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Research Paper

Geochemical Constraints on Sources of Metabolic Energy for Chemolithoautotrophy in Ultramafic-Hosted Deep-Sea Hydrothermal Systems

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ABSTRACT

Numerical models are employed to investigate sources of chemical energy for autotrophic microbial metabolism that develop during mixing of oxidized seawater with strongly reduced fluids discharged from ultramafic-hosted hydrothermal systems on the seafloor. Hydrothermal fluids in these systems are highly enriched in H_2 and CH_4 as a result of alteration of ultramafic rocks (serpentinization) in the subsurface. Based on the availability of chemical energy sources, inferences are made about the likely metabolic diversity, relative abundance, and spatial distribution of microorganisms within ultramafic-hosted systems. Metabolic reactions involving H_2 and CH_4, particularly hydrogen oxidation, methanotrophy, sulfate reduction, and methanogenesis, represent the predominant sources of chemical energy during fluid mixing. Owing to chemical gradients that develop from fluid mixing, aerobic metabolisms are likely to predominate in low-temperature environments (<20–30°C), while anaerobes will dominate higher-temperature environments. Overall, aerobic metabolic reactions can supply up to ~7 kJ of energy per kilogram of hydrothermal fluid, while anaerobic metabolic reactions can supply about 1 kJ, which is sufficient to support a maximum of ~120 mg (dry weight) of primary biomass production by aerobic organisms and ~20–30 mg biomass by anaerobes. The results indicate that ultramafic-hosted systems are capable of supplying about twice as much chemical energy as analogous deep-sea hydrothermal systems hosted in basaltic rocks. Key Words: Hydrothermal systems—Geomicrobiology—Serpentinites. Astrobiology 7, 933–950.

INTRODUCTION

DEEP-SEA HYDROTHERMAL SYSTEMS support prolific biological communities whose trophic structure is based almost entirely on the conversion of chemical energy to biomass by chemolithoautotrophic microorganisms (chemolithoautotrophs are organisms that utilize inorganic chemical energy sources to obtain metabolic energy and use that energy to synthesize their own biomass from CO_2 and other inorganic compounds) (Karl, 1995; Kelley *et al.*, 2002). Because these deep-sea ecosys-

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tems exist with very little input of organic matter from photosynthesis, they are frequently cited as possible analogues for habitable environments on early Earth and other planetary bodies (Boston *et al.*, 1992; Shock, 1997; Fisk and Giovannoni, 1999; McCollom, 1999; Varnes *et al.*, 2003; Schulte *et al.*, 2006). Submarine hydrothermal environments have also been suggested as a possible site for the origin and early evolution of life on Earth (*e.g.*, Holm, 1992; Holm and Andersson, 1998; Shock and Schulte, 1998; Russell *et al.*, 2005).

To date, studies of the microbiology of deep-sea hydrothermal environments have focused almost exclusively on hydrothermal systems hosted in basaltic rocks (Karl, 1995; Kelley *et al.*, 2002). In recent years, however, there has been an increasing interest in hydrothermal systems that are hosted in ultramafic rocks instead of basalts (Kelley *et al.*, 2001, 2005; Früh-Green *et al.*, 2004). Ultramafic rocks, such as peridotite and dunite, are primarily composed of the minerals olivine and pyroxene, and occurrences of ultramafic rocks at the seafloor or shallow subsurface represent blocks of the mantle that have been displaced to near-surface environments by tectonic processes. Olivine and pyroxene are unstable in the presence of water at low temperatures, and exposures of ultramafic rocks subjected to circulating seawater or groundwater undergo aqueous alteration. Ultramafic rocks that have undergone aqueous alteration are often referred to as serpentinites, owing to the predominance of the serpentine group minerals in the alteration mineral assemblage (the term "serpentine" is often used to refer to a family of minerals that includes antigorite, lizardite, chrysotile, and others; for brevity, these will be referred to here collectively as serpentine without implying a particular phase). Other common minerals in serpentinites include talc, brucite, and magnetite. Although once thought to be anomalous, ultramafic-hosted hydrothermal environments are increasingly recognized to be widespread on the seafloor (Bach *et al.*, 2002; Früh-Green *et al.*, 2004).

Owing to differences in mineralogy and bulk chemistry, serpentinization of ultramafic rocks results in substantially different alteration mineralogy and vent fluid compositions than occur during hydrothermal alteration of basaltic rocks (Table 1) (Janecky and Seyfried, 1986; Wetzel and

Table 1 Compositions of Hydrothermal Vent Fluids from Ultramafic-Hosted, Deep-Sea Hydrothermal Systems, with Representative Vent Fluids from Basalt-Hosted Systems and Seawater Included for Reference

	Seawater	*Rainbow MAR*	*Logatchev MAR*	*Lost City off-axis*	*TAG MAR*	*21°N OBS EPR*
Host Rock		ultramafic	ultramafic	ultramafic	basalt	basalt
T (°C)	2	365	352	40–91	321	350
pH	7.8	2.8	3.3	9.0 to ~11	2.8	4.3
$O_{2(aq)}$	0.100	0	0	0	0	0
$H_{2(aq)}$	0.0000004	16	12	<1–15	0.15	1.7
SO_4^{2-}	27.9	~0	~0	1–4	~0	~0
$H_2S_{(aq)}$	0	1.2	0.8	—	6.7	7.3
$CH_{4(aq)}$	0.0000003	2.5	2.1	1–2	0.124	0.07
CO	—	0.005	—	—	—	—
ΣCO_2	2.3	16	10.1	—	2.9	5.72
Na^+	464	553	430	—	584	432
Cl^-	546	750	515	~550	659	490
Br^-	0.84	1.18	0.82	—	0.88	0.8
NO_3^-	0.03	—	—	—	—	—
Ca^{2+}	10.2	66.6	27.3	to 30	26	15.6
Mg^{2+}	52.7	~0	~0	~0	~0	~0
K^+	9.8	20.4	21.9	—	18	23.2
$SiO_{2(aq)}$	0.16	6.9	8.2	—	22.0	17.6
Fe	0.0000015	24.1	2.5	—	1.64	1.66
Mn^{2+}	0	2.25	0.33	—	1.0	0.96
Cu^{2+}	0.000007	0.16	0.05	—	0.15	0.035
Zn^{2+}	0.00001	0.185	0.03	—	0.046	0.10

All concentrations in *m*mol kg^{-1}.

MAR, Mid-Atlantic Ridge; EPR, East Pacific Rise. $\Sigma CO_2 = CO_{2(aq)} + HCO_3^- + CO_3^{2-}$.

Data sources: Rainbow: Charlou *et al.* (2002); Lost City: Kelley *et al.* (2001, 2005); TAG: Campbell *et al.* (1988) and Charlou *et al.* (1996); OBS: Von Damm *et al.* (1985), Welhan and Craig (1983), and Von Damm (1990).

Shock, 2000; Allen and Seyfried, 2003). Of particular concern to microbiology, hydrothermal fluids discharging from serpentinites are highly enriched in H_2 and CH_4, both of which can serve as electron donors for microbial metabolism (Charlou *et al.*, 1998, 2002; Kelley *et al.*, 2001, 2005). Generation of H_2 during serpentinization results from the reaction of water with ferrous Fe-bearing minerals, primarily olivine and pyroxene. In the reaction, ferrous Fe [Fe(II)] is partially oxidized by the water to ferric Fe [Fe(III)], which typically precipitates as magnetite in serpentinites, while hydrogen from water is reduced to H_2. The process can be represented by the generalized reaction:

$$2(FeO)_{rock} + H_2O \rightarrow \underset{\text{Magnetite}}{Fe_3O_4} + H_2, \quad (1)$$

where $(FeO)_{rock}$ refers to the ferrous component of igneous silicate minerals. For instance, H_2 generation during serpentinization of olivine, the predominant mineral in most ultramafic rocks, can be expressed as:

$$\underset{\text{Olivine}}{Mg_{1.8}Fe_{0.2}SiO_4} + 1.37H_2O \rightarrow \underset{\text{Serpentine}}{0.5Mg_3Si_2O_5(OH)_4} + \underset{\text{Brucite}}{0.3Mg(OH)_2} + \underset{\text{Magnetite}}{0.067Fe_3O_4} + 0.067H_2 \quad (2)$$

where Fe(II) is supplied from the olivine, and magnetite and H_2 occur as reaction products.

