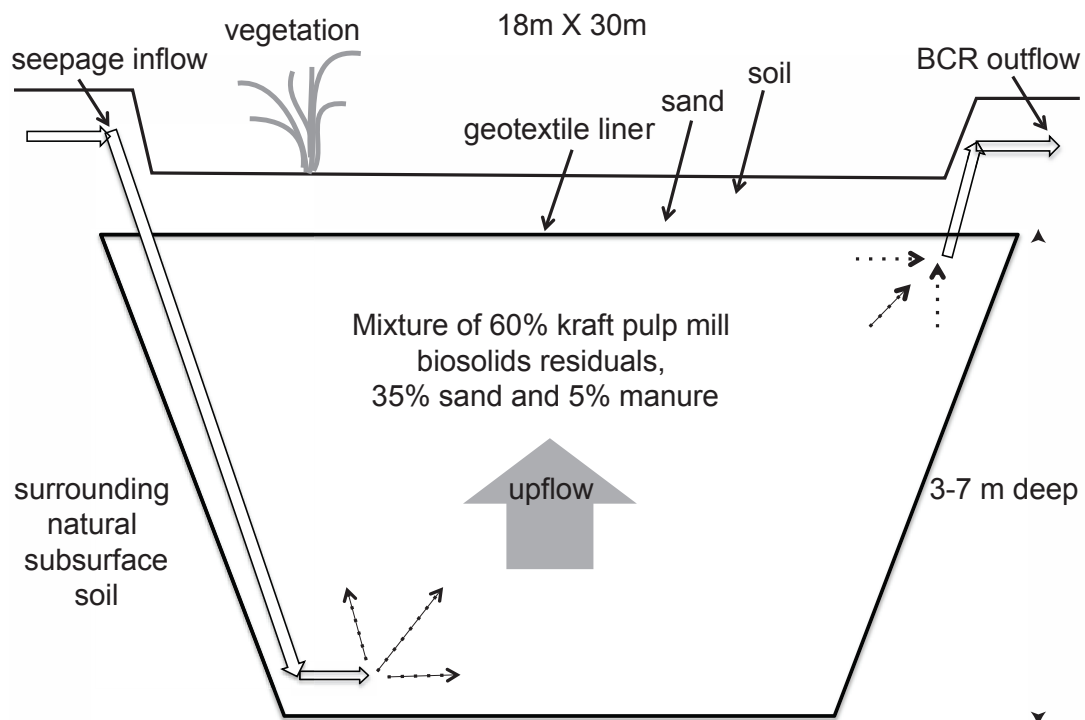


Supplemental Information

Figure S1. Schematic and photograph of the BCR. Diagram adopted from [1]. Photograph taken by Maryam Khoshnoodi on 21 April 2009.



Total Arsenic, Iron and Sulphur Concentrations Measured in the BCR Used to Obtain Concentration Ranges for As-O-H-S-Fe Geochemical Modeling

Table S1. Total arsenic, iron and sulphur in the BCR influent and effluent from 25 June 2008 to 2 October 2009.

