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# Uranium(VI) Reduction by *Anaeromyxobacter dehalogenans* Strain 2CP-C

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**Previous studies demonstrated growth of *Anaeromyxobacter dehalogenans* strain 2CP-C with acetate or hydrogen as the electron donor and Fe(III), nitrate, nitrite, fumarate, oxygen, or *ortho*-substituted halophenols as electron acceptors. In this study, we explored and characterized U(VI) reduction by strain 2CP-C. Cell suspensions of fumarate-grown 2CP-C cells reduced U(VI) to U(IV). More-detailed growth studies demonstrated that hydrogen was the required electron donor for U(VI) reduction and could not be replaced by acetate. The addition of nitrate to U(VI)-reducing cultures resulted in a transitory increase in U(VI) concentration, apparently caused by the reoxidation of reduced U(IV), but U(VI) reduction resumed following the consumption of N-oxyanions. Inhibition of U(VI) reduction occurred in cultures amended with Fe(III) citrate, or citrate. In the presence of amorphous Fe(III) oxide, U(VI) reduction proceeded to completion but the U(VI) reduction rates decreased threefold compared to control cultures. Fumarate and 2-chlorophenol had no inhibitory effects on U(VI) reduction, and both electron acceptors were consumed concomitantly with U(VI). Since cocontaminants (e.g., nitrate, halogenated compounds) and bioavailable ferric iron are often encountered at uranium-impacted sites, the metabolic versatility makes *Anaeromyxobacter dehalogenans* a promising model organism for studying the complex interaction of multiple electron acceptors in U(VI) reduction and immobilization.**

Uranium is one of the most abundant radionuclides found in soils, sediments, and groundwater at U.S. Department of Energy (DOE) sites. Although biological activity cannot destroy or remove uranium, soluble and mobile U(VI) can be microbiologically precipitated and immobilized by its reduction to insoluble U(IV) oxide (2, 22–24, 28). Therefore, in situ microbial reduction of U(VI) to U(IV) is a promising bioremediation strategy for containing uranium plumes. Microbial U(VI) reduction has been observed in two major bacterial groups: the metal-reducing bacteria (e.g., *Geobacter* spp. and *Shewanella* spp.) (22, 23, 28, 36) and the sulfate-reducing bacteria (e.g., *Desulfovibrio* spp.) (33, 34, 40). Some of these microorganisms have been reported to conserve energy for growth from U(VI) reduction (29, 40, 47), while others reduce uranium without apparent energy gain (4, 12, 21, 23, 33, 34, 44, 46).

*Anaeromyxobacter dehalogenans* is the first myxobacterium capable of anaerobic respiration by coupling the oxidation of acetate or hydrogen to the reduction of *ortho*-substituted halophenols, soluble and amorphous ferric iron, nitrate, nitrite, fumarate, humic substances (i.e., anthraquinone-2,6-disulfonate), or oxygen (41). A 16S rRNA gene-based community analysis of Fe(III)-reducing enrichment cultures obtained from uranium-contaminated sediment collected at the U.S. DOE Field Research Center (FRC) near Oak Ridge, TN, suggested the predominance of organisms related to the genus *Anaeromyxobacter* (39). Furthermore, biostimulation push-pull tests at the FRC yielded evidence of metal-reducing microorganisms in acidic subsurface sediments associated with both the *Anaeromyxobacter* and *Geobacter* genera (38).

The geochemistry at the U(VI)-contaminated FRC site is complex, and cocontaminants including nitrate, petroleum hydrocarbons, and chlorinated hydrocarbons (e.g., chlorinated solvents, polychlorinated biphenyls) are encountered (35). Other energetically favorable terminal electron acceptors such as oxygen, nitrate, Fe(III), and chloro-organic compounds can interfere with efficient U(VI) reduction. For instance, oxidized nitrogen species can inhibit U(VI) reduction and reoxidize reduced U(IV) to U(VI) (7, 9, 10, 19, 45). Fe(III) oxides affect uranium speciation and mobility, and these complex interactions are under intensive investigation (9, 20, 37, 42, 43, 48). Further, chloro-organic electron acceptors may affect U(VI) reduction, but these interactions have not been explored. Studies on bacterial uranium reduction have largely focused on the well-known metal- and sulfate-reducing bacteria. Evidence for the presence of *Anaeromyxobacter* spp. in FRC sediments impacted with uranium, nitrate, and chloro-organic compounds and their metabolic versatility warrants exploration of uranium reduction by *Anaeromyxobacter dehalogenans*.

