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Bacterial Biotransformations for the *In situ* Stabilization of Plutonium

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Overview

Plutonium contamination in the environment is generally low-level and may be present and transported in a range of forms (IV, V, VI).

Current remediation strategies are costly, financially and in terms of increased exposure risk to people and the environment.
In situ bacterial biostabilization is a promising alternative.

Aqueous Speciation Related to Environmental Conditions

- Plutonium(VI) *vs* Uranium(VI) Hydrolysis
- Plutonium(IV/III) EDTA Speciation and Stability
- Siderophore Stabilization of Plutonium(IV)
- New Reduction Potentials

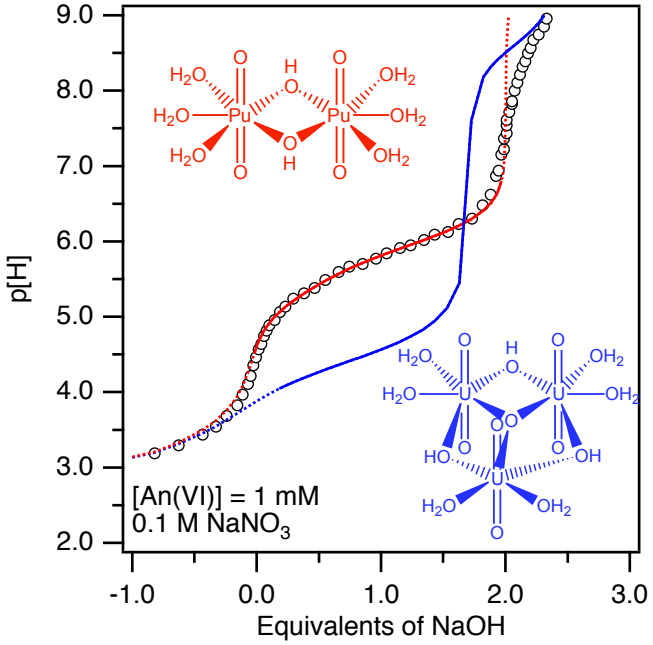
Bacterial Biotransformations

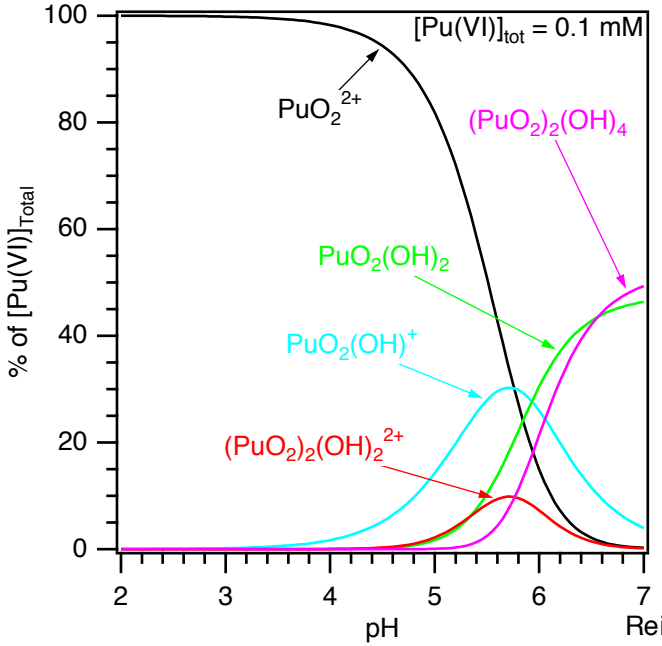
- Siderophore-mediated Accumulation by Aerobic Bacteria
- EPS and Cell Adsorption by Aerobic Bacteria
- Reduction by DMRB

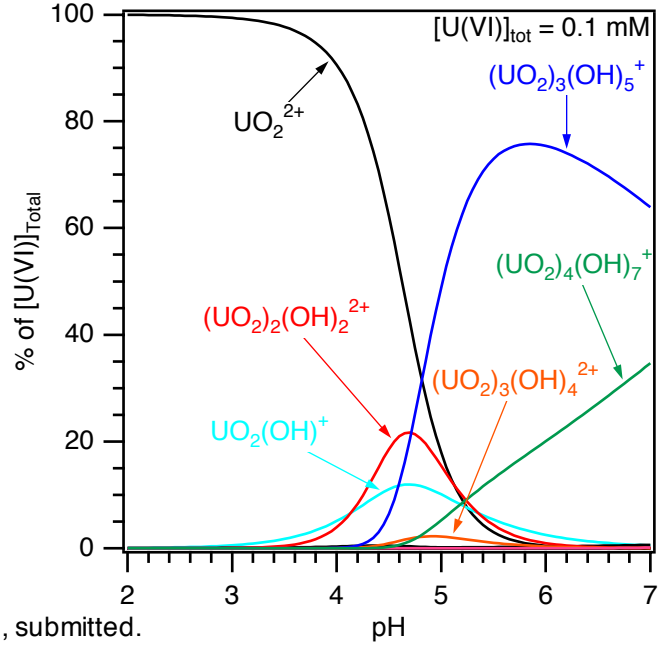
Pu (VI) Hydrolysis

Why biostabilization methods being developed for U, e.g. reduction by DMRB, may or may not work for Pu

~600 papers on U(VI) hydrolysis
5 papers on Pu(VI) hydrolysis
2 papers on Pu(V) hydrolysis



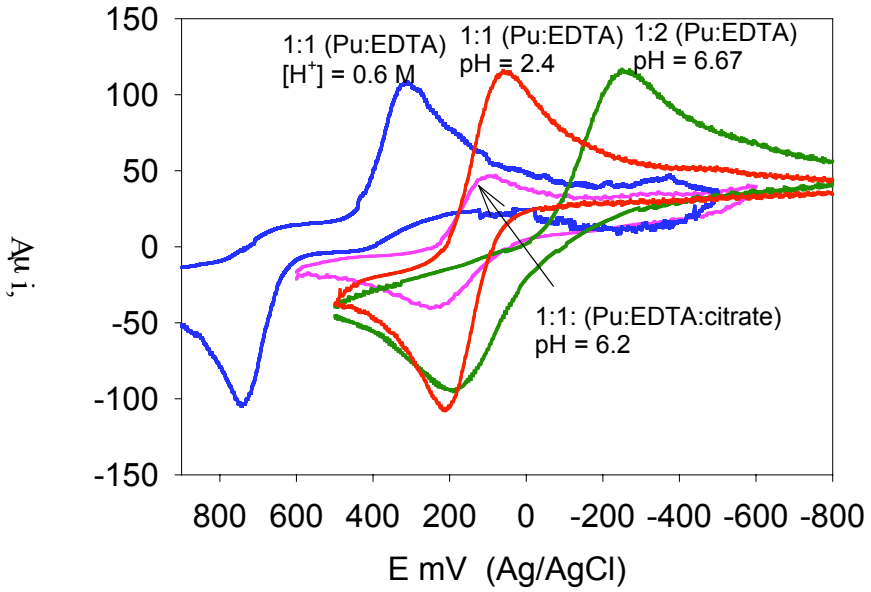




Reilly, Neu, *Inorg. Chem.*, submitted.