Date	Total metal concentrations (mM)					
	BCR influent			BCR effluent		
	As	Fe	S	As	Fe	S
25 June 2008	0.534	0.041	8.33	0.095	0.287	9.38
3 July 2008	0.108	0.008	6.25	0.160	0.502	6.25
8 July 2008	0.071	0.233	6.25	1.028	0.323	6.25
16 July 2008	0.601	0.013	6.25	0.134	0.109	9.38
29 July 2008	0.347	0.008	8.33	0.028	0.066	9.38
13 August 2008	0.561	0.007	8.65	0.011	0.039	5.21
27 August 2008	0.174	0.102	7.29	0.016	0.065	9.38
10 September 2008	0.059	0.002	6.25	0.035	0.073	6.25
24 September 2008	0.160	0.004	6.25	0.587	1.971	6.25
8 October 2008	0.427	0.038	5.21	0.115	0.394	6.25
22 October 2008	0.547	0.109	5.21	0.019	0.030	6.25
5 November 2008	0.174	0.007	8.33	0.160	0.095	9.38
18 November 2008	1.135	0.573	7.29	0.012	0.057	10.42
2 December 2008	0.734	0.013	7.29	0.059	0.059	7.29
17 December 2008	0.267	0.052	6.25	0.079	0.090	7.29
15 January 2009	0.227	0.004	5.21	0.023	0.017	5.21
29 January 2009	0.174	0.008	4.17	0.019	0.010	4.17
11 February 2009	0.280	0.013	7.29	0.053	0.015	6.25
25 February 2009	0.214	0.018	6.67	0.064	0.116	7.08
10 March 2009	0.174	0.006	6.67	0.043	0.018	6.46
24 March 2009	0.320	0.358	6.56	0.507	2.509	7.92
6 April 2009	0.174	0.045	7.19	0.021	0.014	7.08
24 April 2009	2.537	0.048	6.67	0.016	0.006	8.44
5 May 2009	0.587	0.032	6.88	0.019	0.005	7.81
22 May 2009	0.467	0.079	6.67	0.025	0.020	7.08
5 June 2009	0.454	0.007	7.08	0.096	0.043	7.81
19 June 2009	0.387	0.006	6.98	0.020	0.006	7.50
30 June 2009	0.307	0.016	7.60	0.043	0.034	8.85
4 September 2009	0.614	0.065	8.54	0.017	0.011	9.17
2 October 2009	0.467	0.018	nd	0.075	0.039	nd
Maximum	2.537	0.573	8.646	1.028	2.509	10.417
Minimum	0.059	0.002	4.167	0.011	0.005	4.167
Average	0.443	0.064	6.814	0.119	0.234	7.428
Std. dev.	0.46	0.12	1.07	0.22	0.56	1.54

Table S2. Total arsenic, iron and sulphur in the BCR lysimeter.

Date	T/As (mM)	T/Fe (mM)	T/S (mM)
10 September 2008	0.008	0.048	3.13
08 October 2008	0.011	0.043	3.13
12 November 2008	0.010	0.057	3.85
10 December 2008	0.009	0.086	4.38
07 January 2009	0.008	0.077	4.17
13 February 2009	0.007	0.070	4.58
12 March 2009	0.006	0.057	4.58
08 April 2009	0.012	0.143	5.00
09 May 2009	0.010	0.104	5.00
12 June 2009	0.007	0.072	4.79
07 July 2009	0.005	0.034	4.79
10 August 2009	0.006	0.023	4.69
Maximum	0.012	0.143	5.000
Minimum	0.005	0.023	3.125
Average	0.008	0.068	4.340
Std. dev.	0.002	0.03	0.66

Table S3. Total arsenic, iron and sulphur in the borehole pore water.

Sample origin	Date	T/As (mM)	T/Fe (mM)	T/S (mM)
Hole1 pore water	24 July 2008	0.003	13.2 *	2.08
Hole2 pore water	24 July 2008	0.001	16.3 *	0.83
Hole3 pore water	24 July 2008	0.003	2.51	1.56
Hole1 pore water	21 April 2009	0.013	0.14	4.90
Hole2 pore water	21 April 2009	0.005	0.01	6.35
Hole1 pore water	21 October 2009	0.112	4.48	6.25
Hole2 pore water	21 October 2009	0.013	4.30	2.19
Hole3 pore water	21 October 2009	0.036	0.73	2.81
Maximum		0.112	4.48	6.35
Minimum		0.001	0.01	0.83
Average		0.023	2.03	3.37
Std. dev.		0.038	2.04	2.16

Note: * These numbers may be artificially high due to the close proximity of iron pipes.

Geochemical Modeling

Table S4. Aqueous and solid species equilibrium reactions and constants used for geochemical modeling of the As-O-H-S-Fe system with Spana. This is the input file. First column gives the chemical species, second column is the log(K) value and the final five columns give the stoichiometry in terms of the species H^+ , e^- , HS^- , $As(OH)_3$ and Fe^{2+} . Equilibrium constants for As species modified according to Tables 1 and 2 in [2].