A key property of ultramafic rocks that allows abundant H_2 production to occur during serpentinization is that their low silica activity results in the formation of alteration minerals (particularly serpentines and brucite) that have a tendency to exclude Fe(II) from their structure, which leads to the formation of magnetite (and, consequently, H_2). In rocks that have higher silica contents, such as basalt, a greater proportion of Fe(II) is sequestered in alteration minerals such as chlorite; these minerals more readily allow Fe(II) into their structure rather than its being converted to Fe(III). As a result, hydrothermal alteration of basalt generates much lower amounts of H_2 and iron oxides than serpentinization of ultramafic rocks, even though the Fe content of basalt is typically much higher. Thus, ultramafic rocks appear to be somewhat unique in their capacity to generate high H_2 abundances to drive microbial processes, and this capacity depends largely on the relative partitioning of Fe(II) into product minerals (*e.g.*, Bach *et al.*, 2004).

Because of the chemical reactions described above, serpentinization frequently results in very high concentrations of H_2 in hydrothermal fluids. For instance, H_2 concentrations of 12–16 *m*mol/kg have been reported for ultramafic-hosted deep-sea hydrothermal vent fluids (Table 1) (Charlou *et al.*, 2002; Kelley *et al.*, 2005). Even higher H_2 concentrations, which can exceed 100 *m*mol/kg in some circumstances, are indicated by the presence of native metal alloys in many serpentinites (Frost, 1985; Alt and Shanks, 1998) and have been produced in experimental studies of serpentinization (Berndt *et al.*, 1996; McCollom and Seewald, 2001). For comparison, interaction of deep-sea hydrothermal fluids with basalt typically results in H_2 concentrations that are in the 0.05–1.7 *m*mol/kg range (Table 1) (Von Damm, 1995).

Ultramafic-hosted hydrothermal fluids are also typically highly enriched in methane (Table 1). There are several possible sources for methane in hydrothermal vent fluids, including biological metabolism (*e.g.*, methanogenesis, fermentation), thermal degradation of complex organic matter from seawater or indigenous biomass, direct injection of magmatic gases, extraction of gases trapped within basalt or other rocks during fluid circulation, and abiotic organic synthesis (Welhan, 1988). Currently available isotopic and abundance data do not allow the relative contributions of these various potential sources to the methane found in deep-sea hydrothermal systems to be clearly differentiated (see recent review by McCollom and Seewald, 2007). However, given the high concentrations of H_2 that develop during serpentinization, it is likely that reduction of CO_2 is the dominant contributor to methane in these systems, which can be expressed by the reaction:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O. \quad (3)$$

This reaction may proceed biologically through the activity of methanogenic microbes in the lower temperature parts of hydrothermal circulation cells ($< \sim 120°C$), or it may proceed abiotically through catalyzed reactions during serpentinization (Charlou *et al.*, 1998, 2002; Horita and Berndt, 1999; McCollom and Seewald, 2001, 2007).

Hydrothermal fluids that circulate through basalt are highly enriched in H_2S (Table 1), and

oxidation of sulfur compounds is the predominant source of metabolic energy for chemolithoautotrophy in basalt-hosted deep-sea hydrothermal systems (Karl, 1995; McCollom and Shock, 1997; McCollom, 2000; Kelley *et al.*, 2002). Because ultramafic rocks have low sulfur contents, concentrations of H_2S in hydrothermal fluids that circulate through, and react with, ultramafic rocks are typically low relative to their basalt-hosted counterparts (Table 1). In addition, the ultramafic-hosted Rainbow hydrothermal system contains one of the highest concentrations of Fe observed in a deep-sea hydrothermal fluid (Table 1), which may be attributable to a low pH in the subsurface reaction zone (Allen and Seyfried, 2003). However, it is not yet clear whether these high metal contents are anomalous or are common in ultramafic-hosted systems.

The distinct differences in composition between hydrothermal fluids venting from ultramafic-hosted systems and those venting from basalt-hosted systems is likely to be reflected in substantial differences in the populations of chemolithoautotrophic microorganisms that inhabit these two environments. However, while there have been numerous published microbiological studies of basalt-hosted deep-sea hydrothermal environments over the last several decades, there have been only a few microbiological studies of ultramafic-hosted systems published to date (López-García *et al.*, 2003a, 2003b; Miroshnichenko *et al.*, 2003a, 2003b, 2004; Schrenk *et al.*, 2004; Kelley *et al.*, 2005; Nercessian *et al.*, 2005; Voordeckers *et al.*, 2005; Brazelton *et al.*, 2006). As a consequence, the characteristics of the microbial population in ultramafic-hosted systems are much less well known than those of basalt-hosted environments.

Because chemolithoautotrophic microorganisms are dependent on sources of chemical energy supplied by the environment for their metabolism, some insight can be gained into the likely metabolic diversity, spatial distribution, and relative abundances of chemolithoautotrophic microbial populations in hydrothermal systems by examining the availability of chemical energy sources (McCollom and Shock, 1997; McCollom, 2000; Tivey, 2004; Shock and Holland, 2004). Previous studies of the relationship between deep-sea microbial communities and the geochemical energy sources on which they depend have primarily focused on basalt-hosted hydrothermal environments.

Here, numerical models were employed to evaluate constraints on the amounts and types of chemical energy available within mixing environments in ultramafic-hosted deep-sea hydrothermal systems. The results were used to infer some characteristics of the microbial populations that live there and were compared with similar calculations performed for basalt-hosted systems.

CHEMICAL ENERGY SOURCES FOR MICROBIAL METABOLISM

For the most part, sources of metabolic energy for chemolithoautotrophy in deep-sea hydrothermal environments arise from chemical disequilibria that develop during the mixing of

Table 2. Metabolic Energy Sources for Chemolithoautotrophy Considered in this Study

Chemolithoautotrophic energy source	*Overall chemical reaction*	*Limiting reactant*†
Aerobic reactions		
Sulfide oxidation	$H_2S + 2O_2 = SO_4^{2-} + 2H^+$	$H_2S < 17°C$, $O_2 > 17°C$
Methanotrophy (CH_4 oxidation)	$CH_4 + 2O_2 = HCO_3^- + H^+ + H_2O$	$CH_4 < 9°C$, $O_2 > 9°C$
Iron (II) oxidation	$4Fe^{2+} + O_2 + 4H^+ = 4Fe^{3+} + 2H_2O$	$Fe^{2+} < 8°C$, $O_2 > 8°C$
Hydrogen oxidation	$H_2 + {}^1/_2O_2 = H_2O$	$H_2 < 6°C$, $O_2 > 6°C$
Anaerobic reactions		
Methanogenesis	$CO_2 + 4H_2 = CH_4 + 2H_2O$	H_2
Sulfate reduction	$SO_4^{2-} + 2H^+ + 4H_2 = H_2S + 4H_2O$	H_2
Nitrate reduction	$NO_3^- + 2H^+ + 4H_2 = NH_4^+ + 3H_2O$	$H_2 < 5°C$, $NO_3^- > 5°C$
Anaerobic methane oxidation	$SO_4^{2-} + 2H^+ + CH_4 = CO_2 + H_2S + 2H_2O$	CH_4
Anaerobic iron oxidation	$8Fe^{2+} + NO_3^- + 10H^+ = 8Fe^{3+} + NH_4^+ + 3H_2O$	$Fe^{2+} < 6°C$, $NO_3^- > 6°C$

