

Energetics of chemolithoautotrophy in the hydrothermal system of Vulcano Island, southern Italy

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ABSTRACT

The hydrothermal system at Vulcano, Aeolian Islands (Italy), is home to a wide variety of thermophilic, chemolithoautotrophic archaea and bacteria. As observed in laboratory growth studies, these organisms may use an array of terminal electron acceptors (TEAs), including O₂, NO₃⁻, Fe(III), SO₄²⁻, elemental sulphur and CO₂; electron donors include H₂, NH₄⁺, Fe²⁺, H₂S and CH₄. Concentrations of inorganic aqueous species and gases were measured in 10 hydrothermal fluids from seeps, wells and vents on Vulcano. These data were combined with standard Gibbs free energies (ΔG_r°) to calculate overall Gibbs free energies (ΔG_r) of 90 redox reactions that involve 16 inorganic N-, S-, C-, Fe-, H- and O-bearing compounds. It is shown that oxidation reactions with O₂ as the TEA release significantly more energy (normalized per electron transferred) than most anaerobic oxidation reactions, but the energy yield is comparable or even higher for several reactions in which NO₃⁻, NO₂⁻ or Fe(III) serves as the TEA. For example, the oxidation of CH₄ to CO₂ coupled to the reduction of Fe(III) in magnetite to Fe²⁺ releases between 94 and 123 kJ/mol e⁻, depending on the site. By comparison, the aerobic oxidation of H₂ or reduced inorganic N-, S-, C- and Fe-bearing compounds generally yields between 70 and 100 kJ/mol e⁻. It is further shown that the energy yield from the reduction of elemental sulphur to H₂S is relatively low (8–19 kJ/mol e⁻) despite being a very common metabolism among thermophiles. In addition, for many of the 90 reactions evaluated at each of the 10 sites, values of ΔG_r tend to cluster with differences < 20 kJ/mol e⁻. However, large differences in ΔG_r (up to ~ 60 kJ/mol e⁻) are observed in Fe redox reactions, due largely to considerable variations in Fe²⁺, H⁺ and H₂ concentrations. In fact, at the sites investigated, most variations in ΔG_r arise from differences in composition and not in temperature.

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INTRODUCTION

Most known moderate thermophiles and all known hyperthermophiles^a are chemotrophs, gaining metabolic energy by catalysing thermodynamically favoured, but kinetically inhibited redox reactions. In recent years, geochemical models have been used to quantify the amount of metabolic energy

available from the oxidation and reduction of C-, S-, N-, Fe-, H- and O-bearing compounds in deep-sea hydrothermal systems on Earth and in their putative counterparts on the Jovian satellite Europa (McCollom & Shock, 1997; McCollom, 1999, 2000; Zolotov & Shock, 2003). *In situ* analyses of redox-sensitive compounds together with sampling and subsequent laboratory analyses of thermal fluids in Yellowstone National Park (USA) over the past ~ 4 years enabled analogous metabolic energy calculations to be made in a variety of acidic, circumneutral and slightly alkaline continental hot springs (Shock *et al.*, in press). Thermodynamic modelling showed that the autotrophic synthesis of all 20

^aThroughout the text, we use the terms ‘moderate thermophile’ and ‘hyperthermophile’ to denote organisms that grow optimally at 45–80 °C and ≥ 80 °C, respectively. The term ‘thermophile’ is used generically to refer to all high-temperature bacteria and archaea.

