Relative Importance of H$_2$ and H$_2$S as Energy Sources for Primary Production in Geothermal Springs$^\dagger$

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Geothermal waters contain numerous potential electron donors capable of supporting chemolithotrophy-based primary production. Thermodynamic predictions of energy yields for specific electron donor and acceptor pairs in such systems are available, although direct assessments of these predictions are rare. This study assessed the relative importance of dissolved H$_2$ and H$_2$S as energy sources for the support of chemolithotrophic metabolism in an acidic geothermal spring in Yellowstone National Park. H$_2$S and H$_2$ concentration gradients were observed in the outflow channel, and vertical H$_2$S and O$_2$ gradients were evident within the microbial mat. H$_2$S levels and microbial consumption rates were approximately three orders of magnitude greater than those of H$_2$. Hydrogenobaculum-like organisms dominated the bacterial component of the microbial community, and isolates representing three distinct 16S rRNA gene phylotypes (phylogeny = 100% identity) were isolated and characterized. Within a phylotype, O$_2$ requirements varied, as did energy source utilization: some isolates could grow only with H$_2$S, some only with H$_2$, while others could utilize either as an energy source. These metabolic phenotypes were consistent with in situ geochemical conditions measured using aqueous chemical analysis and in-field measurements made by using gas chromatography and microelectrodes. Pure-culture experiments with an isolate that could utilize H$_2$S and H$_2$ and that represented the dominant phylotype (70% of the PCR clones) showed that H$_2$S and H$_2$ were used simultaneously, without evidence of induction or catabolite repression, and at relative rate differences comparable to those measured in ex situ field assays. Under in situ-relevant concentrations, growth of this isolate with H$_2$S was better than that with H$_2$. The major conclusions drawn from this study are that phylogeny may not necessarily be reliable for predicting physiology and that H$_2$S can dominate over H$_2$ as an energy source in terms of availability, apparent in situ consumption rates, and growth-supporting energy.

Thermophiles dominate the deepest and shortest branches of the Bacteria and Archaea domains in the tree of life, suggesting that they are likely ancestors of Earth’s contemporary microbial populations (8, 35). Consequently, these organisms have attracted considerable attention due to interest in the origin of enzymes and metabolic pathways that are thought to have evolved from such organisms. Chemolithotrophic metabolism is foundational to primary productivity in geothermal environments where temperatures exceed the limit of photosynthesis. The bioenergetics of such systems have been examined from the perspective of theoretical energy yield as a way of discussing the relative importance of the various electron donors and acceptors that could support primary productivity (3–5, 22). Other studies have sought to link the inferred physiology of microbial populations with the predicted energy yields obtainable from the inorganic constituents present (4, 18, 26, 28, 33).

Geothermal features vary considerably with respect to temperature, pH, and the presence and concentrations of energy sources such as H$_2$, H$_2$S, S$^0$, Fe(II) and As(III) (18, 29). Acid-sulfate-chloride (ASC) springs (e.g., Fig. 1) are common throughout the Yellowstone geothermal complex, although they are considerably more concentrated in and around the Norris Geyser Basin area. ASC springs are intriguing from a bioenergetics standpoint because they offer a virtual buffet of energy sources for chemolithoautotrophs, including a constant flux of $\mu$M concentrations of dissolved H$_2$S, Fe(II), and As(III), nM concentrations of H$_2$, conspicuous amounts of S$^0$, and often-supersaturating levels of CO$_2$ (10, 18, 21, 26, 37). Discussions regarding the relative importance of these electron donors in supporting primary production in such systems sometimes center on comparisons of potential energy released from their oxidation, with the H$_2$/O$_2$ couple perhaps being favored (37), whereas others urge caution in making such predictions, pointing out that in low-pH systems, the oxidation of H$_2$S could yield nearly as much energy (18).