†Limiting reactant based on mixing of seawater with Rainbow hydrothermal fluid. The limiting reactant is determined from the reactant with the lowest concentration in the mixed fluid as a function of temperature, taking into account reaction stoichiometry.

hydrothermal fluids with seawater (Karl, 1995; McCollom and Shock, 1997; McCollom, 2000). As a result of interaction with rocks in the subsurface, hydrothermal fluids are reducing and enriched in potential electron donors such as H_2, CH_4, H_2S, and Fe^{2+}. In contrast, seawater is oxidized and enriched in electron acceptors, particularly O_2, NO_3^-, and SO_4^{2-}. As the fluids mix, kinetic inhibitions for oxidation-reduction (redox) reactions involving C-, S-, Fe-, H-, O-, and N-bearing compounds result in chemical disequilibria among these electron donors and acceptors, which can then be exploited by microorganisms to gain metabolic energy (McCollom and Shock, 1997). The potential metabolic energy sources considered in this study are summarized in Table 2. Because these reactions encompass the most abundant electron donors and acceptors in hydrothermal mixing environments, they are likely to represent the predominant sources of metabolic energy for chemolithoautotrophy, though other minor elements capable of redox reactions, such as Mn and Cu, may also play a role.

Mixing of hydrothermal fluids with seawater can occur in a variety of environments within deep-sea hydrothermal systems, including within the walls of hydrothermal vent chimneys, in diffuse mixing zones within cracks and pore spaces that surround high-temperature conduits in the subsurface, and in hydrothermal plumes in the water column above the vents. Fluid mixing in these environments, as well as other factors such as conduction and diffusion, will create spatial gradients in fluid chemistry and temperature, which provide habitats for different types of microorganisms (Fig. 1) (Tivey, 1995, 2004; Karl, 1995; McCollom and Shock, 1997; Kelley *et al.*, 2002; Schrenk *et al.*, 2003; Huber *et al.*, 2003). In many instances, substantially different habitats may occur over very fine spatial scales (millimeters or less).

Because it is difficult to make accurate measurements at fine scales and without disturbing the environment, detailed measurements of chemical gradients within mixing environments in deep-sea hydrothermal systems are not yet available. In the absence of such data, numerical geochemical models can provide a framework for evaluating chemical environments during mixing of hydrothermal fluid with seawater (Tivey, 1995, 2004; McCollom and Shock, 1997; McCollom, 2000; Shock and Holland, 2004).

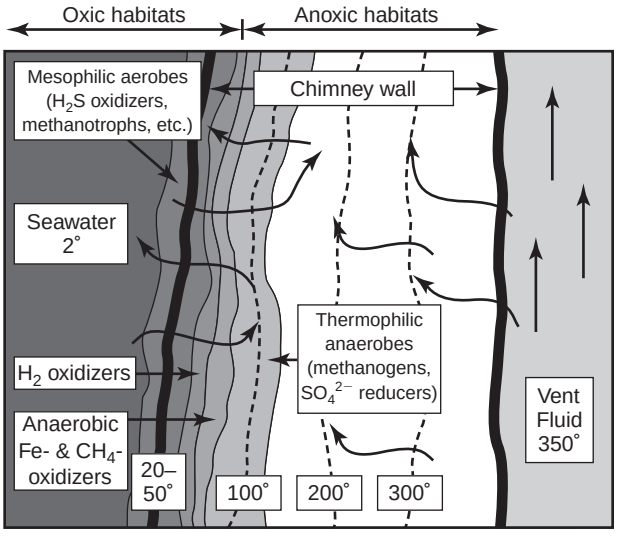

FIG. 1. Schematic cross-section of a deep-sea hydrothermal vent chimney showing spatial distributions of habitats for chemolithoautotrophic microbes based on gradients in fluid chemistry that develop from mixing of components from high-temperature hydrothermal fluid and seawater. The figure is based on hydrothermal fluid compositions for basalt-hosted systems (see McCollom and Shock, 1997).

MODEL METHODS

For this study, geochemical models were employed to examine chemical gradients that develop during mixing of seawater with hydrothermal fluids derived from interaction with ultramafic rocks. The model results were then used to investigate constraints on the distribution, metabolic diversity, and abundance of chemolithoautotrophic microbes in these environments. Basically, the procedure employed was to use numerical models to calculate the fluid composition during mixing and then to use this composition to determine the amount of energy available, in terms of Gibbs energy, from a variety of potential metabolic energy reactions as a function of temperature. The models closely follow methods used previously to examine relationships between geochemical gradients and microbial populations in basalt-hosted deep-sea hydrothermal environments (McCollom and Shock, 1997; McCollom, 2000; Tivey, 2004; Shock and Holland, 2004).

Gradients in fluid chemistry and temperature during mixing of hydrothermal fluids and seawater were investigated by way of geochemical reaction-path models. The models began with 1

kg of hydrothermal vent fluid; then successive increments of seawater were added until a seawater:hydrothermal fluid ratio (SW:HF) of 1000 was reached. The measured composition of hydrothermal fluid from the Rainbow field (Table 1) was used in the models to represent fluids in high-temperature ultramafic-hosted systems. In the models, it was presumed that all redox reactions were kinetically inhibited at the timescale of fluid mixing. All calculations were performed at 250 bars to simulate the elevated pressures at the seafloor and within the shallow subsurface. Because data for ammonium concentrations for Rainbow hydrothermal fluid and bottom seawater are unavailable, we assumed for the mixing calculations a concentration of 10 μmol NH_4^+ kg^{-1} for hydrothermal fluid [the maximum value reported for unsedimented basalt-hosted systems by Lilley *et al.* (1993)] and 50 nmol NH_4^+ kg^{-1} for seawater.

The numerical calculations were performed with the computer program EQ3/6, Version 7.2b (Wolery, 1992; Wolery and Daveler, 1992). The thermodynamic database required for EQ3/6 was generated by the SUPCRT92 computer program (Johnson *et al.*, 1992), with thermodynamic data for aqueous species and complexes from Shock and Helgeson (1988), Shock *et al.* (1989, 1997), Sverjensky *et al.* (1997), and McCollom and Shock (1997). It was assumed that the temperature of the mixed fluid is a linear function of the proportions of seawater and hydrothermal fluid.

The amounts of chemical energy potentially available for various chemolithoautotrophic metabolisms in the mixed fluid as a function of temperature were determined by calculating the Gibbs energy (ΔG) of each of the reactions shown in Table 2. Values for ΔG were calculated according to the familiar equation:

$$\Delta G = \Delta G^\circ + RT \ln Q \qquad (4)$$

where ΔG is the Gibbs energy of reaction, ΔG° the standard free energy, R the universal gas constant, T the temperature, and Q the activity quotient of the compounds involved in the reaction. The latter factor, Q, takes into account the contribution of the fluid composition to the Gibbs energy of each reaction, and values of Q for the metabolic reactions were determined based on the chemical composition of the mixed fluid produced by the reaction path calculations. Chemical reactions with a negative free energy (*i.e.*, exergonic reactions) release energy as they proceed and are potential sources of metabolic energy for chemolithoautotrophs. Values of the standard Gibbs energy (ΔG°) for the chemolithoautotrophic metabolic reactions were calculated by way of SUPCRT92 with data from the sources listed above.

