time PCR results from sediment extracts showed that the cell densities of organisms within the *Geobacteraceae* remained consistent between the reduction (1.8×10^8 copies (g sediment)⁻¹) and oxidation stages (1.2 to 2.6×10^8 copies (g sediment)⁻¹).
- These results suggest that a microbial community capable of continued U reduction remained through the reoxidation phase and that a loss of microbial functionality was not a factor in U(IV) reoxidation.

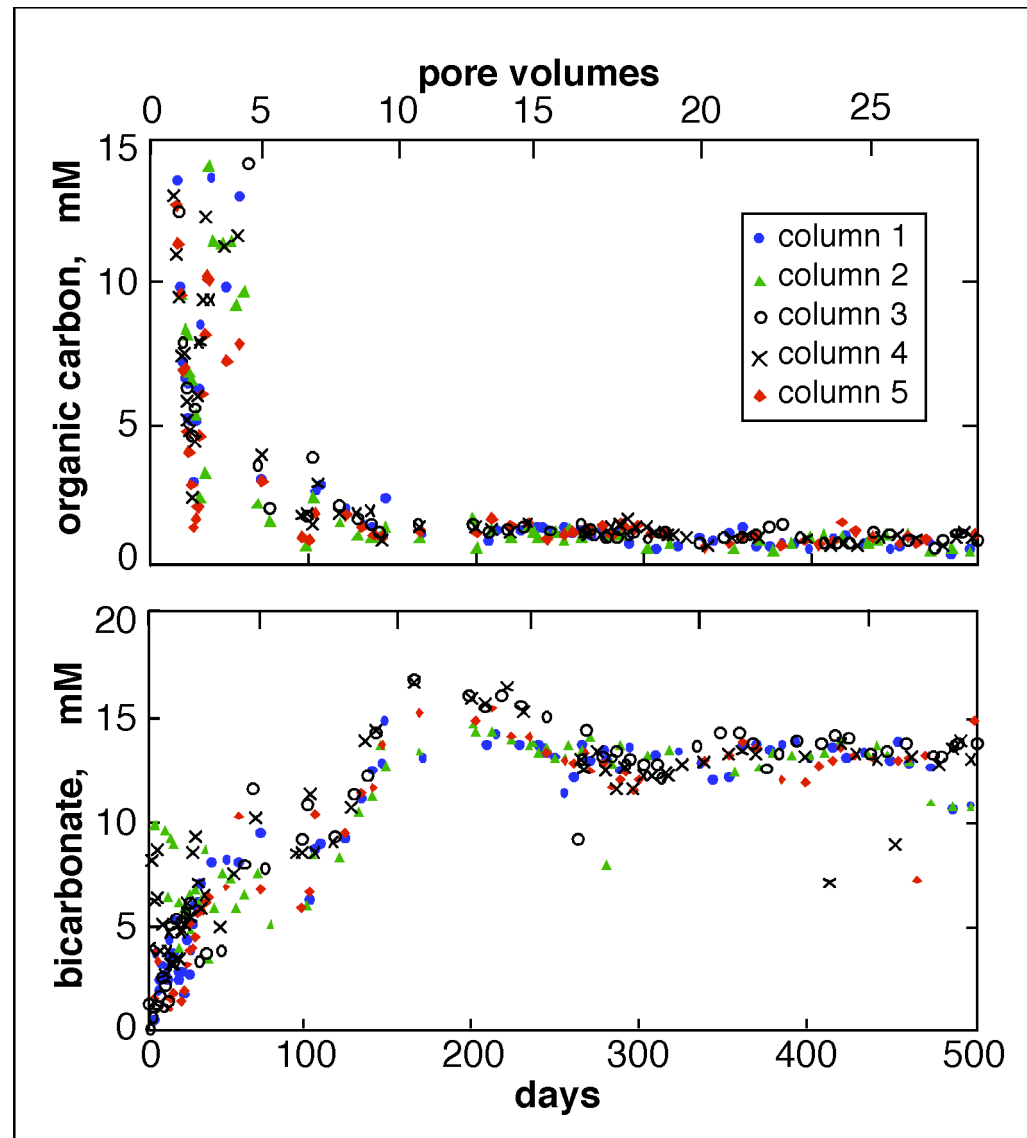
High Density DNA Microarray and qPCR show *Geobacteraceae* populations remain stable through oxidation phase