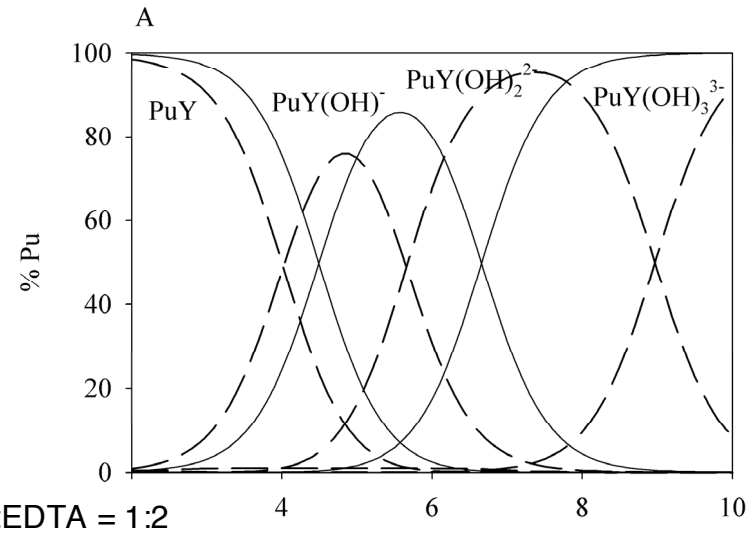
Pu(IV)EDTA Aqueous Speciation

Cyclic Voltammetry

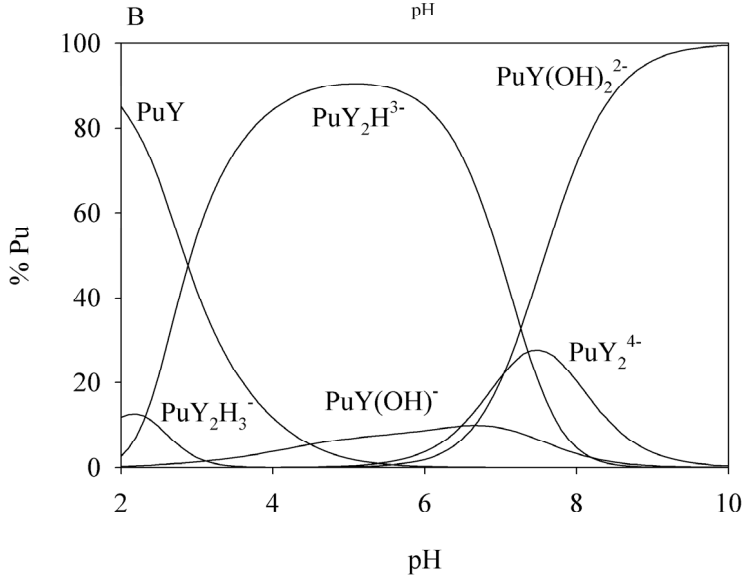


New Species Distributions (---)

Pu:EDTA = 1:1



Pu:EDTA = 1:2



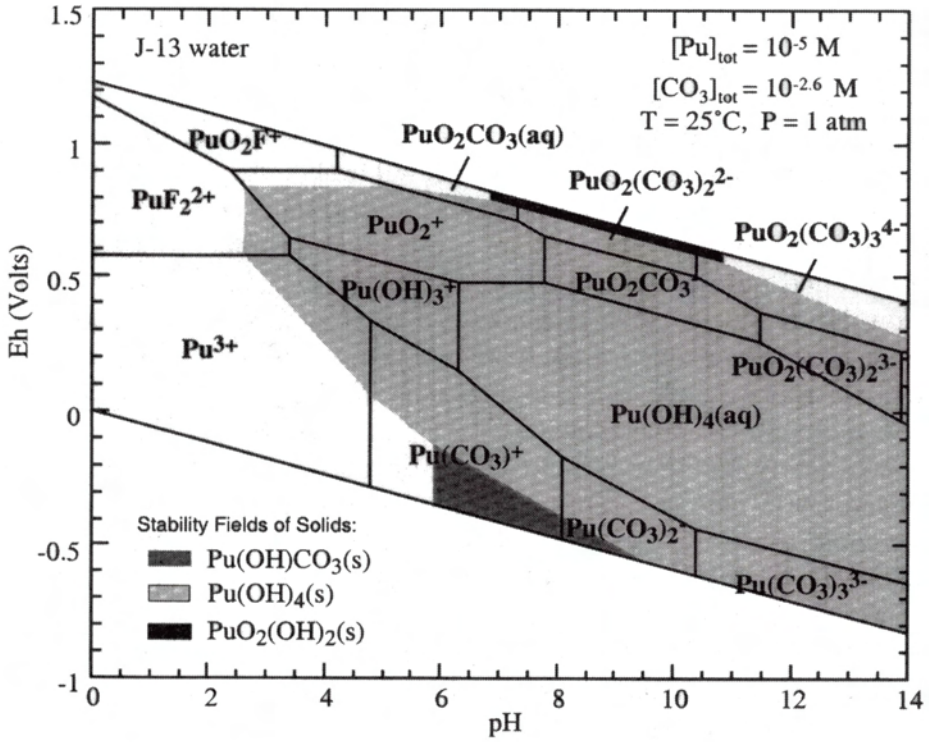
New species are identified including $Pu(EDTA)_2$, log β = 35.43