MODEL RESULTS

Dissolved concentrations of elements relevant to microbial metabolism during mixing of Rainbow hydrothermal fluid with seawater are shown in Fig. 2 (though the mixing calculations begin with hydrothermal fluid at 365°C, this and subsequent figures are limited to 150°C and below in order to focus on the temperature range most relevant to microbial activities). Based on the calculated fluid compositions, the Gibbs energies of chemolithoautotrophic metabolic reactions during mixing were calculated and are shown in Fig. 3a. All of the reactions considered have negative values of ΔG, which indicates that any of the reactions could potentially be utilized as an energy source for microorganisms. It should be noted, however, that the calculations in this figure as-

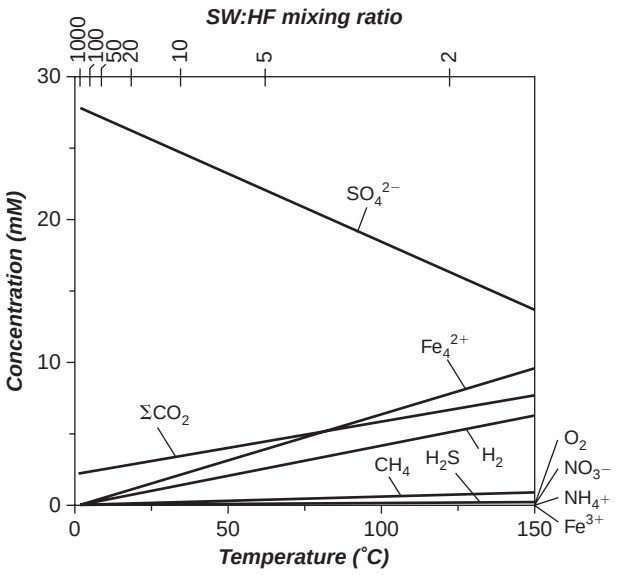

FIG. 2. Gradients in fluid chemistry during mixing of Rainbow hydrothermal vent fluid with seawater. Although the mixing models include all of the elements shown in Table 1, only those redox-labile elements that are potential chemical energy sources are shown. Note that concentrations of dissolved O_2, NO_3^-, NH_4^+, and Fe^{3+} are too low to be discernible on the scale of this diagram. $\Sigma CO_2 = CO_{2(aq)} + HCO_3^-$.

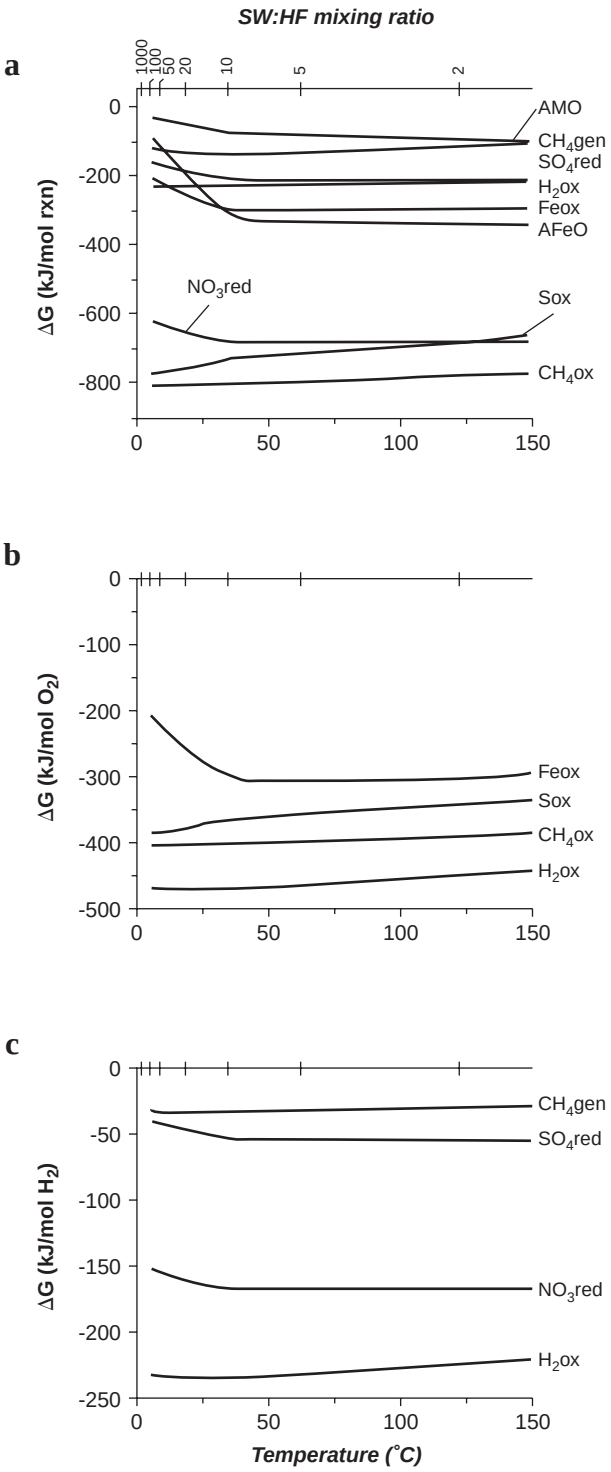

FIG. 3. Calculated Gibbs energies for chemolithoautotrophic metabolic reactions during mixing of Rainbow hydrothermal fluid with seawater. Figure (**a**) includes all reactions considered, while (**b**) compares O_2-consuming reactions and (**c**) compares H_2-consuming reactions. Abbreviations: Sox, sulfide oxidation; CH_4ox, methanotrophy; Feox, iron oxidation; H_2ox, hydrogen oxidation; CH_4gen, methanogenesis; SO_4red, sulfate reduction; NO_3red, nitrate reduction; AMO, anaerobic methane oxidation; AFeO, anaerobic iron oxidation.

sume that all the reactants are consumed only at each given temperature and that the reactants are consumed by only one reaction. Consequently, if electron donors are removed at high temperatures by other biological activities or by chemical processes, the amount of energy available at lower temperatures would be reduced.

Of course, there are other considerations beyond a favorable Gibbs energy that will determine whether a potential metabolic energy source will be utilized by a microorganism. Not all of the metabolic reactions shown in Fig. 3a could necessarily provide energy simultaneously in the same environment, since many of the reactions utilize the same electron donor or acceptor (*e.g.*, O_2 or H_2), and these reactants are present in limited supply. In these cases, organisms would be expected to compete for the limiting reactant. The limiting reactants during fluid mixing for the metabolic reactions under study are listed in Table 2. The limiting reactant for each potential metabolic energy source was assessed by comparing the concentrations of the reactants involved in the reaction as a function of temperature and determining which reactant was present at the lowest concentration for a particular temperature, taking into account the stoichiometry of the reaction. For this determination, it was assumed that H^+ was not limiting for any reaction since additional H^+ is available from sources such as:

$$CO_{2(aq)} + H_2O \rightarrow HCO_3^- + H^+. \quad (5)$$

At temperatures above ~20°C (SW:HF ratios < ~20), O_2 is the limiting reactant for all aerobic reactions (Table 1), and microorganisms will compete for available O_2. Some insight into the likely outcome of this competition can be gained by comparing the Gibbs energies of the aerobic reactions based on the amount of energy available per molecule of O_2, as shown in Fig. 3b. As can be seen in this figure, hydrogen oxidation provides the greatest amount of energy per O_2 molecule, followed by methanotrophy. This result suggests that hydrogen oxidizers should be able to outcompete other aerobes for available O_2 and are likely to predominate over other aerobes in higher-temperature environments if O_2 is present. A similar situation has been observed in terrestrial hydrothermal environments, where hydrogen oxidizers have been found to dominate the microbial populations of hot spring pools

near their source in Yellowstone National Park despite the presence of high concentrations of other electron donors, including H_2S and CH_4 (Spear *et al.*, 2005). Although O_2 is the limiting reactant for aerobic metabolisms at low SW:HF mixing ratios, as seawater continues to mix with hydrothermal fluids there will eventually be enough O_2 available to satisfy all electron donors, and these will become the limiting reactants for aerobic metabolic energy sources (Table 2).