| | | Reduction | Oxidation (Bottom) | Oxidation (Middle) | Oxidation (Top) |
|---|--|------------|--------------------|--------------------|-----------------|
| qPCR analysis | <i>Geobacteraceae</i> 16S rRNA genes (10 ⁸ copies g ⁻¹ sediment) | 1.8 ± 0.43 | 2.6 ± 1.10 | 1.2 ± 0.08 | 1.2 ± 0.48 |
| 16S Microarray analysis (Corrected Hybridization Intensity) | <i>Pelobacter acetylenicus</i> subgroup prokMSA ‡ 79502 | 2034 | 1917 | 1869 | 2022 |
| | <i>Pelobacter acetylenicus</i> subgroup prokMSA 78912 | 1961 | 1957 | 1924 | 1980 |
| | <i>Desulfuromonas acetoxidans</i> subgroup prokMSA 76391 | 2839 | 2764 | 2836 | 2929 |
| | <i>Geobacter metallireducens</i> subgroup prokMSA 60598 | 2102 | 1978 | 2076 | 2231 |
| | <i>Geobacter metallireducens</i> subgroup prokMSA 10717 | 942 | 771 | 781 | 802 |
| | <i>Geobacter metallireducens</i> subgroup prokMSA 10726 | 2250 | 2214 | 2160 | 2224 |
| | <i>Geobacter metallireducens</i> subgroup prokMSA 79548 | 1664 | 1327 | 1354 | 1535 |
| | <i>Geobacter metallireducens</i> subgroup prokMSA 79712 | 2484 | 2364 | 2412 | 2722 |
| | <i>Geobacter metallireducens</i> subgroup prokMSA 71753 | 3674 | 3145 | 3237 | 3340 |
| | <i>Geobacter metallireducens</i> subgroup prokMSA 63255 | 2971 | 2997 | 2742 | 3028 |

‡ prokMSA sequences are available at <http://greengenes.lbl.gov>

Organic carbon mineralization to bicarbonate

- Most of the supplied OC (32 mM at inlet, 0.5 mmol (kg soil)⁻¹ day⁻¹) is consumed within the sediment column.
- Nearly half of this OC is oxidized to dissolved inorganic carbon and is discharged in column effluents (13 mM at outlet, 0.2 mmol (kg soil)⁻¹ day⁻¹).
- Is carbonate production responsible for the observed U concentration increases?



Influence of dissolved (bi)carbonate on U(VI) speciation

| Reactions | Eh° (mV) | Eh (mV) at P _{CO2} = | | |
|--|-------------|-------------------------------|---------------------------|---------------------------|
| | | 10 ^{-3.5} atm | 10 ^{-1.5} atm | 10 ^{-1.0} atm |
| UO ₂ (am) expected as the initial and reactive U(IV) solid phase | | | | |
| 1. UO ₂ CO ₃ + H ⁺ + 2e ⁻ → UO ₂ (am) + HCO ₃ ⁻ | 235 | -57 | -116 | -131 |
| 2. UO ₂ (CO ₃) ₂ ²⁻ + 2H ⁺ + 2e ⁻ → UO ₂ (am) + 2HCO ₃ ⁻ | 337 | -70 | -188 | -218 |
| 3. UO ₂ (CO ₃) ₃ ⁴⁻ + 3H ⁺ + 2e ⁻ → UO ₂ (am) + 3HCO ₃ ⁻ | 485 | -38 | -215 | -259 |
| 4. Ca ₂ UO ₂ (CO ₃) ₃ + 3H ⁺ + 2e ⁻ → UO ₂ (am) + 3HCO ₃ ⁻ + 2Ca ²⁺ | 257 | -177 | -236 | -251 |
| 5. CaUO ₂ (CO ₃) ₃ ²⁻ + 3H ⁺ + 2e ⁻ → UO ₂ (am) + 3HCO ₃ ⁻ + Ca ²⁺ | 400 | -78 | -196 | -225 |

- Carbonate forms very strong aqueous complexes with UO₂²⁺ at pH > 5.
- The stability of these U(VI)-carbonato complexes drives U(IV) oxidation (above). (Usually unrecognized, but we have direct evidence for net U reoxidation.)
- The stability of these U(VI)-carbonato complexes also drives U(VI) desorption. (More recognized, and may be occurring simultaneously.)

