Metal reduction at low pH by a *Desulfosporosinus* species: implications for the biological treatment of acidic mine drainage

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Abstract

We isolated an acid-tolerant sulfate-reducing bacterium, GBSRB4.2, from coal mine-derived acidic mine drainage (AMD)-derived sediments. Sequence analysis of partial 16S rRNA gene of GBSRB4.2 revealed that it was affiliated with the genus *Desulfosporosinus*. GBSRB4.2 reduced sulfate, Fe(III) (hydr)oxide, Mn(IV) oxide, and U(VI) in acidic solutions (pH 4.2). Sulfate, Fe(III), and Mn(IV) but not U(VI) bioreduction led to an increase in the pH of acidic solutions and concurrent hydrolysis and precipitation of dissolved Al^{3+}. Reduction of Fe(III), Mn(IV), and U(VI) in sulfate free-solutions revealed that these metals are enzymatically reduced by GBSRB4.2. GBSRB4.2 reduced U(VI) in groundwater from a radionuclide-contaminated aquifer more rapidly at pH 4.4 than at pH 7.1, possibly due to the formation of poorly bioreducible Ca-U(VI)-CO_3 complexes in the pH 7.1 groundwater.

INTRODUCTION

Acidic mine drainage (AMD) arises when sulfide-rich (primarily iron sulfides as pyrite, FeS_2) rocks that were previously under anoxic conditions are exposed to oxygen rich waters through the mining process. Sulfuric acid is produced via the overall reaction below

$$FeS_2 + 3.5\ O_2 + H_2O \rightarrow 2\ SO_4^{2-} + Fe^{2+} + 2\ H^+ \quad (1)$$

and may be enhanced the activity of sufide- and Fe(II)-oxidizing bacteria and archaea (Johnson, 2002; Baker and Banfield, 2003). These acidic fluids enhance the dissolution of metals (including, but not limited to Al, Cd, Cu, Fe, Mn, Ni, Pb, U, or Zn depending on mining activity and host rock) in the rock matrix (Nordstrom and Alpers, 1999; Selenska-Pobell et al., 2001; Geller et al., 2002; Johnson, 2002; Landa, 2004). The acidity and high metal content of AMD represent environmental hazards to soils, surface waters, and ground waters.

Dissolved metals may be removed from AMD by the activities of sulfate-reducing bacteria (SRB) via a variety of mechanisms. Dissolved uranium (as U(VI)) may be removed from fluids by SRB that also catalyze the reduction of soluble U(VI) to relatively insoluble U(IV) (Wall and Krumholz, 2006). Sulfate respiration involves the conversion of a strong acid (sulfate; $H_2SO_4 \leftrightarrow HSO_4^-$ $pK_a = -3.0$, $HSO_4^- \leftrightarrow SO_4^{2-}$ $pK_a = 1.99$) to a weak acid (sulfide; $H_2S \leftrightarrow HS^-$ $pK_a = 6.9$, $HS^- \leftrightarrow S^{2-}$ $pK_a = 14$), which increases the pH of AMD, leading to the hydrolysis and precipitation of dissolved Al^{3+} (Champagne et al., 2005; Daubert and Brennan, 2007). Biogenic sulfide may also react with dissolved metals such as Fe, Zn, Cu, Cd, Ni, and Pb, leading to the precipitation insoluble metal sulfide phases and consequently, removal of metals from solution (Christensen et al., 1996; Johnson and Hallberg, 2005a). However, these metal sulfides represent a concentrated pool of reduced sulfur that, should oxygen penetrate these

55 sediments, could be oxidized back to sulfate and release fluids that are more acidic than the
56 originally treated AMD (Johnson and Hallberg, 2003; Johnson and Hallberg, 2005b).
57 In light of the concerns regarding concentration of sulfides in treatment systems, an
58 alternative strategy for treatment of Appalachian coal mine-derived AMD has been proposed.
59 Since dissolved Fe^{2+}, Al^{3+}, in some cases Mn^{2+}, and acidity are the contaminants of greatest
60 concern in Appalachian AMD, it may be treated via microbiologically mediated oxidative
61 precipitation of Fe(II) and Mn(II) (as Fe(III) or Mn(IV) (hydr)oxides) (Unz et al., 1979; Kirby et
62 al., 1999; Vail and Riley, 2000; Nicormat et al., 2006; Nengovhela et al., 2004; Johnson and
63 Hallberg, 2005a; Cravotta, 2008; Senko et al., 2008). Fe- and, possibly Mn-free AMD may then
64 be passed through limestone, which neutralizes the pH, causing the hydrolysis and precipitation
65 of dissolved Al^{3+}. This AMD treatment strategy (referred to as an "aeration terrace" (Senko et
66 al., 2008)) eliminates the concentration of reduced sulfur in treatment systems, since it does not
67 rely on SRB activity to remove dissolved metals from solution.
68 However, in such systems, the oxidative precipitation of Fe and Mn from AMD may be
69 reversed by the reductive dissolution of Fe(III) and Mn(IV) (hydr)oxides by Fe(III)- and
70 Mn(IV)-reducing bacterial activities (Tarutis et al., 1992; Tarutis and Unz, 1995; Johnson and
71 Hallberg, 2002; Koschorreck et al., 2007). Such processes may be mediated by enzymatic
72 Fe(III) and Mn(IV) reduction by acidophilic/tolerant microorganisms or by the reaction of
73 sulfide (produced by acidophilic/tolerant SRB) with Fe(III) and Mn(IV) (hydr)oxides (Johnson
74 and McGinness, 1991; Küsel et al., 1999; Bilgin et al., 2004; Adams et al., 2007). Indeed,
75 concurrent Fe(III), Mn(IV), and sulfate reduction (and release of dissolved Fe(II) and Mn(II))
76 have been observed in wetlands designed to maximize the oxidative precipitation of Fe(II) and
77 Mn(II) (Tarutas et al., 1992; Tarutas and Unz, 1995).

While SRB activity in AMD-impacted systems (pH as low as 2.5) is well established (Herlihy and Mills, 1985; Fauville et al., 2004; Luptokova and Kusnierova, 2005; García-Moyano et al., 2007; Rowe et al., 2007), many known SRB are not active at low pH (i.e. pH < 5), and only a few acidophilic/tolerant SRB have been cultured (Tuttle et al., 1969; Hard et al., 1997; Küsel et al., 2001; Kimura et al., 2006; Church et al., 2007). To examine the effect of SRB activity on the solubility of metals in AMD-impacted systems, we isolated an acid-tolerant *Desulfosporosinus* species from AMD-impacted sediments and assessed 1) the geochemical consequences of this organism's activities and 2) the metal reducing activities of this organism.

MATERIALS AND METHODS

Sediment collection. Sediment samples were obtained from an AMD-impacted site in McKean County, Pennsylvania called Gum Boot (41^{o} 41' 02" N; 78^{o} 29' 37" W). Briefly, the pH of emergent AMD at the Gum Boot site is 4.1 and contains 0.05 mM dissolved Al, 0.3 mM dissolved Ca, 0.05 mM dissolved Mn, 0.9 mM dissolved Fe(II), and 1 mM sulfate. AMD flows as a 0.5-cm thick sheet over Fe(III) (hydr)oxide-rich sediments, which result from microbially mediated Fe(II) oxidation and subsequent hydrolysis and precipitation of Fe^{3+}, a process that leads to the complete removal of dissolved Fe(II) from the AMD within 10 m of its emergence. A more detailed description of the Gum Boot system is provided elsewhere (Senko et al., 2008). Sediments were collected approximately 2 m from AMD emergence from the top 2 cm of Fe(III) (hydr)oxide-rich sediments with a sterile spatula, transferred to sterile centrifuge tubes, transported to our laboratory on ice, and stored at 4 ^{o}C before further processing ($\leq$ 2 weeks).

Microbial culture medium, enrichments, and isolation. Initial enrichments were designed to target both Fe(III)- and sulfate-reducing bacteria. The medium used for these enrichments was based on a medium described by Johnson (1995) and contained 10 mM $(NH_4)_2SO_4$, 2 mM

101 $MgSO_4$, 25 mM $Fe_2(SO_4)_3$, 5 mM glucose, 0.5 g/l trypticase soy broth (TSB), vitamins, and trace
102 metals (Tanner, 1997). The pH of the medium was adjusted to 4.2 with NaOH, causing the
103 formation of Fe(III) (hydr)oxide precipitate. Oxygen was removed by bubbling with N_2. The
104 medium was dispensed into serum tubes in an anoxic glovebag (Coy Laboratory Products, Grass
105 Lake, MI) containing 97.5% N_2 and 2.5% H_2. Serum tubes were sealed with rubber stoppers in
106 the glovebag (with a headspace of 97.5% N_2 and 2.5% H_2), and autoclaved. The pH of the
107 medium decreased to 2.5 after autoclaving. Gum Boot inuclula were prepared by suspending
108 sediments in the anoxic medium described above with no Fe(III) (pH adjusted to 4.2 with
109 H_2SO_4), and serial dilution in the same medium. Growth of SRB was indicated by a change in
110 the color of precipitates in the medium from orange to black, suggesting the formation of Fe(II)
111 sulfide. The most dilute enrichment that contained SRB was transferred to media that contained
112 the same constituents described above except that $Fe_2(SO_4)_3$ and headspace H_2 were omitted.
113 Additional sulfate was provided by adding 0.4 ml of filter-sterilized, anoxic $FeSO_4$ (400 mM, pH
114 3.2) to 10 ml of medium. The pH of the media were adjusted to 3.0, 3.5, and 4.5 with 1 M
115 H_2SO_4. The addition of $FeSO_4$ did not alter the pH of the media with initial pH of 3.0 and 3.5,
116 but the addition of $FeSO_4$ decreased the pH of the medium with an initial pH of 4.5 to 4.2. No
117 growth was observed in the media at pH 3.0 and 3.5, but was observed in the medium that had an
118 initial pH of 4.2. This medium (pH 4.2; called aSRBFe) was used for the routine maintenance of
119 SRB cultures. A Fe-free variation of this medium (called aSRB) was prepared as described
120 above, but sulfate was provided as Na_2SO_4 (pH adjusted to 3.2 with H_2SO_4) instead of $FeSO_4$.
121 A pure SRB culture (called GBSRB4.2) was obtained by streaking the enrichment culture
122 on plates of aSRBFe that contained agarose (2%) as a solidifying agent. Plates were prepared
123 and incubated in an anoxic glovebag. Individual colonies (that were black due to the formation

