Supporting Information

Anaerobic biodegradation of citric acid in the presence of Ni and U at alkaline pH; impact on metal fate and speciation

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Section S1. Additional pH, anion and ICP-MS data from the Ni and U experiments

Figure S1. Full geochemical dataset from the 0.01 mM (green), 0.1 mM (blue) and 1 mM (purple) Ni/nitrate-reducing experiments. Solid colour lines – inoculated, dashed grey lines – sterile (no-inoculum) control.



Figure S2. Full geochemical dataset from the 0.01 mM (green), 0.1 mM (blue) and 1 mM (purple) Ni/sulfate-reducing experiments. Solid colour lines – inoculated, dashed grey lines – sterile (no-inoculum) control.



Figure S3. Geochemical data for the U/nitrate-reducing systems, green = 0.01 mM, blue = 0.1 mM, purple = 1 mM, dashed grey = sterile (autoclaved cells). Columns left to right are pH, citrate concentration, nitrate/nitrite concentration. Error bars represent 10 of triplicate samples.



Figure S4 ICP-MS data for the start and end point samples from the U/nitrate-reducing experiments. From left to right 0.01 mM U (green), 0.1 mM U (blue, and 1 mM U (purple). Data indicate no significant change in aqueous U concentration. Error bars represent 1 o of triplicate samples.

Section S2. Geochemical Modelling

To aid in determining the Ni and U speciation in experiments, geochemical modelling was used. Figure 1 summarises the key Ni/U and citrate species predicted by the model for the 0.01, 0.1, or 1 mM added metal systems at the start of the experiment. The speciation predicted by the model did not change significantly based on the electron acceptor added (nitrate or sulfate).



Figure 5. Geochemical model outputs showing speciation vs pH for the Ni (top) and U (bottom) experiments: 0.01 mM (left), 0.1 mM (middle) and 1 mM (right).

Dis	tribution	of species	Saturation	indices	
Spec	ies	Molality	Phase	SI**	log IAP
Ni Ni(C Ni+2 Ni(C Ni(C Ni(F Ni(S NiC] Ni(N Ni(S Ni2(Ni2(Ni4(1. (03) (H)2 (03)2-2 (H)+ (C03)+ (C03)+ (O3)+ (O4)2-2 (OH)+3 (O3)2 (OH)4+4	2000e-05 6.051e-06 1.871e-06 3.206e-07 2.654e-07 2.352e-07 6.493e-08 1.904e-09 4.134e-10 1.239e-10 7.188e-14 2.877e-15 1.002e-15	Bunsenite Ni(CO3)(cr) Ni(CO3):5.5H2O(cr) Ni(cr) Ni3(PO4)2(cr) Ni3(PO4)2(cr) Ni304(s) NiCl2(s) NiCl2:2H2O(s) NiCl2:4H2O(s) NiCl2:6H2O(s)	-0.80 1.37 -2.02 -22.20 0.70 -1.95 -5.02 -19.15 -15.29 -14.10 -13.27	11.98 -9.57 -9.57 -14.02 11.98 8.87 61.94 -10.23 -10.23 -10.23 -10.23

------Distribution of species------Saturation indices-----

				Phase	SI**	Log IAP	
Species	5	Molality	Activity	Clarkeite	-0.88	8.84	
				Metaschoepite	-3.64	1.71	
U(6)	1.000e-0	15		Na2U207:H20(cr)	-6.72	17.68	
U02(C03	3)3-4	9.937e-06	1.391e-06	Rutherfordine	-5.38	-20.13	
U02(C03	3)2-2	6.235e-08	3.811e-08	Sodium-compreignacite	-16.44	24.50	
U02(0H)	3-	1.490e-10	1.306e-10	U207Na2(s)	-5.43	17.68	
U02(C03	3)	6.188e-11	6.188e-11	U02(C03)3Na4(cr)	-8.10	-35.28	
(U02)2((CO3)(OH)3-	5.832e-11	5.158e-11	U02(OH)2(beta)	-3.39	1.71	
U02(0H)	2	2.122e-11	2.122e-11	UO3(alfa)	-8.09	1.71	
U02(0H))+	2.373e-13	2.124e-13	UO3(beta)	-6.85	1.71	
U02(0H)	4-2	1.068e-13	6.535e-14	U03:0.9H20(s)	-3.46	1.71	
(U02)3((CO3)6-6	1.520e-16	1.846e-18	UO4Na2(alfa)	-14.57	15.97	
U02+2		8.224e-17	5.090e-17				
U02(N03	3)+	2.840e-19	2.513e-19				
(U02)3((OH)7-	1.965e-19	1.738e-19				
U02C1+		7.129e-20	6.308e-20				
(U02)3((OH)5+	1.806e-20	1.615e-20				
(U02)2((OH)2+2	7.210e-21	4.467e-21				
U02C12		2.858e-24	2.858e-24				
(U02)4((OH)7+	9.516e-25	8.416e-25				
(U02)3((OH)4+2	1.344e-25	8.369e-26				
(U02)3((CO3)(OH)3+	5.625e-26	4.974e-26				
(U02)2((OH)+3	1.414e-26	4.680e-27				