## MATERIALS AND METHODS

**Bacterial strain and culture conditions.** *Anaeromyxobacter dehalogenans* strain 2CP-C (ATCC BAA-259) was routinely grown in 50-ml glass serum bottles with 20 ml of anoxic, bicarbonate-buffered mineral salt medium. The bottles were closed with butyl rubber stoppers and aluminum crimps. The bicarbonate (30 mM)-buffered medium was prepared as described previously (26) except that sulfide was omitted. The headspaces of culture vessels consisted of nitrogen gas balanced with $CO_2$ to adjust the pH to 7.1. Acetate (5 mM) or hydrogen (2 ml, or 82 μmol) or both were added as electron donors. U(VI) (0.3 mM) was added as uranyl carbonate from an anoxic, filter-sterilized, 30 mM stock solution of uranyl acetate (Spectrum, Gardena, CA) prepared in 30 mM bicarbonate buffer (pH 8.4). Transfers of 1 to 2% (vol/vol) occurred from growing 2CP-C cultures. Duplicate cultures were incubated at 35°C in the dark without agitation. All results were verified in at least one additional, independent experiment with duplicate cultures. Uranium precipitation, sorption, and speciation are influenced by pH (5, 18, 49). Therefore, we monitored pH in selected cultures over the course of the U(VI) reduction experiments. In all cultures, the pH remained

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unchanged from the initial pH of 7.1, indicating that pH variations did not affect the results.

**U(VI) reduction in cell suspensions.** Cells were grown in 100-ml volumes with two feedings of 4 mM acetate and 5 mM fumarate. Once the fumarate had been exhausted, the cell suspensions were distributed in 15-ml aliquots to 24-ml anaerobic culture tubes in an anaerobic chamber containing $H_2$ (5 to 10% of the headspace, vol/vol). Each tube was amended with uranyl chloride to achieve a final concentration of 293 μM and incubated for 8 h. The reduction of U(VI) was monitored every 2 h.

**Effect of hydrogen on U(VI) reduction.** To test the effect of hydrogen on U(VI) reduction by strain 2CP-C, replicate 50-ml serum bottles with 20 ml medium were amended with 5 mM acetate, 0.3 mM uranyl carbonate, and 82 μmol of hydrogen or no hydrogen. Replicate vessels received 1 mM nitrate or 1 mM fumarate and 2% (vol/vol) inocula from acetate-nitrate- and acetate-fumarate-grown cultures, respectively. Duplicate control bottles received filter-sterilized (0.2-μm Acrodisc syringe filter; Pall Corp., Ann Arbor, MI) inocula.

**Effects of alternate electron acceptors on U(VI) reduction.** The influence of nitrate, Fe(III), 2-chlorophenol (2-CP), and fumarate on U(VI) reduction in 50-ml serum bottles containing 20 ml of medium was evaluated.

**(i) Nitrate.** To test the effect of nitrate, uranyl carbonate (6 μmol) and hydrogen (82 μmol) were added to an acetate-nitrate-grown 2CP-C culture after nitrate had been completely consumed. Following the onset of U(VI) reduction, nitrate (0.5 mM) was added to one set of duplicate cultures, and nitrate and U(VI) reduction were monitored.

**(ii) Ferric iron.** To test the influence of different forms of ferric iron on U(VI) reduction, cultures were grown with 5 mM acetate and 2 mM fumarate, and 0.3 mM uranyl carbonate and 82 μmol hydrogen were added when fumarate reduction was complete. Following the onset of uranium reduction, ferric citrate (4 mM), citrate (4 mM), or amorphous Fe(III) oxyhydroxide (4 mM, nominal concentration) was added to sets of duplicate cultures, and U(VI) reduction was monitored. Control vessels were autoclaved at 121°C for 30 min prior to the addition of uranyl carbonate and hydrogen. Amorphous Fe(III) oxyhydroxide (pH 6.95) was prepared from $FeCl_3$ and NaOH as previously described (32). In order to investigate the sorption of U(VI) to amorphous Fe(III) oxide, bottles with sterile mineral salt medium were amended with 0.3 mM uranyl carbonate and Fe(III) oxide at nominal concentrations of 0, 5, 10, and 20 mM and incubated statically at 35°C. The addition of amorphous Fe(III) oxyhydroxide had no effect on the pH of the medium. Samples were taken after 2 and 10 days of incubation.

**(iii) 2-CP.** The effect of 2-CP on U(VI) reduction was tested with cultures that were grown with 5 mM acetate and 0.2 mM nitrate and with cultures grown with 0.1 mM acetate, 82 μmol hydrogen, and 0.2 mM 2-CP. After growth had occurred and the electron acceptors were depleted, 0.3 mM (6 μmol) uranyl carbonate and hydrogen (82 μmol) were added to the cultures. Two culture bottles were autoclaved before the addition of uranyl carbonate and hydrogen and served as controls. Following the onset of uranium reduction, 0.1 mM 2-CP was added to one set of duplicate cultures. A second experiment tested U(VI) reduction in the presence of 0.25 mM 2-CP. U(VI) reduction in 2-CP-amended cultures was compared to replicate cultures that received no 2-CP.