124 of FeS) were restreaked, and the colonies that formed on these plates were transferred to fresh
125 aSRBFe medium. This culture was stable through at least 50 transfers and the purity of the
126 culture was routinely checked by light microscopy.
127 For experiments to test electron donor utilization by GBSRB4.2, glucose-free aSRBFe
128 medium was prepared as described above with and without 0.5 g/l TSB. Electron donors were
129 provided as 60 mM sodium formate, 15 mM sodium acetate, 15 mM sodium lactate, 5 mM
130 glucose, or by pressurizing the headspace of the serum tubes (approximately 18 ml) with 10 ml
131 of H_2 and 10 ml of CO_2. If TSB was omitted from the medium, 0.2 mM KH_2PO_4 was provided
132 as a phosphorous source. For experiments to assess the initial medium pH tolerance of
133 GBSRB4.2, aSRBFe medium was prepared as described above, and the pH was adjusted to 2.5,
134 3.0, and 4.5 with H_2SO_4 and 7.0, 8.0, and 8.5 with 1 M NaOH. The addition of $FeSO_4$ caused
135 the media pH values of 2.5, 3.0, 4.5, 7.0, 8.0, and 8.5 to change to 2.5, 2.9, 4.2, 5.3, 5.8, and 6.3,
136 respectively.
137 **Cell incubations.** Activities of GBSRB4.2 were assessed in synthetic acidic mine drainage
138 (SAMD); a solution buffered at 6.3 with 20 mM Piperazine-1,4-bis(2-ethanesulfonic acid)
139 (PIPES); and radionuclide- and nitrate-contaminated groundwater from the U.S. Department of
140 Energy Environmental Remediation Science Program's Oak Ridge Integrated Field-Scale
141 Research Challenge site (well FW029; referred to here as ORGW) (Saunders and Toran, 1995;
142 Brooks, 2001). SAMD (pH 4.2) contained 5 mM $CaSO_4$, 4 mM $MgSO_4$, 1 mM Na_2SO_4, 0.5 mM
143 $Al_2(SO_4)_3$, and 0.1 mM $(NH_4)_2SO_4$ (Senko et al., 2008). While historically acidic (Saunders and
144 Toran, 1995; Brooks, 2001), the ORGW used for these experiments had a pH of 6.8, likely due
145 to extensive field-scale experiments to stimulate in situ U(VI) reduction (Istok et al., 2004), so
146 we adjusted the pH to 4.4 with nitric acid before cell incubations. The chemical composition of

147 ORGW is shown in Table S3. Oxygen was removed from SAMD, PIPES buffer, and ORGW by
148 bubbling with oxygen-free N_2. Bubbling with N_2 increased the pH of ORGW to 7.1, probably
149 due to the removal of dissolved CO_2. SAMD, PIPES, and ORGW were dispensed into serum
150 bottles that were sealed with rubber stoppers. SAMD and ORGW were filter sterilized in an
151 anoxic glovebag and PIPES buffer was sterilized by autoclaving. Where appropriate, anoxic
152 Fe(III) (hydr)oxide, Mn(IV) oxide, uranyl sulfate, or uranyl acetate were added to solutions from
153 sterile or pasteurized stock solutions or suspensions to achieve concentrations of 2 mmole/l
154 Fe(III), 2 mmole/l Mn(IV), or 250 μM U(VI). The preparation of Fe(III) and Mn(IV)
155 (hydr)oxide suspensions and uranyl sulfate solution is described below. H_2 was provided as an
156 electron donor to SAMD incubations by the addition of 10 ml of H_2 to 70 ml of headspace. H_2
157 was provided to PIPES and ORGW incubations by pressurizing serum bottles to 1.5 atm.
158 Sodium molybdate (20 mM) was added to selected SAMD incubations to inhibit sulfate
159 reduction (Oremland and Capone, 1988), which caused an increase in the pH of the SAMD to
160 6.0.

161 GBSRB4.2 was grown to late log/early stationary phase in aSRB medium, and cells were
162 harvested by centrifugation. Cells were then washed three times and finally resuspended in
163 anoxic SAMD, PIPES buffer, or ORGW (where appropriate). Cells were added to incubations to
164 achieve a density of approximately 1 x 10^8 cell/ml.

165 **Sampling and analytical techniques.** Samples were periodically removed from incubations in
166 an anoxic glovebag using a needle and syringe. Solids were removed by centrifugation, and
167 dissolved U(VI), dissolved Fe(II), dissolved Al, dissolved Mn(II), glucose, organic acids, and
168 sulfate were quantified in the soluble fraction as described below. Fe(II) and Mn(II) were
169 preserved in 0.5 M HCl. Total Fe(II) and Mn(II) (i.e. solid-associated) were solubilized with 0.5

M HCl and solids were removed by centrifugation. To confirm that loss of U(VI) from solution was due to U(VI) reduction and not sorption to cells or formation of insoluble U(VI) phases, solids-associated U(VI) was solubilized using the bicarbonate extraction technique described by Elias et al. (2003a). Samples for sulfide analysis were preserved in anoxic 10% zinc acetate and sulfide was quantified as described below. Samples for pH measurement were placed in centrifuge tubes, removed from the glovebag and the pH was immediately measured using a Thermo-Orion PerpHecT semi-micro combination pH electrode and 550A pH meter (ThermoFisher Scientific, Waltham, MA). To determine protein concentrations in GBSRB4.2 growth experiments, samples were first centrifuged and Fe and sulfide were removed from pellets by washing them three times with 0.5 M HCl (to remove Fe(II) and sulfide), followed by three washes with water, and three washes with 0.3 M ammonium oxalate (to remove Fe(III)) before resuspension in 1 M NaOH to solubilize proteins. Samples were then boiled and protein was quantified using the bicinchoninic acid assay (Pierce Biotechnology, Inc., Rockford, IL).

Fe(II) was quantified with the ferrozine assay (Lovley and Phillips, 1987). Mn(II) was quantified using PAN indicator kits (Hach Co., Loveland, CO). Sulfate was quantified by ion chromatography with conductivity detection (Dionex DX 100 fitted with an AS-4A column; Dionex Corp., Sunnyvale, CA). U(VI) was quantified by kinetic phosphorescence analysis (KPA) on a KPA-11 (ChemChek Instruments, Richland, WA; Brina and Miller, 1992). Sulfide was quantified by methylene blue assay (Cline, 1969). Glucose was quantified by the phenol-sulfuric acid method (Daniels et al., 1994). Organic acids were quantified by high performance liquid chromatography using a Waters (Waters Corp., Milford, MA) 2695 Separations Module fitted with a Bio-Rad HPX-87H organic acid column (Hercules, CA) and Waters 2996 Photodiode array detector. Al, Ca, K, Mg, and Na were quantified by inductively coupled

193 plasma emission spectrometry using a Perkin-Elmer Optima 5300 ICP (Perkin-Elmer Inc.,
194 Waltham, MA). Dissolved inorganic carbon (DIC) in ORGW was quantified using a Shimadzu
195 total organic carbon analyzer TOC-Vcsn (Shimadzu Corp., Columbia, MD).
196 **Preparation of Fe(III) and Mn(IV) (hydr)oxide suspensions and U(VI) solutions.** For
197 experiments to assess the ability of GBSRB4.2 to reduce solid-phase Fe(III) in PIPES buffer,
198 Fe(III) (hydr)oxide was prepared as described by Lovley and Phillips (1986). For experiments to
199 assess the ability of GBSRB4.2 to reduce solid-phase Fe(III) in SAMD, Fe(III) (hydr)oxide was
200 prepared as described above, except that a solution of $Fe_2(SO_4)_3$ (instead of $FeCl_3$) was
201 hydrolyzed with NaOH. For experiments to assess the ability of GBSRB4.2 to reduce solid-
202 phase Mn(IV) in PIPES buffer, Mn(IV) oxide was prepared as described by Feng et al. (2000),
203 except that a solution of $MnCl_2$ was oxidized and hydrolyzed instead of a solution of $Mn(NO_3)_2$.
204 For experiments to assess the ability of GBSRB4.2 to reduce solid-phase Mn(IV) in SAMD,
205 Mn(IV) oxide was prepared as described above, except that a solution of $MnSO_4$ was oxidized
206 and hydrolyzed instead of a solution of $MnCl_2$. For experiments to assess the ability of
207 GBSRB4.2 to reduce U(VI) in PIPES buffer, U(VI) was provided as uranyl acetate. For
208 experiments to assess the ability of GBSRB4.2 to reduce U(VI) in SAMD, U(VI) was provided
209 as uranyl sulfate. Uranyl sulfate was produced by first precipitating U with 1 mM sodium
210 sulfide in anoxic water as described by Beyenal et al. (2004). The resulting precipitate was
211 washed three times with anoxic water and then dissolved with oxygen-saturated, dilute H_2SO_4
212 (pH 4.0). All Fe(III) and Mn(IV) (hydr)oxide suspensions and U(VI) solutions were bubbled
213 with oxygen-free N_2 to remove O_2 and pasteurized (Fe(III) and Mn(IV)) or autoclaved (U(VI)).
214 **Electron microscopy.** For scanning electron microscopy (SEM), samples were prepared in a
215 glove box following a previously published procedure (Zhang et al., 2007). Briefly, cell-mineral