ASC springs are clearly in the latter category and are the focus of the current study, in which we examined microbial utilization of H$_2$S and H$_2$ by using a combination of in-field ex situ assays, microelectrodes, and gas chromatography to document the presence, concentrations, gradients, and consumption of these energy sources. These analyses were combined with molecular-community analysis and with the cultivation of...
which it was found to be 2.8, and the temperatures ranged from 68 to 72°C. The 2-year study. The pH of the source water was 3.1 on all but two occasions, on the Basin, YNP. Water temperature and pH were routinely monitored throughout Yellowstone National Park [YNP] thermal inventory), located in Norris Geyser Basin, YNP. Water temperature and pH were routinely monitored throughout the year. The pH of the source water was 3.1 on all but two occasions, on which it was found to be 2.8, and the temperatures ranged from 68 to 72°C. Dissolved H₂S was measured by using the methylene blue method (9). Spectrophotometric analysis was performed at 380 nm using a UV-2000 portable spectrophotometer (Ocean Optics, Dunedin, FL). The presence of H₂S was determined by using a portable Varian gas chromatograph (model CP-2900) with an Ar and N₂ as carrier gases. The concentration of aqueous H₂S [H₂S(aq)] in each sample was determined by using the headspace gas chromatography method and temperature-corrected Henry’s law constants described previously (18).

Ex situ measurements of H₂S and H₂ consumption activity. Mat samples were collected by using sterile wide-bore pipette tips and were placed into sterile 15-ml conical tubes, homogenized by mixing, and split into two subsamples. One subsample was maintained at in situ temperature and was used to assay for microbial H₂ or H₂S consumption, while the other was boiled for 20 min to serve as a killed control. Subsamples of live and heat-treated mat material were aseptically transferred into autoclaved 50-ml serum bottles containing 40 ml of filter-sterilized spring water. The assay bottles were immediately closed with rubber septa, pressure sealed with aluminum rings, and incubated at in situ temperatures by placing the bottles in the spring at the same location from which the mat material was sampled. H₂O samples were withdrawn at timed intervals to be assayed for H₂S(aq), whereas headspace gas samples were taken to measure H₂. Data for dissolved H₂ and H₂S levels were normalized based on the mat sample dry weight, which was determined by collecting all mat material from each serum bottle onto preweighed 0.2-μm filters, drying it overnight at 65°C, and then weighing it. The maximum sample dry weight variation between samples was 4.1%.

Cultivation and isolation. Mat samples were collected from sites 35, 115, and 350 cm from the discharge point of the spring. The mat material was stored for transportation in 15-ml conical tubes and maintained at 65°C in a Thermos bottle filled with water from the site. Mat samples were resuspended in 10 ml of spring water collected directly above the mat-sampling site, and 100 μl of this suspension was used to inoculate 16-ml serum bottles sealed with Teflon-coated butyl rubber septa (National Scientific, Rockwood, TN). For aerobic H₂ chemolithoautotroph enrichment experiments, the serum bottles were immediately closed with rubber septa, pressure sealed with aluminum rings, and incubated at in situ temperatures by placing the bottles in the spring at the same location from which the mat material was sampled. H₂O samples were withdrawn at timed intervals to be assayed for H₂S(aq), whereas headspace gas samples were taken to measure H₂. Data for dissolved H₂ and H₂S levels were normalized based on the mat sample dry weight, which was determined by collecting all mat material from each serum bottle onto preweighed 0.2-μm filters, drying it overnight at 65°C, and then weighing it. The maximum sample dry weight variation between samples was 4.1%. The pH of the source water was 3.1 on all but two occasions, on which it was found to be 2.8, and the temperatures ranged from 68 to 72°C. Dissolved H₂S was measured by using the methylene blue method (9). Spectrophotometric analysis was performed at 380 nm using a UV-2000 portable spectrophotometer (Ocean Optics, Dunedin, FL). The presence of H₂S was determined by using a portable Varian gas chromatograph (model CP-2900) with an Ar and N₂ as carrier gases. The concentration of aqueous H₂S [H₂S(aq)] in each sample was determined by using the headspace gas chromatography method and temperature-corrected Henry’s law constants described previously (18).