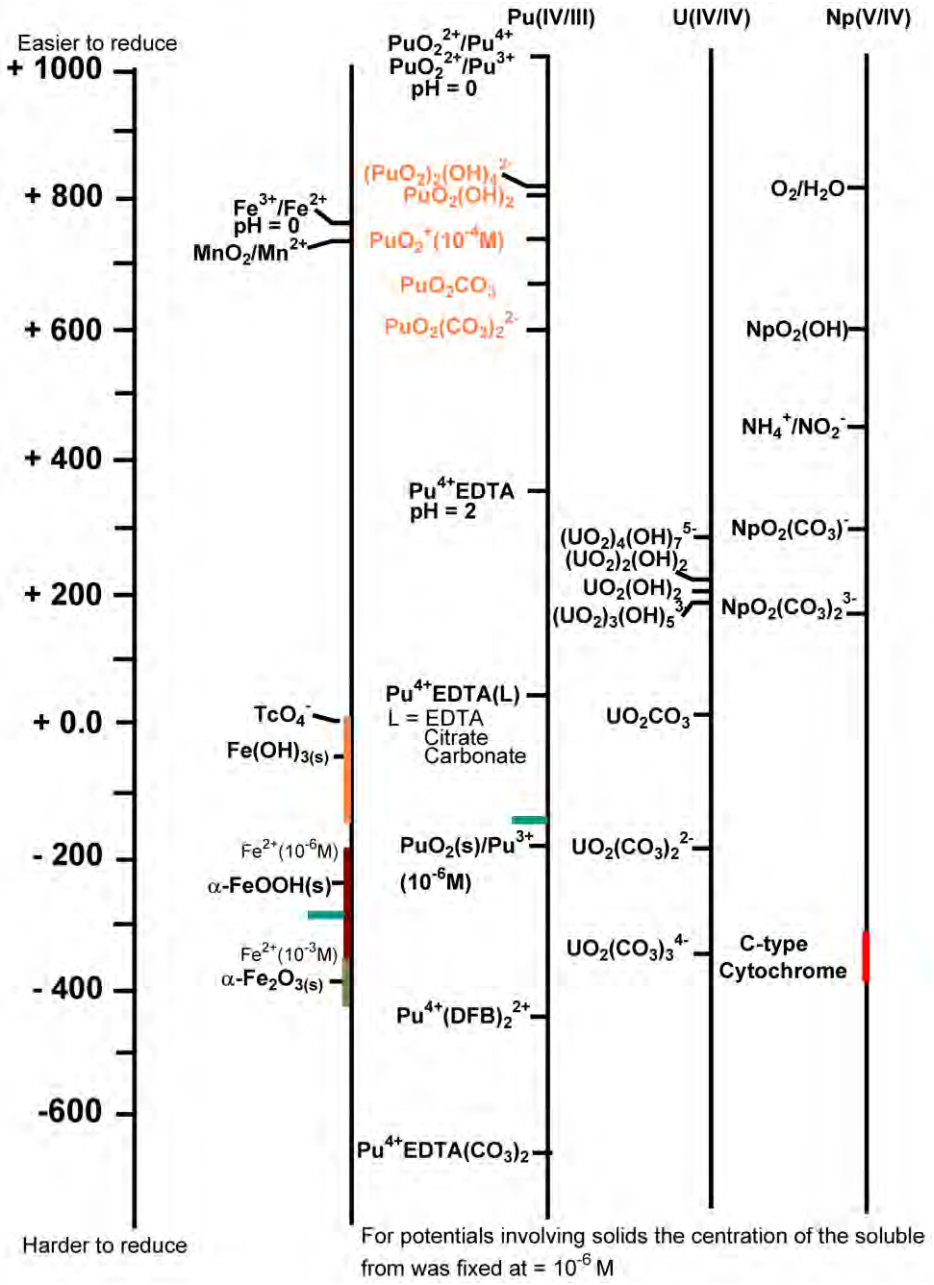
Stability of Pu(IV) is enhanced at environmentally relevant pH by the formation Pu(IV)-EDTA-hydroxo and Pu(IV)-EDTA-L mixed complexes.

Unusual speciation due to high charge and large coordination sphere

Boukhalfa, Reilly, Neu, *Inorg. Chem.*, **2004**, *43(19)*, 5816.

Pu Speciation Under Environmental Conditions





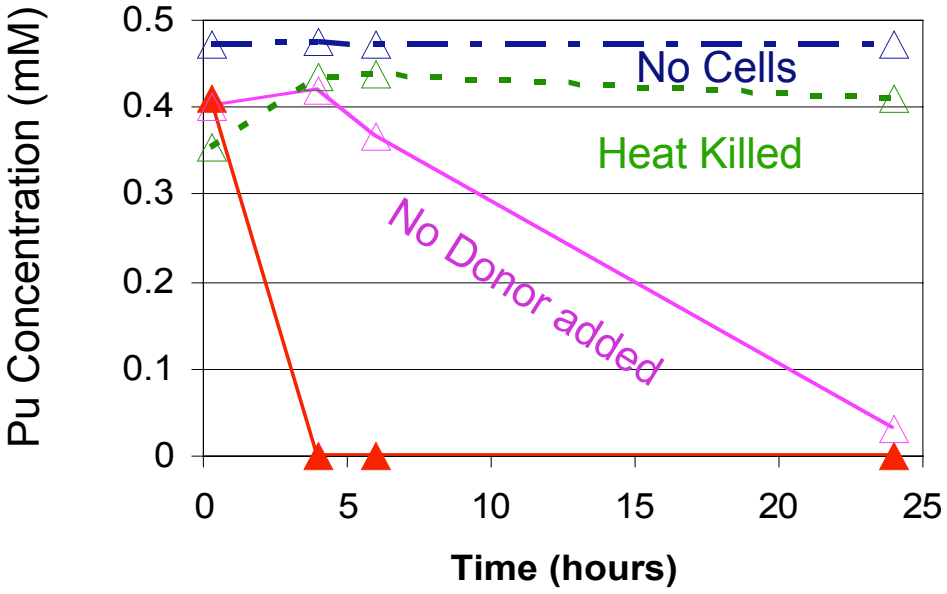
Solubility of primary (hydr)oxide phases

PuO_2^{2+} $\log K_{sp} = 5.5$
PuO_2^{+} $\log K_{sp} = 5.0$
Pu^{4+} $\log K_{sp} = -2.0$
Pu^{3+} $\log K_{sp} = 15.8$