suspensions were fixed in anoxic 2.5% glutaraldehyde, placed on a glass cover slip, and cells and mineral particles were allowed to settle onto the cover slip for 15 min. The particle-coated cover slips were gradually dehydrated in an ethanol series followed by critical point drying (CPD). All sample preparation, except CPD, was performed in an anoxic glovebag to minimize the exposure of samples to O_2. Cover slips were mounted onto a SEM stub and Au coated for observation using a Zeiss Supra 35 FEG-VP SEM at an accelerating voltage of 10 to 15 kV. A short working distance (6 -10 mm) and low beam current (30 – 40 mA) were used to achieve the best image resolution. A longer working distance (8 mm) and higher beam current (50 – 70 mA) were used for qualitative energy dispersive spectroscopy (EDS) analysis. Elemental analysis was performed using an Oxford EDS system equipped with a SiLi detector coupled to the SEM, and analyzed with ISIS software. Images were digitally collected using a Gatan CCD camera and analyzed using Gatan Digital Micrograph.

DNA isolation, PCR amplification, cloning, sequencing, and phylogenetic analysis of isolate. GBSRB4.2 was stored at -80°C until DNA was extracted. Before DNA extraction, Fe and sulfide were removed from cells using 0.5 M HCl and 0.3 M ammonium acetate as described above. The remaining Fe- and sulfide-free cells were then washed three times with TE buffer (10 mM tris-hydroxymethylaminomethane (Tris) and 1 mM ethylene diamine tetraacetic acid (EDTA), pH 8.0), and stored at -20°C before further processing. DNA was extracted from cells using the Qiagen DNEasy Blood and Tissue DNA extraction kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. The 16S rRNA gene of GBSRB4.2 was amplified by polymerase chain reaction (PCR) using bacteria-specific primers based on *Escherichia coli* positions 16S-27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 16S-1492r (5'-TACGGYTACCTTGTTACGACTT -3') (Lane, 1991) purchased from Invitrogen Corp.

(Carlsbad, CA). PCR mixtures contained 2 µl of genomic DNA, 5 µl of 10x HotMaster PCR buffer with 25 mM $MgCl_2$ (Eppendorf Corp., Westbury, NY), 1 µl of 10 mM dNTPs, 3 µl (each) of 10 mM primer, 0.5 µl of 50 mg/ml bovine serum albumin, 0.25 µl of 5 units/µl HotMaster Taq polymerase (Eppendorf Corp., Westbury, NY), and 35.25 µl of molecular biology grade water. PCR cycling in a 2400 Perkin-Elmer thermocycler consisted of an initial denaturation step for 5 min at 94 °C and 30 cycles of 94 ^{o}C for 0.5 min, 54 ^{o}C for 0.5 min, and 72 ^{o}C for 1 min, followed by a final extension step at 72 ^{o}C for 7 min. Fresh PCR products were directly cloned into TOPO-TA vector (Invitrogen) following the manufacturer's instructions. Ten clones were obtained and PCR insert-containing TOPO-TA vectors were prepared for sequencing using TempliPhi rolling circle amplification (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) according to the manufacturer's instructions. DNA sequencing was performed at The Pennsylvania State University's DNA sequencing facility using an ABI Hitachi 3730XL DNA Analyzer. The partial sequence of the 16S rRNA gene from GBSRB4.2 has been submitted to GenBank under accession number EU839714.

For phylogenetic placement, 16S rRNA gene sequences were initially analyzed using Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1997). Sequences were checked for chimeras using the Ribosomal Database Project II's chimera detection function (Cole et al., 2003). Sequences obtained in this work and those obtained from GenBank were downloaded into a Geneious 3.0 software environment (Drummond et al., 2007). Sequences were aligned within the Geneious environment using the ClustalW algorithm (Thompson et al., 1994), and an evolutionary distance tree (neighbor joining algorithm with Jukes-Cantor corrections) was produced using 16S rRNA gene sequences of GBSRB4.2 and selected sequences obtained from GenBank with *Chloroflexus aurantiacus* (GenBank accession number D38365) as an outgroup.

RESULTS AND DISCUSSION

Growth and activities of GBSRB4.2. GBSRB4.2 was isolated from AMD-impacted sediments and used glucose as an electron donor for sulfate reduction to sulfide in medium with an initial pH of 4.2 (Figure 1A, B, and C). Acetate accumulated during glucose oxidation (Figure 1C). Sugar-metabolizing SRB including *Desulfovibrio*, *Desulfolobus*, and *Desulfotomaculum* spp. produce acetate and CO_2 as the primary products of sugar metabolism (Akagi and Jackson, 1967; Klemps et al., 1985; Daumas et al., 1988; Ollivier et al., 1988; Zellner et al., 1989; Trinkerl et al., 1990; Reichenbecher and Schink, 1997; Sass et al., 2002). The ratio of glucose oxidized to acetate produced was 1:1.8 and the ratio of glucose oxidized to sulfide produced was 1:1, suggesting that GBSRB4.2 obtained energy for growth via the reaction:

$$C_6H_{12}O_6 + SO_4^{2-} \rightarrow 2\ CH_3COOH + 2\ CO_2 + S^{2-} + 2\ H_2O \quad (2)$$

No other fermentation products were detected in the medium. At low pH, acetate and other organic acids are present in their protonated form and will easily pass through cell membranes, thus acidifying the cytoplasm (Norris and Ingledew, 1992; Gemmel and Knowles, 2000), an explanation for the necessity of neutralization before AMD treatment by SRB activity (Johnson and Hallberg, 2002; Tsukamoto et al., 2004; Johnson and Hallberg, 2005b; Luptakova and Kusnierova, 2005; Koschorreck et al., 2007) and the poor success in enriching SRB at low pH (Tuttle et al., 1969; Kimura et al., 2006; Rampinelli et al., 2007). This problem may have been avoided during growth by GBSRB4.2, since sulfate reduction led to an increase in the medium pH (Figure 1A and D). However, continued production of acetate did appear to lead to a subsequent decrease in the medium from 5.3 to 4.9 (Figure 1A and C). GBSRB4.2 reached a final protein concentration of 36 μg/ml (Figure 1A). Assuming 155 fg of protein per cell (Madigan et al., 1997), this would correspond to a cell density of approximately 2.3×10^8

cell/ml. Cells of GBSRB4.2 were rods of approximately 5 μm in length (Figure 2A). Besides glucose, GBSRB4.2 was able to use lactate and constituents of TSB as electron donors and carbon sources for growth via sulfate reduction. GBSRB4.2 also grew lithoautotrophically with H_2/CO_2, but did not use acetate or formate as electron donors for sulfate reduction. GBSRB4.2 grew in media with initial pH of 4.0 to 6.3, but not in media with initial pH ≤ 2.9, suggesting that this organism may be best described as acid-tolerant, as opposed to acidophilic.

Loss of dissolved Fe(II) in aSRBFe medium was concurrent with sulfidogenesis and the ratio of dissolved Fe(II) loss to sulfide produced from sulfate was 1:1 (Figure 1A and B). Most FeS precipitates were amorphous, but some exhibited unique "shish kebab" morphologies, where cubic phases appeared to be lanced by other acicular phases (Figure 2 A, B, and C). Energy dispersive spectroscopic (EDS) analysis of these "shish kebab-like" phases revealed the presence of Fe and S (Figure 2D). The presence of Na, C, and O in these precipitates may be attributable to cell biomass or components of TSB.

Phylogenetic analysis of the 16S rRNA gene sequence of GBSRB4.2 revealed that it is a member of the genus *Desulfosporosinus*, and most closely related to *Desulfosporosinus* sp. LauIII, a sulfate-reducing bacterium isolated from AMD-impacted lake sediments that grew in a pH range of 4.9 to 6.1 (pH optimum 5.5; Küsel et al., 2001). *Desulfosporosinus* spp. have been observed in other acidic environments (Küsel et al., 2001; Shelobolina et al., 2003; Suzuki et al., 2004; Geissler and Selenska-Pobell, 2005; Kimura et al., 2006; Church et al., 2007; García-Moyano et al., 2007; Jin et al., 2008), but only *Desulfosporosinus* sp. M1 has been shown to be active in culture at pH < 4.9 (Johnson et al., 2006; Kimura et al., 2006). An acetate-oxidizing *Desulfosporosinus* sp.-containing enrichment culture exhibited sulfidogenic activity at pH 4.35, but sulfidogenesis was quite low (approximately 15 μM) relative to the sulfate concentration

(approximately 33 mM) and compared to the extent of sulfidogenesis by this organism at circumneutral pH (Church et al., 2007).