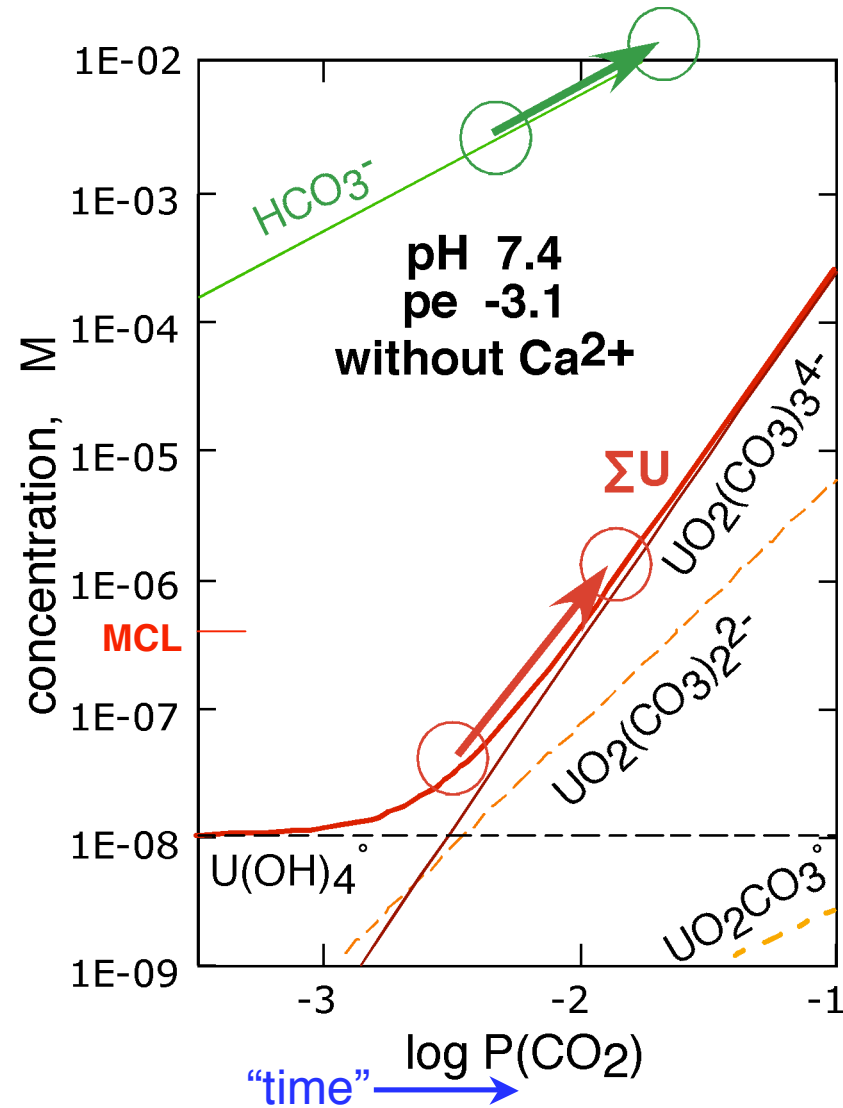
Influence of increased $P(\text{CO}_2)$ on U(VI) concentrations

Increased $P(\text{CO}_2)$ from microbial respiration increases dissolved inorganic carbon (primarily bicarbonate at circum-neutral pH).

High stabilities of U(VI)-carbonato complexes support higher U(VI) concentrations.

For $P(\text{CO}_2)$ levels greater than $\approx 10^{-2.5}$ atm, U solubility becomes highly CO_2 -dependent, primarily because of $\text{UO}_2(\text{CO}_3)_3^{4-}$.

Therefore $P(\text{CO}_2)$ levels need to be controlled in reductive U bioremediation.