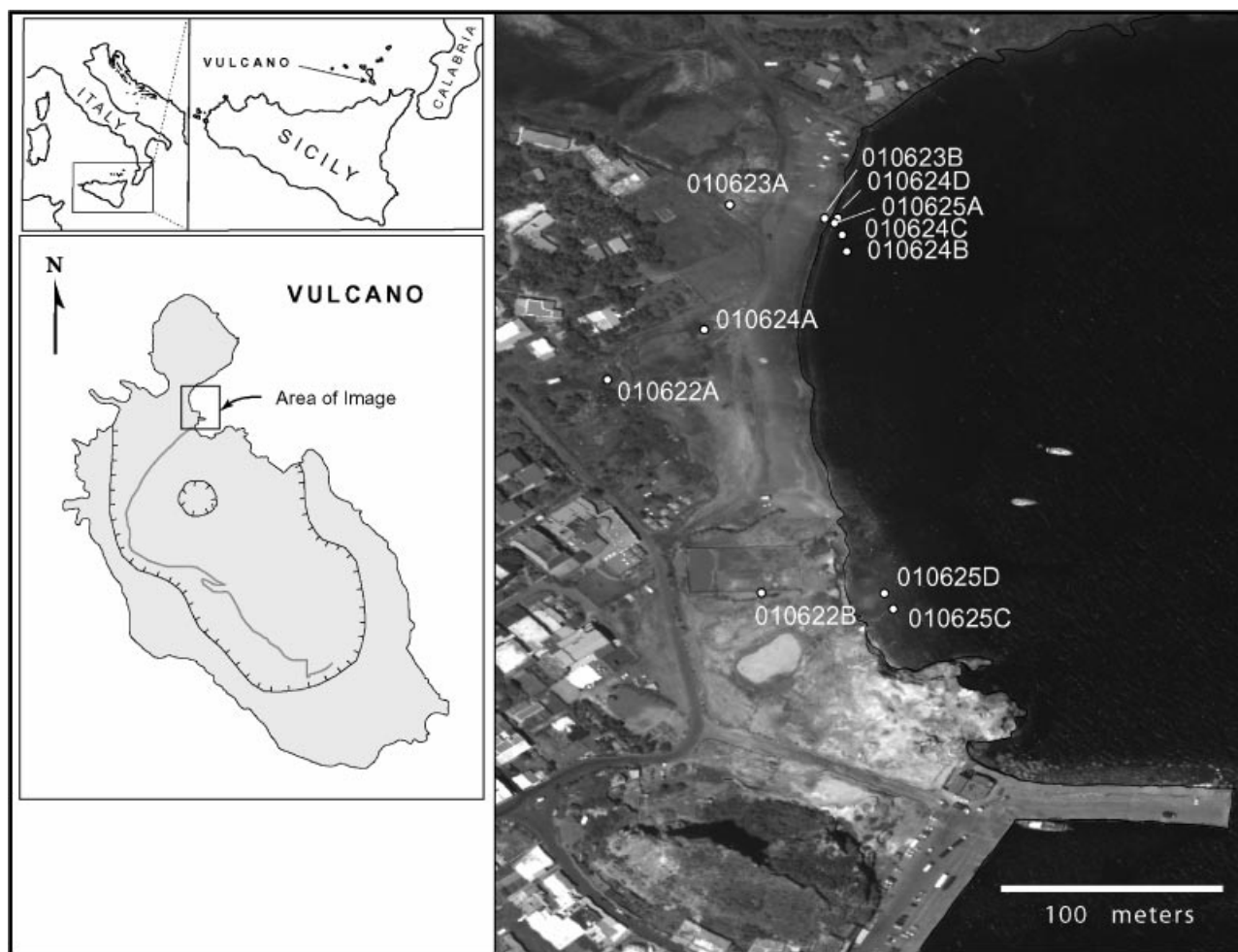


Fig. 1 Aerial photograph of the Baia de Levante on the island of Vulcano (see inset map). Sampling sites are indicated by open circles and samples numbers. Sample 010625B (Pozzo Currò) lies just south of the area shown in the aerial photograph. The latitude and longitude of each site are given in Table 1.

protein-forming amino acids is energetically favoured in hot, moderately reduced, deep-sea hydrothermal solutions relative to the synthesis in cool, oxidized, surface seawater (Amend & Shock, 1998). Owing to the dearth of analytical data for aqueous organic compounds in thermal fluids, the focus was largely on chemolithoautotrophy in the aforementioned studies; only a few publications report the energetics in hydrothermal systems of organic oxidation reactions (chemoorganoheterotrophy) (Amend *et al.*, 1998; Shock *et al.*, 1999). Here, we calculate values of the overall Gibbs free energy (ΔG_r) of redox reactions at the *in situ* temperatures and chemical compositions of 10 seeps, wells and vents in the hydrothermal system of Vulcano, Aeolian Islands (Italy), perhaps the best-studied site of hyperthermophily.

