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Unique Ecophysiology among U(VI)-Reducing Bacteria as Revealed by Evaluation of Oxygen Metabolism in *Anaeromyxobacter dehalogenans* Strain 2CP-C▽†

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***Anaeromyxobacter* spp. respire soluble hexavalent uranium, U(VI), leading to the formation of insoluble U(IV), and are present at the uranium-contaminated Oak Ridge Integrated Field Research Challenge (IFC) site. Pilot-scale *in situ* bioreduction of U(VI) has been accomplished in area 3 of the Oak Ridge IFC site following biostimulation, but the susceptibility of the reduced material to oxidants (i.e., oxygen) compromises long-term U immobilization. Following oxygen intrusion, attached *Anaeromyxobacter dehalogenans* cells increased approximately 5-fold from $2.2 \times 10^7 \pm 8.6 \times 10^6$ to $1.0 \times 10^8 \pm 2.2 \times 10^7$ cells per g of sediment collected from well FW101-2. In the same samples, the numbers of cells of *Geobacter lovleyi*, a population native to area 3 and also capable of U(VI) reduction, decreased or did not change. *A. dehalogenans* cells captured via groundwater sampling (i.e., not attached to sediment) were present in much lower numbers ($<1.3 \times 10^4 \pm 1.1 \times 10^4$ cells per liter) than sediment-associated cells, suggesting that *A. dehalogenans* cells occur predominantly in association with soil particles. Laboratory studies confirmed aerobic growth of *A. dehalogenans* strain 2CP-C at initial oxygen partial pressures (pO_2) at and below 0.18 atm. A negative linear correlation [$\mu = (-0.09 \times pO_2) + 0.051$; $R^2 = 0.923$] was observed between the instantaneous specific growth rate μ and pO_2, indicating that this organism should be classified as a microaerophile. Quantification of cells during aerobic growth revealed that the fraction of electrons released in electron donor oxidation and used for biomass production (f_s) decreased from 0.52 at a pO_2 of 0.02 atm to 0.19 at a pO_2 of 0.18 atm. Hence, the apparent fraction of electrons utilized for energy generation (i.e., oxygen reduction) (f_e) increased from 0.48 to 0.81 with increasing pO_2, suggesting that oxygen is consumed in a nonrespiratory process at a high pO_2. The ability to tolerate high oxygen concentrations, perform microaerophilic oxygen respiration, and preferentially associate with soil particles represents an ecophysiology that distinguishes *A. dehalogenans* from other known U(VI)-reducing bacteria in area 3, and these features may play roles for stabilizing immobilized radionuclides *in situ*.**

The U.S. Department of Energy (DOE) has initiated efforts to remediate 120 uranium-contaminated locations in 36 states and territories that have been impacted by former nuclear weapon production sites (25). Several dissimilatory iron-reducing bacteria (DIRB) are capable of reducing soluble U(VI) to sparingly soluble U(IV), which often precipitates as uraninite (UO_2) (13, 14, 35, 51), and the feasibility of microbial U(VI) reduction and immobilization as a containment strategy has been demonstrated at field sites (57, 58). A remaining challenge is controlling oxic/anoxic interface processes to ensure the long-term stability of the precipitated material because U(IV) is susceptible to reoxidation by oxidants (e.g., oxygen) (22, 52, 56). DIRB grow by coupling the oxidation of organic compounds or H_2 to the reduction of ferric iron and often reduce other oxidized metals such as manganese and U as well (2, 17, 29, 30, 42, 44, 51). Metals reduced by DIRB undergo rapid cycling between reduced and oxidized states at oxic/anoxic transition zones due to fluctuating oxygen gradients (4). Organisms capable of respiring oxygen, as well as oxidized metals, are thus expected to be good competitors in fluctuating redox environments, taking advantage of oxygen and oxidized metals as electron acceptors (11). Several DIRB have been shown to consume oxygen, ranging from facultative aerobes (e.g., *Shewanella* spp.) to those that reduce oxygen only when present at very low partial pressures (e.g., some *Desulfovibrio* spp.) (20, 23).

Recent studies demonstrated that *Anaeromyxobacter dehalogenans* strain 2CP-C and *Geobacter lovleyi* grow with U(VI) as an electron acceptor (44, 54), and *Anaeromyxobacter* and *Geobacter lovleyi* 16S rRNA gene sequences were detected at U-contaminated sites, including the Oak Ridge Integrated Field Research Challenge (IFC) site (5, 36, 39, 48). In the current study, we enumerated *Anaeromyxobacter* and *Geobacter lovleyi* 16S rRNA gene sequences in area 3 at the Oak Ridge IFC site before (i.e., during ethanol biostimulation) and after oxygen

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intrusion to monitor their responses to changing redox conditions.

A few reports have mentioned growth of *Anaeromyxobacter* spp. under aerobic conditions (7, 43, 50), and the analysis of the *A. dehalogenans* strain 2CP-C genome supports observations that this organism is capable of oxygen utilization (49); however, we found that aerobic growth was unreliable (e.g., did not occur when the culture flasks were rigorously shaken). To better understand the oxygen metabolism of *A. dehalogenans*, provide growth yield and growth rate data, and speculate about the potential role of oxygen-consuming DIRB for U bioremediation, detailed laboratory experiments were performed at various oxygen partial pressures (pO_2). The results reveal unique physiological adaptations of *A. dehalogenans* to oxygen, which distinguish members of this bacterial group from other DIRB, and suggest that the ecophysiology of *A. dehalogenans* has implications for *in situ* U immobilization.

MATERIALS AND METHODS

Oak Ridge IFC site samples. Groundwater samples were collected by Oak Ridge National Laboratory (ORNL) personnel from a pilot-scale U bioreduction pilot plot in area 3 (55–57) and processed by Joy Van Nostrand at the University of Oklahoma as described previously (60). Multiport sampling wells FW101 and FW102 are located in the inner circulation loop of the bioreduction test plot (31). FW101 and FW102 wells were sampled before and after oxygen intrusion into the pilot plot area at screened intervals 2 (13.7 m below ground surface [bgs]) and 3 (12.2 m bgs) (see Fig. S1 in the supplemental material) (56). Cessation of electron donor (ethanol) and reductant (sulfite) additions initiated the intrusion of aerobic groundwater, which occurred between days 806 and 884, and samples were taken during test plot operation on days 746, 754, 810, 887, and 901 (see Fig. S1 in the supplemental material) (56). Area 3 sediment samples were collected by ORNL personnel using a well surging technique as described previously (5). Sediment samples from wells FW101 and FW102 were obtained on day 774 during the phase of active biostimulation with ethanol (days 755 to 805), which preceded oxygen intrusion (days 806 to 884) (Fig. S1) (54, 55, 57). Sediment samples were collected again on day 935 during weekly ethanol stimulation, about 7 weeks after termination of the 11-week introduction of aerobic groundwater into the pilot plot (days 806 to 884) (Fig. S1). Sediments from slurries were collected by centrifugation, transferred aseptically to 50-ml Falcon plastic tubes, frozen at −80°C, shipped on dry ice, and stored at −80°C until DNA extraction (5).

Bacterial strains and culture conditions. *A. dehalogenans* strain 2CP-C was routinely grown at 30°C without shaking in 60-ml (nominal capacity) glass serum bottles (Wheaton, Millville, NJ) containing 40 ml of reduced, bicarbonate-buffered (30 mM) mineral salts medium with a N_2/CO_2 headspace (80:20, vol/vol) (8, 27). The bottles were sealed with thick butyl rubber stoppers (Geo-Microbial Technologies, Inc., Ochelata, OK) and aluminum crimp caps (Wheaton). Acetate (5 mM) (Sigma-Aldrich, St. Louis, MO) was provided as an electron donor, and 10 mM fumarate (Sigma-Aldrich) served as an electron acceptor (unless indicated otherwise).