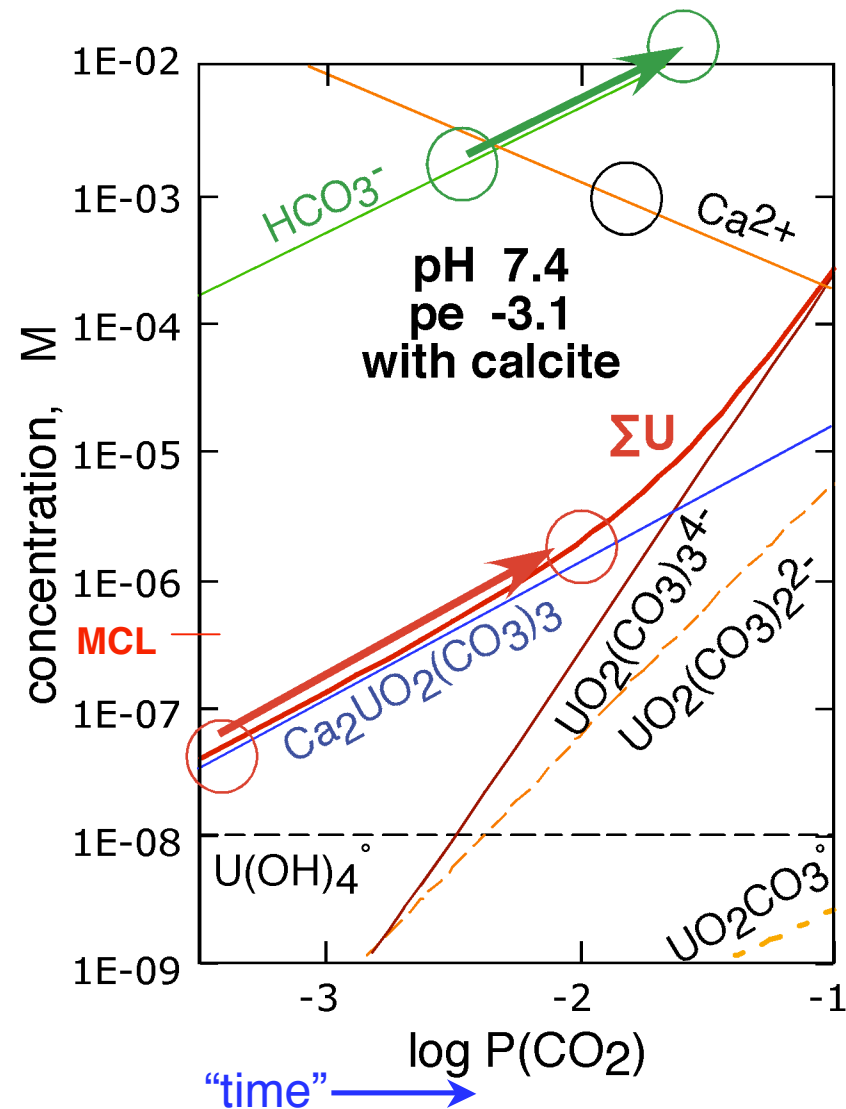
When Ca^{2+} is present at $\approx \text{mM}$ concentrations, the influence of increased $\text{P}(\text{CO}_2)$ on $\text{U}(\text{VI})$ concentrations is even stronger

The extremely strong $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ complex (Bernhard et al. 1996, 2001) supports even higher $\text{U}(\text{VI})$ levels in the circum-neutral pH range.

This suggests that OC-stimulated U bioreduction is a more difficult immobilization strategy in calcareous sediments.

Other impacts of $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$:

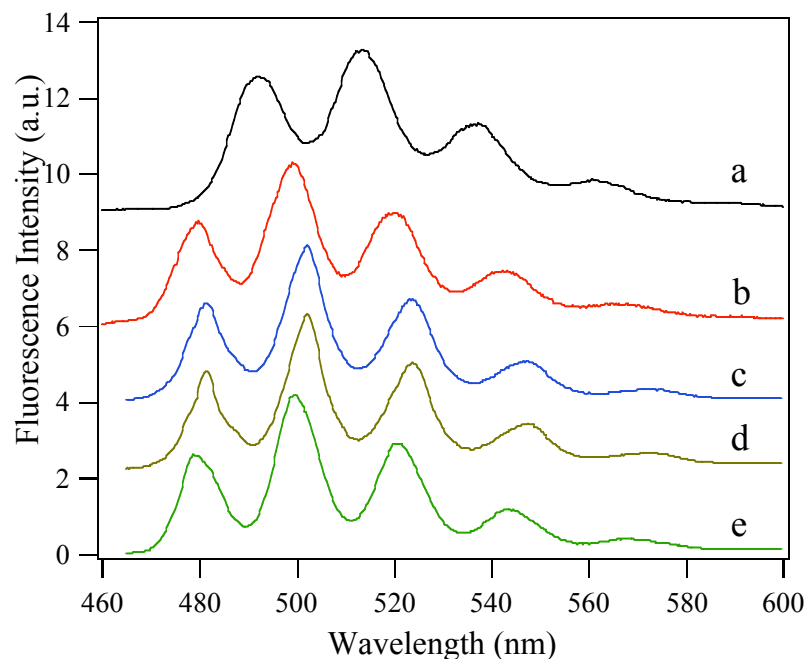
1. can slow $\text{U}(\text{VI})$ bioreduction (Brooks et al., 2003).
2. diminishes $\text{U}(\text{VI})$ sorption (Zheng et al., 2003).
3. enhances $\text{U}(\text{VI})$ diffusion (Tokunaga et al., 2004).