In-field microelectrode assays. Electrochemical field analysis of H₂S concentrations was accomplished by using a portable DLK-60 potentiostat controlled by a laptop computer (Analytical Instrument Systems, Inc.) and micromanipulator. Voltammetry scans (1,000 mV/sec; initial holding potential of −0.1 V between −0.1 and −1.8 V [versus that for Ag/AgCl]) were acquired via a glass Au-amalgam solid-state working electrode, a platinum wire counter electrode, and a Ag/AgCl reference electrode constructed like that of Brendel and Luther (7). The H₂S signal assignment and calibrations were conducted by using spring water doped with freshly prepared standards (16). Vertical measurements of O₂ were carried out with a Clark-type microsensor and micromanipulator, using field methods, calibration techniques, and temperature compensation as described previously (32). Scanning electron microscopy/energy-dispersive X-ray spectroscopy and sulfite analyses. Cultures were grown under the conditions described above, and the yellow precipitate that is common to these cultures was removed and separated for further analysis. The pH of the source water was 3.1 on all but two occasions, on which it was found to be 2.8, and the temperatures ranged from 68 to 72°C. Dissolved H₂S was measured by using the methylene blue method (9). Spectrophotometric analysis was performed at 380 nm using a UV-2000 portable spectrophotometer (Ocean Optics, Dunedin, FL). The presence of H₂S was determined by using a portable Varian gas chromatograph (model CP-2900) with Ar and N₂ as carrier gases. The concentration of aqueous H₂S [H₂S(aq)] in each sample was determined by using the headspace gas chromatography method and temperature-corrected Henry’s law constants described previously (18).
rated from the culture media by centrifugation at 13,000 × g for 5 min and then dried under vacuum conditions. The resulting material was applied to carbon tape on a sample stage and analyzed on a JEOL model 6100 scanning electron microscope (JEOL, Tokyo, Japan) equipped with an X-ray detector. The sample was subjected to 20 keV of incident energy, and measurements were made over a 50-s interval.

Production levels of sulfate by isolate 3684 were monitored in vitro by growing 10 ml of cells under standard conditions to 5 × 10^7 cells ml^-1 and then concentrating the cells by centrifugation at 5,000 × g for 5 min. Cells were resuspended in 1 ml of a synthetic medium [10 mM (NH₄)₂PO₄, 0.2 mM KH₂PO₄, 2 mM trace element solution (2), 5 mM citric acid, pH 3.0] and used to inoculate 5.0 ml of the same medium. Na₂S was added to a final concentration of 200 mM NaCl, 1 mM MgCl₂, 0.5 mM CaCl₂, 50 µM (NH₄)₂SO₄, 1.2 µM FeCl₃, 0.5 ml trace element solution (2), 5 mM citric acid, pH 3.0] and used to inoculate 59 ml of the same medium. Na₂S was added to a final concentration of 200 mM, and 5.5-ml samples were removed at 12-h intervals and measured for H₂S utilization as described above, with 5.0 ml filtered through a 0.2-µm polytetrafluoroethylene filter and measured for SO₄ by using a Dionex ion chromatograph.

**RESULTS**

**Spring chemistry.** In a preceding study, sulfide constraints on the population distribution of an As(III) chemolithotroph were studied (10). Mapping H₂S gradients showed that measurable H₂S was primarily found in the yellow solid-phase zone (Fig. 1), which is a microbial mat comprised of filamentous microorganisms (primarily *Hydrogenobaculum* [19]) interwoven with a mineral phase made of up S₀ (21). At the point of discharge of the spring, H₂S(aq) was found to be 75 to 80 µM; it declined to 5 to 10 µM at a distance of 3.5 m in the outflow channel at transect position 5 (Fig. 1) and was below detection at approximately 5 m (10). In the same study, O₂ gradients were also measured, and concentrations were found to be essentially opposite to those of H₂S; i.e., O₂(aq) was undetectable in the first 100 cm in the outflow channel but then increased rapidly from there on.