Chemical species	log(K)	H^+	e^-	HS^-	$As(OH)_3$	Fe^{2+}
$H_2AsO_3^-$	-9.17	-1	0	0	1	0
$HAsO_3^{2-}$	-23.27	-2	0	0	1	0
AsO_3^{3-}	-38.27	-3	0	0	1	0
$H_4AsO_3^+$	-0.305	1	0	0	1	0
$As_3S_4(HS)_2^-$	72.23	5	0	6	3	0
$AsS(OH)(HS)^-$	18.008	1	0	2	1	0
$H_2AsO_4^-$	-21.65	-3	-2	0	1	0
$HAsO_4^{2-}$	-28.64	-4	-2	0	1	0
AsO_4^{3-}	-40.43	-5	-2	0	1	0
H_3AsO_4	-19.35	-2	-2	0	1	0
Fe^{3+}	-13.02	0	-1	0	0	1
$Fe(HS)_2$	8.95	0	0	2	0	1
$Fe(HS)_3^-$	10.987	0	0	3	0	1
$Fe(OH)_2$	-20.8	-2	0	0	0	1
$Fe(OH)_3^-$	-33.4	-3	0	0	0	1
$Fe(OH)_4^{2-}$	-46.35	-4	0	0	0	1
$FeOH^+$	-10.2	-1	0	0	0	1
H_2	-3.15	2	2	0	0	0
$H_2(g)$	0	2	2	0	0	0
H_2O_2	-59.601	-2	-2	0	0	0
H_2S	6.994	1	0	1	0	0
$H_2S(g)$	7.991	1	0	1	0	0
H_2S_4	7.879	-2	-6	4	0	0
H_2S_5	8.994	-3	-8	5	0	0
HS^{4-}	3.678	-3	-6	4	0	0
HS^{5-}	5.094	-4	-8	5	0	0
O_2	-86.08	-4	-4	0	0	0
$O_2(g)$	-83.12	-4	-4	0	0	0
O_3	-156.05	-6	-6	0	0	0
$O_3(g)$	-153.25	-6	-6	0	0	0
OH^-	-14	-1	0	0	0	0
S^{2-}	-19	-1	0	1	0	0
S^{2-}	-5.903	-2	-3	2	0	0
S_2^{2-}	-12.734	-2	-2	2	0	0
$S_2O_3^{2-}$	-28.793	-8	-8	2	0	0
S_3^{2-}	-7.065	-3	-4	3	0	0
S_4^{2-}	-3.022	-4	-6	4	0	0
S_5^{2-}	-1.007	-5	-8	5	0	0

Table S4. Cont.

Chemical species	log(K)	H ⁺	e [−]	HS [−]	As(OH) ₃	Fe ²⁺
S ₆ ^{2−}	0.844	−6	−10	6	0	0
SO ₃ ^{2−}	−37.089	−7	−6	1	0	0
SO ₄ ^{2−}	−33.692	−9	−8	1	0	0
Fe(OH) ₂ ⁺	−18.69	−2	−1	0	0	1
Fe(OH) ₃	−25.58	−3	−1	0	0	1
Fe(OH) ₄ [−]	−34.62	−4	−1	0	0	1
Fe(SO ₄) ₂ [−]	75.024	−18	−17	2	0	1
Fe ₂ (OH) ₂ ⁴⁺	−28.99	−2	−2	0	0	2
Fe ₃ (OH) ₄ ⁵⁺	−45.36	−4	−3	0	0	3
FeHSO ₄ ²⁺	−42.244	−8	−9	1	0	1
FeHSO ₄ ⁺	−30.624	−8	−8	1	0	1
FeO ₄ ^{2−}	−125.87	−8	−4	0	0	1
FeOH ²⁺	−15.21	−1	−1	0	0	1
FeS ₂ O ₃ ⁺	−39.833	−8	−9	2	0	1
FeSO ₄	−31.442	−9	−8	1	0	1
FeSO ₄ ⁺	−42.672	−9	−9	1	0	1
H ₂ S ₂ O ₃	−26.521	−6	−8	2	0	0
H ₂ SO ₄	−33.692	−7	−8	1	0	0
HO ^{2−}	−71.251	−3	−2	0	0	0
HS ₂ O ₃ [−]	−27.108	−7	−8	2	0	0
HSO ₃ [−]	−29.869	−6	−6	1	0	0
HSO ₄ [−]	−31.712	−8	−8	1	0	0
S ₂ O ₆ ^{2−}	−75.428	−14	−14	2	0	0
S ₂ O ₈ ^{2−}	−133.645	−18	−18	2	0	0
S ₄ O ₆ ^{2−}	−54.881	−16	−18	4	0	0
SO ₂ (aq)	−28.029	−5	−6	1	0	0
SO ₂ (g)	−28.349	−5	−6	1	0	0
FeAsS (c)	25.629	2	3	1	1	1
As (c)	12.5253	3	3	0	1	0
As ₂ S ₃ (c)	46.3	3	0	3	2	0
AsS (c)	20.11	2	1	1	1	0
Fe (c)	−16.097	0	2	0	0	1
Fe(OH) ₂ (c)	−12.996	−2	0	0	0	1
Fe ₃ O ₄ (c)	−37.077	−8	−2	0	0	3
FeS (am)	3.915	−1	0	1	0	1
FeS (c)	4.648	−1	0	1	0	1
FeS ₂ (c)	18.479	−2	−2	2	0	1
S (c)	2.145	−1	−2	1	0	0
As ₂ O ₃ (s)	1.432	0	0	0	2	0
As ₂ O ₅ (c)	−46.96	−4	−4	0	2	0
Fe(OH) ₃ (am)	−17.911	−3	−1	0	0	1
Fe ₂ (SO ₄) ₃ (c)	−130.696	−27	−26	3	0	2
Fe ₂ O ₃ (cr)	−26.448	−6	−2	0	0	2
Fe ₃ (OH) ₈ (c)	−46.262	−8	−2	0	0	3
Fe ₃ S ₄ (c)	18.995	−4	−2	4	0	3