Oxygen growth experiments. For cultivation with oxygen as an electron acceptor, 50 mM HEPES (4-[2-hydroxyethyl]-1-piperazine-ethanesulfonic acid [pH 7.2]; Sigma-Aldrich) replaced the bicarbonate buffer system, the acetate concentration was increased to 10 mM, and fumarate was omitted. Medium (25 ml) was dispensed into 160-ml glass serum bottles, resulting in a N_2/CO_2 headspace (80:20, vol/vol) of 135 ml. Filter-sterilized (0.2-μm membrane syringe filter; Pall Life Sciences, Ann Arbor, MI) air (16, 30, and 60 ml [i.e., 3.4, 6.3, and 13 ml oxygen, respectively]) was added using a 60-ml syringe to achieve initial pO_2 values of 0.02, 0.03, and 0.07 atm, respectively. Nitrogen gas was added to balance pressure differences across treatments. To achieve higher initial pO_2 values, serum bottles containing sterilized, anaerobic medium were opened, loosely covered with sterile aluminum foil, shaken at 220 rpm for 3 h exposed to air, and resealed with sterile rubber stoppers. Sterile air (60 ml) was added via syringe to achieve equal overpressure in all treatments. The initial pO_2 in these culture vessels was 0.178 ± 0.008 atm (referred to as the 0.18-atm O_2 treatment). Triplicate uninoculated vessels corresponding to each pO_2 treatment served as controls to account for oxygen loss and abiotic oxygen reduction. Triplicate, oxygen-free control cultures received 60 ml of sterile nitrogen gas instead of oxygen. Growth at atmospheric pO_2 was analyzed in foam-stoppered 250-ml Erlenmeyer flasks containing 25 ml medium. For the quantitative growth experiments, triplicate cultures for each pO_2 treatment and oxygen-free controls received 4% (vol/vol) inocula from stationary-phase, fumarate-grown cultures. Oxygen-grown cultures were repeatedly transferred to aerobic mineral salts medium amended with acetate as an electron donor to verify that the ability to respire oxygen was maintained. All culture vessels were shaken at 220 rpm and incubated at 30°C in the dark. Samples were analyzed periodically for organic acids (including acetate), oxygen, and cell numbers via quantitative real-time PCR (qPCR; see below).

DNA extraction. DNA from laboratory cultures was extracted using InstaGene matrix (Bio-Rad Laboratories, Hercules, CA). Aliquots (0.5 ml) from laboratory cultures were transferred to 1.5-ml plastic microcentrifuge tubes (Westbury, NY), and cells were collected by centrifugation at 16,000 × *g* for 3 min at room temperature. The supernatant was decanted, and the pellets were frozen at −20°C for at least 24 h. InstaGene matrix solution (0.2 ml; Bio-Rad Laboratories, Hercules, CA) was added to each frozen pellet, and DNA was prepared according to the manufacturer's specifications. The DNA was utilized immediately or stored at −20°C until qPCR analysis.

DNA extraction from area 3 sediment samples used 500-mg aliquots of sediment with the FastDNA SPIN kit for soil (Bio 101, Vista, CA), and dry DNA aliquots (100 ng each) were shipped to the Georgia Institute of Technology. The DNA was suspended in 100 μl of sterile, nuclease-free, deionized water to yield a homogeneous solution with a DNA concentration of 1 ng μl^{-1}.

qPCR analysis. The SYBR green approach was used to quantify organisms closely related to *Geobacter lovleyi* strain SZ as described previously (1). TaqMan reactions targeting the *A. dehalogenans* strain 2CP-C 16S rRNA gene were performed as described previously (48). To quantify all *Anaeromyxobacter* 16S rRNA gene sequences in field samples, genus level-targeted TaqMan-based qPCR tools were utilized (48). For all TaqMan reactions, qPCR 1-fold-concentrated master mix (Applied Biosystems [ABI], Foster City, CA), 125 nM probe, and 100 nM (each) primer were combined in sterile, nuclease-free water. Aliquots (18 μl) of the reaction mix were dispensed into an ABI MicroAmp Fast Optical 96-well reaction plate held on ice. Template DNA (2 μl) was added to each tube, and the tubes were sealed with an ABI optical adhesive cover. The quantification limit was considered to be the lowest standard in a linear standard curve that produced measurable fluorescence. The quantification limit for *Anaeromyxobacter* 16S rRNA gene-targeted TaqMan qPCR was two to three gene copies per reaction, which equates to about 2×10^2 16S rRNA gene copies/liter of groundwater. Detection of *G. lovleyi* 16S rRNA genes used SYBR green chemistry and required 1×10^3 16S rRNA gene copies per reaction, or about 1×10^5 *G. lovleyi* 16S rRNA gene copies/liter of groundwater to yield positive signals.

Calculation of growth rate, growth yield, and f_e and f_s values. Growth rate values were determined using nonlinear regression analysis by plotting the number of 16S rRNA gene copies per ml of culture over time. Instantaneous specific growth rates (μ) were determined using the exponential growth equation $y = y_0 \times e^{\mu t}$ where y is the number of 16S rRNA gene copies per ml at time t and y_0 is the number of 16S rRNA gene copies per ml at $t = 0$. Triplicate cultures were used to determine growth rates at pO_2 values of 0.02 atm, 0.03 atm, 0.07 atm, and 0.18 atm. Growth yields were determined on the basis of initial and final numbers of 16S rRNA gene copies per ml of culture. 16S rRNA gene copies were used as an approximation of cell numbers. The four sequenced *Anaeromyxobacter* genomes (GenBank accession numbers CP001131, CP000769, CP000251, and ABKC00000000) harbor duplicate rRNA operons (49), suggesting that members of the *Anaeromyxobacter* group contain two 16S rRNA gene copies per cell. The apparent fraction of electrons utilized for energy generation (i.e., oxygen reduction) (f_e) and the fraction of electrons released in electron donor oxidation and used for biomass production (f_s) were calculated from growth yields and the electron balance of cell biosynthesis associated with oxygen reduction by the methods of McCarty (33) and Criddle et al. (9).

Analytical methods and calculations. Organic acids were quantified using a Waters model 1525 high-performance liquid chromatography system equipped with an Aminex HPX-87H ion exclusion column and connected to a Waters model 2487 dual-wavelength absorbance detector (15). Oxygen was quantified using an Agilent Technologies model 6890N gas chromatograph equipped with a thermal conductivity detector as described previously (37). Oxygen quantification used a 5-point calibration curve established by injecting an ambient air sample and samples from 160-ml bottles containing 25 ml of reduced medium (initial N_2/CO_2 headspace [80:20, vol/vol] of 135 ml) amended and equilibrated with 0, 16, 30, and 60 ml of air. Standards and samples were withdrawn using a 1,000-μl glass syringe without a valve, and 100-μl samples equilibrated to atmospheric pressure were injected. Equivalent amounts of nitrogen were injected