The study described herein occurred approximately 18 months subsequent to that study, and H₂S levels were spot-checked to verify that the same gradient patterns were still present (results not shown). In addition, to better understand potentially important geochemical gradients that may be of value for understanding in situ microbial metabolism and for designing ecologically relevant cultivation conditions, microelectrodes were used to examine H₂S and O₂ concentrations in the vertical dimension (Fig. 2). At a location approximately corresponding to transect site 3 (Fig. 1), H₂S concentrations in the water and within the upper 1 mm of mat appeared to remain nearly constant at ~50 µM but then decreased sharply to the detection limit within ~2.5 mm (Fig. 2A). Vertical O₂ profiles were similarly steep, with O₂ levels being saturated 4 mm above the mat surface and rapidly changing to anaerobic conditions 2 to 3 mm inside the mat (Fig. 2B). Dissolved H₂S concentrations at the spring source were ~13 nM and decreased rapidly for approximately 50 cm. Aqueous H₂ then increased briefly, due to mixing with geothermal water from a second downstream source (Fig. 1), but again declined with distance (Fig. 3), presumably due to off-gassing and or microbial consumption.

**Ex situ assays.** H₂S and H₂ consumption rates were studied to determine their relative importance as electron donors to the microorganisms inhabiting the S₀ deposition zone. Rates of H₂S loss (i.e., apparent consumption) in the sealed assay containers ranged from 35 to 50 µmol min⁻¹ g dry mat⁻¹, whereas H₂ consumption rates were approximately three orders of magnitude lower: 1 to 4 nmol min⁻¹ g dry mat⁻¹ (Fig. 4; see also Fig. S1 in the supplemental material). In ex situ assays using heat-treated mat samples, the apparent H₂ and H₂S consumption was much lower or absent. For H₂S consumption results in heat-killed samples, incomplete heat killing cannot be ruled out, although much-slower abiotic oxidation likely also occurred due to the presence of oxygen unavoidably introduced during sample preparation at springside.

**Phylogenetic analysis.** A previous phylogenetic survey of the spring that included S₀ mat material from a position equivalent to site 5 (Fig. 1) found that *Hydrogenobaculum*-like signatures dominated the 16S rDNA clone libraries (19). To better understand the microbial community structure in the regions of the spring where H₂S and H₂ consumption were examined in the current study, additional 16S rRNA gene clones were am-
plified from mat DNA extracted from transect sites 2 and 4 within the central flow channel of the S0 deposition zone (Fig. 1). Of the 90 nearly full-length clones sequenced, 94% represented Hydrogenobaculum-like organisms (>99% identity to Hydrogenobaculum acidophilum, GenBank accession number D16296), falling into two distinct phylotypes with >99.9% nucleotide identity to one another; phylotype I comprised 71% of the Hydrogenobaculum-like clones, and the remainder were referred to as phylotype II.

**Culturing and isolation.** Filter-sterilized spring water was used as a basal medium in cultivation experiments aimed at isolating H2S and H2 chemolithoautotrophs from the mat exhibiting H2S and H2 consumption. Though the O2 electrode experiments (Fig. 2B) indicated a continuum of aerobic, microaerobic, and anaerobic environments, only aerobic and microaerobic enrichments resulted in growth. Following several subcultures, clonal cultures were derived from positive enrichments by three rounds of dilution to a theoretical 0.5 cell·ml−1. DGGE analysis was used to monitor isolation progress throughout; upon continued subculture, DGGE profiles were progressively less complex, reduced to a single band in each of the late-stage enrichment subcultures and remaining as such following dilution to extinction subcultures. A total of 30 isolates that were phylogenetically very similar to Hydrogenobaculum (>99% identity to H. acidophilum; GenBank accession number D16296) and that formed three distinct 16S phylotypes (see Fig. S2 in the supplemental material) were obtained. Isolate phylotypes I and II were identical to the above-mentioned phylotypes amplified from total mat DNA, whereas the 16S rRNA signature of phylotype III isolates was novel relative to amplicons derived from total mat DNA. Phylotypes I and III deviated from each other by 2 nucleotides, whereas phylotype II deviated from phylotypes I and III by 8 nucleotides over a 1,431-nucleotide region of the 16S gene.

