

1 **Supporting Information**

2

3 **Influences of Organic Carbon Supply Rate on Bioreduction of Uranium in Contaminated**
4 **Sediment**

5 Tetsu K. Tokunaga, Jiamin Wan, Yongman Kim, Rebecca A. Daly, Eoin L. Brodie, Terry C.
6 Hazen, Don Herman, and Mary K. Firestone.

7

8 **Table S1.** Chemical composition of sediment.

9 **Table S2.** Characteristic chemical composition of sediment column effluent solutions.

10 **Table S3.** Thermodynamic constants of major U species.

11 **Table S4.** Surface complexation reaction constants.

12 **Table S5.** Identified iron-reducing bacteria used for NMDS ordination.

13 **Table S6.** Identified sulfate-reducing bacteria used for NMDS ordination.

14 **Figure S1.** Nonmetric multidimensional scaling (NMDS) ordinations of hybridization intensity
15 scores for detected bacteria.

16 **DNA extraction procedure.**

17 **References for Supporting Information**

Mg	Al	Si	K	Ca	CaCO ₃	Ti
%	%	%	%	%	%	%
0.78	11.57	29.80	3.63	0.37	0.20 ±0.10	0.52
Mn	Fe: Total				P	Sr
mmol/kg	mmol/kg	HCl-extr. mmol/kg	Hydr-red. mmol/kg	citr-dith. mmol/kg	mg/kg	mg/kg
49.1	1,185	0.23	5.91	248	179	69.9
U: Total	exchang.	OC	pH			
mmol/kg	mmol/kg	mmol/kg	-			
1.08	0.64	369	7.8			

19

20 **Table S1.** Major elemental composition (X-ray fluorescence analyses), calcium carbonate
 21 equivalent (pressure calcimetry with 2 M HCl), (bi)carbonated exchangeable U, 0.5 M HCl-
 22 extractable Fe²⁺, hydroxylamine reducible Fe³⁺, citrate-dithionite extracted Fe, and pH (1:1
 23 sediment:water extract) of the Area 2 sediment. Note that the extractable Fe fractions are
 24 relatively small.

	OC <u>in</u>	Effluent							
		pH	HCO ₃ ⁻	pCO ₂	Ca	Mn	Fe	S	U
	mM		mM		mM	μM	μM	mM	μM
PV = 2	0					1	n.d.	0.70	10
	3					n.d.	n.d.	0.90	12
	10					125	n.d.	1.01	12
	30					39	n.d.	0.50	44
	100					790	n.d.	0.11	37
PV = 4	0				3.6	3	0.9	1.04	5
	3				1.7	66	1.6	0.61	6
	10				1.7	47	1.6	0.64	17
	30				3.3	170	1.8	0.20	13
	100				4.9	260	3.8	n.d.	2
PV = 5.8	0				1.9	1	n.d.	0.37	7
	3				1.7	10	n.d.	0.17	8
	10				1.7	17	0.2	0.09	18
	30				2.6	48	6	0.011	0.7
	100				4.3	23	9	0.025	0.7
PV =12.8	0		2	10 ^{-2.3}	1.7	1	n.d.	0.56	5
	3		4	10 ^{-2.1}	2.0	8	n.d.	0.12	6
	10		6	10 ^{-1.9}	1.2	15	8	0.01	28
	30		16	10 ^{-1.6}	0.4	19	7	n.d.	0.6
	100		50	10 ^{-1.1}	0.4	8	9	n.d.	2.5

26 **Table S2.** Characteristic concentrations of elements/species in column effluent solutions. Ca
 27 values are interpolated from measurements at nearest PV values.