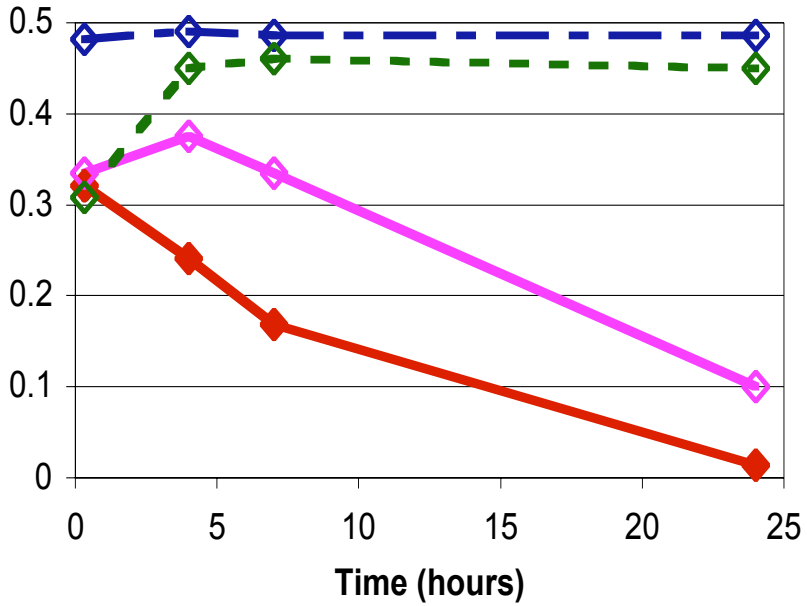
e.g., $Pu^{4+}/PuO_{2(hyd)}$ $[H^+]^4$

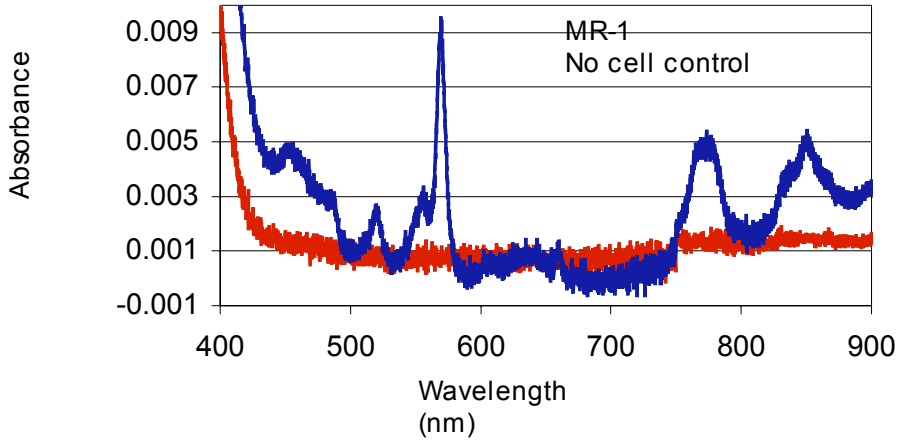
Pu(VI) as a Terminal Electron Acceptor for DMRB

Shewanella oneidensis MR1
10 mM Lactate
5 x 10^8 Cells/mL



Geobacter metallireducens GS15
10 mM Acetate
5 x 10^8 Cells/mL





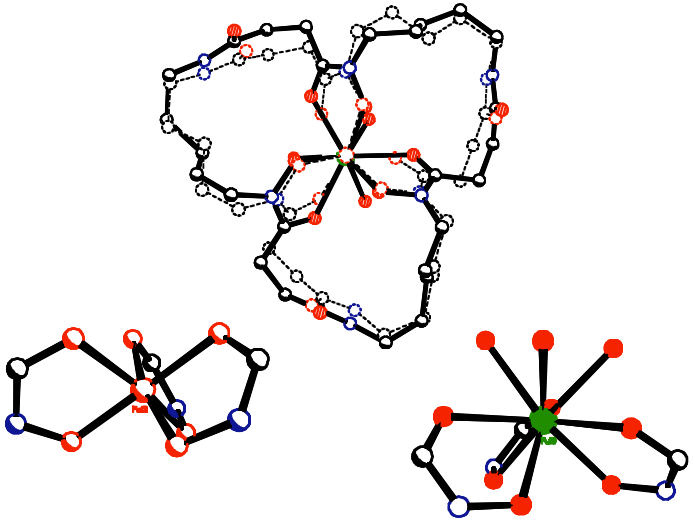
DMRB reduce Pu(VI) and Pu(V)

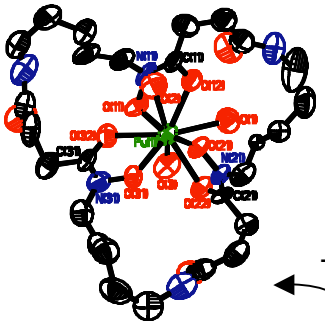
Product appears to be $PuO_{2\ (hyd)}$

Characterization and dissolution studies in progress

Siderophore-Mediated Pu Accumulation

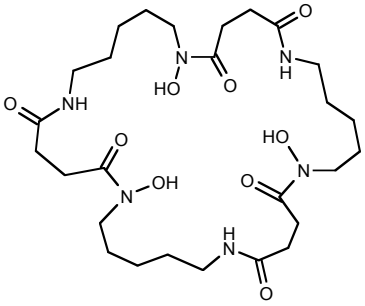
Fe(III) and Pu(IV) siderophore structures





+ Pu(IV)

Gram +
A. flavescens
JG-9



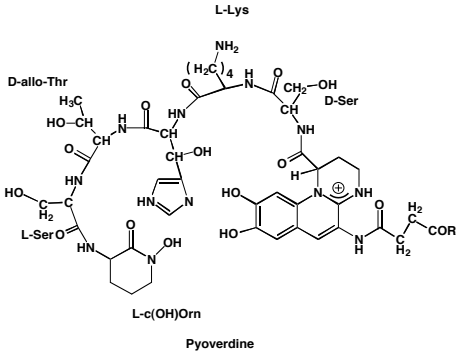
DFE

+ Pu(IV)

+ Fe(III)