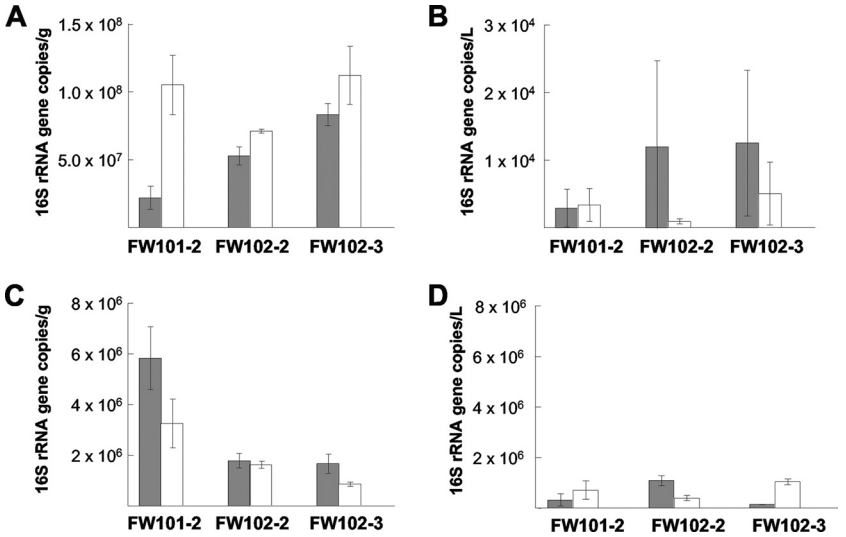

FIG. 1. Oxygen effects on *Anaeromyxobacter dehalogenans* and *Geobacter lovleyi* populations native to the Oak Ridge IFC area 3 U(VI) reduction treatment zone. 16S rRNA genes of target organisms were monitored by qPCR in samples collected from multilevel sampling well FW101 at screened interval 2 (13.7 m bgs), and well FW102 at screened intervals 2 (13.7 m bgs) and 3 (12.2 m bgs). The top panels show the total population sizes indicated by 16S rRNA gene copy numbers of attached (A) and unattached (B) *Anaeromyxobacter* spp. before (shaded bar) and after (white bar) oxygen intrusion. The bottom panels show attached (C) and unattached (D) *Geobacter lovleyi* strain SZ 16S rRNA gene copies. Note the different *y*-axis scales. Error bars represent standard deviations between multiple time points for groundwater samples and between multiple quantitative analyses for sediment samples.

into the bottles at each sampling event to maintain constant overpressure. The detector response (i.e., peak area) was correlated to the calculated pO_2 in the headspaces of the 160-ml bottles based on the calibration curves. Peak areas from all samples and standards were corrected to account for the analytical method baseline response, which was determined by injecting oxygen-free nitrogen gas. pO_2 is reported in atmosphere (atm) and in total micromoles per bottle. The total mass of oxygen per bottle (n, in micromoles) was calculated from pO_2 (atm) according to the ideal gas law [$n = (pO_2 \times V \times 10^6)/(R \times T)$, where V equals the culture headspace volume (0.135 liter), R is the ideal gas constant (0.082057 liter atm K^{-1} mol^{-1}), T is the incubation temperature in Kelvin (i.e., 303 K), and the multiplier 10^6 converts moles to micromoles]. For example, a pO_2 of 0.02 atm equates to 110 μmol of oxygen per bottle. Due to oxygen's very low Henry's law constant (K_H) of 1.26×10^{-3} M/atm, dissolved oxygen did not significantly contribute to the total amount of oxygen present in each bottle (e.g., less than 1% of the total amount of oxygen would be dissolved in the aqueous phase of the experimental system at a pO_2 of 0.02 atm).

Statistical analysis. Nonlinear and linear regression analyses, including significance tests for the regressions, were performed using GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA). For modeling exponential growth of strain 2CP-C, the instantaneous specific growth rate μ was constrained to less than 1, and default settings were used for all other analyses.

RESULTS

Response of two DIRB populations to oxygen intrusion at the Oak Ridge IFC site. Analysis of Oak Ridge IFC area 3 sediment and groundwater samples collected before and after oxygen intrusion (5, 56) suggested a change in the *Anaeromyxobacter* population size (Fig. 1). The number of *Anaeromyxobacter* 16S rRNA gene copies per g of sediment increased as much as 5-fold in response to oxygen intrusion. For example, the number of *Anaeromyxobacter* cells increased from $2.2 \times 10^7 \pm 8.6 \times 10^6$ to $1.0 \times 10^8 \pm 2.2 \times 10^7$ cells per g of sediment collected from well FW101-2 (i.e., FW101 at screened interval 2 [13.7 m bgs]). The number of *Anaeromyxobacter* cells in sediment samples ranged from 1×10^7 to 1×10^8 cells per g (Fig. 1A) and were substantially higher than the cell titers in groundwater, which ranged from 1×10^3 to 1×10^4 cells per liter (Fig. 1B), suggesting that the majority of *Anaeromyxobacter* cells were attached to the aquifer solids. The number of unattached *Anaeromyxobacter* cells either remained relatively constant (well FW101-2) or decreased (FW102-2 and FW102-3) after oxygen intrusion (Fig. 1B). In contrast to observations made with *Anaeromyxobacter*, the number of sediment-associated cells of *G. lovleyi*, a U(VI)-reducing deltaproteobacterium present at area 3 at the Oak Ridge IFC site (1), decreased by as much as 50% following the intrusion of oxygenated groundwater (Fig. 1C). Conversely, the percentage of unattached *G. lovleyi* cells increased in two of the three samples (FW101-2 and FW102-3) following oxygen intrusion (see Table 2) (Fig. 1D).

Oxygen consumption in laboratory cultures. Laboratory experiments were performed under precisely controlled conditions to study the different behaviors of *Anaeromyxobacter dehalogenans* and *Geobacter lovleyi* in more detail. Stationary-phase *A. dehalogenans* strain 2CP-C cultures previously grown with 10 mM fumarate consumed three consecutive oxygen feedings (each at a pO_2 of 0.02 atm) within 50 h per addition (Fig. 2A). Oxygen reduction coincided with acetate oxidation (Fig. 2A), and neither substrate was consumed in uninoculated controls (data not shown). No measurable net increase in biomass occurred during oxygen reduction in the stationary-phase cultures as indicated by constant 16S rRNA gene copy numbers (Fig. 2A). The ability to consume oxygen in stationary-phase cultures was not shared by *G. lovleyi* strain SZ (Fig. 2B). Under the conditions tested, neither oxygen reduction nor acetate consumption was observed in cultures of strain SZ, and cell numbers decreased by an order of magnitude during exposure to oxygen (pO_2 of 0.02 atm) (Fig. 2B). Previous studies have shown that *A. dehalogenans* and *Geobacter lovleyi* use acetate as an elec-

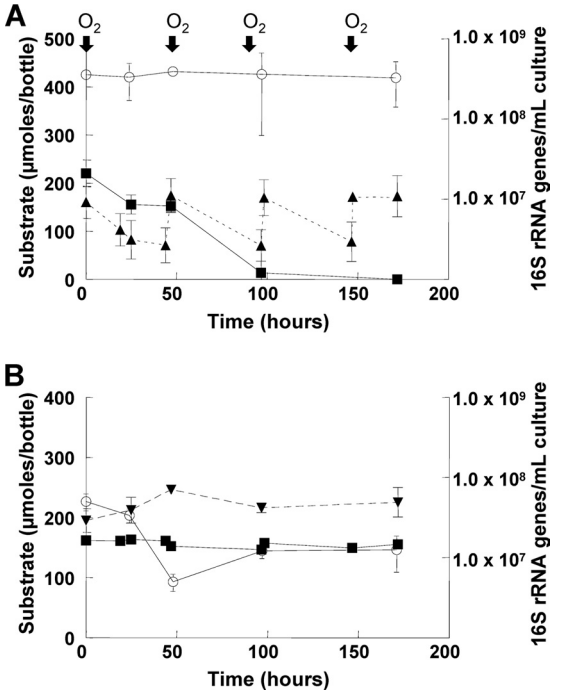

FIG. 2. Response of *A. dehalogenans* strain 2CP-C and *G. lovleyi* to O_2. Stationary-phase cultures of *A. dehalogenans* strain 2CP-C (A) and *G. lovleyi* (B) were exposed to an oxygen-containing headspace (initial pO_2 was 0.02 atm). Acetate (filled squares), 16S rRNA gene copy numbers (open circles) and oxygen (filled triangles) were monitored over 10 days in cultures amended with 0.02 atm of oxygen. The arrows indicate additional 0.02-atm oxygen amendments to cultures of *A. dehalogenans* strain 2CP-C. *G. lovleyi* strain SZ did not receive additional oxygen, since consumption was negligible. Values shown are means ± standard deviations (error bars) for triplicate cultures.