**(iv) Fumarate.** To explore the effects of fumarate on U(VI) reduction, cells grown with 4 mM acetate and 5 mM fumarate were harvested by centrifugation (12,100 × *g* for 10 min at room temperature) when fumarate had been consumed. The cells were washed twice under anoxic conditions in 1 ml of fresh medium before the biomass was suspended in 10 ml of medium in 24-ml glass vials. The cell concentration was approximately $8 \times 10^8$ ml$^{-1}$, as determined by *Anaeromyxobacter* 16S rRNA gene-targeted quantitative real-time PCR (see below). The vials were amended with 3 μmol uranyl carbonate and 41 μmol (1 ml) hydrogen. Replicate vials received 10 μmol of fumarate or no fumarate. Control vials received the same additions except cells.

**Impact of uranyl carbonate on 2-CP dechlorination.** Serum bottles containing 20 ml fresh medium were amended with 0.1 mM acetate, 2 ml hydrogen (82 μmol), and 0.2 mM (4 μmol) 2-CP and inoculated from a 2-CP-grown culture. Uranyl carbonate (0.3 mM) was added to one set of replicate cultures. After 2-CP was completely reduced to phenol, additional 0.25 mM 2-CP (5 μmol) was fed, and its depletion was monitored. The U(VI)-amended cultures contained about 0.22 mM U(VI) at the time of the second 2-CP addition.

**Analytical methods.** U(VI) was determined by a modified laser excitation spectrofluorescence method (3). A 1:1 (vol/vol) mixture of 40 mM sodium hypophosphite and 80 mM sodium pyrophosphate was used as a complexing agent for U(VI) analysis. Samples (0.1 ml) containing U(VI) were withdrawn from the cultures by syringe, mixed with 0.9 ml of filtered, deionized water and 0.03 ml of the complexing solution, and immediately analyzed at 498.5 nm using a luminescence spectrometer (LS50B; Perkin-Elmer). The reduction of U(VI) to U(IV) was confirmed by measuring the increase in U(VI) concentrations in samples exposed to air for at least 1 h. The concentrations of U(VI) measured following air oxidation of reduced U(IV) ranged from 65 to 70% of the amount of U(VI) added to the cultures. Nitrate and nitrite were quantified with a DX-100 ion chromatograph (Dionex Corp., Sunnyvale, CA) equipped with an AS14A IonPac column. Fe(II) was analyzed using the ferrozine assay following HCl extraction as described previously (30, 31). 2-CP and phenol were analyzed with a high-performance liquid chromatography (HPLC) system equipped with a 717 Plus autosampler (10-μl injection volume) (Waters Corp., Milford, MA) and a Nova-Pac $C_{18}$ column (4 μm, 3.9 by 50 mm; Waters). The eluent was methanol-water (60/40, vol/vol) containing 0.1% (vol/vol) phosphoric acid, which was pumped at 1.5 ml/min. Phenolic compounds were detected at 275.6 nm on a Waters 2996 photodiode array detector. Samples (1 ml) were withdrawn from the cultures by syringe, made basic with 10 μl of 2 M NaOH, and filtered through Acrodisc syringe filters (13-mm diameter, nylon membrane with 0.45-μm pore size) prior to transfer to autosampler vials and HPLC analysis. Fumarate and succinate were monitored by HPLC with a Waters HPLC system equipped with a Waters 2487 dual-wavelength absorbance detector set at 210 nm and a Waters 717 Plus autosampler (20-μl injection volume) as described previously (15). 2CP-C cells were enumerated by quantifying the organism's 16S rRNA gene using quantitative real-time PCR (qPCR). DNA was extracted with an InstaGene matrix (Bio-Rad Laboratories, Hercules, CA) by following the manufacturer's instructions. Primers targeting 16S rRNA genes of *Anaeromyxobacter dehalogenans* strain 2CP-C were designed using Primer Express software (Applied Biosystems). Primer F112 (5′GTA ATC TGC CCT AGA GTC CGG A-3′) and primer R227 (5′AGA GCG ATA GCT TGT GTA CAG AGG-3′) were used to amplify 16S rRNA gene sequences of *Anaeromyxobacter dehalogenans* strain 2CP-C. Primer specificities were verified using the Ribosomal Database Project (6) and GenBank databases (1). qPCR was performed in a total reaction volume of 30 μl with 15 μl of SYBR green master mixture (Applied Biosystems, Foster City, CA), a 100 nM concentration of each primer, and 10-fold-diluted template DNA. The PCR conditions were as follows: 2 min at 50°C and 15 min at 95°C, followed by 40 cycles of 30 s at 94°C and 60 s at 60°C. qPCR was carried out in an ABI Prism 7000 sequence detection system (Applied Biosystems). Since each *Anaeromyxobacter dehalogenans* cell possesses two 16S RNA gene copies (strain 2CP-C, GenBank accession number CP000251), the gene copy number determined by qPCR was divided by 2 to calculate the cell number.