Figure S6. PHREEQC outputs for Top right: 0.01 mM Ni/nitrate-reducing experiments, after 0.8 mM citrate has been degraded to produce 4.8 mM HCO₃⁻. Top left- aqueous Ni speciation at pH 9, top right- PHREEQC output showing saturation indices for relevant solid Ni phases (positive saturation index indicates the solution is oversaturated and a solid phase is expected to precipitate). Bottom left: 0.01 mM U/nitrate-reducing experiments, after 0.7 mM citrate was degraded to produce 4.2 mM HCO₃⁻. Bottom right: PHREEQC output showing saturation indices for relevant solid U phases (positive saturated and a solid phase is expected to precipitate).

Table S1. PHREEQC predicted species distribution as a % of total citrate or Ni or U species at the start of each experiment (T_0) . Also includes saturation indices for U species predicted to form, where positive saturation index indicates the solution is oversaturated and a solid phase is expected to precipitate.

		% citrate species			_{i)} or U _(aq) spe	cies
	0.01 mM Ni	0.1 mM Ni	1 mM Ni	0.01 mM Ni	0.1 mM Ni	1 mM Ni
[Cit] ³⁻	99	90	4	-	-	-
[Ni(Cit)] ⁻	1	9	92	91	89	92
[Ni(Cit) ₂] ⁴⁻	0	1	1	9	11	1
other	-	-	3	-	-	3

	0.01 mM	0.1 mM U	1 mM U	0.01 mM U	0.1 mM U	1 mM U
	U					
[Cit] ³⁻	99	99	99	-	-	-
[UO ₂ (Cit)] ⁻	0.03	0.08	0.1	3	0.8	0.2
$[(UO_2)_2(Cit)_2]^{2-1}$	0	0	0	0	0	0
[(UO ₂) ₃ (OH) ₇] ⁻	-	-	-	17	22	19
[UO ₂ (OH) ₃] ⁻	-	-	-	31	7	1
other	-	-	-	3	7	11

Uranium saturation indices at T0, pH 9 (before cells added)			
Phase	SI		
Clarkeite	3.39		
Metaschoepite	0.67		
$Na_2U_2O_7:H_2O(cr)$	1.81		
Sodium-compreignacite	9.33		
$U_2O_7Na_2(s)$	3.1		
$UO_2(OH)_2(beta)$	0.92		
UO ₃ (alfa)	-3.78		
UO ₃ (beta)	-2.54		
UO ₃ :0.9H ₂ O(s)	0.85		
UO₄Na₂(alfa)	-10.35		

	0.01 mM Ni
	(with 0.01 mM Cit ³⁻ & 1 mM S ²⁻)
Aqueous species (mM)	
[Cit]3-	0.006
[Ni(Cit)]-	0.004
Sulfide species (S ₅ ²⁻ , S ₄ ²⁻ S ₃ ²⁻ , HS ⁻)	0.12
Saturation indices	
Millerite (NiS)	-3.2
Vaesite (NiS ₂)	5.7
Polydymite (Ni ₃ S ₄)	0.48
Heazlewoodite (Ni ₃ S ₂)	-24
Ni(OH) ₂	0.36
NiCO ₃	1.1
Ni ₃ (PO ₄) ₂	-2.3

Table S2. PHREEQC predicted aqueous Ni, citrate and sulfide species distribution and relevant saturation indices for the end point samples from the $0.01 \text{ mM Ni/SO}_4^{2^\circ}$ reducing experiment.