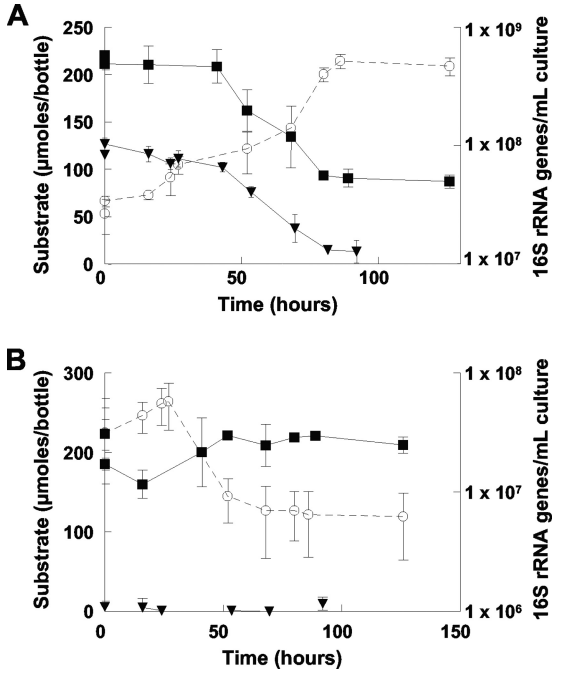

FIG. 3. Oxygen-dependent growth of *A. dehalogenans* strain 2CP-C. (A) Closed vessels containing defined mineral salts medium amended with acetate as an electron donor and oxygen (pO_2 of 0.02 atm) as an electron acceptor received a 4% inoculum from a fumarate-grown culture and were incubated at 30°C. (B) Neither acetate consumption nor growth occurred in no-oxygen controls. Squares and inverted triangles indicate acetate and oxygen, respectively (left-hand *y* axis). Open circles indicate 16S rRNA gene copies per ml culture (right-hand *y* axis). Values shown are means ± standard deviations (error bars) for triplicate cultures.

tron donor, which both organisms oxidize to carbon dioxide (16, 17, 47). Our analyses supported full oxidation of acetate, as no organic breakdown products were detected in any of the cultures.

Microaerophilic growth by *A. dehalogenans* strain 2CP-C. While strain 2CP-C grew readily under anaerobic conditions, growth in liquid medium at atmospheric oxygen tension (i.e., pO_2 of 0.21 atm and cultures shaken in foam-stoppered Erlenmeyer flasks) was unreliable and occurred only in about one third of the cultures after long lag phases of 3 to 5 weeks (data not shown). To quantify oxygen depletion and to correlate oxygen consumption to biomass production, experiments in closed serum bottles were performed. At an initial pO_2 of 0.02 atm, oxygen consumption began after a 45-hour lag phase and 110 ± 29 μmol of oxygen were consumed after 82 h of incubation (Fig. 3A; see Fig. S2 in the supplemental material). Oxygen was not consumed in uninoculated control bottles (data not shown), indicating that strain 2CP-C cells were responsible for oxygen consumption. *A. dehalogenans* strain 2CP-C grew during oxygen reduction as indicated by increases in the number of 16S rRNA gene copies from $2.66 \times 10^7 \pm 8.78 \times 10^6$ to $5.18 \times 10^8 \pm 7.20 \times 10^7$ per ml of culture suspension (Fig. 3A). In culture vessels not amended with oxygen, the number of *A. dehalogenans* 16S rRNA gene copies decreased from $3.16 \times 10^7 \pm 9.16 \times 10^6$ (cells introduced with the inoculum) to $6.48 \times 10^6 \pm 3.64 \times 10^6$ per ml of culture suspension over the 86 h of incubation period (Fig. 3B), indicating that cell growth in the oxygen-amended cultures was attributable to oxygen respiration (Fig. 3A). The mean numbers of 16S rRNA gene copies in experimental and control cultures increased from $2.7 \times 10^7 \pm 8.8 \times 10^6$ to $6.9 \times 10^7 \pm 1.2 \times 10^7$ and from $3.2 \times 10^7 \pm 9.2 \times 10^6$ to $5.8 \times 10^7 \pm 2.5 \times 10^7$ 16S rRNA gene copies per ml culture, respectively, in the first 27 h with no measurable decrease in acetate or oxygen concentration during the first 45 h. The modest increases in cell numbers were likely due to residual growth and were within experimental error (i.e., standard deviations of individual data points) and, thus, within the error reported for biomass calculations. Following 45 h of incubation, oxygen and the electron donor acetate were consumed concomitantly (Fig. 3A). No acetate consumption occurred in control cultures without oxygen (Fig. 3B), and oxygen consumption ceased in bottles that had consumed all acetate (data not shown). Growth of *A. dehalogenans* was also observed after comparable lag times at pO_2 values of 0.03, 0.07, and 0.18 atm (see Fig. S2 in the supplemental material). When oxygen-grown cells were transferred to fresh medium, oxygen reduction and growth resumed with a lag time of <45 h, and the ability to grow with oxygen as the sole electron acceptor was maintained over repeated transfers.

Effect of pO_2 on growth. Increasing pO_2 affected growth rates of strain 2CP-C (see Table S1 and Fig. S2 in the supple-

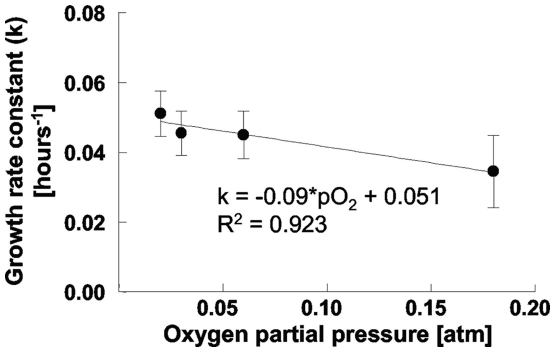

FIG. 4. Linear regression analysis of *A. dehalogenans* instantaneous specific growth rate μ at varying pO_2. The regression indicates an inverse linear correlation of μ and pO_2. Error bars represent standard errors of the mean growth rate. Regression coefficients are presented in Table S1 in the supplemental material.

mental material), and the instantaneous specific growth rate μ decreased linearly [$\mu = (-0.09 \times pO_2) + 0.051$] from 0.051 $\pm$ 0.006 h^{-1} at a pO_2 of 0.02 atm to 0.034 $\pm$ 0.010 h^{-1} at a pO_2 of 0.18 atm (i.e., a 33% decrease) (Fig. 4). The slope of the μ-pO_2 regression line in Fig. 4 was significantly not zero ($P < 0.05$). Doubling times of fumarate-grown and oxygen-grown (pO_2 of 0.02 atm) cultures were not statistically different, with 95% confidence intervals of 10 to 19 h and 12 to 16 h, respectively (see Table S1 in the supplemental material). In addition to growth rate, the O_2 utilization efficiency of *A. dehalogenans* strain 2CP-C decreased with increasing pO_2 (Table 1). The growth yield of strain 2CP-C at an initial pO_2 of 0.02 atm was 6 $\pm$ 2 g of biomass (dry weight) per mol of electrons consumed, which is comparable to the growth yield with fumarate (5.7 $\pm$ 0.6 g of cells per mol of electrons consumed; Table 1). When pO_2 was increased to 0.18 atm, the growth yield decreased by 78% to 1.3 $\pm$ 0.5 g per mol of electrons consumed. The measurement of biomass revealed that the fraction of electrons released in acetate oxidation and used for cell growth (f_s) decreased from 0.52 to 0.19 as the pO_2 values increased from 0.02 to 0.18 atm. Accordingly, the fraction of electrons used for oxygen reduction (f_e) increased from 0.48 to 0.81 as the pO_2 increased from 0.02 atm to 0.18 atm (Table 1).