Table S4. Cont.

Chemical species	log(K)	H ⁺	e ⁻	HS ⁻	As(OH) ₃	Fe ²⁺
FeAsO ₄ ·2H ₂ O (c)	-27.35	-5	-3	0	1	1
FeOOH (cr)	-14.02	-3	-1	0	0	1
FeSO ₄ ·7H ₂ O (c)	-26.08	-9	-8	1	0	1
H ₃ OFe ₃ (SO ₄) ₂ (OH) ₆ (c)	-101.054	-23	-19	2	0	3
FeAsO ₄	-39.32	-5	-3	0	1	1
FeHAsO ₄	-31.76	-4	-3	0	1	1
FeH ₂ AsO ₄ ²⁺	-30.47	-3	-3	0	1	1
FeH ₂ AsO ₄ ⁺	-18.94	-3	-2	0	1	1
FeHAsO ₄	-25.34	-4	-2	0	1	1
FeAsO ₄ ⁻	-32.88	-5	-2	0	1	1
FeH ₂ AsO ₃ ²⁺	-14.98	-1	-1	0	1	1

Figure S2. Eh-pH diagrams for As-Fe-S-O-H at 250 °C and 1 atm pressure. Predominant arsenic species predicted for the chemistry of the BCR pore water at total concentration of arsenic 0.44 mM; sulfur 7.00 mM and total iron (A) 0.002 mM, (B) 0.064 mM, (C) 1.29 mM, and (D) 2.51 mM. The Eh and pH ranges of the BCR are within the dashed rectangle.