Section S3. ESI-MS data from Ni experiment



Figure S7. Mass spectra and model isotope pattern in the 246.8 - 247.0 m/z region (by ESI-MS) relevant to the chemical species [Ni(Cit)]⁻ (246.9389 g mol⁻¹) for the nitrate-reducing/0.01 mM Ni system. The peak representing [Ni(Cit)]⁻ is highlighted in green. Bottom: isotope pattern showing expected peak for [⁵⁸Ni(Cit)]⁻ species; middle: spectrum of sample taken from un-inoculated media on day 0 (T_0), top: spectrum of sample taken on day 162 (T_{162}). The peak representing [Ni(Cit)]⁻ in the T_0 sample matched the isotope pattern with $\Delta ppm = -4$), this peak was not identified in the T_{162} sample.



Figure S8 Mass spectra and model isotope pattern in the 246.8 - 247.0 m/z region (by ESI-MS) relevant to the chemical species [Ni(Cit)]⁻ (246.9389 g mol⁻¹) for the sulfate-reducing/0.01 mM Ni system. The peak representing [Ni(Cit)]⁻ is highlighted in green. Bottom: isotope pattern showing expected peak for [⁵⁸Ni(Cit)]⁻ species; middle: spectrum of sample taken from un-inoculated media on day 0 (T_0), top: spectrum of sample taken on day 100 (T_{100}). The peak representing [Ni(Cit)]⁻ in the T_0 sample matched the isotope pattern with $\Delta ppm = -4$), this peak was not identified in the T_{100} sample



Figure S9 Mass spectra and model isotope pattern in the 246.8 - 247.0 m/z region (by ESI-MS) relevant to the chemical species [Ni(Cit)]- (246.9389 g mol-1) for the sulfate-reducing/0.1 mM Ni system. The peak representing [Ni(Cit)]- is highlighted in bl

Section S4. Electron microscopy data from the Ni experiments



Figure S10. SEM image and element maps (Ni, S, Ca, O) of precipitates from inoculated 0.01 mM and 0.1 mM Ni/sulfatereducing experiment. Co-location of Ca, S and O on the element maps suggest $CaSO_4$ crystals had formed in both systems. Indeed, low levels of calcium were present in the experiment from the mineral mix added to the growth medium. The data from the 0.1 mM Ni system did not indicate accumulation of Ni.



Figure S11. Electron microscopy data showing the morphology and composition of black precipitate samples obtained from the inoculated 0.01 mM Ni/ sulfate-reducing systems. Panel A: Low and high magnification SEM images (top left) and EDS spectra for region [1] (bottom left); Panel B: TEM image (top right), EDS spectra for region [2] and element wt % (bottom right).

Section S5. EXAFS fitting parameters for the U experiments

Table S3. Fitting parameters for non-crystalline U(IV) model. All coordination numbers were fixed, and best fits were reached through manually varying these numbers. Amplitude reduction factor $(S_0^2) = 0.8$; Shift in the Fermi level $(E_0) = 5.5(11)$; R-factor = 0.009; Bond valence sum (BVS) = 3.90; Fitting windows: k-range = 3 - 12 and R-range = 1.3 - 4.2. F-test result for addition of P1 and P2 shells shows a statistically significant better fit for the data than for nano-uraninite (Downward et al., 2007).

Path	N	σ²	R (Å)	F-test (%)
01	4.5	0.006(1)	2.31(1)	-
02	3.5	0.008(3)	2.49(2)	99.9
P1	1.5	0.011(4)	3.14(3)	95.0
P2	1	0.006(5)	3.64(5)	100.0
U1	2.5	0.012(4)	3.84(3)	99.3
01 MS	24	0.016	3.97	-

Table S4. Fitting parameters for nano-uraninite model. All coordination numbers were fixed, and best fits were reached through manually varying these numbers. $S_0^2 = 0.8$; $E_0 = 3.1(39)$; R-factor = 0.0524; BVS = 4.02; Fitting windows: k-range = 3 – 12 and R-range = 1.3 - 4.2.

Path	Ν	σ²	R (Å)
01	4.5	0.008(2)	2.32(2)
02	3.5	0.031(24)	2.43(19)
U1	2.5	0.009(4)	3.80(5)
01 MS	24	0.062	3.86

References

Downward, L.; Booth, C. H.; Lukens, W. W.; Bridges, F. A (2007) Variation of the F-Test for Determining Statistical Relevance of Particular Parameters in EXAFS Fits. In AIP Conference Proceedings; AIP, 2007; Vol. 882, pp 129–131.