## RESULTS

Cell suspension of fumarate-grown *Anaeromyxobacter dehalogenans* strain 2CP-C cells readily reduced U(VI) to U(IV). In these cultures U(VI) was reduced by 90% within 8 h of incubation (data not shown). Suspensions of washed, fumarate-grown 2CP-C cells amended with acetate and hydrogen reduced U(VI) following a lag time of <3 days, while loss of soluble U(VI) in the killed controls was negligible (data not shown).

No U(VI) reduction was observed in growing cultures lacking hydrogen (Fig. 1 and 2). In cultures amended with uranyl carbonate and nitrate, nitrate was readily reduced to ammonium but the reduction of U(VI) required the addition of hydrogen (Fig. 1). Similarly, fumarate was reduced to succinate in cultures amended with acetate available as an electron donor, but the reduction of U(VI) in the same cultures did not occur in the absence of hydrogen (Fig. 2). These findings suggest that growing cultures of strain 2CP-C require hydrogen to reduce uranium, whereas the same organism couples the reduction of other electron acceptors such as fumarate and nitrate to the oxidation of both acetate and hydrogen.

**Effects of alternative electron acceptors on U(VI) reduction.** Many uranium-impacted sites contain cocontaminants, such as nitrate and chloro-organic compounds, or contain other electron acceptors that may interfere with U(VI) reduction. Hence, the effects of other oxidants on U(VI) reduction were

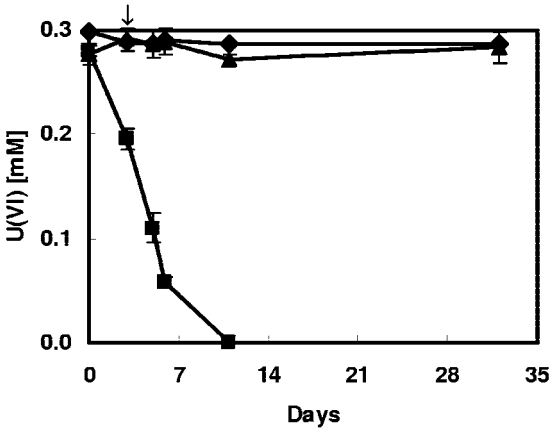


FIG. 1. U(VI) reduction in cultures amended with acetate, nitrate, and uranyl carbonate with or without hydrogen. ■, with hydrogen; ▲, no hydrogen; ◆, no cells. The culture vessels were inoculated with acetate-nitrate-grown 2CP-C cells. Nitrate reduction in live cultures was complete after 3 days, as indicated by the arrow. The data points represent the averages of duplicate cultures, with error bars showing the standard deviations.

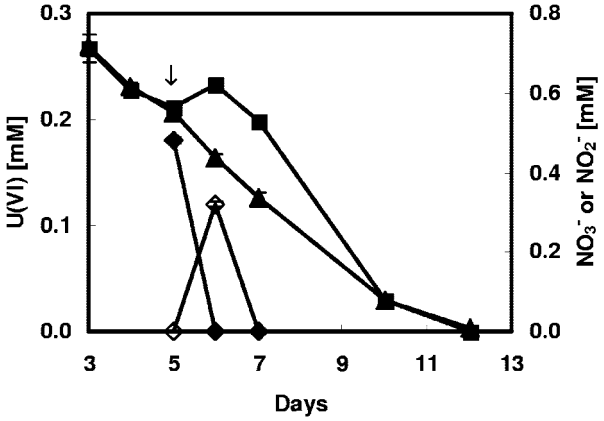


FIG. 3. Effect of nitrate on U(VI) reduction. ■, U(VI) plus nitrate; ▲, U(VI) with no nitrate; ◆, nitrate; ◇, nitrite. Strain 2CP-C was grown with acetate-nitrate, and complete nitrate reduction had occurred by day 3 when the cultures were amended with uranyl carbonate and hydrogen. The arrow indicates the addition of 0.5 mM nitrate following the onset of U(VI) reduction. Results are the averages of duplicate cultures, with error bars showing the standard deviations.

explored in cultures of *Anaeromyxobacter dehalogenans* strain 2CP-C.