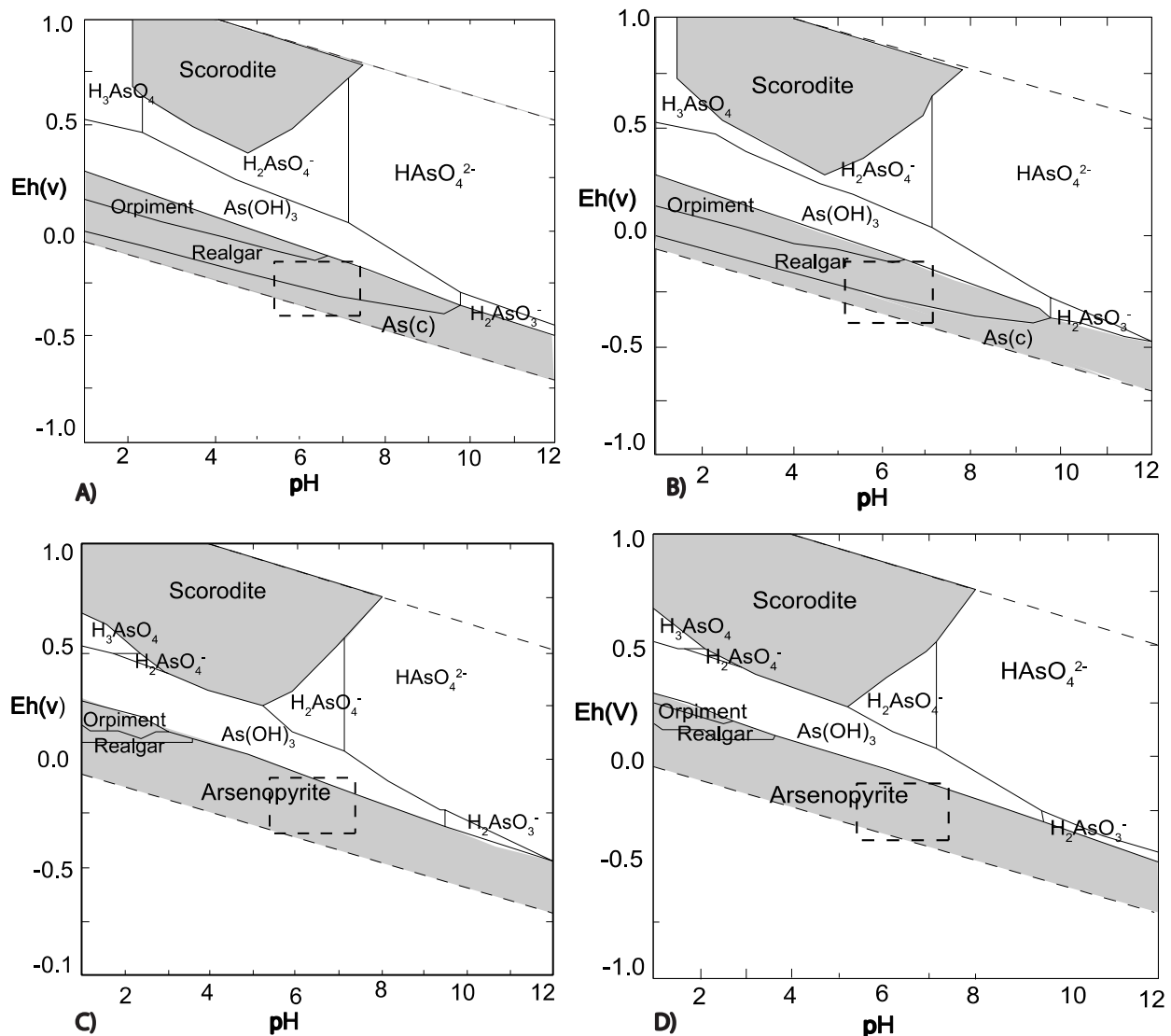


Table S5. Results of geochemical modeling of the As-O-H-S-Fe system using the equilibrium data in Table S4 over the concentration ranges measured in the BCR (Tables S1–S3).

Concentrations (mM)			Predominant Arsenic solid species at BCR conditions (pH 5.6–7.5, ORP –100 to –300 mV)		
As(OH) ₃	Fe ²⁺	HS [–]			
0.000011	0.002509	0.007	FeAsS		
0.000011	0.000064	0.007	FeAsS		
0.000011	0.000234	0.007	FeAsS		
0.000011	0.000002	0.007		AsS	As
0.002537	0.002509	0.007	FeAsS		
0.002537	0.000064	0.007		AsS	As
0.002537	0.000234	0.007		AsS	As
0.002537	0.000002	0.007		AsS	As
0.000443	0.000002	0.007			
0.000119	0.000002	0.007			
0.000443	0.002509	0.007	FeAsS		
0.000119	0.002509	0.007	FeAsS		
0.000443	0.000234	0.007	FeAsS		
0.000119	0.000234	0.007	FeAsS		
0.000443	0.000064	0.007		AsS	As

Table S6. Predominance of high metal concentration associated microbial groups in the BCR core samples.