Speciation of U in Effluents from Sediments

Direct speciation of U in these effluents is difficult or impossible by most spectroscopic methods because of the relatively low U concentrations ($\approx 1 \mu\text{M}$).

Laser fluorescence spectroscopy provided direct evidence for UO_2^{2+} occurring primarily in complexes as $\text{UO}_2(\text{CO}_3)_3^{4-}$ and as $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$.



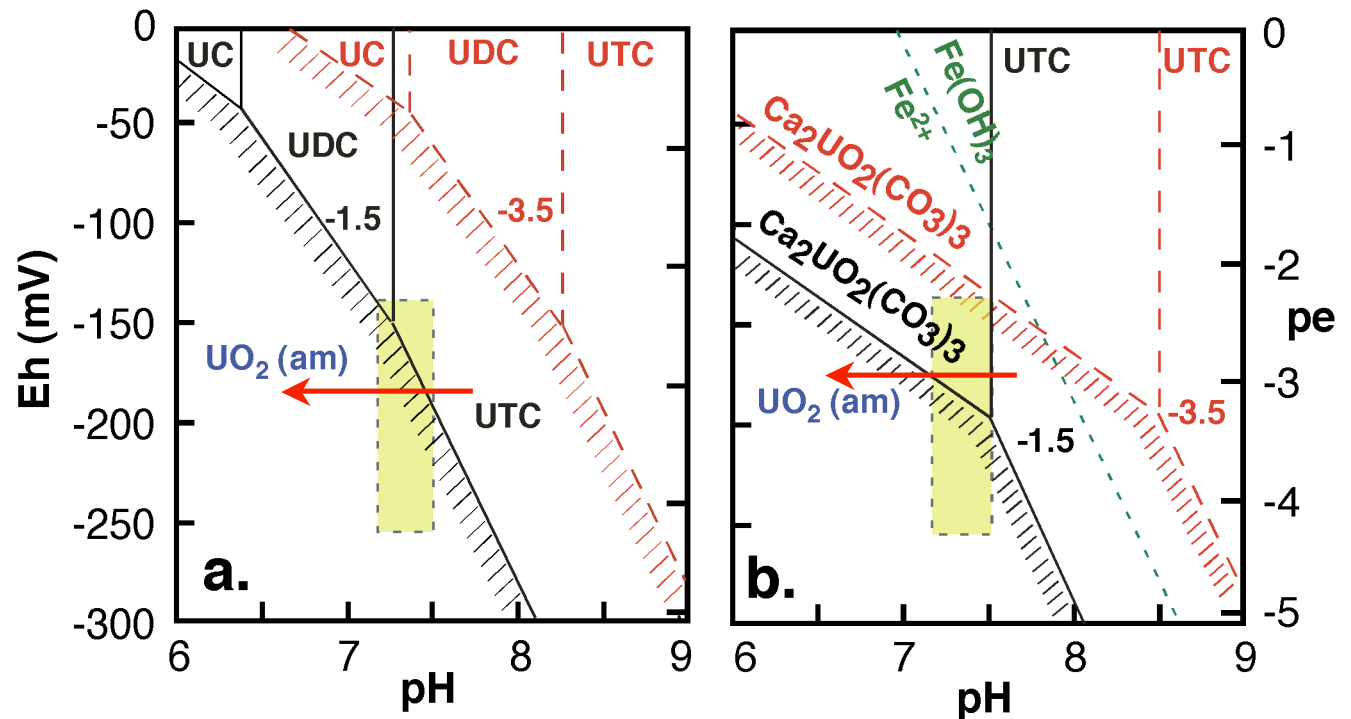
Fluorescence spectra of aqueous uranyl complexes at 6 K, with $\lambda_{\text{ex}} = 415 \text{ nm}$. **a**, uranyl ion $[\text{UO}_2^{2+}]$; **b**, $(\text{UO}_2)(\text{CO}_3)_3^{4-}$; **c**, $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$; **d**, effluent sample collected before reduction; **e**, effluent sample collected during the steady-state reoxidation stage.