**(i) Nitrate.** Complete reduction of nitrate occurred within 3 days of incubation in cultures provided with acetate as an electron donor. U(VI) reduction occurred 1 day after uranyl carbonate and hydrogen were added to the acetate-nitrate-grown cultures (Fig. 3). The addition of nitrate caused an apparent increase in soluble U(VI) concentration (Fig. 3). The increase in U(VI) concentration coincided with the intermediate formation of nitrite, suggesting that nitrate-reducing conditions are associated with U(IV) oxidation. Following complete consumption of nitrate and nitrite, U(VI) reduction resumed (Fig. 3).

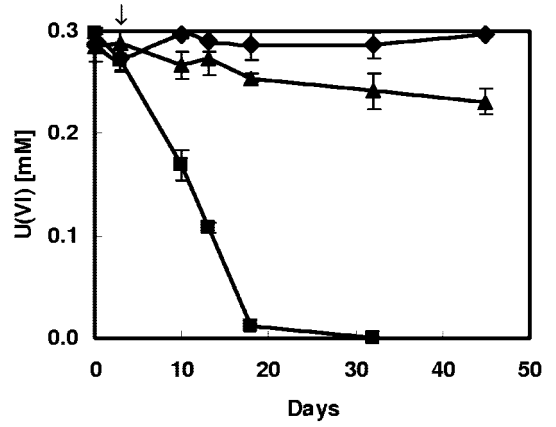


FIG. 2. U(VI) reduction in cultures amended with acetate, fumarate, and uranyl carbonate with or without hydrogen. ■, with hydrogen; ▲, no hydrogen; ◆, no cells. The culture vessels were inoculated with acetate-fumarate-grown 2CP-C cells. In live cultures, fumarate was completely reduced after 3 days, as indicated by the arrow. Results are the averages of duplicate cultures, with error bars showing the standard deviations.

**(ii) Ferric iron.** The effect of ferric citrate was explored in acetate-fumarate-grown cultures that had consumed all fumarate (1 day following inoculation). In the presence of hydrogen, U(VI) was readily reduced without apparent lag phase, but the addition of ferric citrate caused the immediate cessation of U(VI) reduction (Fig. 4). In contrast, complete Fe(III)-to-Fe(II) reduction occurred within 1 day following the addition of ferric citrate, suggesting that the cells were metabolically active. In a parallel experiment, sodium citrate, instead of ferric citrate, was added following the onset of U(VI) reduction. The addition of citrate alone was sufficient to prevent U(VI) reduction (Fig. 4), suggesting that ferric iron was not

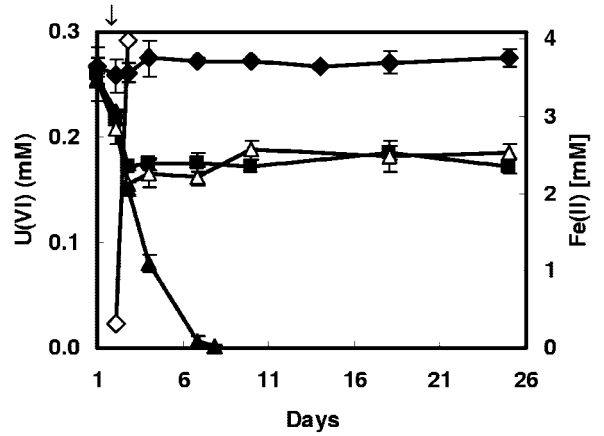


FIG. 4. Effects of ferric citrate and citrate on U(VI) reduction. ■, U(VI) plus ferric citrate; ▲, U(VI) with no additions; △, U(VI) plus citrate; ◆, U(VI) plus killed inoculum; ◇, Fe(II). Strain 2CP-C cultures were grown with acetate-fumarate. Fumarate was consumed after 1 day, and then the cultures were amended with uranyl carbonate and hydrogen. The arrow indicates the addition of ferric citrate or sodium citrate to duplicate sets of cultures following the onset of U(VI) reduction. The data shown represent averaged values of duplicate cultures, with error bars showing the standard deviations.

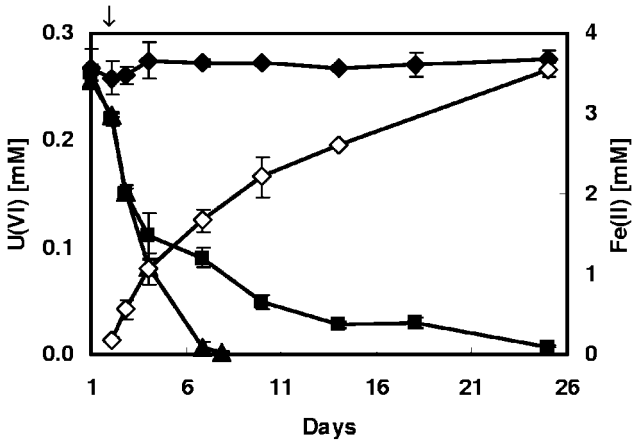


FIG. 5. Effect of amorphous Fe(III) oxide on U(VI) reduction. ■, U(VI) plus amorphous Fe(III) oxide; ▲, U(VI) with no amorphous Fe(III) oxide; ◆, U(VI) plus killed cells; ◇, Fe(II). Acetate-fumarate-grown strain 2CP-C cultures consumed fumarate within 1 day, at which time the cultures were amended with uranyl carbonate and hydrogen. The arrow indicates the addition of amorphous Fe(III) oxide. The data points represent the averages generated with duplicate cultures, with error bars showing the standard deviations.