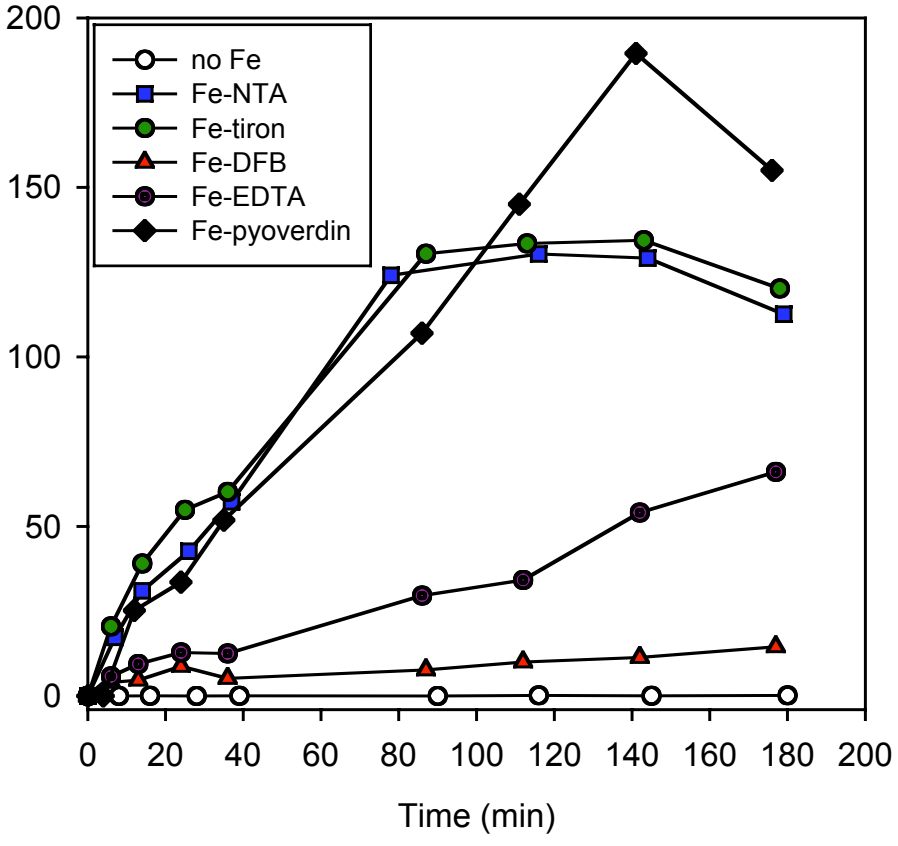
???

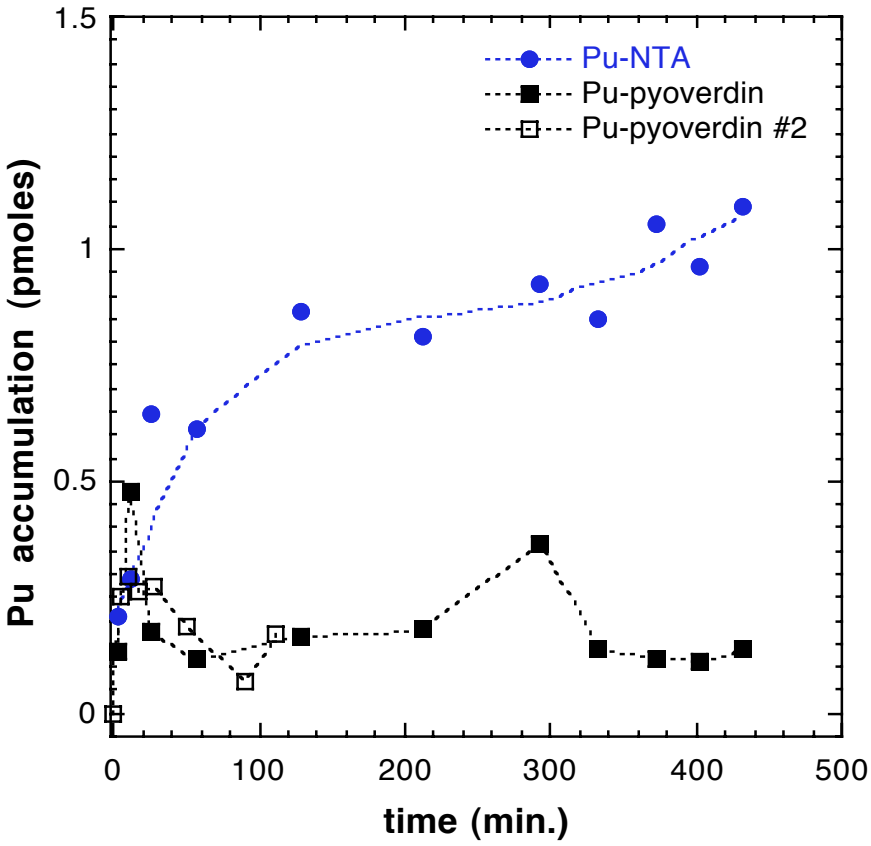
Gram -
P. putida



Can Pu be accumulated by other bacteria via other types of siderophores?

P. putida Metal Acquisition from Multiple Chelates



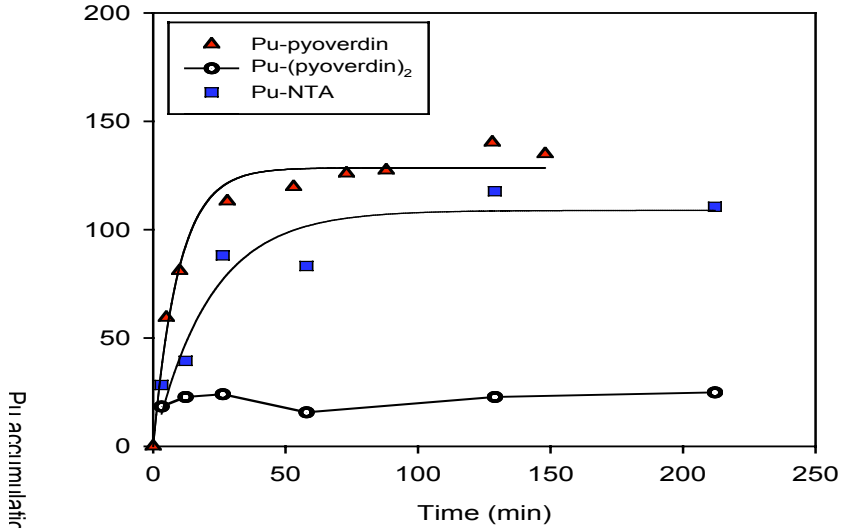


Uptake inversely proportional to Fe-L complexes stability.