Sulfate and metal reducing activities of GBSRB4.2. Given the ability of *Desulfosporosinus* and related *Desulfotomaculum* and *Desulfosporomusa* spp. to reduce metals (Tebo and Obraztsova, 1998; Robertson et al., 2001; Sass et al., 2004; Suzuki et al., 2004; Muyzer and Stams, 2008), we tested the activities of GBSRB4.2 (including metal reduction) under chemical conditions characteristic of Appalachian coal mine-derived AMD-impacted systems (i.e. low pH and millimolar concentrations of Al^{3+} and Ca^{2+}). When we incubated GBSRB4.2 in synthetic acidic mine drainage (SAMD) with H_2 as an electron donor, sulfate reduction (as indicated by sulfidogenesis) led to an increase in pH due to the conversion of sulfate to sulfide (reaction 3), and concurrent hydrolysis and precipitation of dissolved Al^{3+} (Figure 4A and B).

$$4\ H_2 + SO_4^{2-} + 2\ H^+ \rightarrow H_2S + 4\ H_2O \quad (3)$$

Such activity is exploited for the neutralization of AMD and subsequent removal of dissolved Al^{3+} (Champgne et al., 2005; Daubert and Brennan, 2007).

GBSRB4.2 reduced Fe(III) (hydr)oxide and sulfate concurrently in SAMD (Figure 4C and D). Sulfide accumulated to levels comparable to those of Fe(III)-free incubations (Figure 4A and D). Activity of GBSRB4.2 lead to the reductive solubilization of Fe(II), despite abundant sulfide (Figure 4C), suggesting that the activities of SRB in AMD-impacted systems will lead to the release of previously immobilized Fe. Sulfate and Fe(III) reduction led to an increase in pH (Figure 4E) via reaction 3 and sulfide-mediated (4) or enzymatic (5) Fe(III) reduction shown below:

$$S^{2-} + 2\ Fe(OH)_3 + 6\ H^+ \rightarrow 2Fe^{2+} + S^0 + 6\ H_2O \quad (4)$$

$$H_2 + 2\ Fe(OH)_3 + 4\ H^+ \rightarrow 2\ Fe^{2+} + 6\ H_2O \quad (5)$$

331 which are proton-consuming reactions. Increased pH led to the hydrolysis and precipitation of
332 dissolved Al^{3+} (Figure 4D).

333 GBSRB4.2 completely reduced Mn(IV) oxide in SAMD via the reaction:

334 $$H_2 + MnO_2 + 2\ H^+ \rightarrow Mn^{2+} + 2\ H_2O \quad (6)$$

335 which is also a proton-consuming reaction and led to an increase in pH (Figure 4F and H).
336 However, the pH only increased to approximately 5.4 in Mn(IV)-amended incubations,
337 compared to pH ≥ 6 in the unamended and Fe(III)-amended incubations (Figure 4B, E, and H),
338 and consequently, Al^{3+} was incompletely removed from solution (Figure 4G). No sulfidogenesis
339 was observed in Mn(IV)-amended SAMD incubations, suggesting that Mn(IV) reduction was not
340 mediated by biogenic sulfide, but rather was an enzymatic process (Figure 4G and F). We point
341 out that based on these data, we can not conclusively exclude the possibility that the reaction
342 between sulfide and Mn(IV) was so rapid that sulfide accumulation could not be observed
343 (Burdige and Nealson, 1986), and we address this topic below.

344 GBSRB4.2 reduced U(VI) in SAMD (Figure 4I), by the reaction:

345 $$H_2 + UO_2^{2+} \rightarrow UO_2 + 2\ H^+ \quad (7)$$

346 but since protons are not consumed by this reaction, no alteration of SAMD pH was observed
347 (Figure 4K), and consequently, no Al^{3+} precipitation occurred (Figure 4J). While Fe(III) and
348 sulfate reduction occurred concurrently in SAMD, no sulfidogenesis was observed in SAMD
349 while U(VI) or Mn(IV) reduction occurred (Figure 4). This finding may be explained by the
350 greater thermodynamic favorability of U(VI) and Mn(IV) reduction relative to sulfate reduction
351 compared to the difference in thermodynamic favorability between Fe(III) reduction and sulfate
352 reduction (Table 1).

While GBSRB4.2 did not reduce Fe(III) or Mn(IV) in molybdate-amended incubations (not shown), it did reduce U(VI) (Figure 4I). This suggests that U(VI) reduction is mediated by enzymes other than those involved in sulfate, Fe(III), or Mn(IV) reduction. Since molybdate is considered a "specific" inhibitor of sulfate reduction (Oremland and Capone, 1988), the lack of Fe(III) or Mn(IV) reduction by GBSRB4.2 in molybdate-amended incubations initially suggests that the Fe(III) and Mn(IV) reduction that we observed in SAMD incubations is mediated by biogenic sulfide and not via enzymatic activity. Indeed, molybdate does not inhibit Fe(III) reduction by *Desulfovibrio desulfuricans* (Lovley et al., 1993). However, molybdate has also been shown to inhibit the growth of *Geobacter metallireducens* under nitrate-reducing conditions (Martínez Murillo et al., 1999), casting doubt on the "specificity" of molybdate as an inhibitor of sulfate reduction, particularly at the high molybdate concentration to which we exposed GBSRB4.2 (20 mM; Oremland and Capone, 1988).

We incubated GBSRB4.2 in sulfate-free PIPES buffer with H_2 as an electron donor to test its ability to reduce Fe(III), Mn(IV), and U(VI) enzymatically and independent of sulfate reduction. GBSRB4.2 completely reduced dissolved U(VI), Fe(III) (hydr)oxide, and Mn(IV) oxide in the absence of sulfate (Figure 5), suggesting that this organism is capable of the enzymatic reduction of all three of these metals. It is also notable that all reduced Fe and Mn were released into solution as Fe^{2+} or Mn^{2+}, and no secondary mineral phases (e.g. magnetite; Lovley et al., 1987) were observed visually. This is the first report of enzymatic Mn(IV) oxide reduction by a *Desulfosporosinus* sp. of which we are aware, though it remains unknown whether GBSRB4.2 exploits energy from Mn(IV) respiration for growth. While a related *Desulfotomaculum* sp. is capable Mn(IV) respiration (Tebo and Obraztsova, 1998), *Desulfosporosinus* and *Desulfosporomusa* spp. have been shown to be capable of Fe(III)

respiration, but not Mn(IV) respiration (Robertson et al., 2001; Sass et al., 2004; Ramamoorthy et al., 2006).

The robust sulfate- and metal-reducing activity of GBSRB4.2 in the presence 1 mM dissolved Al^{3+} (SAMD) is striking, since comparable Al^{3+} concentrations have been shown to be quite toxic to other SRB (Amonette et al., 2003), a finding which has been invoked as an explanation for minimal SRB activity in aluminosilicate-rich sediments (Ulrich et al., 1998; Elias et al., 2003b; Wong et al., 2004). However, acidophilic bacteria have been recovered from acidic systems that tolerate Al^{3+} concentrations as high as 200 mM (Kawai et al., 2000). There is evidence that acidophilic bacteria have inducible Al^{3+} resistance mechanisms (Fischer et al., 2002), suggesting that organisms present in microbial communities associated with AMD may possess unique mechanisms of Al^{3+} tolerance.

Acidic, metal-contaminated fluids also arise from radionuclide processing, including groundwater at the U.S. Department of Energy Environmental Remediation Science Program's Oak Ridge Integrated Field-Scale Research Challenge (IFRC) site (Saunders and Toran, 1995; Brooks, 2001). Given the enrichment of a *Desulfosporosinus* sp. from Oak Ridge IFRC sediments (Shelobolina et al., 2003), the routine detection of *Desulfosporosinus* and *Desulfotomaculum* spp. in U(VI)-contaminated sediments (Chang et al., 2001; Nevin et al., 2003; Suzuki et al., 2003; Geissler and Selenska-Pobell, 2005; Chandler et al., 2006), and the robust U(VI)-reducing activity at low pH by GBSRB4.2, we tested its ability to reduce U(VI) in groundwater from this site (referred to as ORGW) at pH 4.4 and 7.1.

GBSRB4.2 reduced U(VI) in ORGW more rapidly at pH 4.4 than at pH 7.1 (Figure 6). None of the nitrate present in ORGW (Table S3) was reduced by GBSRB4.2 and no change in ORGW pH resulted from U(VI) reduction. After complete reduction of U(VI), GBSRB4.2

399 reduced sulfate (not shown). One explanation for the faster rate of U(VI) reduction in pH 4.4
400 ORGW than in pH 7.1 ORGW is that the pH of the latter solution is outside the optimally active
401 range of GBSRB4.2. Lovley and Phillips (1992) observed that U(VI) reduction by *Desulfovibrio*
402 *desulfuricans* proceeded at comparable rates in mine waters at pH 4.0 and 7.4. The wide pH
403 range at which GBSRB4.2 is active suggests that factors other than pH may influence the rate of
404 U(VI) reduction by this organism. A more attractive explanation for the pH-dependent
405 differences in U(VI) reduction rates may be differences in U(VI) speciation among the various
406 solutions used here to test U(VI) bioreduction. Aqueous speciation modeling of SAMD using
407 Visual MINTEQ (Gustafson, 2007) revealed that the predominant aqueous U(VI) species were
408 UO_2SO_4 (aq) (68%) and UO_2^{2+} (23%), and the predominant U(VI) species in PIPES-buffered
409 incubations were $(UO_2)_3(OH)_5^+$ (73%) and $(UO_2)_4(OH)_7^+$ (24%) (Tables S1 and S2). Similarly,
410 the predominant U(VI) species in ORGW at pH 4.4 were UO_2SO_4 (aq) (69%) and UO_2^{2+} (23%)
411 (Table S4), but in ORGW at pH 7.1, the predominant U(VI) species were $Ca_2UO_2(CO_3)_3$ (aq)
412 (73%) and $CaUO_2(CO_3)_3^{2-}$ (24%) (Table S5). Indeed, previous work has shown that U(VI)
413 present in Ca-U(VI)-CO_3 complexes is poorly reducible by several U(VI) reducing
414 microorganisms (Brooks et al., 2003; Stewart et al., 2007), and Suzuki et al. (2004) showed that
415 a *Desulfosporosinus* sp. did not reduce U(VI) in bicarbonate-buffered solution but did reduce
416 U(VI) in distilled water.
417 **Environmental implications.** The activities of acidophilic/tolerant SRB may significantly alter
418 the geochemical conditions of AMD-impacted systems. For instance, the oxidative precipiation
419 of Fe and Mn through "aeration terraces" is an attractive strategy for the removal of those
420 elements from AMD since it avoids the concentration of metal sulfides in sediments associated
421 with AMD treatment systems (Unz et al., 1979; Kirby et al., 1999; Vail and Riley, 2000;