Within each phylotype, cell morphology was invariant, and all isolates were identical with respect to 16S rRNA gene sequences and the cloned 350-bp ITS region. However, within each phylotype, the isolates displayed widely varying growth phenotypes. Some isolates would grow only on H2S and some only on H2, whereas others could grow with either energy source (Table 1). Further, some were found to grow best under microaerobic conditions, whereas others preferred fully aerobic conditions. Phylotype II contained isolates having all combinations of H2S/H2/O2 utilization/requirement patterns. An aerobic isolate (strain YNP3684) capable of growth on either electron donor was maintained on sulfide for a period of 2 years, encompassing 32 culture transfers (1:50 dilution each) in the absence of H2 (effective dilution = 1052). Following this period of extended dilution and culturing, YNP3684 retained its ability to grow autotrophically on H2, providing evidence that it was not a mixed culture.

**Pure culture studies.** Given that YNP3684 was phylogenetically identical to the dominant community sequence type and was capable of growth using either H2S or H2 as an electron donor and CO2 as a carbon source, it was viewed to be ecologically relevant and ideal for modeling studies that would more closely examine H2S or H2 utilization under spring-relevant conditions. One set of experiments examined whether H2S or H2 exerted catabolite regulatory controls over the other and thus potentially influenced in situ utilization patterns. YNP3684 was first cultured separately with H2S or H2 (in filter-sterilized spring water) to acclimate the cells to a single energy source, and then late-log-phase cells were transferred to the same medium that now contained H2S (30 μM) and H2 (25 nM). In such experiments, consumption of both H2S and H2 was immediate and without a discernible lag (Fig. 5A). Initial consumption rates (first three time points) were 0.25 μmol·min−1·106 cells and 0.32 nmol·min−1·106 cells for H2S and H2, respectively. H2S consumption appeared to re-

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**TABLE 1.** Metabolic differences among Hydrogenobaculum-like isolates

| Phylotype | No. of isolates | No. of isolates capable of using indicated electron donor: | No. of microaerobic isolates* | No. of aerobic isolates* |
|-----------|-----------------|---------------------------------------------------------|-------------------------------|------------------------|
| I         | 9               | H2S H2 H2S/H2                                           | 0                            | 9                      |
| II        | 18              | 3 2 4                                                  | 6                            | 12                     |
| III       | 3               | 2 0 1                                                  | 0                            | 3                      |
| **Total** | **30**          | **20** 2 8                                              | **6**                         | **24**                 |

*Isolated listed as microaerobic were incapable of growth with a headspace O2 content of >2%, while aerobic isolates exhibited the best growth at a headspace O2 content of 5.5% and are capable of growth with >10% O2.
Dissolved H$_2$S following inoculation with 2 H$_2$(aq) decreased to
Growth rates with spring-relevant levels of H$_2$S were intermediate between these extremes (doubling time of 13.7 ± 1.8 h), and growth with H$_2$S and H$_2$ (doubling time of ~12.9 ± 0.9 h) did not significantly improve growth statistically beyond that which occurred with H$_2$S alone (Fig. 5B).