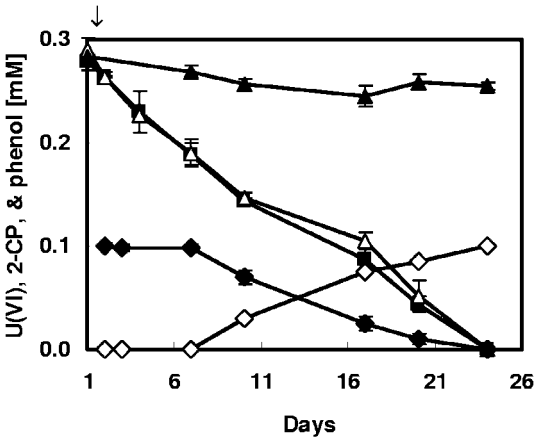


FIG. 6. Effect of 2-chlorophenol on U(VI) reduction. ■, U(VI) with no 2-CP; △, U(VI) plus 2-CP; ▲, U(VI) plus killed cells; ◆, 2-CP; ◇, phenol. Strain 2CP-C was pregrown with acetate-nitrate. Nitrate reduction was complete after day 1, when uranyl carbonate and hydrogen were added. The arrow indicates when 2-CP was added after the onset of U(VI) reduction. Results are the averages of duplicate cultures, with error bars showing the standard deviations.

the cause for the observed inhibition. To further explore the effects of ferric iron on U(VI) reduction, amorphous Fe(III) oxide was added to U(VI)-reducing cultures. The addition of Fe(III) oxide slowed down the rate of U(VI) reduction relative to control cultures not receiving amorphous iron; however, U(VI) reduction proceeded to completion (Fig. 5). Concomitant with U(VI) reduction, Fe(II) was formed, suggesting that both ferric iron and U(VI) were reduced simultaneously. The addition of amorphous iron increased the time required to completely reduce the initial amount of U(VI) about threefold compared to cultures not receiving amorphous ferric iron (Fig. 5). To verify that the loss of U(VI) was due to microbial reduction, U(VI) sorption to Fe(III) oxide under the culture conditions applied was evaluated. Table 1 shows the change in U(VI) concentrations in sterile mineral salt medium amended with different amounts of Fe(III) oxide. Only a small amount (<2%) of the total U(VI) sorbed to 5 mM (nominal concentration) amorphous Fe(III) oxide following a 10-day incubation period, suggesting that the loss of U(VI) in the live cultures was due to reduction by strain 2CP-C rather than physical (abiotic) sorption processes. The medium pH remained at 7.1 over the 10-day incubation period, suggesting that no pH-induced changes in the surface characteristics of the Fe(III) oxide occurred.

TABLE 1. Loss of U(VI) in mineral salt medium amended with amorphous Fe(III) oxide

| Amorphous Fe(III) oxide concn[a] (mM) | U(VI) remaining in solution (%) after: | |
|---|---|---|
| | 2 days | 10 days |
| 0 | 100 | 100 |
| 5 | 100 | 98 |
| 10 | 93 | 91 |
| 20 | 79 | 71 |

[a] Nominal concentration.

**(iii) 2-CP.** The effects of 2-CP on U(VI) reduction were initially explored in acetate-nitrate-grown cultures that had consumed all nitrate. U(VI) was reduced at similar rates in the 2-CP-amended cultures and the controls lacking 2-CP, suggesting that 2-CP did not influence U(VI) reduction (Fig. 6). 2-CP was dechlorinated to phenol following a 5-day lag phase in the cultures pregrown with nitrate. A second, independent experiment with cultures that had consumed 0.2 mM 2-CP confirmed that 2-CP and U(VI) were reduced concomitantly, and the presence of 2-CP had no effect on U(VI) reduction rates. In the 2-CP-pregrown cultures, phenol was produced without a lag time following the addition of 2-CP. Similarly, U(VI) had no effect on 2-CP dechlorination to phenol, and both 2-CP and U(VI) were reduced simultaneously (data not shown).