Nicormat et al., 2006; Nengovhela et al., 2004; Johnson and Hallberg, 2005a; Senko et al., 2008), but for such systems to be effective, anaerobic processes associated with AMD must be considered. Since sulfate, Fe(III), and/or Mn(IV) will represent the most abundant anaerobic terminal electron acceptors in such systems, the stability of Fe(III) and Mn(IV) (hydr)oxides may be threatened under anoxic conditions, and we show here that the activities of acid-tolerant SRB may lead to the reductive release of previously oxidized and immobile Fe and Mn despite the production of abundant sulfide, which itself is an undesirable process in some AMD treatment systems (Johnson and McGinness, 1991; Tarutis et al., 1992; Tarutis and Unz, 1995; Küsel et al., 1999; Johnson and Hallberg, 2002; Bilgin et al., 2004; Adams et al., 2007; Koschorreck et al., 2007).

While the activities of GBSRB4.2 may lead to lead to undesirable outcomes of reductive solubilization of previously immobilized Fe and Mn and near-surface concentration sulfide, it also mediates the beneficial outcomes of AMD neutralization, precipitation of dissolved Al^{3+}, and reductive precipitation of U. The reductive precipitation of U at low pH is particularly promising, given the prevalence of acidic U-contaminated systems (Saunders and Toran, 1995; Selenska-Pobell et al., 2001; Suzuki et al., 2003; Landa, 2004). The exploitation of the U(VI)-reducing activities of acidophilic/tolerant microorganisms like GBSRB4.2 may eliminate the need to neutralize U(VI)-contaminated waters to stimulate U(VI) reduction (Istok et al., 2004; Peacock et al., 2004; Wu et al., 2006; Spain et al., 2007).

ACKNOWLEDGEMENTS

We thank Pam Milavec and Jon Smoyer from the Pennsylvania Department of Environmental Protection for directing us to the Gum Boot system. This work was supported by the Pennsylvania Department of Environmental Protection, Bureau of Abandoned Mine

445 Reclamation, the Pennsylvania Water Resources Research Center at the Pennsylvania State
446 University, the Environmental Remediation Science Program (ERSP), Office of Biological and
447 Environmental Research (OBER), Office of Energy Research, US Department of Energy (DOE)
448 grant no. DE-FG02-04ER63914, and the National Science Foundation and US DOE OBER
449 under grant no. CHE-0431328.
450

450 **Table 1.** Gibbs free energies of reactions coupling the oxidation of H_2 to the reduction of

451 various terminal electron acceptors at pH 4.2[a].

Reaction	**$\Delta G_R^{o'}$ (kJ/mol)**
$H_2 + 0.25\ SO_4^{2-} + 0.5\ H^+ \rightarrow 0.25\ H_2S + H_2O$	-47
$H_2 + 2\ Fe(OH)_3 + 4\ H^+ \rightarrow 2\ Fe^{2+} + 6\ H_2O$	-75
$H_2 + UO_2^{2+} \rightarrow UO_2 + 2\ H^+$	-126
$H_2 + MnO_2 + 2\ H^+ \rightarrow Mn^{2+} + 2\ H_2O$	-189

452 [a]Values calculated from Dean (1985).

453

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714 **Figure Captions**

715 **Figure 1.** Growth and activities of GBSRB4.2 in medium with an initial pH of 4.2. Sulfide
716 concentration (○), protein concentration (□), and pH (●) are shown in panel A. Sulfate (■),
717 dissolved Fe(II) ($Fe(II)_{sol}$; ◇), and total Fe(II) ($Fe(II)_{tot}$; ◆) concentrations are shown in panel B.
718 Glucose (△) and acetate (▲) concentrations are shown in panel C. Panel D shows sulfide (○)
719 and proton (as calculated from pH; ●) concentrations in the first 7 days of growth.
720 **Figure 2.** Scanning electron micrographs of GBSRB4.2 and associated mineral phases (panels
721 A-C) and EDS spectrum of mineral phases (D) formed during growth in medium with an initial
722 pH of 4.2. The white arrow in panel A points out a GBSRB4.2 cell. The striped arrow in panel
723 B points out amorphous mineral phases produced during growth. The black arrows in panels A,
724 B, and C point out mineral phases produced during growth that exhibit "shish kebab"
725 morphology. The EDS spectrum was determined at the arrow in panel C. Scale bars in panels A
726 and B = 4 μm; scale bar in panel C = 1 μm.
727 **Figure 3.** Neighbor-joining tree showing the phyolgenetic relatedness of GBSRB4.2 to selected
728 Firmicutes bacterial sequences obtained from GenBank. Organism names in bold type represent
729 *Desulfosporosinus* species observed in acidic environments or acidophilic/tolerant SRB that have
730 been cultured. GenBank accession numbers are provided in parentheses. Bootstrap values (%)
731 were determined on the basis of results for 1,000 replicates and are shown for branches with
732 more than 50% bootstrap support.
733 **Figure 4.** Activities of GBSRB4.2 in synthetic acidic mine drainage (SAMD) and SAMD
734 amended with Fe(III) (hydr)oxide, Mn(IV) oxide, or uranyl sulfate (U(VI)). In panels A, D, G,
735 and J, sulfide concentrations of inoculated and uninoculated incubations are represented by □
736 and ■, respectively, and dissolved aluminum concentrations of inoculated and uninoculated

737 incubations are represented by ◇ and ◆, respectively. In panels B, E, H, and K, pH of
738 inoculated and uninoculated incubations are represented by ○ and ●, respectively. In panels C
739 and F, dissolved Fe(II) or Mn(II) concentrations of inoculated and uninoculated Fe(III)
740 (hydr)oxide- or Mn(IV) oxide-amended incubations are represented by △ and ▲, respectively
741 and total (0.5 M HCl-extractable) Fe(II) or Mn(II) concentrations of inoculated and uninoculated
742 Fe(III) (hydr)oxide- or Mn(IV) oxide-amended incubations are represented by ▽ and ▼,
743 respectively. In panel I, for U(VI)-amended incubations, dissolved U(VI) concentration is
744 represented by △ (inoculated) and ▲ (uninoculated), and for U(VI)- and molybdate-amended
745 incubations dissolved U(VI) concentration is represented by ▽ (inoculated) and ▼
746 (uninoculated). Error bars represent one standard deviation.
747 **Figure 5.** Soluble Fe(II) (◇ and ◆), Mn(II) (△ and ▲), and U(VI) (○ and ●) concentrations in
748 20 mM PIPES buffer (pH 6.3) amended with Fe(III) (hydr)oxide, Mn(IV) oxide, or uranyl
749 acetate, respectively. Open shapes represent soluble metal concentrations in incubations
750 inoculated with GBSRB4.2 and closed shapes represent soluble metal concentrations in
751 uninoculated incubations. Error bars represent one standard deviation.
752 **Figure 6.** Soluble U(VI) concentrations in Oak Ridge Integrated Field-Scale Research
753 Challenge site groundwater (ORGW) at pH 7.1 (○ and ●) or 4.4 (◇ and ◆). Open shapes
754 represent soluble metal concentrations in incubations inoculated with GBSRB4.2 and closed
755 shapes represent soluble metal concentrations in uninoculated incubations. Error bars represent
756 one standard deviation.
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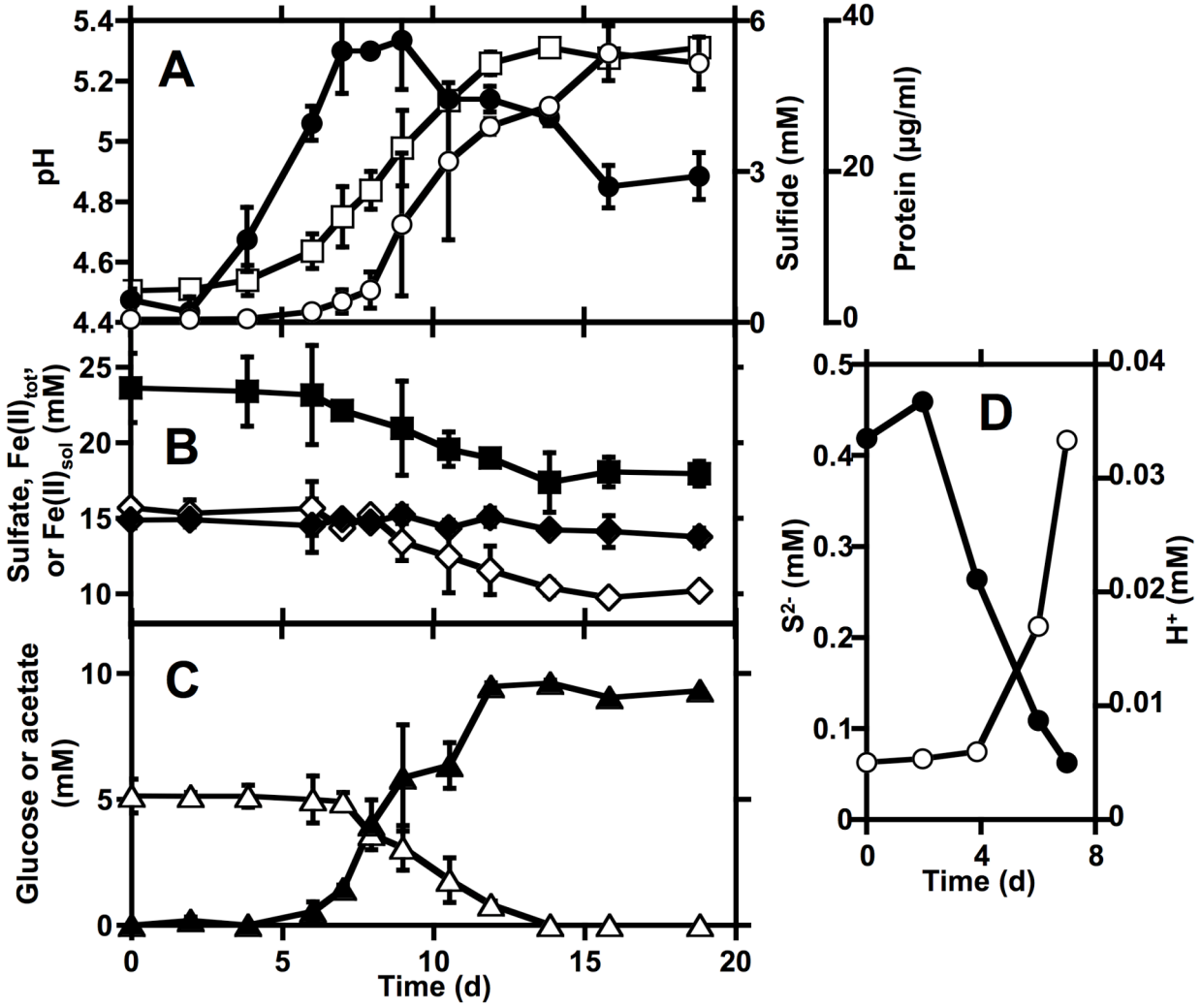
758 **Figure 1.**