Hydrogen oxidizers must also compete with other organisms, including methanogens, sulfate reducers, and nitrate reducers, for available H_2. Per mole of H_2, hydrogen oxidation would yield more energy than any of these other metabolisms, followed by nitrate reduction, sulfate reduction, and methanogenesis in decreasing order of energy yields (Fig. 3c). This comparison would suggest that hydrogen oxidizers should be able to outcompete these other organisms for H_2 if O_2 is present. In high-temperature mixing environments, however, there is far too little O_2 available to utilize all of the H_2 provided by the hydrothermal fluid (Fig. 2), so even if hydrogen oxidizers are present, they are likely to constitute only a minor fraction of the H_2-consuming population. Nitrate and sulfate reducers should also be able to outcompete methanogens for H_2 and should represent the most abundant chemolithoautotrophs at high temperatures, provided that nitrate and sulfate are not removed during mixing by mineral precipitation or biological processes at lower temperatures. If nitrate and sulfate are depleted, methanogens should be the predominant chemolithoautotrophs at high temperatures.

To obtain the results shown in Fig. 3, it was assumed that oxidized seawater is available to mix with hydrothermal fluids at all temperatures. This is probably most relevant to situations where high-temperature fluids discharge directly into seawater and mix rapidly. In environments such as the walls of hydrothermal chimneys or in the subsurface where mixing takes place in confined spaces such as in rock fractures, the O_2 in seawater may be somewhat depleted by microbial processes or redox reactions prior to mixing with hydrothermal fluids. In such cases, chemical energy from hydrogen oxidation and other O_2-requiring reactions may be restricted to lower temperature environments than is reflected in the calculations shown in Fig. 3.

Conversely, much of the H_2 present in hydrothermal fluids may be consumed by microbial processes at high temperatures before it reaches lower-temperature environments. Although energy would be available from H_2-utilizing reactions at low temperatures, much of the H_2 may be utilized at higher temperatures so that these metabolisms will be primarily restricted to high-temperature environments, as they appear to be in basalt-hosted systems. Nevertheless, the high H_2 concentrations that exist in ultramafic-hosted systems present the possibility that nominally anaerobic metabolisms (methanogenesis, sulfate reduction) might persist to mesophilic and even psychrophilic temperatures where O_2 may be present.

The presence of high levels of methane in ultramafic-hosted hydrothermal fluids and the limited availability of O_2 at high temperatures suggest the possibility that anaerobic methane oxidation (AMO) could play a significant role in the consumption of methane in high-temperature mixing environments. Although methanotrophy with O_2 yields much more energy than AMO (Fig. 3a), the low abundance of O_2 means that only a small fraction of the methane could be consumed by methanotrophy at high temperatures. Methanotrophs are also likely to be outcompeted by hydrogen oxidizers for available O_2 in high-temperature environments. Because the concentration of sulfate is about 300 times greater than that of O_2 in seawater and is less likely to be consumed, it is more likely to be available in high-temperature environments with low mixing ratios. Furthermore, owing to the high levels of sulfate in seawater, even if only a fraction of the sulfate is available, electron donors (H_2 and CH_4) will be the limiting reactants for sulfate reduction and AMO, so that these metabolisms would not have to compete for sulfate. As a result, AMO could proceed in the same environments where methanogenesis and reduction of sulfate with H_2 is occurring.

Nitrate represents another possible electron acceptor for anaerobic methane or H_2 oxidation, but the low abundance of nitrate relative to sulfate in seawater suggests it is likely to be a less significant process. Methane could also be oxidized by ferric iron (Fe^{3+}). Although concentrations of dissolved Fe^{3+} are extremely low in seawater and hydrothermal fluids, ferric iron [Fe(III)] generated by iron-oxidizing microbes and present as either dissolved species or precipitated as iron oxide/hydroxide minerals could provide a source of electron acceptors for methane oxidation (or oxidation

of other organic compounds). However, because the abundance of oxidized iron compounds within mixing zones is not well constrained, it is difficult to place quantitative constraints on the potential contribution of this process.

Based on the Gibbs energies and calculated composition of the fluid during mixing, the total amount of metabolic energy potentially available from the chemolithoautotrophic reactions can be estimated as shown in Fig. 4. Values shown in this figure are calculated by multiplying the ΔG for each reaction by the concentration of the limiting reactant at each temperature, after accounting for reaction stoichiometry, and then multiplying by the total amount of mixed fluid. Consequently, the values represent the total amount of energy available from a particular reaction if all of the reactants in the mixed fluid were consumed at a given temperature, expressed in terms of the energy available per kilogram of hydrothermal fluid.

At SW:HF mixing ratios less than about 10 (temperature $>$ ~35°C), conditions within the mixed fluid are dominated by the anoxic conditions of the hydrothermal fluids, and anaerobic reactions are the major source of chemical energy (sulfate reduction, methanogenesis, and anaerobic methane or iron oxidation). Since sulfate reduction and methanogenesis compete for limited H_2, the total energy available from these reactions cannot be used simultaneously but will instead be limited by competition for H_2. On the other hand, anaerobic methane oxidation does not compete with these reactions, so it can proceed in parallel with H_2-consuming metabolisms. The calculations shown in Fig. 4 indicate that a total of about 1 kJ per kilogram of hydrothermal fluid is available from all anaerobic reactions combined.

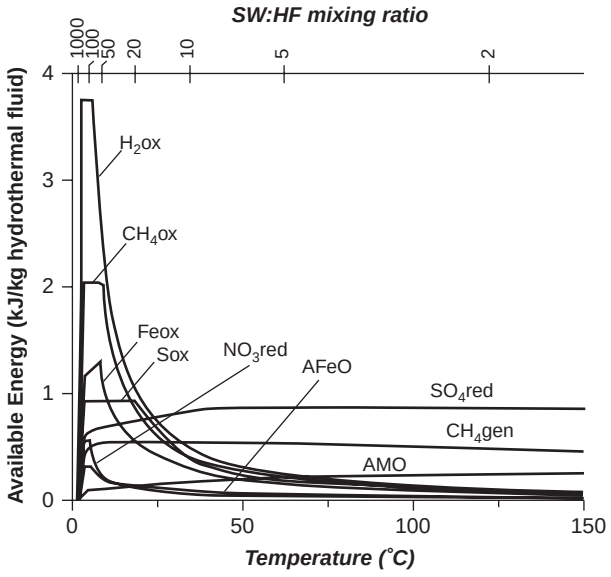

FIG. 4. Total energy available from chemolithoautotrophic reactions as a function of temperature. Values represent the energy available if all of the reactants were consumed at a particular temperature, taking account of the limiting reactant for the reaction at that temperature. Abbreviations as in Fig. 3.

