Reduction of Uranium by Clostridium sp. in Acidic Groundwater Samples at the Oak Ridge Field Research Center

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Introduction

We tested the ability of *Clostridium* sp., a fermentative bacterium capable of metal reduction, to reduce uranium under conditions found at the FRC site.

Methodology

Groundwater samples. FW-024-000233 and FW-026-000343 collected from Area 3 at the FRC site were characterized for pH, Al, Ca, Cl, Fe, K, Mg, Mn, Na, Ni, NO_3^-, SO_4^{2-}, Tc, U, and total and inorganic carbon.

Uranium speciation: Determined by KPA, XPS, XANES, and EXAFS analysis.

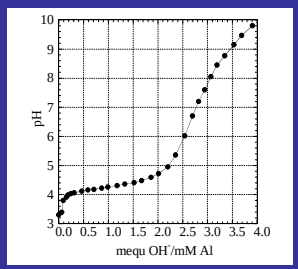
Microbial experiments. The ability of indigenous bacteria to reduce U was tested on "as received" and pH adjusted groundwater.

- The ability of *Clostridium* sp. to reduce U was tested in the presence of various concentrations of groundwater, uranium, and aluminum.

Chemical Characterization of FRC Groundwater

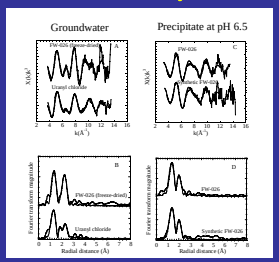
Parameter	"as received"	pH adjusted
	(mM)	
pH	3.4	6.5
Al	17.5	0.37
Ca	22.4	20.8
Cl*	7.02	na
Fe*	0.04	na
K*	2.40	na
Mg	6.38	6.09
Mn*	2.33	na
Na*	36.0	na
Ni*	0.21	na
NO_3^-*	121	na
SO_4^{2-}*	10.1	na
Tc*	1.1×10^{-4}	na
U	0.18	<0.002
TOC*	71 ppm	na
TIC*	273 ppm	na

Groundwater is acidic (pH 3.4); contains high levels of Al, U, NO_3^-. Adjustment to pH 6.5 results in precipitation of Al and U.



Potentiometric titration of FW-024 groundwater indicates a buffer region between pH 4 and 5 due to formation of solid-phase Gibbsite [$Al(OH)_3$] species. At pH 9.0 Al precipitated as a whitish floc.

EXAFS Analysis



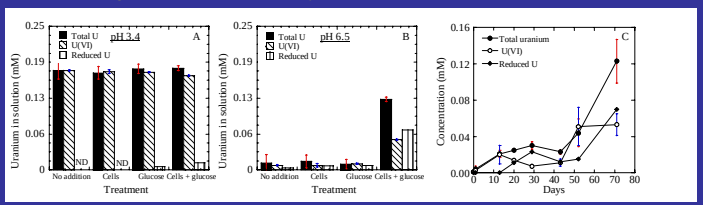
EXAFS analysis of freeze-dried FRC groundwater shows formation of uranyl chloride species (A, B); FRC precipitate formed at pH 6.5 indicates association of hydrated uranyl ion in coprecipitated form with Al (C, D). Experimental data (-); fitted data (---).

Fitting parameters for EXAFS data

Sample	Atom	N	R(Å)	σ^2	E_0
Uranyl chloride	UO_{ax}	2.0	1.76±0.01	0.003±0.001	12.2±1.0
	UO_{eq}	2.0±0.6	2.39±0.02	0.006±0.001	10.5±2.3
	U-Cl	3.0±1.0	2.72±0.02	0.009±0.002	10.1±3.3
FRC groundwater (FW-026)*	UO_{ax}	2.0	1.76±0.01	0.002±0.001	11.3±0.9
	U-Cl	4.4±1.2	2.69±0.03	0.006±0.002	10.2±1.9
Synthetic groundwater (pH 6.5)	UO_{ax}	2.0	1.80±0.02	0.002±0.001	9.8±1.2
	UO_{eq}	5.5±1.1	2.40±0.02	0.008±0.003	9.3±1.5
pH 6.5 adjusted groundwater	UO_{ax}	2.0	1.81±0.02	0.002±0.001	10.5±0.9
	UO_{eq}	5.3±1.5	2.40±0.03	0.009±0.004	10.9±0.6

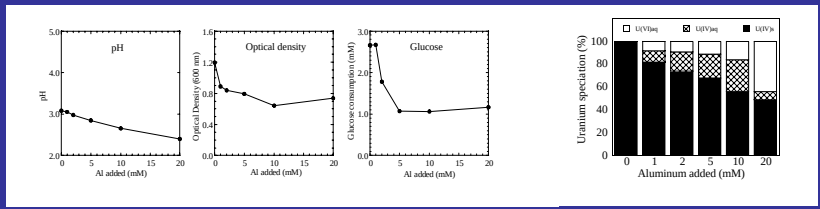
EXAFS fitting parameters show association of uranium in freeze-dried groundwater with chloride and in coprecipitated form with Al following pH adjustment.