The shallow seeps, wells and vents in and around the Baia di Levante on Vulcano (Fig. 1) have yielded more genera of hyperthermophiles than any other hydrothermal environment.

In fact, of the more than two dozen known hyperthermophilic genera from continental and marine systems worldwide, at least 10 are represented at Vulcano. These include members of the bacteria *Aquifex* (e.g. *Aq. aeolicus*) and *Thermotoga* (*Tt. maritima*), the euryarchaeota *Archaeoglobus* (*Ag. fulgidus*), *Ferroglobus* (*Fg. placidus*), *Palaeococcus* (*Pa. helgesonii*), *Pyrococcus* (*Pc. furiosus*), and *Thermococcus* (*Tc. celer*), and the crenarchaeota *Pyrodicticum* (*Pd. occultum*), *Staphylothermus* (*St. marinus*) and *Thermoplasma* (*Tp. volcanium*) (Stetter, 1982, 1988, 1996; Zillig *et al.*, 1983; Fiala & Stetter, 1986; Fiala *et al.*, 1986; Huber *et al.*, 1986; Stetter *et al.*, 1990; Hafenbradl *et al.*, 1996; Deckert *et al.*, 1998; Amend *et al.*, 2003). Among the ranks of 'Vulcano thermophiles' are the first fully characterized isolate with an optimum growth temperature $> 100^\circ\text{C}$ (*Pd. occultum*; Stetter, 1982) and several isolates for which the complete genomes have now been sequenced (*Aq. aeolicus*, *Tt. maritima*, *Ag. fulgidus*, *Pc. furiosus* and *Thermoplasma volcanium*; Klenk *et al.*, 1997; Deckert *et al.*, 1998; Nelson

et al., 1999; Kawashima *et al.*, 2000; Robb *et al.*, 2001). In addition, *Archaeoglobus* is the only known archaeon and the only marine hyperthermophile able to gain energy by sulphate reduction.^b Finally, *Pa. helgesonii* is the first, and so far only, member of the Thermococcales that is not obligately anaerobic; this archaeon is able to grow in aqueous media under an atmosphere of N₂ : air (P_{O₂} ≤ 0.18 bar) (Amend *et al.*, 2003).

Despite this wealth of physiological, phylogenetic and genomic diversity, very little is known about the *in situ* metabolic strategies used by thermophiles at Vulcano. Culturing studies have shown that many of these thermophiles are obligately anaerobic heterotrophs, but aerobes and autotrophs are not uncommon. Furthermore, many of the known isolates grow best in the laboratory in the presence of elemental sulphur (S⁰). To begin to investigate Vulcano's thermophiles in the context of their geochemical environment, we have combined chemical analyses of samples collected in the spring of 2001 with thermodynamic calculations to evaluate the energetics of a wide variety of chemolithoautotrophic reactions. The sampling sites include shallow submarine hydrothermal vents in the Baia di Levante, seeps in the associated heated beach sands and shallow geothermal wells on the isthmus (see Fig. 1). Ninety redox reactions in the chemical system H–O–N–S–C–Fe are considered here for their potential as energy-yielding processes of thermophiles at Vulcano.