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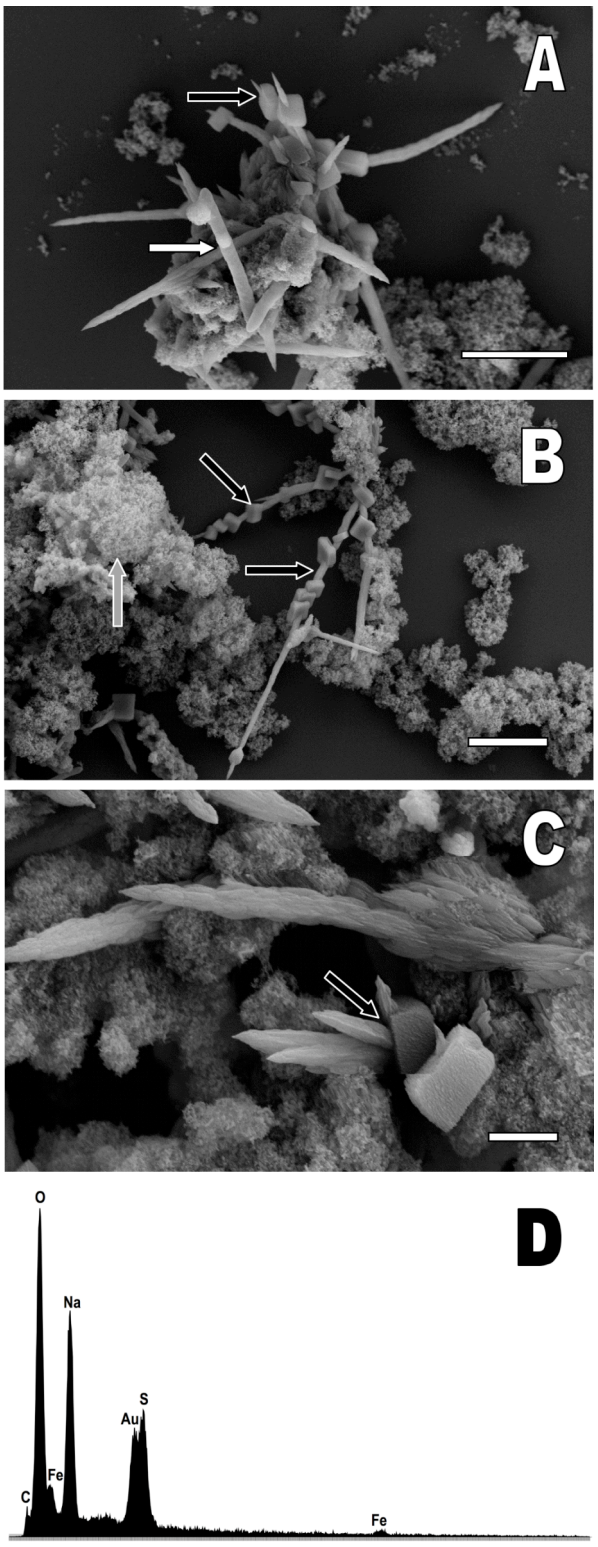
760 **Figure 2.**



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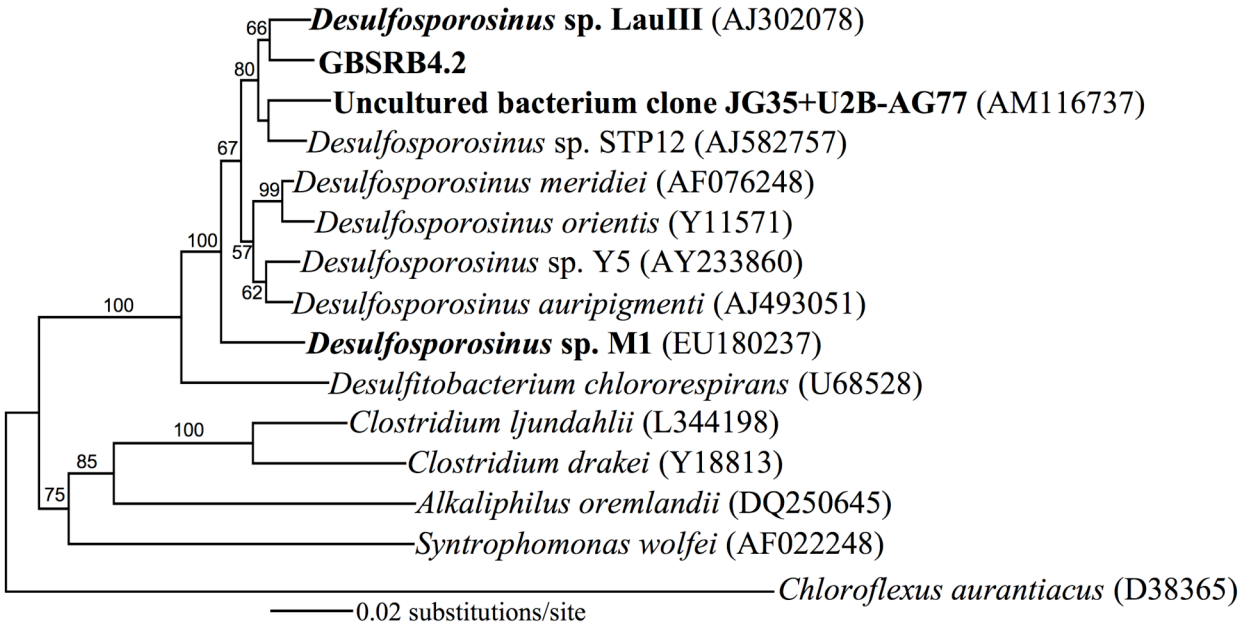
762 **Figure 3.**