At SW:HF mixing ratios above about 20 ($<$ ~20°C), aerobic reactions begin to dominate the available chemical energy. Until the SW:HF mixing ratio reaches ~100, each of the aerobic reactions is limited by the availability of O_2. Under these conditions, chemolithoautotrophs that utilize different electron donors must compete for O_2, and the total amount of energy available will be restricted by competition for O_2. As mixing continues, the amount of O_2 available in the mixed fluid will eventually become sufficient to satisfy all electron donors so that energy from all of the aerobic reactions will be available concurrently. However, because of the high SW:HF mixing ratios required, these conditions might not be attained until the mixed fluids are vented into the overlying water column, where dilution and dispersion may make complete biological utilization of the electron donors problematic. For intermediate mixing ratios of 20–100, the calculations shown in Fig. 4 indicate that a total of about 2–3 kJ of energy are available per kg of hydrothermal fluid from aerobic reactions, while more than 7 kJ of energy are available per kg if all of the electron donors are consumed at high mixing ratios.

The calculations shown in Fig. 4 also give some indication of the relative magnitudes of the contribution of the various chemolithoautotrophic reactions to the overall chemical energy available. Hydrogen oxidation potentially provides the greatest amount of chemical energy, with methanotrophy capable of providing a little over half as much energy as hydrogen oxidation. Iron and sulfide oxidation can provide sub-equal amounts of energy, and each is capable of providing about one quarter of that available from hydrogen oxidation.

The available chemical energy can be used to make rough estimates of the amount of biomass that could potentially be produced by chemolithoautotrophy in these environments. Analyses of laboratory growth yields indicate that organisms that utilize H_2 as electron donor (methanogens,

sulfate reducers, and H_2-oxidizers) require 38 kJ to produce each gram of biomass (dry weight), while aerobes and other organisms that must employ reverse electron flow to fix carbon require about 152 kJ per gram of biomass produced (Heijnen and van Dijken, 1992). Based on these values and the available chemical energy, the amount of biomass that could potentially be produced from the chemolithoautotrophic reactions considered here were calculated as summarized in Table 3. Calculations of potential biomass shown in this table are based on the assumption that each reaction proceeds only at the temperature of maximum energy yield and that the reactions are completely efficient in consuming the reactants and converting them to biomass. Thus, the values represent the maximum possible biomass yields and would have to be adjusted downward for incomplete consumption of the energy sources or lower efficiency in converting energy to biomass. However, if these factors are similar for organisms using different metabolisms, the relative amounts of biomass production for the various energy-sources should be similar to those shown in the table even if the absolute amounts are not representative. The potential biomass yields shown in Table 3 indicate that up to ~120 mg of biomass could be produced from utilization of the electron donors in each kg of Rainbow hydrothermal fluid by aerobic chemolithoautotrophs, with hydrogen oxidation providing the bulk of the biomass potential. When competition for H_2 is taken into account, up to ~20–30 mg of biomass per kg hydrothermal fluid could be produced by anaerobic chemolithoautotrophs.

Even if the efficiency of microbial utilization of these energy sources for biomass production is relatively low, potential yields for chemolithoautotrophic biomass production in these systems is substantial because of the large fluxes of hydrothermal fluid. For instance, if we assume a 10% efficiency for the maximum biomass yields and a typical fluid flux for a hydrothermal vent of 5×10^5 kg/hr, chemolithoautotrophs could potentially produce on the order of ~8 kg of biomass every hour from a single hydrothermal vent. Clearly, there is sufficient chemical energy available in mixing environments to support the prolific biological communities found in these environments.

DISCUSSION

The model calculations suggest some significant differences between the microbial popula-

Table 3. Geochemical Energy Sources and Potential Biomass Yields Resulting from Mixing of Seawater with Hydrothermal Fluids through the Walls of Vent Chimneys and Flanges

	Ultramafic-hosted system (Rainbow)		*Basalt-hosted system (21°N EPR)*[a]	
Chemolithoautotrophic energy source	*Maximum available energy*[b]	*Biomass potential*[c]	*Maximum available energy*[b]	*Biomass potential*[c]
Hydrogen oxidation	3.7	97[d]	0.39	11[d]
Methanotrophy	2.1	14	0.05	0.3
Iron (II) oxidation	1.3	9	0.05	0.3
Sulfide oxidation	0.9	6	3.2	21
Methanogenesis	0.5	13[d]	0.03	0.7[d]
Sulfate reduction	0.9	24[d]	0.04	1.1[d]
Nitrate reduction	0.6	16[d]	—	—
Anaerobic methane oxidation	0.3	8	0.003	0.4
Anaerobic iron oxidation	0.3	2	—	—

[a]Data from McCollom and Shock (1997). Values for nitrate reduction and anaerobic iron oxidation were not reported.

[b]Maximum chemical energy available in kJ per kg hydrothermal fluid.

[c]Biomass potential in mg dry wt. of biomass per kg hydrothermal fluid. Calculations assume that hydrogen oxidation, methanogenesis, sulfate reduction, nitrate reduction, and anaerobic methane oxidation require 38 kJ per g dry wt. biomass and all other reactions require 152 kJ per g dry wt. biomass (Heijnen and van Dijken, 1992).

[d]Hydrogen oxidation, methanogenesis, and sulfate reduction, and nitrate reduction are all limited by the availability of H_2, so the total biomass potential from these 3 sources will be limited by competition for available H_2.

EPR, East Pacific Rise.

tions of ultramafic-hosted deep-sea hydrothermal systems and those of basalt-hosted systems, as well as some similarities. As in basalt-hosted systems (McCollom and Shock, 1997), there is likely to be a sharp differentiation in ultramafic-hosted mixing environments between anoxic conditions dominated by anaerobic organisms at higher temperatures and oxic conditions dominated by aerobic organisms at low temperatures. For the modeled conditions, this transition occurs at 20–30°C, but the temperature of the boundary between aerobic- and anaerobic-dominated environments will be sensitive to factors such as hydrothermal fluid composition, chemical diffusion, and thermal conduction (Tivey, 2004).

The constraints imposed by fluid compositions indicate that high-temperature mixing environments in ultramafic-hosted systems (such as within chimney walls or deep within diffuse subsurface mixing zones) will be dominated by thermophilic anaerobes, including sulfate reducers and methanogens, nitrate reducers, and possibly anaerobic methane and iron oxidizers. If O_2 is not consumed before reaching high-temperature environments, moderately thermophilic hydrogen oxidizers may also be present but should be less abundant than the anaerobes. Microbial populations in low-temperature environments should be predominantly composed of mesophilic and psychrophilic aerobes, including organisms capable of oxidizing H_2, CH_4, H_2S, and Fe^{2+}.

Overall, the total amount of energy available and maximum potential biomass yields are estimated to be substantially larger in ultramafic-hosted systems than in basalt-hosted systems, by a factor of 2 or more (Table 3). In addition, the distribution of energy sources among different metabolic energy sources is substantially different in the two environments. While sulfide oxidation is the predominant source of metabolic energy available in low-temperature habitats in basalt-hosted systems, hydrogen oxidation and methanotrophy are the most abundant sources of chemical energy in the ultramafic-hosted system, with only a modest contribution from sulfide oxidation. The amount of energy available from hydrogen oxidation in ultramafic-hosted systems is roughly equivalent to that supplied by sulfide oxidation in basalt-hosted systems. However, if analyses of laboratory growth yields that indicate that hydrogen oxidizers may be more efficient than sulfide oxidizers in converting energy to biomass are correct (Heijnen and van Dijken, 1992), the potential biomass yields for hydrogen oxidation could be substantially greater in ultramafic-hosted environments even though the energy available in both types of ecosystems is about the same. The high concentrations of CH_4 in ultramafic-hosted hydrothermal fluids indicate that methanotrophs will represent a larger fraction of the microbial population, with potential biomass yields only slightly lower than those of sulfide oxidizers in basaltic systems. Iron oxidation may also make a significant contribution, at least for the Fe-rich Rainbow hydrothermal system.