Fumarate did not inhibit U(VI) reduction, and both fumarate and U(VI) reduction occurred concomitantly (data not shown). In the experiments described above with live, hydrogen-amended cultures, U(VI) was reduced without an apparent lag phase following its addition to nitrate-, ferric iron-, 2-CP-, or fumarate-grown cells. These observations suggest that anaerobically grown cells of strain 2CP-C possess a constitutive pathway (or pathways) for U(VI) reduction.

## DISCUSSION

*Anaeromyxobacter dehalogenans* strain 2CP-C couples the reduction of *ortho*-substituted halophenols, oxygen, nitrate, nitrite, soluble and insoluble forms of ferric iron, humic substances (e.g., anthraquinone-2,6-disulfonate), and fumarate to the oxidation of hydrogen and several organic electron donors including acetate, succinate, pyruvate, formate, and lactate (41). In this study, we demonstrate that strain 2CP-C also reduces soluble and mobile U(VI) to insoluble and less-mobile U(IV). Strain 2CP-C uses hydrogen as the electron donor for U(VI) reduction but not acetate, which is surprising because

acetate serves as electron donor for other growth-supporting electron acceptors. The reasons why strain 2CP-C cannot couple acetate oxidation to U(VI) reduction are unclear but could be related to energy considerations. The free energy ($\Delta G^{\circ\prime}$) produced by $H_2$ oxidation is greater by 13 kJ per mole of electrons than that produced by acetate oxidation. Since the free energy associated with U(VI) reduction may be quite minimal depending on the pathway, this extra energy from $H_2$ could be sufficient to allow the reaction to proceed while with acetate the available free energy is insufficient to drive the reaction in strain 2CP-C. Members of the extensively studied *Geobacter* group use acetate as an electron donor for U(VI) reduction, indicating that this process is feasible and is not constrained entirely by energetics (23). The biochemistry of U(VI) reduction and the diversity of pathways are not completely understood, and the observed differences in electron donor requirements may indicate that *Geobacter* spp. and *Anaeromyxobacter* spp. use mechanistically different U(VI) reduction pathways. For instance, the sulfate reducer *Desulfovibrio vulgaris* has been shown to have divergent catabolic pathways when grown with lactate or with hydrogen as the electron donor (8). Understanding the detailed biochemistry of the process and the specific requirements of U(VI)-reducing bacteria is relevant to successful implementation and management of bioremediation efforts at uranium-impacted sites, such as the FRC.

U(VI)-contaminated FRC sediments contain high concentrations of nitrate because nitric acid was used in the original processing of uranium. Nitrate is an energetically favorable electron acceptor used by many metal-reducing bacteria, including *Anaeromyxobacter* species. The addition of nitrate to U(VI)-reducing 2CP-C cultures resulted in the transient formation of nitrite and an increase in U(VI) concentration. The observed increase in U(VI) concentration under nitrate-reducing conditions is consistent with findings of other studies that reported that oxidized nitrogen species must be removed for U(VI) reduction to proceed (7, 9, 10, 19, 45). *Anaeromyxobacter* species grow with nitrate as the electron acceptor, reducing it completely to ammonium (41), and our experiments demonstrated that nitrate-grown cells immediately reduce U(VI) following nitrate and nitrite consumption. Hence, a promising site remedy could involve the addition of an electron donor to remove nitrate and to increase the size of the *Anaeromyxobacter* population, which could ultimately result in enhanced U(VI) reduction rates.

Fe(III) is one of the most abundant electron acceptors for organic-matter decomposition in many aquatic sediments and subsurface environments (27) and may interfere with U(VI) reduction. Wielinga et al. (48) found that 52% of the initial U(VI) remained in solution containing cell suspensions of *Shewanella algae* in the presence of ferrihydrite compared with <5% in the absence of ferrihydrite or in the presence of goethite. The authors suggested that iron hydroxides compete with uranium as the terminal electron acceptor and retard its reduction and precipitation. Our studies with *Anaeromyxobacter dehalogenans* strain 2CP-C showed that U(VI) reduction occurred at reduced rates following the addition of Fe(III) oxide. Competitive inhibition is likely the cause for the decreased U(VI) reduction rates in strain 2CP-C cultures amended with Fe(III) oxide. Fredrickson et al. (13) observed no inhibition of U(VI) in *Shewanella putrefaciens* CN32 cell suspensions containing 4.5 g liter$^{-1}$ goethite. Jeon et al. (20) reported that the rate and extent of U(VI) reduction by *Geobacter sulfurreducens* in the presence of synthetic Fe(III) oxides (hydrous ferric oxide, goethite, and hematite at 50 mmol/liter of ferric iron) were comparable to those observed during reduction of aqueous U(VI) in the absence of Fe(III) oxides. These observations suggest that Fe(III) oxides affect organisms differently, and no general statements on the effects of amorphous ferric iron forms on microbial radionuclide reduction activity are possible.