DISCUSSION

The implication of *in situ* growth of *Anaeromyxobacter* spp. following oxygen intrusion in area 3 at the Oak Ridge IFC site motivated detailed laboratory studies of the oxygen metabolism of *A. dehalogenans* strain 2CP-C. Oxygen growth studies revealed specific adaptations of *A. dehalogenans* to environments with fluctuating redox conditions. The fraction of electrons released during electron donor oxidation and directed toward reduction of the terminal electron acceptor (f_e) indicates if the electron acceptor is used for energy generation (i.e., respiration) or if electron acceptor reduction is uncoupled from growth (e.g., to achieve detoxification or to serve as a fortuitous electron sink). Previous studies have demonstrated that an organism's metabolic efficiency, as measured by f_e and f_s, is constant for a given set of substrates (9, 33). For example, characteristic f_e values for aerobic respiration range between 0.4 and 0.88 (depending on the electron donor) (32) and are governed by the energetics of the energy-generating redox reaction (33). The growth yield (f_s) and efficiency of oxygen utilization (f_e) in *A. dehalogenans* strain 2CP-C at a pO_2 of 0.02 atm are comparable to those observed with fumarate or nitrate as an electron acceptor (Table 1) and are consistent with other acetate-utilizing aerobes (16, 17). At a pO_2 of 0.18 atm, less biomass was produced per mole of transferred electrons, and f_e and f_s values were in line with those measured for soluble ferric iron respiration (Table 1), an energetically less favorable process than aerobic respiration (28). Growth experiments performed at atmospheric oxygen concentrations indicated that oxygen toxicity and detoxification are a likely explanation for the observed shift in the use of reducing power by the cells. The fraction of reducing power generated from acetate oxidation and used for oxygen reduction (i.e., f_e) increased significantly under high pO_2 conditions and reached values of >0.8.

TABLE 1. *Anaeromyxobacter dehalogenans* strain 2CP-C growth yields and f_e values for growth with oxygen at different pO_2 and alternate electron acceptors[a]

EA (concn)	No. of cells per μmol of e^- transferred to EA ($\times 10^7$)	Cell yield[b] (g [dry wt] per mol of e^-)	Mol of e^- of cells per mol of EA[c]	f_s[d]	f_e[e]
Oxygen (0.18 atm)	3.5 ± 1.4	1.3 ± 0.5	0.23 ± 0.09	0.19	0.81
Oxygen (0.07 atm)	4.27 ± 0.72	1.6 ± 0.3	0.28 ± 0.05	0.22	0.78
Oxygen (0.03 atm)	13.1 ± 3.5	5 ± 1	0.88 ± 0.18	0.47	0.53
Oxygen (0.02 atm)	16.0 ± 5.8	6 ± 2	1.1 ± 0.35	0.52	0.48
Fumarate[f] (10 mM)	14.9 ± 1.2	5.7 ± 0.6	1.0 ± 0.11	0.50	0.50
Nitrate[f] (2 mM)	17.3 ± 0.17	6.6 ± 0.5	1.17 ± 0.09	0.54	0.46
Ferric iron[g] (4 mM)	4.3	1.6	0.28	0.22	0.80
2-Chlorophenol[g] (0.2 mM)	7.74	2.9	0.52	0.34	0.66

[a] EA, electron acceptor; e^-, electrons.
[b] Cell yield is reported per mole of electrons transferred to the electron acceptor based on oxygen reduction to water, 2-chlorophenol (2-CP) reduction to phenol, and fumarate reduction to succinate. Cell yield estimates are calculated by multiplying the number of cells per micromoles of electrons transferred to the electron acceptor times the dry weight of a single cell ($3.80 \pm 0.27 \times 10^{-14}$ g) estimated previously for fumarate-grown *Anaeromyxobacter* cells (44).
[c] Mol of e^- of cells is based on the model formula and molecular weight of cell biomass of $C_5H_7O_2N$ and 113 g/mol, respectively.
[d] f_s is the fraction of electrons released in electron donor oxidation and used for biomass synthesis. It is calculated as follows: f_s = (mol of e^- of cells per mol of EA)/(1 + mol of e^- of cells per mol of EA).
[e] f_e is the fraction of electrons released in electron donor oxidation and transferred to the electron acceptor ($f_e = 1 - f_s$).
[f] Data from reference 44.
[g] Data from reference 17.

TABLE 2. Relative attachment status of *Anaeromyxobacter* spp. and *G. lovleyi* in subsurface materials from the ORNL area 3 pilot test plot before and after oxygen intrusion

Location	*Anaeromyxobacter* spp.			*Geobacter lovleyi*		
	Ratio of attached (10^6 cells/g) to unattached cells (10^6 cells/g)[a]		Δ factor in ratio[b]	Ratio of attached (10^6 cells/g) to unattached cells (10^6 cells/g)		Δ factor in ratio
	Before O_2	After O_2		Before O_2	After O_2	
FW101-2	6.9	27	+3.9	180	46	−3.9
FW102-2	4.4	74	+16.8	16	41	+2.56
FW102-3	6.6	22	+3.3	120	8.3	−14.5

[a] Values show the average value for the number of cells/g of sediment (attached cells) to the number of cells/g of groundwater (unattached cells).
[b] Δ factor values refer to the fold change in attached to unattached cell ratios before O_2 to after O_2 intrusion.

This high f_e does not indicate an increase in metabolic energy gain but reflects an apparent f_e in the sense that not all the free energy release associated with the redox reaction is coupled to growth. A significant fraction of the oxygen is not respired but is consumed without energy capture, presumably to avoid oxygen toxicity. Hence, the true f_e linked to metabolic energy gain from oxygen respiration is observed only with a low pO_2 when electron consumption for oxygen detoxification is minimal. Consequently, the evaluation of f_e for an aerobic process should be determined at different pO_2 values to ensure that a true f_e, as opposed to an apparent f_e, is measured. This procedure is pertinent for microorganisms whose relationships to oxygen are unclear.

Genome analysis of *A. dehalogenans* strain 2CP-C suggested that two oxygen-reducing pathways exist: one that shares homology with anaerobes and microaerophiles and includes a cytochrome cbb_3 oxidase and a second pathway that includes a cytochrome aa_3 oxidase characteristic of the *Myxobacteria*, which are primarily aerobes (49). Cytochrome oxidases can play roles in both respiratory (energy-yielding) and/or oxygen-detoxifying (non-energy-yielding) reactions (40). The *Alphaproteobacteria Bradyrhizobium japonicum* and *Paracoccus denitrificans* both possess cytochrome aa_3 oxidases as well as cytochrome cbb_3 oxidases and are thought to maintain both protein complexes for quick response to changing pO_2 (3, 41). It is conceivable that one cytochrome oxidase is used for detoxification while a separate, respiratory cytochrome oxidase is involved in the organism's energy metabolism at lower pO_2. Instantaneous oxygen reduction by anaerobically grown *A. dehalogenans* strain 2CP-C cells that had reached stationary phase suggests a constitutive oxygen detoxification pathway, an adaptation beneficial for anaerobic or microaerophilic organisms near fluctuating oxic/anoxic interfaces. *Geobacter* spp. represent another group of environmentally relevant metal-reducing deltaproteobacteria that are found at the oxic/anoxic interface but whose relationship to oxygen remains unclear. *G. lovleyi* strain SZ failed to consume oxygen under the conditions tested, but growth under aerobic conditions has been reported for *Geobacter sulfurreducens*; however, growth of *G. sulfurreducens* with oxygen could not be maintained through repeated transfers without intermediate, anoxic cultivation with Fe(III) as an electron acceptor (24). In contrast, *A. dehalogenans* grows with oxygen as the sole electron acceptor over repeated transfers. On the basis of the existing genome annotation for *G. lovleyi* strain SZ (GenBank accession number CP001089), the electron transport chain of strain SZ contains a cytochrome *bd*-type oxidase, but not the aa_3 type, present in both *G. sulfurreducens* (24) and *A. dehalogenans*, or the cbb_3 type present in *A. dehalogenans*. Therefore, differences in oxygen metabolism between *G. lovleyi*, *G. sulfurreducens*, and *A. dehalogenans* would be expected.