Taxonomic classification	Number of sequences (Reads)									
	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TS9	Total
Methanocorpusculum	0	1	2	32	2	1	11	3	6	58
Methanospirillum	0	3	36	1913	255	1042	468	358	663	4738
Methanosarcina	0	1	3	162	9	18	539	1	209	942
Bacteroidetes SB-1	0	0	3	3	0	0	2	0	2	10
M2PB4-65 termite group	5	16	166	263	504	86	129	29	37	1235
VadinHA17 anaerobic digester group	0	0	6	90	8	30	25	4	8	171
TM6	0	0	0	9	0	0	0	2	8	19
19RF3	7	2	16	78	3	4	5	11	12	138
WS6	0	5	6	165	4	722	480	2	70	1454
Victivallaceae	0	0	1	6	1	16	13	2	8	47
Synergistales	0	0	5	57	2	2	1	1	1	69
Total number of reads in each sample	4000	4000	4000	4000	4000	4000	4000	4000	4000	
Arsenic Conc (ppm)	2.7	2.9	1.8	>250	16.6	18.9	45.8	9.3	18.9	
Zinc conc (ppm)	108	122	118	1735	180	177	705	127	380	

Table S7. SRB-related and iron-reducing bacteria enrichment culture related OTUs found in the BCR.

OTU No.	Number of sequences	Taxonomic classification according to Blastn to Silva111 database	Most closely related cultured species	Percent similarity
2066	44	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfobacterales; Desulfobulbaceae; Desulfobulbus; uncultured bacterium	<i>Desulfobulbus elongatus</i> strain FP	99
6440	10	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfobacterales; Nitrospinaceae; uncultured; uncultured bacterium	<i>Desulfovibrio mexicanus</i> strain Lup1	84
1605	31	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio; Desulfovibrio idahonensis	<i>Desulfovibrio mexicanus</i> strain Lup1	96
4663	10	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio; Desulfovibrio idahonensis	<i>Desulfovibrio mexicanus</i> strain Lup1	97
259	2	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio; Desulfovibrio paquesii	<i>Desulfovibrio paquesii</i> strain SB1	98
3241	8	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio; Desulfovibrio sp. A-1	<i>Desulfovibrio aminophilus</i> strain ALA-3	97
728	9	Bacteria; Proteobacteria; Deltaproteobacteria; GR-WP33-30; uncultured bacterium	<i>Geobacter daltonii</i> FRC-32	90
4896	724	Bacteria; Proteobacteria; Deltaproteobacteria; Sh765B-TzT-29; uncultured bacterium	<i>Halothiobacillus neapolitanus</i> c2	85
6722	217	Bacteria; Proteobacteria; Deltaproteobacteria; Sh765B-TzT-29; uncultured delta proteobacterium	<i>Halothiobacillus neapolitanus</i> c2	85
850	10	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; Bacteroides; iron-reducing enrichment clone Cl-A12	<i>Bacteroides graminisolvens</i> strain XDT-1	93
4336	18	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; Bacteroides; iron-reducing enrichment clone Cl-A12	<i>Bacteroides graminisolvens</i> strain XDT-1	97
5861	113	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Petrimonas; iron-reducing bacterium enrichment culture clone HN-HFO84	<i>Proteiniphilum acetatigenes</i> strain TB107	94
11776	10	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Petrimonas; iron-reducing bacterium enrichment culture clone HN-HFO84	<i>Proteiniphilum acetatigenes</i> strain TB107	95
4452	53	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Rikenellaceae; vadinBC27 wastewater-sludge group; iron-reducing bacterium enrichment culture clone HN7	<i>Marinifilum fragile</i> CECT 7942 Strain JC2469	87
6640	92	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Rikenellaceae; vadinBC27 wastewater-sludge group; iron-reducing bacterium Enrichment Culture Clone HN7	<i>Marinifilum fragile</i> CECT 7942 Strain JC2469	87

Table S7. Cont.