Because background levels of S$^{0}$ (solid phase) (Fig. 1) and SO$_4$ (1.2 mM) (21) in the spring prohibited experiments designed to track the fate of H$_2$S in the ex situ assays, experiments were undertaken to determine the fate of H$_2$S in pure culture. Ion chromatography of culture fluids indicated that ~80% of added H$_2$S was converted to sulfate after 24 h of incubation with a cell culture at 1 · 10$^5$ cells ml$^{-1}$ (see Fig. 3A in the supplemental material). YNP3684 culture fluids were visibly yellow when grown on H$_2$S, whereas uninoculated media remained clear, suggesting that S$^{0}$ may occur as an intermediate during enzymatic oxidation by this organism. Scanning electron microscopy/energy-dispersive X-ray spectroscopy analysis of solid phase culture constituents confirmed the presence of a sulfur-containing solid phase, consistent with the oxidation of H$_2$S to S$^{0}$ (see Fig. 3B in the supplemental material).

**DISCUSSION**

Predictive studies concerning the energy sources that fuel primary production in geothermal environments have been very useful as first approximations. Molecular (16S rDNA) approaches estimate in situ metabolism based on physiologies inferred from phylogenetically closely related organisms that have been characterized (28, 33, 37), and thermodynamic predictions have been derived from free-energy yields calculated from the ion activities of electron donors and acceptors found in geothermal systems (4, 5, 18, 27, 33, 37). The present study had similar goals, while focusing on H$_2$ and H$_2$S; however, the experiments were designed to take a more-directed approach: i.e., quantifying presence, measuring utilization, and examining the characteristics of microorganisms capable of growth on these highly exergonic chemolithotrophic substrates.

A previous aqueous chemical analysis (10) agrees with the data obtained for this report (Fig. 2A), with both demonstrating significant levels of H$_2$S, and time course ex situ assays provide evidence of rapid microbe-based H$_2$S consumption (Fig. 4A). H$_2$S- and O$_2$-sensitive microelectrode experiments illustrated that H$_2$S penetration into the mat was limited, rapidly decreasing to below detection within the oxygenated region of the mat (Fig. 2), suggesting that H$_2$S consumption is linked to the presence of O$_2$. Similar experiments also demonstrated H$_2$ gradients (Fig. 3) and microbial consumption (Fig. 4B). The vertical and horizontal gradients of H$_2$S, H$_2$, and O$_2$ suggest a continuum of chemical energy gradients, providing numerous niche opportunities for populations having specialized or flexible metabolic needs. The latter was confirmed by the organisms obtained in the isolation exercises (Table 1) and, indeed, demonstrated optimum O$_2$ requirements exceeding that previously documented for Hydrogenobaculum (1, 12, 36).

The Hydrogenobaculum 16S rDNA signatures that dominated the community clone libraries were ~99% identical to those of *H. acidophilum*, which was characterized as being a microaerophile requiring H$_2$ and S$^{0}$ for growth (36). Thus, physiologic inference would predict that organisms inhabiting the yellow S$^{0}$ deposition zone would be engaged in H$_2$ oxidation and utilize the abundant S$^{0}$ present in this region of the spring outflow channel (Fig. 1) (21). Results of the cultivation work, however, illustrate the potential problems associated with physiologic inference in general and, more specifically, the incorrect conclusions drawn if it is applied to the Hydrogenobaculum-like populations inhabiting this spring. Isolates...
sharing identical 16S rRNA gene sequences (and, indeed, ITS sequences) differed in important ways with respect to the ecologically relevant electron donors they were capable of utilizing (Table 1). Such observations affirm those previously reported for organisms that are phylogenetically closely related (38) or, indeed, identical (20) but which exhibit widely varying physiologies.

The isolates obtained in this study were also important for other reasons. First, we drew attention to the observation that the overwhelming majority (90%) of the Hydrogenobaculum isolates were phylogenetically identical to the two major phylotypes (94% of all PCR clones) obtained from total community DNA. The use of filter-sterilized spring water as the basal growth medium along with O2 concentrations predicted from the microelectrode work likely provided a more natural growth environment than synthetic media, which has historically tended to exclude the growth of ecologically relevant microorganisms (for an example, see reference 13).