Effect of Indigenous Bacterial Activity on Uranium Reduction in FRC Groundwater



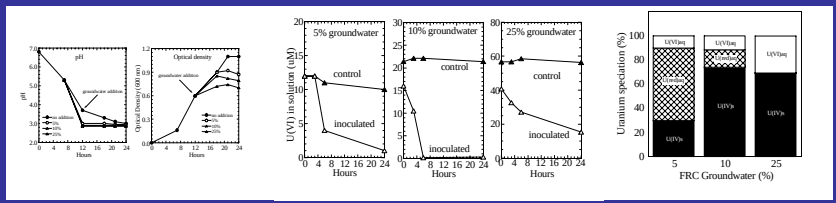
Uranium reduction was not detected by indigenous bacterial activity at pH 3.5 up to 73 days (A). In the pH adjusted samples the indigenous bacteria reduced 2 to 4% of the total uranium to U(IV) (B). *Clostridium* sp. in the presence of glucose enhanced reduction and solubilization of uranium in the acidic and the pH adjusted sample (C).

Effect of Aluminum Addition on Uranium Reduction by *Clostridium* sp.



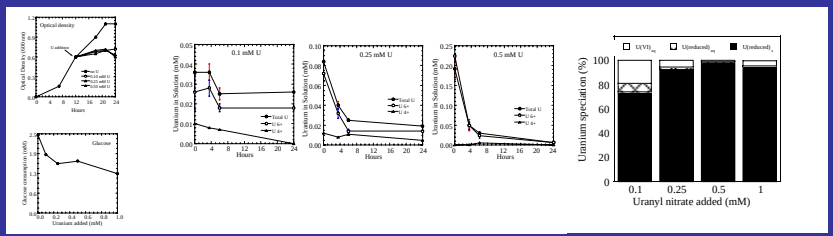
Addition of aluminum chloride to growing culture of *Clostridium* sp. inhibited growth of bacteria due to low pH as shown by OD and glucose consumption; however, uranium reduction occurred at up to 20 mM Al. The partitioning of uranium between the solution and solid phase was affected with increasing uranium solubilization observed at up to 10 mM Al.

Effect of FRC Groundwater on Uranium Reduction by *Clostridium* sp.



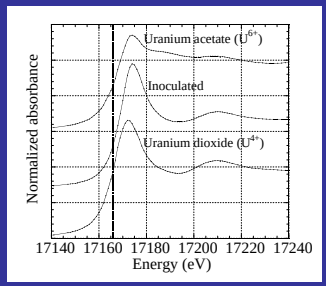
Addition of varying amount of FRC groundwater to actively growing culture of *Clostridium* sp. resulted in a decrease in pH to <3 and decrease in bacterial growth (OD). Rapid reduction and precipitation of uranium was observed in all treatments.

Effect of *Clostridium* sp. on Speciation of Uranium Added as Uranyl Nitrate



Growth of *Clostridium* sp. was affected with increasing addition of U. U(VI) was reduced to U(IV) in the presence of up to 1.0 mM U and it precipitated from solution.

XANES Analysis of Uranium Reduction by *Clostridium* sp.



XANES analysis shows shift in absorption edge for uranium from 17168 to 17166 eV in the presence of *Clostridium* sp. indicating reduction of U(VI) to U(IV).

Summary

- Uranium is present in FRC groundwater as hexavalent form.
- Indigenous bacteria at pH 3.4 do not reduce U. In pH adjusted (pH 6.5) sample 2-4% U is reduced.
- *Clostridium* sp. reduced up to 90% of the U present in FRC groundwater to U(IV) at pH <3.0.
- Nitrate and pH do not interfere in the reduction of U by *Clostridium* sp.
- The mechanism of U reduction by *Clostridium* sp. appears to be direct enzymatic action.