Owing to the high H_2 and CH_4 concentrations in ultramafic-hosted hydrothermal fluids, there are substantially greater amounts of energy available for anaerobic metabolism in high-temperature environments than in basalt-hosted systems. For the conditions considered here, the amount of energy available and potential biomass yields are about tenfold higher in the ultramafic-hosted system (Table 3). This result indicates that populations of thermophilic chemolithoautotrophs in anoxic environments in ultramafic systems, such as within the walls of hydrothermal chimneys and in diffuse subsurface mixing zones, may be an order of magnitude larger than populations in analogous environments in basalt-hosted systems (*e.g.*, Huber *et al.*, 2003; Schrenk *et al.*, 2003).

It should be noted, however, that if anaerobic sulfate reducers and methanogens are efficient in consuming H_2 from hydrothermal fluids, this will alter the availability of energy sources for aerobic organisms at lower temperatures and may reduce the overall amount of biomass produced by chemolithoautotrophy. Any H_2 consumed by the anaerobes at high temperatures will reduce the amount available to hydrogen oxidizers at lower temperatures and, since the energy yield from hydrogen oxidation is substantially higher than from other H_2-consuming reactions (Fig. 3b), will reduce the overall amount of energy available. At the same time, however, the products of anaerobic metabolisms (CH_4 and/or H_2S) may enhance the energy available to methanotrophs and sulfide oxidizers. Consequently, if H_2 is consumed efficiently by sulfate reducers and methanogens at high temperatures (low SW:HF mixing ratios), methanotrophy and sulfide oxidation may displace hydrogen oxidation as the most abundant source of metabolic energy and biomass potential at lower temperatures.

The recent discovery of warm hydrothermal fluids (up to 90°C) discharging from serpentinites

in an off-axis setting at Lost City has indicated that ultramafic-hosted environments are not limited to high-temperature vents along the mid-ocean ridge axis but may instead be more diverse and widespread on the seafloor (Kelley *et al.*, 2001, 2005; Früh-Green *et al.*, 2004). Measured concentrations of H_2 and CH_4 in fluids at Lost City are comparable to those of higher-temperature axial ultramafic-hosted fluids (Table 1), which indicates that the availability of chemical energy sources and potential biomass yields at Lost City will be similar to those indicated by the calculations shown here. However, because the temperature of the hydrothermal fluids is lower (~90°C versus >350°C for axial systems), the temperatures of metabolic reactions will be shifted downward accordingly. In particular, at Lost City, anaerobic-dominated environments may persist to lower-temperature, possibly mesophilic, conditions.

Another important feature of the Lost City hydrothermal fluids that distinguishes them from fluids in axial systems is their strongly alkaline pH (measured pH values = 9 to ~11). The high pH will affect the speciation of the fluid and may impact the energetics of metabolic energy sources to a significant degree. Perhaps more critical, however, is the impact of the high pH of the fluid on dissolved inorganic carbon sources. At the strongly alkaline pH of the Lost City fluids, inorganic carbon is present primarily as carbonate (CO_3^{2-}), and precipitation of carbonate minerals reduces its abundance to very low levels (Kelley *et al.*, 2005), which presents a challenge to autotrophic microorganisms that require uptake of inorganic carbon for their metabolism. In addition, low concentrations of carbonate would reduce the metabolic energy yield from methanogenesis and also limit the extent to which methanogenesis may proceed. Organisms living within these strongly alkaline environments might also face increased energetic costs for growth at high pH.

In addition to seafloor systems, there has also been increasing interest in recent years in the chemistry and microbiology of alkaline springs that discharge from serpentinites in terrestrial settings, such as those at The Cedars in California (Morrill *et al.*, 2006; Johnson *et al.*, 2006). Although alkaline spring waters usually discharge at low temperatures (<40°C), their chemistry indicates extensive reaction with ultramafic rocks in the subsurface (*e.g.*, Barnes *et al.*, 1967; Barnes and O'Neil, 1969). The basic chemical reactions that produce abundant electron donors during serpentinization (primarily H_2 and CH_4; Reactions 2 and 3) are the same in both terrestrial and seafloor settings, so springs discharging from serpentinites on land often show enrichments in these same compounds (*e.g.*, Neal and Stanger, 1983; Abrajano *et al.*, 1988, 1990). Like the moderate-temperature system at Lost City, spring waters that discharge from serpentinites on land frequently have very high pH (10 or higher) as a result of serpentinization reactions (*e.g.*, Barnes and O'Neil, 1969), and the high pH severely limits the abundance of dissolved inorganic carbon (DIC; predominantly CO_3^{2-}), owing to precipitation of carbonates within the serpentinites. The methane in these systems might arise from abiotic synthesis (Abrajano *et al.*, 1988, 1990) or microbial methanogenesis in the subsurface, but the progress of these processes may be limited in extent by the low levels of DIC in the high pH groundwater.

As in seafloor systems, the electron donors in alkaline springs represent a potential source of metabolic energy for chemolithoautotrophic organisms such as hydrogen oxidizers, methanotrophs, and methanogens. In this case, however, the primary source of electron acceptors will be dissolution of gases from the atmosphere into the spring waters rather than addition of dissolved volatiles owing to mixing with oxidized seawater. In hot spring pools at Yellowstone, competition for O_2 dissolving into the hydrothermal fluids appears to be a primary control on the composition of the microbial population (Spear *et al.*, 2005), and rates of diffusion of O_2 and CO_2 into spring waters may similarly be an important constraint on the utilization of H_2 and CH_4 in terrestrial alkaline springs. The limited solubility of H_2 and CH_4 at low temperatures and pressures also suggests that a significant fraction of these electron donors may escape into the atmosphere before they can be utilized by microorganisms. Nevertheless, the energy supplied by exposure of ultramafic-hosted spring waters to the oxidized atmosphere is likely to support significant chemolithoautotrophic populations, and further study of these environments will provide additional insight into the diversity of microbial habitats supported by serpentinization of ultramafic rocks.

At present, there have been very few studies published on the microbiology of ultramafic-

hosted deep-sea hydrothermal systems with which to compare the model results. However, several species capable of chemolithoautotrophic growth have been isolated from chimney sulfide samples taken from the Rainbow and Logatchev vent fields, including the species *Deferribacter abyssi* (Miroshnichenko *et al.*, 2003b), *Caldithrix abyssi* (Miroshnichenko *et al.*, 2003a), *Caminibacter profundus* (Miroshnichenko *et al.*, 2004), and *Caminibacter mediatlanticus* (Voordeckers *et al.*, 2005). These organisms grow in culture chemolithoautotrophically using H_2 as electron donor and nitrate, sulfur, and, in some cases, Fe(III) as electron acceptor. *Caminibacter profundus* was also able to grow with O_2 as an electron acceptor under microaerobic conditions (Miroshnichenko *et al.*, 2004), but *C. mediatlanticus* did not grow with O_2 (Voordeckers *et al.*, 2005), which suggests that this organism may have been involved in anaerobic hydrogen oxidation (*i.e.*, nitrate or sulfur reduction). The ability of *D. abyssi* and *C. abyssi* to utilize O_2 as electron acceptor has yet to be reported.