Such isolates are also useful for pure-culture modeling studies, where results of ex situ experiments can be studied in more detail and under more-controlled conditions. When isolate YNP3684 (representing the dominant mat phylotype) was cultured in spring water with an average spring concentration of H2S (30 nM) but H2 levels (30 nM) considerably exceeding the maximum value observed at any location in the spring, H2S consumption rates exceeded those of H2 by three orders of magnitude, an observation completely consistent with the ex situ measurements (Fig. 4). Regardless of the substrate to which the cells had been preconditioned, utilization of both substrates was immediate and without a lag phase (Fig. 5A), suggesting constitutive expression of the enzymes involved. In some geothermal systems where microorganisms can be bathed in a continuous flux of multiple energy sources, constitutive utilization of several available energy sources may be the norm.

Another important observation derived from the pure-culture experiments speaks to the significance of enzyme kinetics when considering the relative importance of various energy sources in natural settings. Theoretical energy yields derived from calculating ΔG°rxn fail to account for enzyme properties, the importance of which cannot be overstated. H2 concentrations in the range of 5 to 10 nM have been shown to support microbial growth (24, 25, 37) and are similar to the concentrations observed in several Yellowstone geothermal features (Fig. 3) (37). However, these concentrations are considerably lower than the published Km values derived from purified uptake hydrogenases (e.g., 0.92 μM H2 for Pyrodictium brockii [30]; 19 μM for an Anabaena sp. [17]). The H2 and H2S consumption profiles exhibited by YNP3684 (Fig. 5A) suggest that over the range of concentrations of both substrates directly measured in the spring, utilization of H2S surpassed that of H2. Further, when spring-relevant concentrations of H2 and H2S were provided, consumption rates appeared closely related to actual growth, with this particular organism growing better with H2S (Fig. 5B). Regular analysis of headspace gases in these experiments showed that the H2-cultured cells never experienced H2 concentrations lower than what were measured in the yellow SO4 deposition zone (Fig. 3), indicating that the observed culture growth rates may exceed even that occurring in situ. Superior growth with H2 was observed only with saturating concentrations of H2, levels that should be considered unrealistic given the concentrations measured in Dragon Spring and elsewhere in Yellowstone (29, 37).

While it is clear that the aerobic respiration of H2 can provide substantial energy to chemolithotrophs, the potential energy gained from the oxidation of sulfide depends on the extent to which the sulfide is oxidized and may, in fact, be quite similar to that gained through H2 oxidation under the spring conditions examined in this study (18). Several organisms, including Thiobacillus thioparus (23, 41) and Desulfovibalis propionicus (15), are capable of oxidizing H2S to SO4 under aerobic conditions. SO4 production by this Hydrogenobaculum isolate (see Fig. S3B in the supplemental material) appears to be intermediate, as SO4 production was also observed (see Fig. S3A in the supplemental material). While Rowe et al. (34) suggested that the presence of elemental sulfur was a major niche determinant for Hydrogenobaculum-like organisms they encountered in thermal tributaries feeding Lemonade Creek, our results indicate that the occurrence of SO4 is due, at least in part, to the activity of the organisms present, with rates of abiotic H2S oxidation apparently being a relatively minor contributor (Fig. 4).

In summary, this study directly examined the relative importance of H2S and H2 as energy sources in support of primary productivity in a geothermal spring. Field experiments quantified both and measured consumption rates and were combined with cultivation and pure-culture assays to determine the dynamics of electron donor usage by an environmentally relevant isolate. The major conclusions drawn from this study are that phylogeny may not necessarily be a reliable predictor of physiology and that regardless of thermodynamic estimates, H2S can dominate H2 as an energy source in terms of availability, apparent in situ consumption rates, and growth-supporting energy.

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