OTU No.	Number of sequences	Taxonomic classification according to Blastn to Silva111 database	Most closely related cultured species	Percent similarity
2065	274	Bacteria; Firmicutes; Clostridia; Clostridiales; Gracilibacteraceae; Gracilibacter; iron-reducing bacterium enrichment culture clone FEA_2_F1	<i>Gracilibacter thermotolerans</i> JW/YJL-S1	97
1197	63	Bacteria; Firmicutes; Clostridia; Clostridiales; Gracilibacteraceae; Gracilibacter; iron-reducing bacterium enrichment culture clone FEA_2_F1	<i>Gracilibacter thermotolerans</i> JW/YJL-S1	97
1550	70	Bacteria; Firmicutes; Clostridia; Clostridiales; Gracilibacteraceae; Gracilibacter; iron-reducing bacterium enrichment culture clone FEA_2_F1	<i>Gracilibacter thermotolerans</i> JW/YJL-S1	97
4012	10	Bacteria; Firmicutes; Clostridia; Clostridiales; Gracilibacteraceae; Gracilibacter; iron-reducing bacterium enrichment culture clone FEA_2_F1	<i>Gracilibacter thermotolerans</i> JW/YJL-S1	97
2714	12	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Caldicoprobacter; iron-reducing bacterium enrichment culture clone FEA_2_A2	<i>Tindallia texcoconensis</i> strain IMP-300	90
1398	13	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; uncultured; iron-reducing bacterium enrichment culture clone HN109	<i>Bacillus thuringiensis</i> Bt407	88
4392	140	Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Incertae Sedis; iron-reducing bacterium enrichment culture clone FEA_2_H3	<i>Clostridium sufflavum</i> strain CDT-1	98
7291	53	Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Incertae Sedis; iron-reducing bacterium enrichment culture clone FEA_2_H3	<i>Clostridium sufflavum</i> strain CDT-1	98
980	28	Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; uncultured; iron-reducing bacterium enrichment culture clone HN3	<i>Oscillibacter valericigenes</i> Sjm18---20	89
Several	31	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Desulfosporosinus; uncultured bacterium		
Several	13	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Desulfotomaculum; uncultured <i>Bacillus</i> sp.		

Table S8. Most prevalent Synergistetes related OTUs found in the BCR.

OTU No.	Number of sequences	Taxonomic classification according to Blastn to Silva111 database	Most closely related cultured species	Percent similarity
8578	178	Bacteria; Synergistetes; Synergistia; Synergistales; Synergistaceae; uncultured; uncultured bacterium	<i>Thermanaerovibrio acidaminovorans</i> DSM 6589	90
8930	123	Bacteria; Synergistetes; Synergistia; Synergistales; Synergistaceae; uncultured; uncultured bacterium	<i>Thermanaerovibrio acidaminovorans</i> DSM 6589	90
12548	68	Bacteria; Synergistetes; Synergistia; Synergistales; Synergistaceae; uncultured; uncultured bacterium	<i>Thermanaerovibrio acidaminovorans</i> DSM 6589	92
3668	10	Bacteria; Synergistetes; Synergistia; Synergistales; Synergistaceae; uncultured; uncultured bacterium	<i>Aminobacterium colombiense</i> DSM 12261	91
4310	32	Bacteria; Synergistetes; Synergistia; Synergistales; Synergistaceae; uncultured; uncultured bacterium	<i>Thermovirga lienii</i> DSM 17291	94
6986	46	Bacteria; Synergistetes; Synergistia; Synergistales; Synergistaceae; uncultured; uncultured bacterium	<i>Thermanaerovibrio acidaminovorans</i> DSM 6589	89
Total	13600			

Note: These data were obtained from sequencing of a total of 48 samples from the BCR, including the nine that were used for metal and mineralogical analysis.

References

1. Duncan, W.F.A.; Mattes, A.G.; Gould, W.D.; Goodazi, F. Multi-stage Biological Treatment System for Removal of Heavy Metal Contaminants. In Proceedings of 43rd Annual Conference of Metallurgists (COM 2004), Hamilton, ON, Canada, 22–25 August 2004.
2. Lu, P.; Zhu, C. Arsenic Eh-pH diagrams at 25 °C and 1 bar. *Environ. Earth Sci.* **2011**, *62*, 1673–1683.

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