Other members of the *Deltaproteobacteria*, including sulfate-reducing *Desulfovibrio* spp., flourish at the oxic/anoxic interface in the presence of sulfide/sulfate and are capable of oxygen detoxification, although they were classically considered strict anaerobes (21). Aerobic growth of *Desulfovibrio* spp. has not been demonstrated, but cultures of *Desulfovibrio desulfuricans* ATCC 27774 consumed oxygen, and the expression of catalase and superoxide dismutase increased when grown with nitrate under a high pO_2 (up to 0.18 atm) (26). Several transcriptomic and proteomic studies with *Desulfovibrio* spp. have been performed to identify the metabolic changes in response to oxygen (12, 38, 45, 59), including a study indicating that exposure to air (pO_2 of 0.21 atm) caused downregulation of central metabolic pathways and that the organisms dedicated less reducing power for biomass production (34). Growth measurements of *A. dehalogenans* strain 2CP-C indicated that the respiratory efficiency decreased at higher pO_2, resulting in less biomass synthesis (Table 1). Downregulation of central metabolic pathways in response to high pO_2, as seen in *D. vulgaris*, could explain the decreasing biomass synthesis observed in *A. dehalogenans* strain 2CP-C cultures.

A major physiological difference between *A. dehalogenans* and other metal-reducing *Deltaproteobacteria* is its demonstrated use of type IV pilus-based surface motility (49). Consistent with the classification of *Anaeromyxobacter* as a myxobacterium with surface motility, the quantitative analysis of area 3 sediment and groundwater samples demonstrated that *Anaeromyxobacter* cells occur predominantly associated with the soil surfaces. In area 3, *Anaeromyxobacter* cells were 1,000- to 10,000-fold more prevalent on solids than *Geobacter lovleyi* cells (Fig. 1 and Table 2). In contrast, metal-reducing *Geobacter* spp., which use flagellar motility (6, 47), were more evenly distributed between solids and the aqueous phase (Table 2), similar to what was observed in another U-contaminated aquifer (Rifle site, CO) (18). The ratio of attached to unattached *G. lovleyi* cells decreased in two out of three samples following oxygen intrusion (Table 2). A possible explanation is detachment of *G. lovelyi* cells from the solids in response to oxygen intrusion due to toxicity or a shift of lifestyle from a solid-associated lifestyle to an unattached lifestyle. Thus, the apparent increase in the number of *G. lovleyi* cells in ground-

water may reflect a redistribution of cells from the attached state to the unattached state rather than aerobic growth of unattached cells. The uneven distribution of DIRB between solids and groundwater emphasizes the need for investigation of how organism-specific characteristics and environmental factors influence attached versus unattached growth. Obviously, the analysis of *Anaeromyxobacter* population dynamics based on groundwater sampling data alone will provide an incomplete picture of the true abundance of members of this group.

Bioreduction and immobilization of U are a promising treatment strategy, but reoxidation of immobilized U(IV) affects stability and mobility of the mineralized material and complicates long-term site management. In anaerobic subsurface environments, different DIRB coexist (5, 10, 39, 46, 53), and area 3 at the Oak Ridge IFC site harbors at least *Geobacter* spp., *Anaeromyxobacter* spp., and *Desulfovibrio* spp. Ecological exclusion theory predicts that organisms occupying the same ecological niche do not cooccur in the same environment (19). Therefore, physiological features must distinguish the DIRB present in area 3, and a relevant distinguishing factor is their response to oxygen. *A. dehalogenans* responds quickly to oxygen intrusion via a constitutive detoxification system, grows with oxygen under microaerophilic conditions, predominantly associates with solids, and uses oxidized metals and radionuclides, including U(VI), as respiratory electron acceptors. These combined characteristics are unique and not shared with other known bacterial populations contributing to U(VI) reduction in area 3 at the Oak Ridge IFC site. Continued monitoring of U concentrations in the area 3 bioreduction plot suggested that U initially oxidized by oxygen was quickly immobilized (Wei-min Wu, personal communication), possibly related to DIRB activity. Future efforts should therefore explore not only the abilities of organisms to reduce U(VI) under anaerobic conditions but also focus on the ecophysiologies of the organisms, as those features may affect the long-term stability of reduced radionuclides.

