Unique Ecophysiology among U(VI)-Reducing Bacteria as Revealed by Evaluation of Oxygen Metabolism in *Anaeromyxobacter dehalogenans* Strain 2CP-C$^{\dagger}$

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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 $\mu$ 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 ($f_e$) 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) ($f_o$) 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 (pO₂). 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 Tennessee (UT). Oak Ridge IFC site samples are located in the inner circulation loop of the bioreduction test plot (31).

FW101 and FW102 were sampled before and after oxygen intrusion to monitor their responses to changing redox conditions. FW102 are located in the inner circulation loop of the bioreduction test plot (31). Pilot plot in area 3 (55–57) and processed by Joy Van Nostrand at the University of Tennessee (UT). Oak Ridge IFC site samples.

**Materials and Methods.** Oxygen metabolism in *Anaeromyxobacter* spp. under aerobic conditions (7, 43, 50), and the analysis of *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 (pO₂). 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.

Oxidation of aerobic cultures was measured 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, nucleic-free, deionized water to yield a homogenous 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 the *A. dehalogenans* 16S rRNA gene sequences in field samples, genus-level targeted TaqMan-based qPCR tools were utilized (48). For all TaqMan reactions, qPCR 10-fold concentrated master mix (Applied Biosystems [ABI], Foster City, CA), 125 nM probe, and 100 nM (each) primer were combined in sterile, nucleic-free water. Aliquots (18 µl) of the reaction mix were dispensed into an ABI MicroAmpl Fast Optical 96-well reaction plate held on ice. Template DNA (2 µl) was added to each well, and the plates were sealed with an ABI optiSeal adromy 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 × 10⁵ 16S rRNA gene copies/liter of groundwater. Detection of *G. lovleyi* 16S rRNA genes used SYBR green chemistry and required 1 × 10⁵ 16S rRNA gene copies per reaction, or about 1 × 10⁶ *G. lovleyi* 16S rRNA gene copies/liter of groundwater to yield positive signals.

**Calculation of growth rate, growth yield, and f₀ and f₁ values.** Growth 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 phase. Growth yield (Y₉₀) was calculated by counting the number of 16S rRNA gene copies per ml at time t and is the number of 16S rRNA gene copies per ml × t⁻¹. Triplicate cultures were used to determine growth rates at pO₂ values of 0.02 atm, 0.03 atm, and 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 ABKCO00000000) harbor duplicate rRNA operons (49), suggesting that members of the *Anaeromyxobacter* group contain two 16S rRNA gene copies per cell. The apparent fraction of cells utilized for energy generation (i.e., oxygen reduction) (f₀) and the fraction of electrons released in electron donor oxidation and used for biomass production (f₁) 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 Amexc 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 N₂/CO₂ 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 at atmospheric pressure were injected. Equivalent amounts of nitrogen were injected.
into the bottles at each sampling event to maintain constant overpressure. The detector response (i.e., peak area) was correlated to the calculated pO2 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. pO2 is reported in atmosphere (atm) and in total micromoles per bottle. The total mass of oxygen per bottle (n, in micromoles) was calculated from pO2 (atm) according to the ideal gas law \[ n = \frac{(pO_2 \times V \times 10^6)}{(R \times T)}, \] 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 pO2 of 0.02 atm equates to 110 \( \mu \)mol of oxygen per bottle. Due to oxygen’s very low Henry’s law constant \( (K_H) \) of 1.26 \( \times \) \( 10^{-3} \) 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 pO2 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 \( \mu \) 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* sediment samples ranged from \( 1 \times 10^7 \) to \( 1 \times 10^8 \) cells per g (Fig. 1A) and were substantially higher than the cell titers in groundwater, which ranged from \( 1 \times 10^3 \) to \( 1 \times 10^4 \) 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 pO2 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 (pO2 of 0.02 atm) (Fig. 2B). Previous studies have shown that *A. dehalogenans* and *G. lovleyi* use acetate as an elec-

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.
Stationary-phase cultures of A. dehalogenans strain 2CP-C and G. lovleyi to O2. Stationary-phase cultures of A. dehalogenans strain 2CP-C (A) and G. lovleyi (B) were exposed to an oxygen-containing headspace (initial pO2 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.

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., pO2 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 pO2 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 2.6 × 107 ± 8.78 × 106 to 5.18 × 109 ± 7.20 × 107 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 × 109 ± 8.8 × 108 to 6.9 × 107 ± 1.2 × 107 and from 3.2 × 109 ± 9.2 × 108 to 5.8 × 108 ± 2.5 × 107 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).

Effect of pO2 on growth. Increasing pO2 affected growth rates of strain 2CP-C (see Table S1 and Fig. S2 in the supple-
specific growth rate \( \mu \) at varying \( pO_2 \). The regression indicates an inverse linear correlation of \( \mu \) and \( pO_2 \). Error bars represent standard errors of the mean growth rate. Regression coefficients are presented in Table S1 in the supplemental material.

Doubling times of fumarate-grown and oxygen-grown \( pO_2 \) of 0.02 atm to 0.03 atm (i.e., a 33% decrease) (Fig. 4). The slope of the \( \mu-pO_2 \) 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 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 \( (f_e) \) 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 \( (f_a) \) 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 \( f_a \), 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 \( (\mu) \) 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 mol of transferred electrons, and \( f_e \) and \( f_a \) 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

| EA (conc) | No. of cells per \( \mu \) mol 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\(^c\) | \( f_e^{d} \) | \( f_a^{e} \) |
|----------|----------------------------------|----------------------------------|----------------------------------|---|---|
| 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\(^h\) (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 mol 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 \( (3.80 ± 0.27 \times 10^{-14} \text{ g}) \) 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 \( \text{C}_5\text{H}_7\text{O}_2\text{N} \) and 113 g/mol, respectively.

\(^d\) \( f_e \) is the fraction of electrons released in electron donor oxidation and used for biomass synthesis. It is calculated as follows: \( f_e = \frac{\text{mol of e}^{-} \text{ of cells per mol of EA}}{1} \).

\(^e\) mol of e\(^{-}\) of cells per mol of EA.

\(^f\) Data from reference 44.

\(^g\) Data from reference 17.

\(^h\) Data from reference 18.

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

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