A few studies observed that loss of U(VI) from solution is caused by sorption of U(VI) to Fe(III) oxides (20, 42, 43). Investigation of U(VI) reduction by *Geobacter sulfurreducens* in the presence of synthetic or natural Fe(III) oxides showed that more than 95% of the added U(VI) sorbed quickly to the synthetic Fe(III) oxides and Fe(III) oxide-enriched natural materials (20). Studies with *Desulfovibrio desulfuricans* G20 indicated that the extent of U(VI) sorption onto Fe(III) (hydr)oxides depends on the buffer system and the type of Fe(III) (hydr)oxides present (43). The sorption of U(VI) onto the minerals increased significantly in 1,4-piperazine-diethanesulfonate (PIPES) buffer compared with bicarbonate-buffered systems. We evaluated the impact of Fe(III) oxides in our test systems and found that U(VI) loss due to sorption was negligible under the experimental conditions used in this study (Table 1).

In contrast to experiments with amorphous Fe(III) oxide, the addition of ferric citrate to U(VI)-reducing 2CP-C cultures caused an immediate and complete inhibition of U(VI) reduction. Similarly, the introduction of citrate to U(VI)-reducing cultures resulted in cessation of U(VI) reduction. Francis et al. (11) described a $([UO_2]_2\ \text{citrate}_2)^{2-}$ binuclear complex that was not degraded by the citrate-metabolizing bacterium *Pseudomonas fluorescens*, though the complex was not toxic to the organism. Therefore, we speculate that the formation of stable U-citrate or Fe-U-citrate complexes may be the reason for the cessation of U(VI) reduction in the presence of citrate. Ganesh et al. (14) observed that the presence of citrate influenced the initial rate of U(VI) reduction by *Desulfovibrio desulfuricans* and *Shewanella algae*. While citrate decreased U(VI) reduction rates in cultures of *Desulfovibrio desulfuricans*, *Shewanella algae* cultures exhibited higher U(VI) reduction rates in the presence of citrate. Apparently, the ability of microbes to reduce citrate-complexed U(VI) differs among U(VI)-reducing bacteria.

Chloro-organic compounds (e.g., chlorinated solvents, polychlorinated biphenyls, etc.) are cocontaminants at the FRC site (35), but their effects on U(VI) reduction have not been explored. Our results demonstrate that strain 2CP-C reduces U(VI) and 2-CP simultaneously, suggesting that U(VI) and hazardous chloro-organic compounds can be remediated concomitantly by the same organism. Since the ability to use chloro-organic compounds as electron acceptors (i.e., chlororespiration) is not rare among bacteria (25), the search for dechlorinating metal reducers should intensify to generate a better understanding of the diversity and metabolic range of microbes capable of detoxifying metals, radionuclides, and chloro-organic contaminants simultaneously.

*Anaeromyxobacter* 16S rRNA gene sequences have been de-

tected in uranium-contaminated FRC sediments (38, 39). As discussed above, the presence of alternate, energetically favorable electron acceptors such as oxygen, nitrate, Fe(III), and chlorinated compounds at uranium-contaminated sites can interfere with efficient U(VI) reduction or result in reoxidation of microbially reduced uranium. The respiratory versatility of *Anaeromyxobacter* species presents opportunities to study these interactions and to possibly design enhanced bioremediation technologies targeting multiple contaminants. *Anaeromyxobacter* species are promising candidates for FRC site remediation because members of this group reduce several toxic electron acceptors simultaneously, maintain reducing conditions, and contribute to U(VI) plume stabilization. Further, the biomass produced in nitrate reduction may switch quickly to U(VI) reduction when the consumption of oxidized nitrogen species is complete. Hence, *Anaeromyxobacter dehalogenans* is an excellent model organism for studying reductive detoxification and the complex interactions of nitrate, Fe(III), U(VI), and halo-organic compounds at sites contaminated with mixed wastes, such as the FRC.

Previous studies demonstrated that 2-CP induces dechlorination activity whereas the pathway for the reduction of soluble ferric iron is constitutively expressed in anaerobically grown *Anaeromyxobacter dehalogenans* strain 2CP-C cells (16, 17). It is currently unclear if *Anaeromyxobacter* species possess multiple metal reduction pathways and if all underlie common regulatory networks. Obviously, knowledge on the regulation of genes and pathways involved in detoxification is relevant for the successful implementation of bioremediation at contaminated sites. The genome sequence of strain 2CP-C has become available (www.jgi.doe.gov), and future efforts will take advantage of the genomic information to elucidate the metal reduction pathways and their regulation in this versatile organism.

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