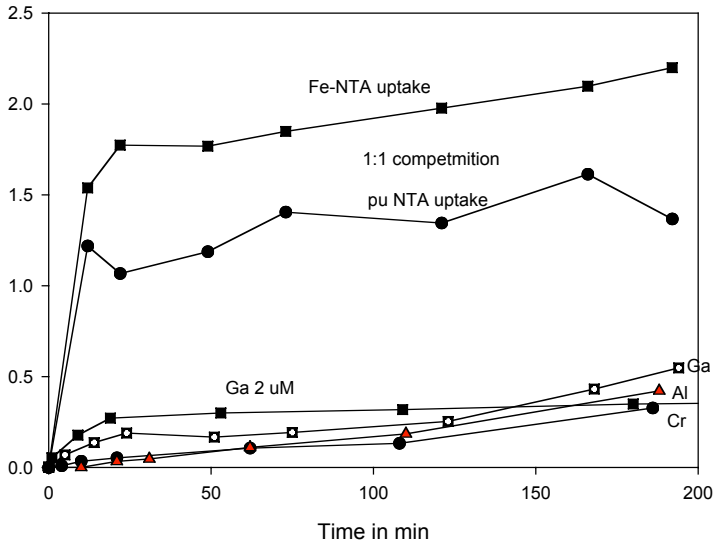
Fe uptake profile suggests that exogenous ligands release iron to the siderophore either in solution or at the membrane surface.

Requirements for Metal–Siderophore Uptake

Siderophore binding, membrane protein recognition, metal shuttle, intra-cellular release thought to require specific physico-chemical characteristics.



P. Putida cells take up NTA and pyoverdin complexes, both in the presence and absence of Fe.



P. Putida cells pre-incubated with 2 μM of pyoverdin complexes of Cr(III), Ga(III) and Al(III) unable to acquire Fe from NTA, EDTA or pyoverdin complexes.

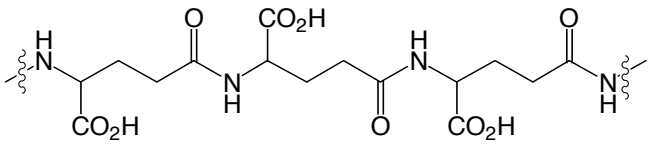
Characteristics required for complete translocation?

specific radii, trivalent charge, specific molecular conformation, neutral molecular charge, metal reduction, ligand exchange (solution or membrane)….

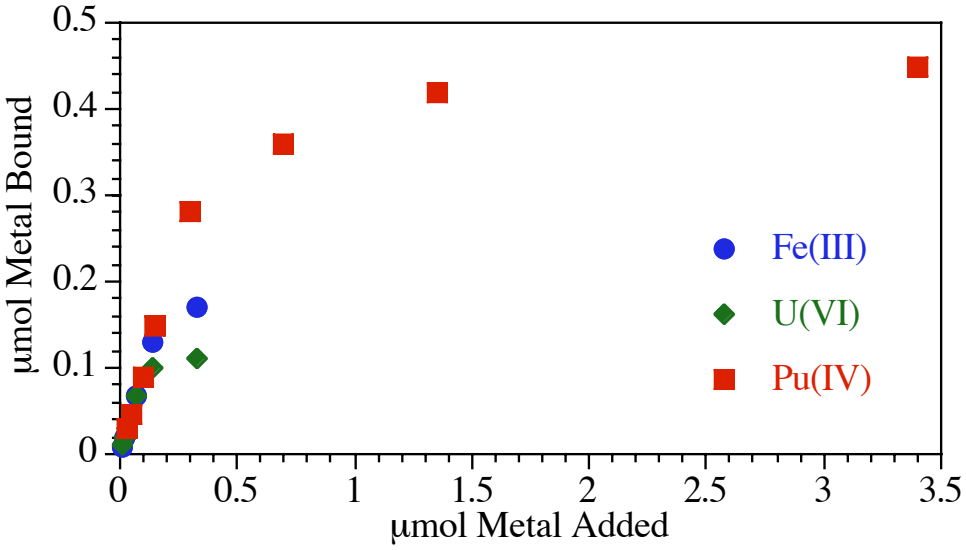
Pu(IV) and Th(IV) uptake reveal combination of ligand exchange and reduction is key

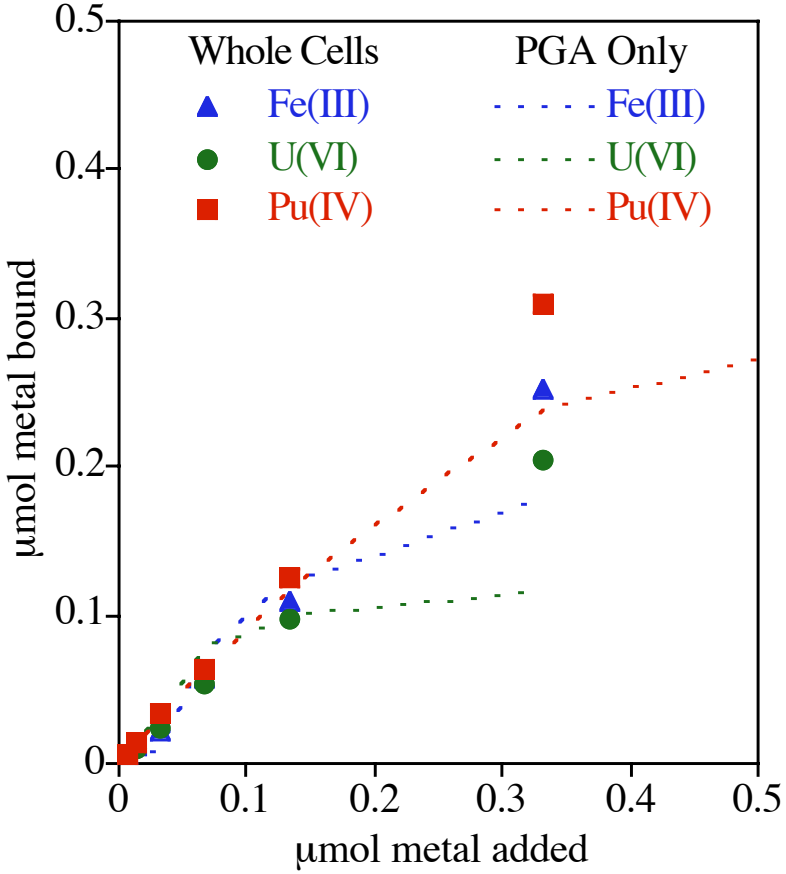
Metal Binding of Microbial Extracellular Polymers