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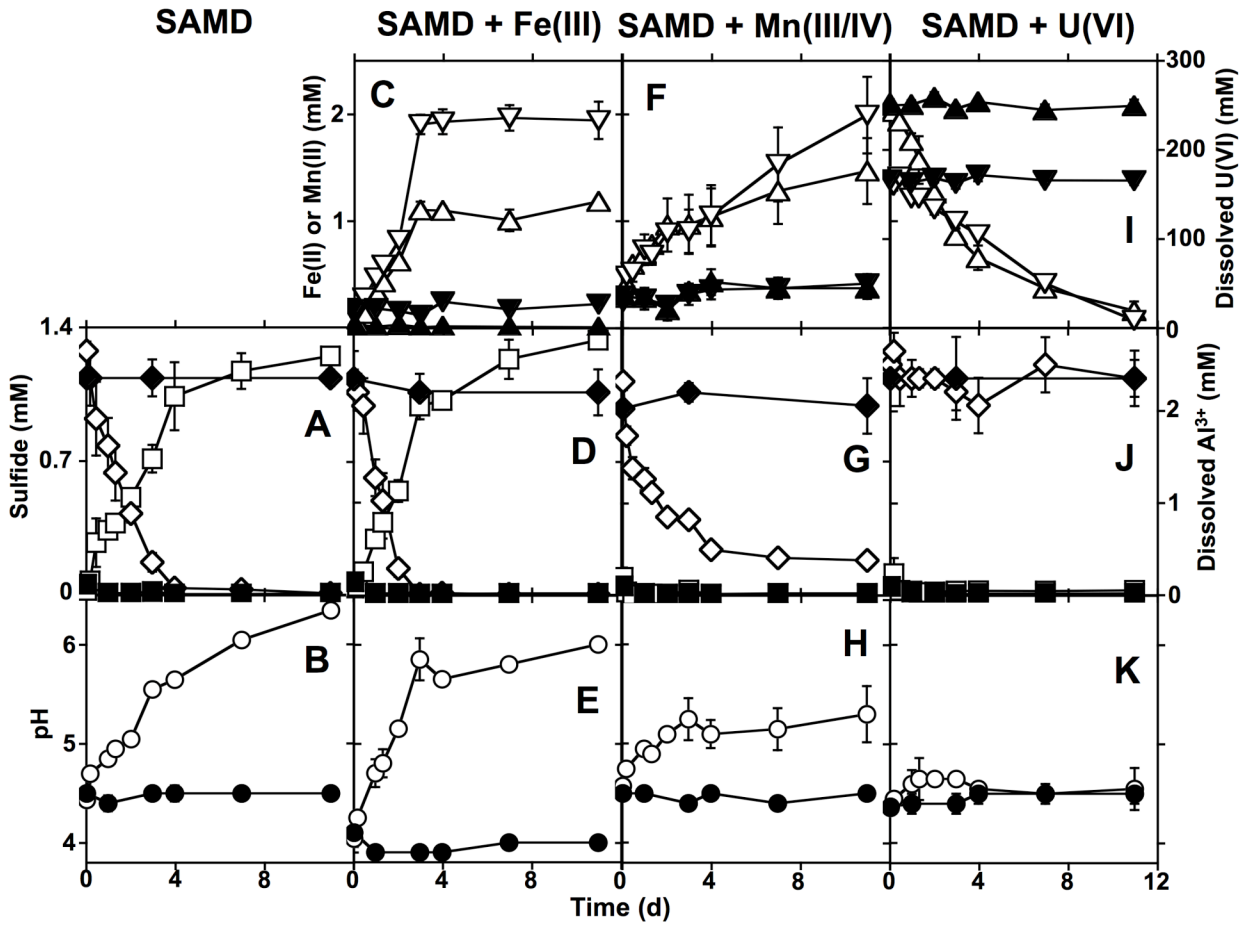
764 **Figure 4.**



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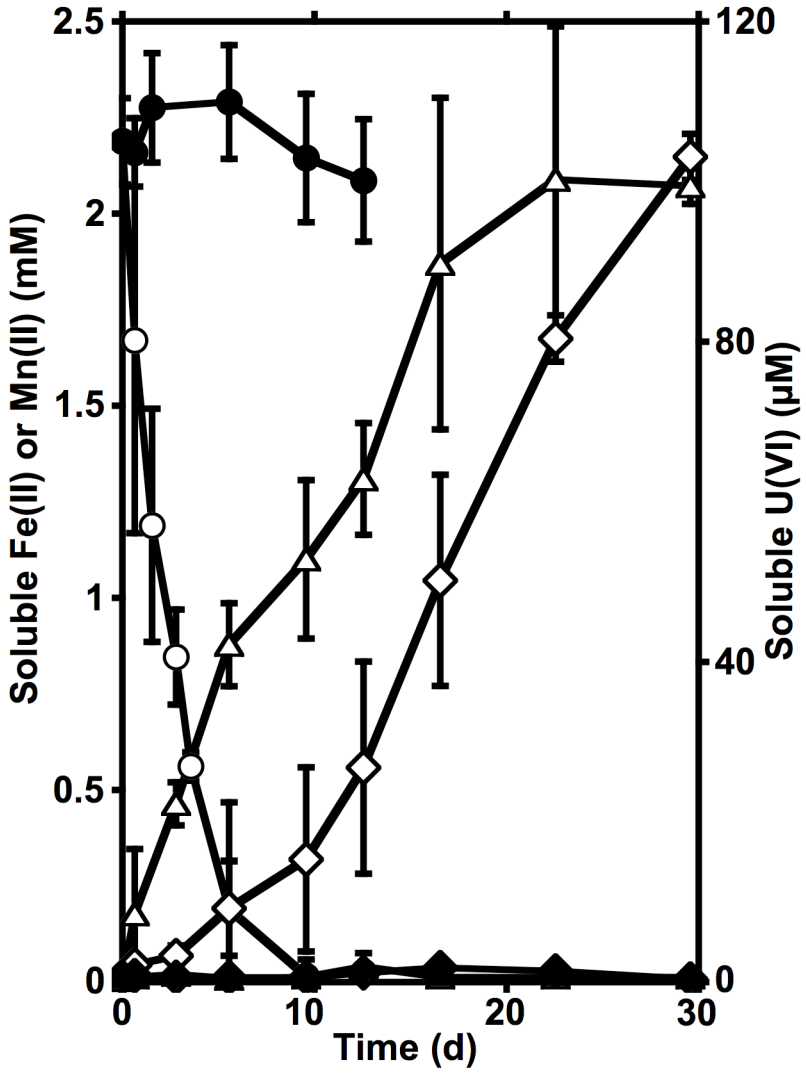
766 **Figure 5.**



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768 **Figure 6.**

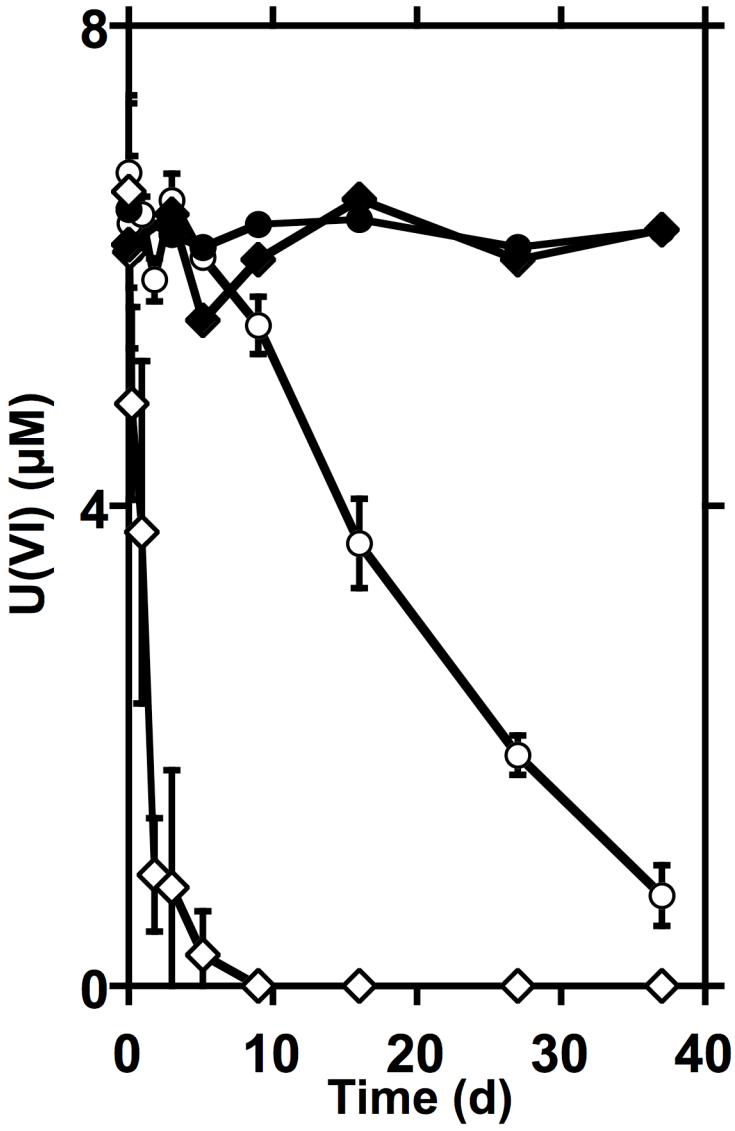

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Table S2. List of aqueous species predicted by MINTEQ in SAMD (pH 4.2) amended with uranyl sulfate.

Species	Concentration (mM)	Species	Concentration (mM)
$(UO_2)_2(OH)_2^{2+}$	1.06×10^{-3}	HSO_4^-	2.95×10^{-2}
$(UO_2)_2OH^{3+}$	1.27×10^{-4}	$Mg(NH_3)_2^{2+}$	9.87×10^{-18}
$(UO_2)_3(OH)_4^{2+}$	4.25×10^{-6}	Mg^{2+}	2.90×10^{0}
$(UO_2)_3(OH)_5^+$	9.22×10^{-6}	$MgOH^+$	1.07×10^{-7}
$(UO_2)_3(OH)_7^-$	5.19×10^{-14}	$MgSO_4$ (aq)	1.10×10^{0}
$(UO_2)_4(OH)_7^+$	3.13×10^{-8}	Na^+	1.96×10^{0}
$Al(OH)_2^+$	3.32×10^{-4}	NaOH (aq)	3.31×10^{-10}
$Al(OH)_3$ (aq)	1.78×10^{-6}	$NaSO_4^-$	4.37×10^{-2}
$Al(OH)_4^-$	1.64×10^{-8}	NH_3 (aq)	1.46×10^{-6}
$Al(SO_4)_2^-$	1.64×10^{-1}	NH_4^+	1.92×10^{-1}
Al^{3+}	9.67×10^{-2}	$NH_4SO_4^-$	8.35×10^{-3}
$Al_2(OH)_2^{4+}$	3.42×10^{-5}	OH^-	1.88×10^{-7}
$Al_3(OH)_4^{5+}$	5.31×10^{-7}	SO_4^{2-}	7.83×10^{0}
$AlOH^{2+}$	6.80×10^{-3}	$UO_2(OH)_2$ (aq)	5.35×10^{-6}
$AlSO_4^+$	7.32×10^{-1}	$UO_2(OH)_3^-$	7.99×10^{-10}
$Ca(NH_3)_2^{2+}$	5.77×10^{-18}	$UO_2(OH)_4^{2-}$	1.47×10^{-17}
Ca^{2+}	3.38×10^{0}	$UO_2(SO_4)_2^{2-}$	1.33×10^{-2}
$CaNH_3^{2+}$	7.94×10^{-9}	UO_2^{2+}	5.83×10^{-2}
$CaOH^+$	6.58×10^{-9}	UO_2OH^+	3.18×10^{-3}
$CaSO_4$ (aq)	1.62×10^{0}	UO_2SO_4 (aq)	1.73×10^{-1}
H^+	7.43×10^{-2}		

772 **Table S2**. List of aqueous U(VI) species predicted by MINTEQ in 20 mM PIPES buffer (pH
773 6.3) amended with uranyl acetate.