Thus far, the sort of culture-independent molecular phylogenetic studies that have been employed to survey the microbial populations of fluid-mixing environments in chimneys and diffuse subsurface mixing zones at other deep-sea hydrothermal systems (*e.g.*, Takai *et al.*, 2001; Schrenk *et al.*, 2003; Huber *et al.*, 2003) have not yet been published for Rainbow or Logatchev. [Molecular phylogenetic analyses have been performed on inactive metalliferous sediments from the Rainbow vent field, but this environment is not relevant to the fluid-mixing calculations shown here (López-García *et al.*, 2003b; Nercessian *et al.*, 2005).] Consequently, it remains unclear whether the cultured species represent a major component of the microbial communities or what other metabolic capabilities (methanogenesis, sulfate reduction, AMO, etc.) might be present in the population. However, the model results suggest that oxidation of H_2 with O_2 and nitrate represents two of the largest sources of metabolic energy in mixing environments, which suggests that the cultured species represent the sort of organisms that should make up a large fraction of the microbial community.

López-García *et al.* (2003a) used molecular methods to examine the bacterial diversity of organisms colonizing three artificial substrates placed in the effluent of a hydrothermal vent at the Rainbow field and found that each of the substrates was colonized predominantly by members of the ε-proteobacteria. Because close relatives to the clones found in their studies are sulfide oxidizers, López-García *et al.* (2003a) inferred that the organisms colonizing their samplers may have been involved in sulfide oxidation. Since hydrogen oxidation and methane oxidation would appear to be capable of supplying significantly more energy than sulfide oxidation in this environment (Fig. 4), it is not clear why sulfide oxidizers should strongly dominate the colonizers. It is uncertain, however, whether the organisms colonizing the artificial substrates are really engaged in sulfide oxidation or whether the communities colonizing these substrates are representative of those that inhabit the natural system.

To date, there have been no reports of organisms cultured from the Lost City field, but the composition of the microbial community has been investigated by way of culture-independent molecular methods. The interiors of active chimneys at Lost City have been observed to be covered with biofilms several tens of microns thick, and 16S rRNA-based phylogenetic analyses of the chimney interiors indicate that the microbial community is dominated by a single archaeal phylotype from the order *Methosarcinales* (Schrenk *et al.*, 2004; Kelley *et al.*, 2005; Brazelton *et al.*, 2006). Also identified by molecular methods in chimney samples was methyl coenzyme M reductase (*mcrA*), which is involved in methane metabolism by archaea (Kelley *et al.*, 2005). Although inferences about the metabolic activities of these uncultured organisms based on related cultured representatives must be made with caution, the predominant phylotype at Lost City is most closely related to a group of organisms involved in anaerobic methane oxidation (AMO). The archaeal community of the cooler, less active parts of the chimneys was dominated by a different phylotype closely related to ANME-1, another group of organisms associated with AMO in a variety of marine environments (Kelley *et al.*, 2005; Brazelton *et al.*, 2006).

The prevalence of organisms with an affinity to known anaerobic methane oxidizers suggests the possibility that similar processes provide the primary support for microbial communities within the chimneys of Lost City. However, since other close relatives to the Lost City clones are also involved in autotrophic methanogenesis, it remains unclear whether the organisms living in the chimneys are involved in methane consump-

tion or methane synthesis (Schrenk *et al.*, 2004; Brazelton *et al.*, 2006). Also, in other marine environments where anaerobic methane oxidation is occurring, the archaeal organism involved occurs in a symbiotic relationship with a eubacterial sulfate reducer, but no evidence of a sulfate-reducing symbiont was found in the Lost City samples (Brazelton *et al.*, 2006). Since both AMO and methanogenesis would appear to be energetically favorable during mixing of hydrothermal fluid with seawater within the chimney walls, either process could be plausibly reconciled with the occurrence of microorganisms within the active chimneys.

The eubacterial community of active Lost City chimneys exhibits a much greater phylogenetic diversity, including organisms closely related to known methylotrophic members of the γ-proteobacteria and to *Thiomicrospiria*, a group of sulfide-oxidizing organisms found in many basalt-hosted hydrothermal vent environments (*e.g.*, Jannash *et al.*, 1985; Wirsen *et al.*, 1998), as well as organisms with no clear metabolic affiliation (Brazelton *et al.*, 2006). Curiously, no clones closely related to known hydrogen oxidizing microorganisms such as the *Aquificales* were identified in active chimney samples (Brazelton *et al.*, 2006). These types of organisms have been found in other hydrogen-rich hydrothermal environments (*e.g.*, Gotz *et al.*, 2002; Takai *et al.*, 2004; Spear *et al.*, 2005), including Rainbow (*e.g.*, Miroshnichenko *et al.*, 2004; Voordeckers *et al.*, 2005), and would be expected to occur at the Lost City field, given the high abundance of H_2 in the hydrothermal fluids and the large amount of energy that would be available from H_2 oxidation relative to other energy sources. It is not immediately apparent why hydrogen-oxidizing organisms were not found in the samples; it may be that some unrecognized factor prevents the utilization of H_2 in this system or, perhaps more likely, organisms capable of hydrogen oxidation may be present but were not recognized among the species identified by molecular methods.

One particularly notable aspect of the Lost City studies is the extremely low diversity of the microbial community in the interiors of the active chimneys (Schrenk *et al.*, 2004; Kelley *et al.*, 2005; Brazelton *et al.*, 2006). Natural environments that exhibit such low diversity are very rare, but one other environment with similarly low diversity is the strongly acidic habitat associated with acid mine drainage (Edwards *et al.*, 2000; Baker and Banfield, 2003). The low diversity found at these opposite ends of the pH spectrum may reflect the difficulty of adaptation to extremes of pH. Alternatively, the low diversity may be attributable to limitations on the diversity of metabolic energy sources in these oligotrophic environments. The model results (Figs. 3 and 4) suggest that the internal spaces of chimneys in ultramafic-hosted systems, where mixing of oxidized seawater with hydrothermal fluids is limited, should be dominated by only 1 or 2 sources of chemolithoautotrophic energy.

Given the current limitations on knowledge of the microbial population of ultramafic-hosted deep-sea hydrothermal systems, it is difficult at present to make detailed comparisons between the predictions about the structure of the microbial community from the mixing models and the structure observed in the natural system. Nevertheless, the models demonstrate that it is possible to make quantitative predictions about the abundance and distribution of metabolic energy sources; these predictions can be useful in interpreting the structure of the microbial community. As more information on the chemical and microbial composition of these interesting systems becomes available, refinements of the models should prove useful in understanding the factors that control the abundance, activity, and spatial distribution of the microbial population.

CONCLUDING REMARKS

Geochemical models can represent a framework with which to understand the abundance, spatial distribution, and metabolic diversity of the microbial populations in deep-sea hydrothermal systems. The models presented here are only the initial attempt at developing a comprehensive biogeochemical model of these environments and leave considerable room for improvement. Nevertheless, these simple models appear to provide a good first-order overview of the principal processes that control the spatial distribution of chemolithoautotrophic microbial habitats and should be useful in guiding future sampling efforts as well as in interpreting species diversity and population distributions in mixing environments. These models could be substantially improved by a better knowledge of the flow pathways of fluids and mixing processes that occur in hydrothermal chimneys and subsurface environ-

ments surrounding hydrothermal upflow zones. Also helpful would be measurements of chemical gradients in these environments; however, attainment of such measurements may be difficult or impossible to achieve in the confined spaces where such mixing occurs, without disturbing the gradient being measured. Additionally, future models that take into account changes in fluid composition due to microbial metabolic processes may produce more realistic chemical gradients, since organisms likely have a substantial impact on redox reactions that occur in hydrothermal environments.

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ABBREVIATIONS

AMO, anaerobic methane oxidation; redox, oxidation-reduction; SW:HF, seawater:hydrothermal fluid ratio.

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