PGA of *B. licheniformis*



~800 kDa, forms soluble metal complexes,generally >10:1 glu to M





~0.12 mmol metal bound per mg PGA (alone)

Pu(IV) remains associated with PGA during repeated pH cycling 2-12

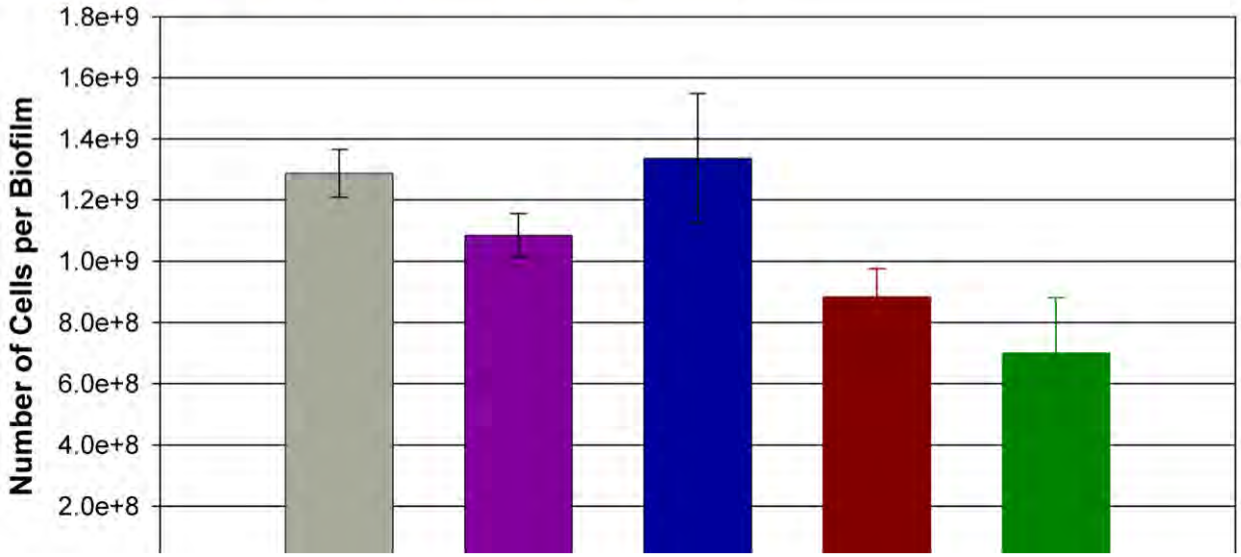
Whole cells (in culture media) take up more Pu per mass than does polyglutamate

Siderophores and EPS associate Pu with cells

Effect of U(VI) on *P. putida* Biofilms

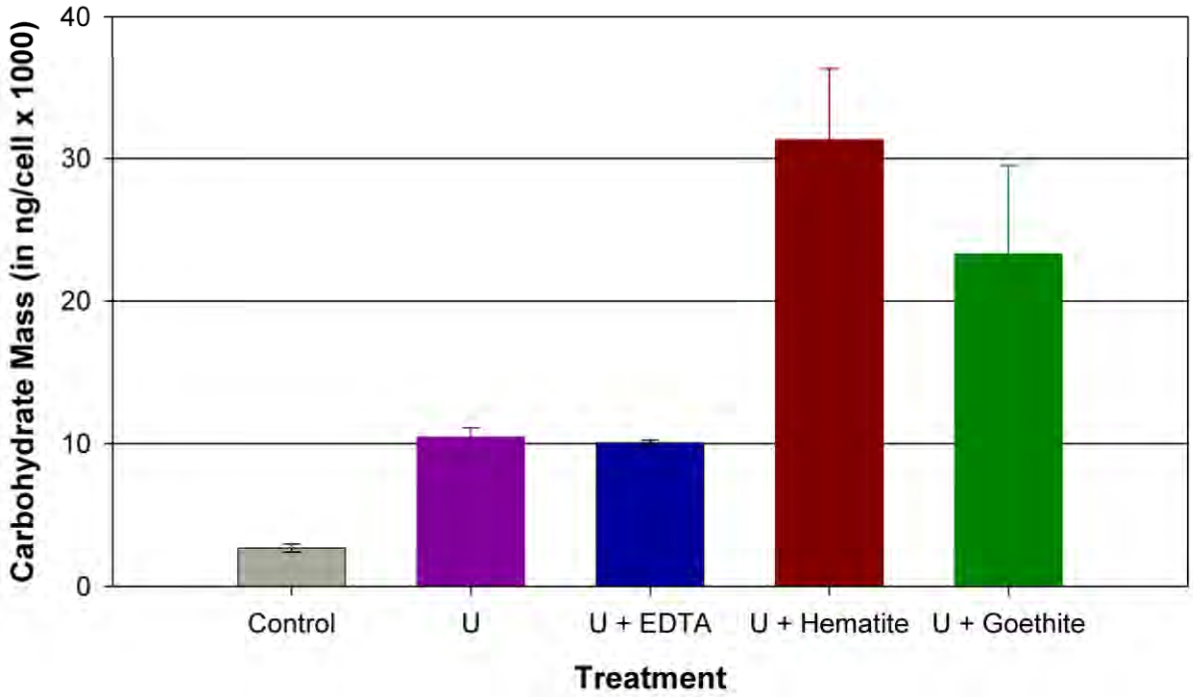
P. Putida grown on membrane discs on U-containing agar with minimal nutrients

[U] = 10^{-4} M
or 50 μg/g
72 hr growth
Fractionation by cent.