Species	Concentration (mM)
$(UO_2)_2(OH)_2^{2+}$	1.95×10^{-4}
$(UO_2)_2OH^{3+}$	8.63×10^{-8}
$(UO_2)_3(OH)_4^{2+}$	5.66×10^{-5}
$(UO_2)_3(OH)_5^{+}$	2.44×10^{-2}
$(UO_2)_3(OH)_7^{-}$	2.17×10^{-6}
$(UO_2)_4(OH)_7^{+}$	6.04×10^{-3}
CH_3COO^{-}	1.94×10^{-1}
H^{+}	5.07×10^{-4}
CH_3COOH (aq)	5.50×10^{-3}
OH^{-}	2.03×10^{-5}
$UO_2(CH_3COO)_2$ (aq)	5.63×10^{-7}
$UO_2(CH_3COO)_3^{-}$	1.15×10^{-8}
$UO_2(OH)_2$ (aq)	3.93×10^{-4}
$UO_2(OH)_3^{-}$	6.30×10^{-6}
$UO_2(OH)_4^{2-}$	9.23×10^{-12}
UO_2^{2+}	1.46×10^{-4}
$UO_2CH_3COO^{+}$	3.49×10^{-5}
UO_2OH^{+}	1.58×10^{-3}

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Table S3. Composition of ORGW at pH 4.4 and 7.1. The valence of Al, Ca, K, Mg, and Na could not be determined by ICP-AES, but were assumed. Dissolved organic carbon (DIC) could not be detected in ORGW pH 4.4.

Component	ORGW pH 4.4 (mM)	ORGW pH 7.1 (mM)
Al^{3+}	0.004	0.007
Ca^{2+}	6.75	5.75
K^{+}	0.28	0.27
Mg^{2+}	2.42	2.42
Na^{+}	10.44	6.87
NO_3^{-}	15.0	0.51
SO_4^{2-}	13.0	13.0
UO_2^{2+}	0.006	0.006
DIC	ND	2.95
pH	4.4	7.1

Table S4. Aqueous speciation of constituents of ORGW pH 4.4 (from Table S3) determined by modeling using MINTEQ.

Species	Concentration (mM)	Species	Concentration (mM)
$(UO_2)_2(OH)_2^{2+}$	1.32×10^{-6}	K^+	2.69×10^{-1}
$(UO_2)_2OH^{3+}$	1.13×10^{-7}	KNO_3 (aq)	1.75×10^{-3}
$(UO_2)_3(OH)_4^{2+}$	2.78×10^{-10}	KOH (aq)	9.69×10^{-11}
$(UO_2)_3(OH)_5^+$	8.84×10^{-10}	KSO_4^-	8.78×10^{-3}
$(UO_2)_3(OH)_7^-$	1.25×10^{-17}	Mg^{2+}	1.74×10^{0}
$(UO_2)_4(OH)_7^+$	1.59×10^{-13}	$MgOH^+$	9.48×10^{-8}
$Al(OH)_2^+$	2.85×10^{-6}	$MgSO_4$ (aq)	6.76×10^{-1}
$Al(OH)_3$ (aq)	2.35×10^{-8}	Na^+	1.01×10^{1}
$Al(OH)_4^-$	3.54×10^{-10}	$NaNO_3$ (aq)	2.87×10^{-2}
$Al(SO4)_2^-$	7.17×10^{-4}	NaOH (aq)	2.64×10^{-9}
Al^{+3}	4.06×10^{-4}	$NaSO_4^-$	2.56×10^{-1}
$Al_2(OH)_2^{4+}$	1.44×10^{-9}	NO_3^-	1.49×10^{1}
$Al_3(OH)_4^{5+}$	2.37×10^{-13}	OH^-	3.06×10^{-7}
$AlOH^{2+}$	3.98×10^{-5}	SO_4^{2-}	9.84×10^{0}
$AlSO_4^+$	2.83×10^{-3}	$UO_2(OH)_2$ (aq)	2.82×10^{-7}
$Ca(NO_3)_2$ (aq)	9.90×10^{-9}	$UO_2(OH)_3^-$	6.87×10^{-11}
Ca^{+2}	4.47×10^{0}	$UO_2(OH)_4^{2-}$	2.16×10^{-18}
$CaNO_3^+$	9.83×10^{-2}	$UO_2(SO4)_2^{2-}$	3.99×10^{-4}
$CaOH^+$	1.28×10^{-8}	UO_2^{2+}	1.36×10^{-3}
$CaSO_4$ (aq)	2.18×10^{0}	$UO_2NO_3^+$	1.89×10^{-5}
H^+	4.81×10^{-2}	UO_2OH^+	1.09×10^{-4}
HSO_4^-	2.17×10^{-2}	UO_2SO_4 (aq)	4.11×10^{-3}

Table S5. Aqueous speciation of constituents of ORGW pH 7.1 (from Table S3) determined by modeling using MINTEQ.

Species	Concentration (mM)	Species	Concentration (mM)
$(UO_2)_2(OH)_2^{2+}$	2.72×10^{-10}	HSO_4^-	4.62×10^{-5}
$(UO_2)_2CO_3(OH)_3^-$	2.81×10^{-5}	K^+	2.61×10^{-1}
$(UO_2)_2OH^{3+}$	4.26×10^{-14}	KNO_3 (aq)	5.99×10^{-5}
$(UO_2)_3(CO_3)_6^{6-}$	6.40×10^{-13}	KOH (aq)	4.80×10^{-8}
$(UO_2)_3(OH)_4^{2+}$	4.30×10^{-13}	KSO_4^-	9.24×10^{-3}
$(UO_2)_3(OH)_5^{2+}$	7.22×10^{-10}	Mg^{2+}	1.66×10^{0}
$(UO_2)_3(OH)_7^-$	2.56×10^{-12}	$Mg_2CO_3^{2+}$	1.29×10^{-6}
$(UO_2)_3CO_3(OH)_3^+$	1.73×10^{-14}	$MgCO_3$ (aq)	1.65×10^{-4}
$(UO_2)_4(OH)_7^+$	9.70×10^{-13}	$MgHCO_3^+$	4.13×10^{-3}
$Al(OH)_2^+$	1.92×10^{-4}	$MgOH^+$	4.78×10^{-5}
$Al(OH)_3$ (aq)	8.09×10^{-4}	$MgSO_4$ (aq)	7.54×10^{-1}
$Al(OH)_4^-$	5.99×10^{-3}	Na^+	6.68×10^{0}
$Al(SO_4)_2^-$	2.27×10^{-7}	$NaCO_3^-$	2.98×10^{-5}
Al^{3+}	9.47×10^{-8}	$NaHCO_3$ (aq)	1.14×10^{-3}
$Al_2(OH)_2^{4+}$	2.04×10^{-11}	$NaNO_3$ (aq)	6.70×10^{-4}
$Al_2(OH)_2CO_3^{2+}$	6.23×10^{-7}	NaOH (aq)	8.91×10^{-7}
$Al_3(OH)_4^{5+}$	1.96×10^{-13}	$NaSO_4^-$	1.84×10^{-1}
$AlOH^{2+}$	5.07×10^{-6}	NO_3^-	5.06×10^{-1}
$AlSO_4^+$	8.26×10^{-7}	OH^-	1.51×10^{-4}
$Ca(NO_3)_2$ (aq)	1.04×10^{-11}	SO_4^{2-}	9.97×10^{0}
Ca^{2+}	3.64×10^{0}	$UO_2(CO_3)_2^{2-}$	8.84×10^{-5}
$Ca_2UO_2(CO_3)_3$ (aq)	4.34×10^{-3}	$UO_2(CO_3)_3^{4-}$	2.85×10^{-5}
$CaCO_3$ (aq)	7.22×10^{-4}	$UO_2(OH)_2$ (aq)	2.11×10^{-6}
$CaHCO_3^+$	1.12×10^{-2}	$UO_2(OH)_3^-$	2.53×10^{-7}
$CaNO_3^+$	2.93×10^{-3}	$UO_2(OH)_4^{2-}$	3.78×10^{-12}
$CaOH^+$	5.50×10^{-6}	$UO_2(SO_4)_2^{2-}$	1.31×10^{-8}
$CaSO_4$ (aq)	2.08×10^{0}	UO_2^{2+}	3.78×10^{-8}
$CaUO_2(CO_3)_3^{2-}$	1.44×10^{-3}	UO_2CO_3 (aq)	3.93×10^{-5}
CO_3^{2-}	4.78×10^{-4}	$UO_2NO_3^+$	1.92×10^{-11}
H^+	9.44×10^{-5}	UO_2OH^+	1.60×10^{-6}
$H_2CO_3^*$ (aq)	7.20×10^{-2}	UO_2SO_4 (aq)	1.33×10^{-7}
HCO_3^-	4.82×10^{-1}		