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REFERENCES

1. **Amos, B. K., Y. Sung, K. E. Fletcher, T. J. Gentry, W. M. Wu, C. S. Criddle, J. Zhou, and F. E. Löffler.** 2007. Detection and quantification of *Geobacter lovleyi* strain SZ: implications for bioremediation at tetrachloroethene- and uranium-impacted sites. Appl. Environ. Microbiol. **73:**6898–6904.
2. **Arnold, R. G., M. R. Hoffmann, T. J. Dichristina, and F. W. Picardal.** 1990. Regulation of dissimilatory Fe(III) reduction activity in *Shewanella putrefaciens*. Appl. Environ. Microbiol. **56:**2811–2817.
3. **Baker, S. C., S. J. Ferguson, B. Ludwig, M. D. Page, O. M. H. Richter, and R. J. M. van Spanning.** 1998. Molecular genetics of the genus *Paracoccus*: metabolically versatile bacteria with bioenergetic flexibility. Microbiol. Mol. Biol. Rev. **62:**1046–1078.
4. **Brune, A., P. Frenzel, and H. Cypionka.** 2000. Life at the oxic-anoxic interface: microbial activities and adaptations. FEMS Microbiol. Rev. **24:**691–710.
5. **Cardenas, E., W. M. Wu, M. B. Leigh, J. Carley, L. S. Carrol, T. Gentry, J. Luo, D. Watson, B. Gu, M. Ginder-Vogel, P. K. Kitanidis, P. M. Jardine, J. Zhou, C. S. Criddle, T. L. Marsh, and J. M. Tiedje.** 2008. Microbial communities in contaminated sediments, associated with bioremediation of uranium to submicromolar levels. Appl. Environ. Microbiol. **74:**3718–3729.
6. **Childers, S. E., S. Ciufo, and D. R. Lovley.** 2002. *Geobacter metallireducens* accesses insoluble Fe(III) oxide by chemotaxis. Nature **416:**767–769.
7. **Cole, J. R., A. L. Cascarelli, W. W. Mohn, and J. M. Tiedje.** 1994. Isolation and characterization of a novel bacterium growing via reductive dehalogenation of 2-chlorophenol. Appl. Environ. Microbiol. **60:**3536–3542.
8. **Cole, J. R., B. Z. Fathepure, and J. M. Tiedje.** 1995. Tetrachloroethene and 3-chlorobenzoate dechlorination activities are co-induced in *Desulfomonile tiedjei* DCB-1. Biodegradation **6:**167–172.
9. **Criddle, C. S., L. A. Alvarez, and P. L. McCarty.** 1991. Microbial processes in porous media, p. 639–691. *In* J. Bear and M. Y. Corapcioglu (ed.), Transport processes in porous media. Kluwer Academic Publishers, Dordrecht, The Netherlands.
10. **Cummings, D. E., O. L. Snoeyenbos-West, D. T. Newby, A. M. Niggemyer, D. R. Lovley, L. A. Achenbach, and R. F. Rosenzweig.** 2003. Diversity of *Geobacteraceae* species inhabiting metal-polluted freshwater lake sediments ascertained by 16S rDNA analyses. Microb. Ecol. **46:**257–269.
11. **DiChristina, T. J., J. K. Fredrickson, and J. M. Zachara.** 2005. Enzymology of electron transport: energy generation with geochemical consequences. Rev. Mineral Geochem. **59:**27–52.
12. **Fournier, M., C. Aubert, Z. Dermoun, M. C. Durand, D. Moinier, and A. Dolla.** 2006. Response of the anaerobe *Desulfovibrio vulgaris* Hildenborough to oxidative conditions: proteome and transcript analysis. Biochimie **88:**85–94.
13. **Gadd, G. M.** 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma **122:**109–119.
14. **Gorby, Y. A., and D. R. Lovley.** 1991. Electron transport in the dissimilatory iron reducer, GS-15. Appl. Environ. Microbiol. **57:**867–870.
15. **He, J. Z., K. M. Ritalahti, M. R. Aiello, and F. E. Löffler.** 2003. Complete detoxification of vinyl chloride by an anaerobic enrichment culture and identification of the reductively dechlorinating population as a *Dehalococcoides* species. Appl. Environ. Microbiol. **69:**996–1003.
16. **He, Q., and R. A. Sanford.** 2004. Acetate threshold concentrations suggest varying energy requirements during anaerobic respiration by *Anaeromyxobacter dehalogenans*. Appl. Environ. Microbiol. **70:**6940–6943.
17. **He, Q., and R. A. Sanford.** 2003. Characterization of Fe(III) reduction by chlororespiring *Anaeromyxobacter dehalogenans*. Appl. Environ. Microbiol. **69:**2712–2718.
18. **Holmes, D. E., R. A. O'Neil, H. A. Vrionis, L. A. N′Guessan, I. Ortiz-Bernad, M. J. Larrahondo, L. A. Adams, J. A. Ward, J. S. Nicoll, K. P. Nevin, M. A. Chavan, J. P. Johnson, P. E. Long, and D. R. Lovley.** 2007. Subsurface clade of *Geobacteraceae* that predominates in a diversity of Fe(III)-reducing subsurface environments. ISME J. **1:**663–677.
19. **Hutchinson, G. E.** 1961. The paradox of the plankton. Am. Nat. **95:**137–145.
20. **Johnson, M. S., I. B. Zhulin, M.-E. R. Gapuzan, and B. L. Taylor.** 1997. Oxygen-dependent growth of the obligate anaerobe *Desulfovibrio vulgaris* Hildenborough. J. Bacteriol. **179:**5598–5601.
21. **Krekeler, D., A. Teske, and H. Cypionka.** 1998. Strategies of sulfate-reducing bacteria to escape oxygen stress in a cyanobacterial mat. FEMS Microbiol. Ecol. **25:**89–96.
22. **Langmuir, D.** 1978. Uranium solution-mineral equilibria at low temperatures with applications to sedimentary ore deposits. Geochim. Cosmochim. Acta **42:**547–569.
23. **Lemos, R. S., C. M. Gomes, M. Santana, J. LeGall, A. V. Xavier, and M. Teixeira.** 2001. The 'strict' anaerobe *Desulfovibrio gigas* contains a membrane-bound oxygen-reducing respiratory chain. FEBS Lett. **496:**40–43.
24. **Lin, W. C., M. V. Coppi, and D. R. Lovley.** 2004. *Geobacter sulfurreducens* can grow with oxygen as a terminal electron acceptor. Appl. Environ. Microbiol. **70:**2525–2528.
25. **Lloyd, J. R., and J. C. Renshaw.** 2005. Bioremediation of radioactive waste: radionuclide-microbe interactions in laboratory and field-scale studies. Curr. Opin. Biotechnol. **16:**254–260.
26. **Lobo, S. A. L., A. M. P. Melo, J. N. Carita, M. Teixeira, and L. M. Saraiva.** 2007. The anaerobe *Desulfovibrio desulfuricans* ATCC 27774 grows at nearly atmospheric oxygen levels. FEBS Lett. **581:**433–436.
27. **Löffler, F. E., R. A. Sanford, and J. M. Tiedje.** 1996. Initial characterization of a reductive dehalogenase from *Desulfitobacterium chlororespirans* Co23. Appl. Environ. Microbiol. **62:**3809–3813.
28. **Löffler, F. E., J. M. Tiedje, and R. A. Sanford.** 1999. Fraction of electrons consumed in electron acceptor reduction and hydrogen thresholds as indicators of halorespiratory physiology. Appl. Environ. Microbiol. **65:**4049–4056.
29. **Lovley, D. R., S. J. Giovannoni, D. C. White, J. E. Champine, E. J. P. Phillips, Y. A. Gorby, and S. Goodwin.** 1993. *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic-compounds to the reduction of iron and other metals. Arch. Microbiol. **159:**336–344.
30. **Lovley, D. R., P. K. Widman, J. C. Woodward, and E. J. P. Phillips.** 1993.