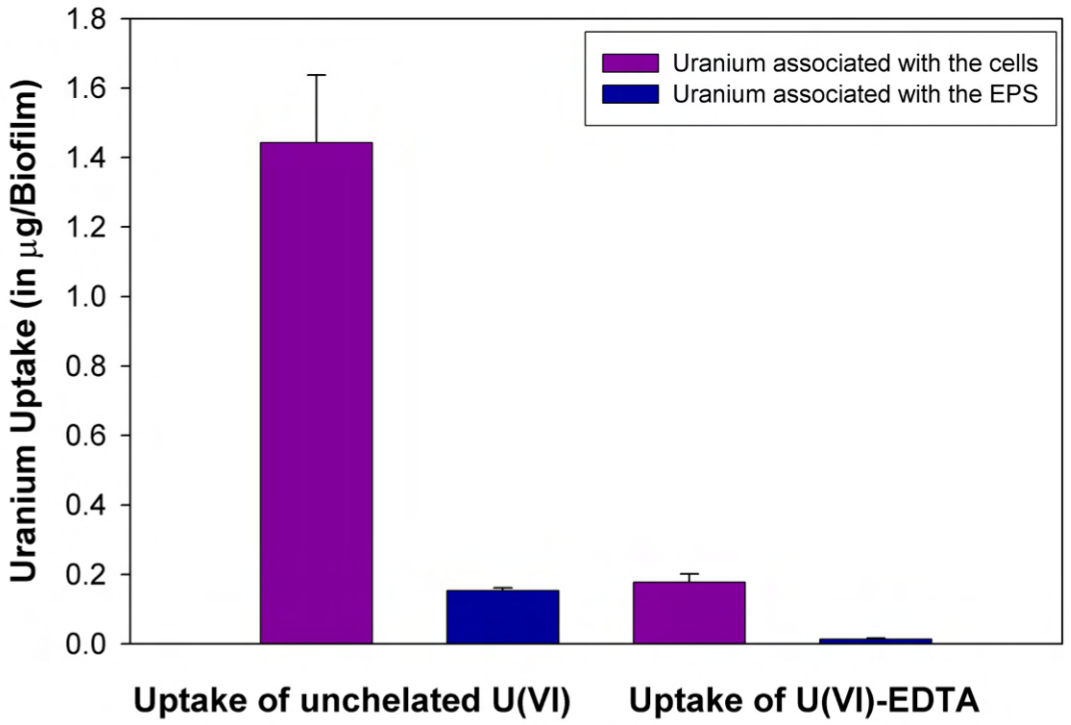


EPS, but not cell growth, affected by U.

U(VI) adsorbed onto Fe(III) minerals increased EPS produced by *P. putida*



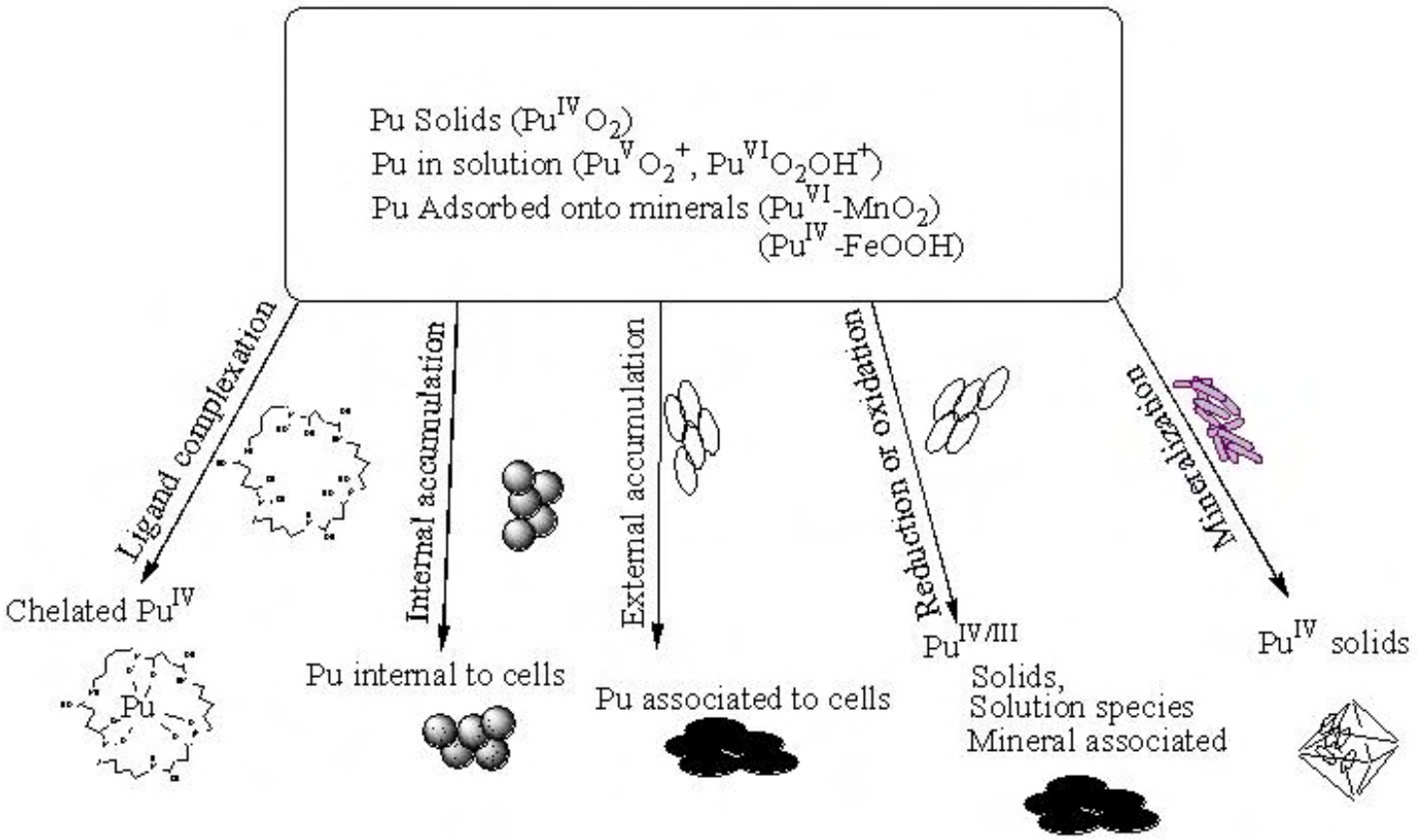
Distribution of U(VI) within *P. putida* Biofilms



Less U removed from substrate by P. *putida* in the presence of EDTA

U preferentially associated with cell fraction

Pu Biogeochemistry



Advances in Pu aqueous geochemistry

Solubilization, biosorption, bioaccumulation, mineralization biotransformation mechanisms all affect Pu

Stability of products, rates of combined processes,…?

Acknowledgements

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