28 Log P_{CO2} values for data were estimated based on carbonate equilibrium as

29
$$\text{Log } P_{\text{CO}_2} = -\text{pH} + (\log [\text{HCO}_3^-]) / (\text{K}_{\text{CO}_2} \text{K}_1) \quad ,$$

30 where $\text{K}_{\text{CO}_2} = 10^{-1.47}$ is the Henry's law constant for CO₂ at 25°C, and $\text{K}_1 = 10^{-6.35}$ is the first
 31 dissociation constant for H₂CO₃.

species	ΔG_f° kJ/mol	source
UO_2^{2+}	-952.6	G
$\text{UO}_2\text{CO}_3^\circ$	-1537.2	G
$\text{UO}_2(\text{CO}_3)_2^{2-}$	-2103.2	G
$\text{UO}_2(\text{CO}_3)_3^{4-}$	-2660.9	G
$(\text{UO}_2)_2\text{CO}_3(\text{OH})_3^-$	-3139.5	G
$\text{CaUO}_2(\text{CO}_3)_3^{2-}$	-3231.8	DB
$\text{Ca}_2\text{UO}_2(\text{CO}_3)_3^\circ$	-3817.1	DB
$\text{UO}_2(\text{am})$	-995.8	G
U^{4+}	-529.9	G
$\text{U}(\text{OH})_4(\text{aq})$	-1421.3	G
H_2O	-237.14	G
OH^-	-157.22	G
HCO_3^-	-586.85	G
CO_3^{2-}	-527.9	G
Ca^{2+}	-552.81	G

33

34 **Table S3.** Thermodynamic constants for major U species (298.15 K).

35 Sources: (G) Guillaumont et al. (s1), (DB) from log K of Dong and Brooks (s2), combined with

36 ΔG_f° values from (s1).

U(VI) surface complexation reaction	log K (I =0)	source
$\text{Hfo_sOH} + \text{H}^+ = \text{Hfo_sOH}_2^+$	7.18	DM, PA
$\text{Hfo_sOH} = \text{Hfo_sO}^- + \text{H}^+$	-8.82	DM, PA
$\text{Hfo_wOH} + \text{H}^+ = \text{Hfo_wOH}_2^+$	7.18	DM, PA
$\text{Hfo_wOH} = \text{Hfo_wO}^- + \text{H}^+$	-8.82	DM, PA
$\text{Hfo_sOH} + \text{Ca}^{+2} = \text{Hfo_sOHCa}^{+2}$	4.97	DM, PA
$\text{Hfo_wOH} + \text{Ca}^{+2} = \text{Hfo_wOCa}^+ + \text{H}^+$	-5.85	DM, PA
$\text{Hfo_sOH} + \text{CO}_3^{-2} + \text{H}^+ = \text{Hfo_sCO}_3^- + \text{H}_2\text{O}$	12.78	A
$\text{Hfo_wOH} + \text{CO}_3^{-2} + \text{H}^+ = \text{Hfo_wCO}_3^- + \text{H}_2\text{O}$	12.78	A
$\text{Hfo_sOH} + \text{CO}_3^{-2} + 2\text{H}^+ = \text{Hfo_sHCO}_3 + \text{H}_2\text{O}$	20.37	A
$\text{Hfo_wOH} + \text{CO}_3^{-2} + 2\text{H}^+ = \text{Hfo_wHCO}_3 + \text{H}_2\text{O}$	20.37	A
$2\text{Hfo_sOH} + \text{UO}_2^{+2} = (\text{Hfo_sO})_2\text{UO}_2 + 2\text{H}^+$	-2.35	P
$2\text{Hfo_sOH} + \text{UO}_2^{+2} + \text{CO}_3^{-2} = (\text{Hfo_sO})_2\text{UO}_2\text{CO}_3^{-2} + 2\text{H}^+$	4.33	P
$2\text{Hfo_wOH} + \text{UO}_2^{+2} = (\text{Hfo_wO})_2\text{UO}_2 + 2\text{H}^+$	-6.06	P
$2\text{Hfo_wOH} + \text{UO}_2^{+2} + \text{CO}_3^{-2} = (\text{Hfo_wO})_2\text{UO}_2\text{CO}_3^{-2} + 2\text{H}^+$	-0.24	P
sites	$\text{m}^2 \text{g}^{-1}$	moles
Hfo_sOH	600	1.96e-3
Hfo_wOH	600	7.83e-2

38

39 **Table S4.** Surface complexation reaction constants.

40 Sources: (DM) Dzombak and Morel (s3), (PA) Parkhurst and Appelo (s4), (A) Appelo et al.

41 (s5), (P) Payne (s6).