Reduction of uranium by cytochrome-c_3 of *Desulfovibrio vulgaris*. Appl. Environ. Microbiol. **59:**3572–3576.
31. **Luo, J., F.-A. Weber, O. A. Cirpka, W.-M. Wu, J. L. Nyman, J. Carley, P. M. Jardine, C. S. Criddle, and P. K. Kitanidis.** 2007. Modeling *in situ* uranium(VI) bioreduction by sulfate-reducing bacteria. J. Contam. Hydrol. **92:**129–148.
32. **McCarty, P. L.** 1988. Bioengineering issues related to in situ remediation of contaminated soils and groundwater, p. 143–162. *In* G. S. Omenn (ed.), Environmental biotechnology: reducing risks from environmental chemicals through biotechnology. Plenum Press, New York, NY.
33. **McCarty, P. L.** 1971. Energetics and bacterial growth, p. 495–531. *In* S. D. Faust and J. V. Hunter (ed.), Organic compounds in aquatic environments. Marcel Dekker, New York, NY.
34. **Mukhopadhyay, A., A. M. Redding, M. P. Joachimiak, A. P. Arkin, S. E. Borglin, P. S. Dehal, R. Chakraborty, J. T. Geller, T. C. Hazen, Q. He, D. C. Joyner, V. J. J. Martin, J. D. Wall, Z. K. Yang, J. Zhou, and J. D. Keasling.** 2007. Cell-wide responses to low-oxygen exposure in *Desulfovibrio vulgaris* Hildenborough. J. Bacteriol. **189:**5996–6010.
35. **Nealson, K. H., A. Belz, and B. McKee.** 2002. Breathing metals as a way of life: geobiology in action. Antonie Van Leeuwenhoek **81:**215–222.
36. **North, N. N., S. L. Dollhopf, L. Petrie, J. D. Istok, D. L. Balkwill, and J. E. Kostka.** 2004. Change in bacterial community structure during in situ biostimulation of subsurface sediment cocontaminated with uranium and nitrate. Appl. Environ. Microbiol. **70:**4911–4920.
37. **Okutman Tas, D., and S. G. Pavlostathis.** 2005. Microbial reductive transformation of pentachloronitrobenzene under methanogenic conditions. Environ. Sci. Technol. **39:**8264–8272.
38. **Pereira, P. M., Q. He, A. V. Xavier, J. Z. Zhou, I. A. C. Periera, and R. O. Louro.** 2008. Transcriptional response of *Desulfovibrio vulgaris* Hildenborough to oxidative stress mimicking environmental conditions. Arch. Microbiol. **189:**451–461.
39. **Petrie, L., N. N. North, S. L. Dollhopf, D. L. Balkwill, and J. E. Kostka.** 2003. Enumeration and characterization of iron(III)-reducing microbial communities from acidic subsurface sediments contaminated with uranium(VI). Appl. Environ. Microbiol. **69:**7467–7479.
40. **Pitcher, R. S., and N. J. Watmough.** 2004. The bacterial cytochrome cbb_3 oxidases. Biochim. Biophys. Acta **1655:**388–399.
41. **Preisig, O., R. Zufferey, L. ThonyMeyer, C. A. Appleby, and H. Hennecke.** 1996. A high-affinity cbb_3-type cytochrome oxidase terminates the symbiosis-specific respiratory chain of *Bradyrhizobium japonicum*. J. Bacteriol. **178:** 1532–1538.
42. **Roden, E. E., and D. R. Lovley.** 1993. Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*. Appl. Environ. Microbiol. **59:**734–742.
43. **Sanford, R. A., J. R. Cole, and J. M. Tiedje.** 2002. Characterization and description of *Anaeromyxobacter dehalogenans* gen. nov., sp. nov., an aryl halorespiring facultative anaerobic myxobacterium. Appl. Environ. Microbiol. **68:**893–900.
44. **Sanford, R. A., Q. Wu, Y. Sung, S. H. Thomas, B. K. Amos, E. K. Prince, and F. E. Löffler.** 2007. Hexavalent uranium supports growth of *Anaeromyxobacter dehalogenans* and *Geobacter* spp. with lower than predicted biomass yields. Environ. Microbiol. **9:**2885–2893.
45. **Santana, M.** 2008. Presence and expression of terminal oxygen reductases in strictly anaerobic sulfate-reducing bacteria isolated from salt-marsh sediments. Anaerobe **14:**145–156.
46. **Stein, L. Y., M. T. La Duc, T. J. Grundl, and K. H. Nealson.** 2001. Bacterial and archaeal populations associated with freshwater ferromanganous micronodules and sediments. Environ. Microbiol. **3:**10–18.
47. **Sung, Y., K. F. Fletcher, K. M. Ritalaliti, R. P. Apkarian, N. Ramos-Hernandez, R. A. Sanford, N. M. Mesbah, and F. E. Löffler.** 2006. *Geobacter lovleyi* sp. nov. strain SZ, a novel metal-reducing and tetrachloroethene-dechlorinating bacterium. Appl. Environ. Microbiol. **72:**2775–2782.
48. **Thomas, S. H., E. P. Crespo, P. M. Jardine, R. A. Sanford, and F. E. Löffler.** 2009. Diversity and distribution of *Anaeromyxobacter* strains in a uranium-contaminated subsurface environment with nonuniform flow. Appl. Environ. Microbiol. **75:**3679–3687.
49. **Thomas, S. H., R. D. Wagner, A. K. Arakaki, J. Skolnick, J. R. Kirby, L. J. Shimkets, R. A. Sanford, and F. E. Löffler.** 2008. The mosaic genome of *Anaeromyxobacter dehalogenans* strain 2CP-C suggests an aerobic common ancestor to the delta-Proteobacteria. PLoS One **3:**e2103.
50. **Treude, N., D. Rosencrantz, W. Liesack, and S. Schnell.** 2003. Strain FAc12, a dissimilatory iron-reducing member of the *Anaeromyxobacter* subgroup of Myxococcales. FEMS Microbiol. Ecol. **44:**261–269.
51. **Wall, J. D., and L. R. Krumholz.** 2006. Uranium reduction. Annu. Rev. Microbiol. **60:**149–166.
52. **Wan, J. M., T. K. Tokunaga, E. Brodie, Z. M. Wang, Z. P. Zheng, D. Herman, T. C. Hazen, M. K. Firestone, and S. R. Sutton.** 2005. Reoxidation of bioreduced uranium under reducing conditions. Environ. Sci. Technol. **39:**6162–6169.
53. **Weber, K. A., M. M. Urrutia, P. F. Churchill, R. K. Kukkadapu, and E. E. Roden.** 2006. Anaerobic redox cycling of iron by freshwater sediment microorganisms. Environ. Microbiol. **8:**100–113.
54. **Wu, Q., R. A. Sanford, and F. E. Löffler.** 2006. Uranium(VI) reduction by *Anaeromyxobacter dehalogenans* strain 2CP-C. Appl. Environ. Microbiol. **72:** 3608–3614.
55. **Wu, W.-M., J. Carley, M. Fienen, T. Mehlhorn, K. Lowe, J. Nyman, J. Luo, M. E. Gentile, R. Rajan, D. Wagner, R. F. Hickey, B. H. Gu, D. Watson, O. A. Cirpka, P. K. Kitanidis, P. M. Jardine, and C. S. Criddle.** 2006. Pilot-scale *in situ* bioremediation of uranium in a highly contaminated aquifer. 1. Conditioning of a treatment zone. Environ. Sci. Technol. **40:**3978–3985.
56. **Wu, W.-M., J. Carley, J. Luo, M. A. Ginder-Vogel, E. Cardenas, M. B. Leigh, C. A. C. Hwang, S. D. Kelly, C. M. Ruan, L. Wu, J. V. Nostrand, T. Gentry, K. Lowe, T. Mehlhorn, S. Carroll, W. Luo, M. W. Fields, B. H. Gu, D. Watson, K. M. Kemner, T. Marsh, J. Tiedje, J. Zhou, S. Fendorf, P. K. Kitanidis, P. M. Jardine, and C. S. Criddle.** 2007. *In situ* bioreduction of uranium(VI) to submicromolar levels and reoxidation by dissolved oxygen. Environ. Sci. Technol. **41:**5716–5723.
57. **Wu, W. M., J. Carley, T. Gentry, M. A. Ginder-Vogel, M. Fienen, T. Mehlhorn, H. Yan, S. Caroll, M. N. Pace, J. Nyman, J. Luo, M. E. Gentile, M. W. Fields, R. F. Hickey, B. H. Gu, D. Watson, O. A. Cirpka, J. Z. Zhou, S. Fendorf, P. K. Kitanidis, P. M. Jardine, and C. S. Criddle.** 2006. Pilot-scale *in situ* bioremedation of uranium in a highly contaminated aquifer. 2. Reduction of U(VI) and geochemical control of U(VI) bioavailability. Environ. Sci. Technol. **40:**3986–3995.
58. **Yabusaki, S. B., Y. Fang, P. E. Long, C. T. Resch, A. D. Peacock, J. Komlos, P. R. Jaffe, S. J. Morrison, R. D. Dayvault, D. C. White, and R. T. Anderson.** 2007. Uranium removal from groundwater via *in situ* biostimulation: field-scale modeling of transport and biological processes. J. Contam. Hydrol. **93:**216–235.
59. **Zhang, W. W., D. E. Culley, M. Hogan, L. Vitiritti, and F. J. Brockman.** 2006. Oxidative stress and heat-shock responses in *Desulfovibrio vulgaris* by genome-wide transcriptomic analysis. Antonie Van Leeuwenhoek **90:**41–55.
60. **Zhou, J., M. A. Bruns, and J. M. Tiedje.** 1996. DNA recovery from soils of diverse composition. Appl. Environ. Microbiol. **62:**316–322.