U bioreduction is expected to be more sustainable under slightly acidic conditions

The U(IV) stability region extends to higher Eh as pH decreases.

Trade-offs:

- weaker U(VI) sorption.
- U(IV) soluble at $\text{pH} \leq 4$.



pH-Eh U predominance diagrams for equilibrium with amorphous UO_2 , (a) without Ca^{2+} , and (b) in equilibrium with calcite. UC, UDC, and UTC denote $\text{UO}_2\text{CO}_3^{\circ}$, $\text{UO}_2(\text{CO}_3)_2^{2-}$, and $\text{UO}_2(\text{CO}_3)_3^{4-}$, respectively. $\sum \text{U(VI)} = 10^{-6}$ M. Boundaries are shown for $\text{PCO}_2 = 10^{-1.5}$ atm (black) and $10^{-3.5}$ atm (red). The yellow rectangular area denotes our measured experimental conditions.

What oxidant(s) are responsible for U(IV) reoxidation under the reducing condition?

We suspect Fe(III) is the TEA for several reasons:

- High concentration of poorly crystalline Fe(III) contained in the sediment: 3,650 mg kg⁻¹ in weak acid extractable form initially.
- Because of kinetic limitations some of this Fe(III) may remain during the U reoxidation stage.
- High Fe²⁺ concentrations in effluents (45 μM) during the U reoxidation stage were measured. This is supersaturated with respect to siderite, and much greater than effluent U(VI) concentrations (1 μM).
- Newly precipitated FeOOH was identified in the reducing columns.

Our current studies will provide a better understanding of the oxidant(s).

Conclusions

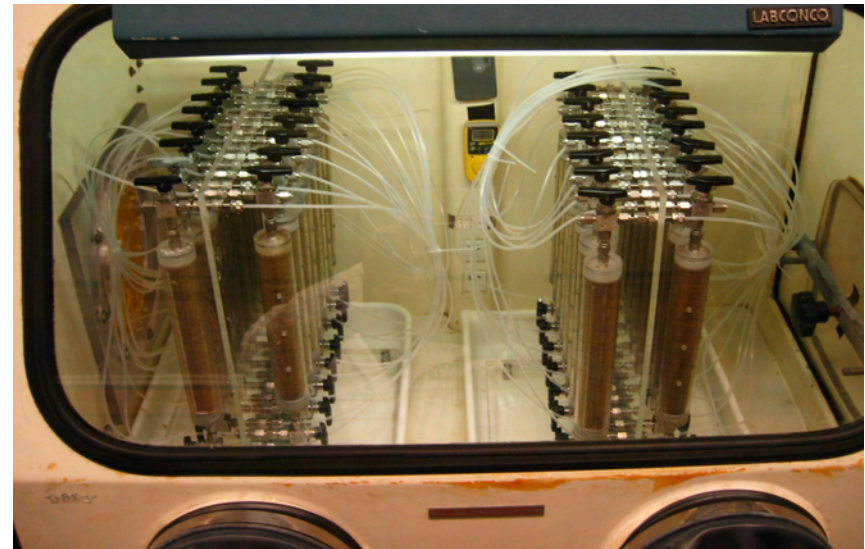
- **Bioreduced U(IV) was reoxidized to U(VI) under sustained reducing (methanogenic) conditions.**
- **A microbial community capable of continued U reduction remained through the U reoxidation phase.**
- **Increased carbonate concentrations resulting from microbial respiration drives formation of very stable U(VI)-carbonato complexes. This unanticipated consequence of OC-stimulation of microbial activity can make it difficult to sustain U bioreduction at neutral and alkaline pH.**

Current and Future Studies to Understand Long-term U Bioreduction Stability

Columns and Batches

32 new columns recently started

- Identify oxidant(s) responsible for U reoxidation at low Eh
- Identify optimal conditions for sustained U bioreduction
- Determine time-scales for $UO_2(am)/UO_2(c)$ transformations



Questions

- **How is OC-based U immobilization to be maintained? Is it realistic to sustain U bioreduction for centuries? Who is going to keep the pumps running?**
- **Are there more practical, low-maintenance approaches we need to consider?**