Phylum	Class	Order	Family	Sub-family	Rep OTU	Rep organism
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	sf_14	386	Geothrix fermentans
Deferribacteres	Deferribacter	Unclassified	Unclassified	sf_1	797	Deferribacter abyssi str. DR
Firmicutes	Clostridia	Clostridiales	Peptococc/Acidaminococc	sf_11	212	Desulfitobacterium frappieri str. TCP-
Firmicutes	Clostridia	Clostridiales	Peptococc/Acidaminococc	sf_11	816	Desulfitobacterium frappieri
Firmicutes	Clostridia	Clostridiales	Peptococc/Acidaminococc	sf_11	832	Desulfitobacterium hafniense str. DCB-2
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	sf_1	9711	Desulfovibrio profundus str. DSM 11384
Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonaceae	sf_1	10166	Pelobacter propionicus str. OttBd1 DSM2379
Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	sf_1	10209	Geobacter brementis str. Dfr1
Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	sf_1	10216	Geobacter pelophilus str. Dfr2
Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	sf_1	10288	Geobacter metallireducens str. GS-15
Proteobacteria	Deltaproteobacteria	Myxococcales	Anaeromyxobacter	sf_1	10088	Anaeromyxobacter dehalogenans str. 2CP-3
Proteobacteria	Gammaproteobacteria	Acidithiobacillales	Acidithiobacillaceae	sf_1	8249	Acidithiobacillus ferrooxidans str. DSM 2392
Proteobacteria	Gammaproteobacteria	Acidithiobacillales	Acidithiobacillaceae	sf_1	8552	Acidithiobacillus ferrooxidans str. D2
Proteobacteria	Gammaproteobacteria	Acidithiobacillales	Acidithiobacillaceae	sf_1	9497	Acidithiobacillus ferrooxidans str. ATCC 19859
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	sf_1	8916	Shewanella algae str. 43940
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	sf_1	9067	Shewanella algae str. ACM 4733
Proteobacteria	Gammaproteobacteria	Shewanella	Unclassified	sf_1	9344	Shewanella algae str. ATCC 51192
Thermodesulfobacteria	Thermodesulfobacteria	Thermodesulfobacteriales	Thermodesulfobacteriaceae	sf_1	667	Geothermobacterium ferrireducens

43 **Table S5.** Identified iron-reducing bacteria used for NMDS ordination. Representative OTUs may contain more than one sequence
44 and only a representative sequence is shown here. ProkMSA identifiers for all sequences present in each OTU can be found at
45 <http://greengenes.lbl.gov>.

Phylum	Class	Order	Family	Sub-family	Rep OTU	Rep organism
Firmicutes	Clostridia	Clostridiales	Peptococc/Acidaminococc	sf_11	177	Desulfosporosinus sp. str. PFB
Firmicutes	Clostridia	Clostridiales	Peptococc/Acidaminococc	sf_11	242	Desulfosporosinus orientis str. DSMZ 7493
Firmicutes	Clostridia	Clostridiales	Peptococc/Acidaminococc	sf_11	264	Desulfosporosinus sp. str. Blif
Firmicutes	Desulfotomaculum	Unclassified	Unclassified	sf_1	2351	Desulfotomaculum thermobenzoicum str. DSM 6193
Firmicutes	Desulfotomaculum	Unclassified	Unclassified	sf_1	2443	Desulfotomaculum thermoacetoxidans str. DSM 5813
Firmicutes	Desulfotomaculum	Unclassified	Unclassified	sf_1	2490	Desulfotomaculum solfataricum str. V21
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	sf_5	10046	Desulfobacterium cetonicum str. DSM 7267 oil recovery water
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	sf_5	10083	Desulfobacter curvatus str. DSM 3379
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfomicrobiaceae	sf_1	10079	Desulfomicrobium baculatum str. DSM 1742
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfomicrobiaceae	sf_1	10370	Desulfomicrobium baculatum str. X; VKM B-1378; DSM 4
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	sf_1	10059	Desulfovibrio hydrothermalis str. AM13
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	sf_1	10071	Desulfovibrio desulfuricans
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	sf_1	10075	Desulfovibrio sp. str. ABHU2SB
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	sf_1	10248	Desulfovibrio giganteus str. DSM 4370
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	sf_1	10262	Desulfovibrio sp. str. Ac5.2
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	sf_1	10263	Desulfovibrio brasiliensis str. LVform1
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	sf_1	10292	Desulfovibrio subsp. desulfuricans str. MB; ATCC27774 subsp.
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	sf_1	10361	Desulfovibrio fairfieldensis
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	sf_1	9711	Desulfovibrio profundus str. DSM 11384
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	sf_3	10285	Desulfomonile limimaris str. DCB-F
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	sf_1	10294	Desulfacinum hydrothermale str. MT-96

47 **Table S6.** Identified sulfate-reducing bacteria used for NMDS ordination. Representative OTUs may contain more than one sequence
48 and only a representative sequence is shown here. ProkMSA identifiers for all sequences present in each OTU can be found at
49 <http://greengenes.lbl.gov>.

73

74 **DNA extraction.** Sediment samples were collected from the set of columns designated for
75 microbial analysis at 5.9 and 12.1 PV relative to OC start, near the outlet of the columns.
76 Triplicate sediment samples (0.5 g wet, frozen weight) were aliquoted into Lysing Matrix E
77 Tubes with 978 μ l Sodium Phosphate Buffer and 4 μ l Superase-In Rnase Inhibitor (Ambion,
78 Austin, TX), beat at 5.5 m/s for 30 s in a FastPrep Instrument (MP Biomedicals, Irvine, CA) and
79 centrifuged at 16.1k x g for 5 min at 4°C (all centrifugations were performed at this speed unless
80 otherwise stated). The supernatant was transferred to another tube, 300 μ l PPS reagent was
81 added to the supernatant, mixed by hand and centrifuged for 5 min at 4°C. The supernatant was
82 transferred to another tube and 0.8 volumes of isopropanol was added, mixed by hand, and
83 precipitated at -20°C overnight. The crude nucleic acid was pelleted by centrifugation for 15 min
84 at 4°C. The supernatant was removed, pellets were washed with cold (-20°C) 70% ethanol and
85 pelleted by centrifugation for 5 min at 4°C. The supernatant was removed and pellets air-dried
86 for 2 min and resuspended in 20 μ l nuclease-free water. Nucleic acids from triplicate samples
87 were pooled and DNA-RNA were separated and purified by adding 600 μ l buffer RLT Plus from
88 an AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA) and proceeding with the column
89 binding step. The remainder of the manufacturer's protocol was followed, excepted that DNA
90 was eluted from the column using 50 μ l of buffer EB. DNA was quantified by absorbance at
91 260nm using a NanoDrop ND-1000.

92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113

References for Supporting Information

(s1) Guillaumont, R.; Fanghanel, T.; Fuger, J.; Grenthe, I.; Neck, V.; Palmer, D. A.; Rand, M. H.; Mompean, F. J.; Illemassene, M.; Domenechi-Orti, C. Update on the Chemical Thermodynamics of Uranium, Neptunium, Plutonium, Americium, and Technicium. *Elsevier* **2003**.

(s2) Dong, W. M.; Brooks, S. C. Determination of the formation constants of ternary complexes of uranyl and carbonate with alkaline earth metals (Mg²⁺, Ca²⁺, Sr²⁺, and Ba²⁺) using anion exchange method. *Environmental Science and Technology* **2006**, *40*, 4689-4695.

(s3) Dzombak, D. A.; Morel, F. M. M. *Surface Complex Modeling- Hydrous Ferric Oxide.*; Wiley: New York, 1990.

(s4) Parkhurst, D. L.; Appelo, C. A. J. User's Guide to PHREEQC (Version 2)--A Computer Program for Speciation, Batch-Reaction, One-Dimensional Transport, and Inverse Geochemical Calculations. http://wwwbrr.cr.usgs.gov/projects/GWC_coupled/phreeqc/ **2005**.

(s5) Appelo, C. A. J.; van der Weiden, M. J. J.; Tournassat, C.; Charlet, L. Surface complexation of ferrous iron and carbonate on ferrihydrite and the mobilization of arsenic. *Environmental Science and Technology* **2002**, *36*, 3096-3103.

(s6) Payne, T. E. In *School of Civil and Environmental Engineering*; University of New South Wales, 1999; Vol. Ph. D., p 332.