The hydrothermal system at Vulcano

Vulcano is the southernmost of the seven islands that form the Aeolian archipelago in the southern Tyrrhenian Sea (Fig. 1). Since its last eruption in 1888–1890, volcanic activity on Vulcano has been characterized by high-temperature fumaroles concentrated near the active edifice of La Fossa crater and diffuse emissions of CO₂-rich gases in and around the Baia di Levante. The temperature of the gases at La Fossa currently ranges between 100 and 450 °C (our unpublished data), but the fluid emissions at the Baia di Levante are significantly cooler, with temperatures ranging from ambient to ~ 100 °C. Compositionally, the crater fumaroles are typical of high-temperature magmatic fluids; CO₂ is the main component, and significant concentrations of HCl, SO₂, H₂S, HF and CO are also reported (Badalamenti *et al.*, 1991a; Chiodini *et al.*, 1991, 1993, 1995; Capasso *et al.*, 1997; Nuccio *et al.*, 1999). The diffuse emissions at the Baia di Levante are more typical of hydrothermal fluids, with higher CH₄ and H₂S contents than the crater fumaroles, lower CO concentrations and no measurable amount of SO₂ (Badalamenti *et al.*, 1984, 1988, 1991b; Baubron *et al.*,

1990; Carapezza & Diliberto, 1993). As discussed below, the fluid compositions at different sites are quite variable, however. The variations in space and time of the chemical as well as isotopic composition of thermal fluids are functions of active tectonism and frequent changes in the exhaling activity of the crater (Capasso *et al.*, 1997).

Over the last two decades, several mixing models were proposed to explain the chemical and isotopic variations of the Vulcano hydrothermal fluids. Each of these models involves mixing between a deep magmatic and a shallow fluid source. Carapezza *et al.* (1981) hypothesized the existence of an over-pressured two-phase reservoir of meteoric origin feeding the fumaroles, and Cioni & D'Amore (1984) suggested a dry, single-phase model. Several investigators proposed that the shallow source originates from the total evaporation of seawater and/or saline hydrothermal groundwaters seeping laterally from an aquifer (Cioni & D'Amore, 1984; Chiodini *et al.*, 1991, 1993, 1995; Panichi & Noto, 1992). Capasso *et al.* (1992), however, argued against a primary magmatic component and suggested that the shallow component is a mixture of marine brine and meteoric water. Bolognesi & D'Amore (1993) discounted seawater as a direct component of the system and concluded that the fumaroles owe their composition to the mixing of magmatic vapour and thermal meteoric water. Capasso *et al.* (1997) noted that the mixing of a positive δ¹⁸O end-member of deep origin and two different meteoric end-members can account for the isotopic composition of the fumarolic steam. More recently, Capasso *et al.* (2001) formulated a model for the fumaroles at the Baia di Levante, in which ascending volcanic gases come into contact and react with a deep (~ 200 m) thermal aquifer. In the process, thermal energy is lost, the more soluble species are dissolved in the water and the depleted residual gas phase condenses in the shallower aquifer. Finally, Capaccioni *et al.* (2001) argue that CO₂-rich emissions in the Baia di Levante fall into several categories, depending on proximity to the crater, and differences in their calculated equilibrium temperatures, H₂S contents and concentrations of hydrocarbons.

It is abundantly clear from the long list of mixing models that the volcanic, hydrological and tectonic processes responsible for the temperature and composition of the hydrothermal fluids at Vulcano, and specifically the Baia di Levante, are difficult to interpret. Part of the difficulty lies in the highly variable chemical composition of fluids from various sample sites in the Baia di Levante. These same variations are largely responsible for the often considerable differences in chemical energy released by microbially catalysed redox processes, and may be largely responsible for the biological diversity of thermophiles from Vulcano. The purpose of this study is to calculate the chemical energy of these microbially catalysed processes and to interpret the differences among them. Specifically, the energetics of chemolithoautotrophy were investigated at 10 Vulcano sites; the names, types and locations of these sites are given in Table 1.

^bSeveral high-temperature, non-marine sulphate reducers are known, including the bacteria *Thermodesulfobacterium commune*, *Th. hvaragerdense*, *Th. hydrogenophilum* and *Thermodesulfovibrio yellowstonii*.

Table 1 Hydrothermal sites at Vulcano

Site	Sample no.	Type	Latitude 38 °N	Longitude 14 °E
Punto Uno	010623A	S	25°6.66'	57°33.63''
Punto Sette	010624A	S	25°4.21'	57°33.17''
Stinky Surf Rock	010623B	S	25°6.39'	57°35.51''
Grip	010625A	V	25°6.34'	57°35.68''
Stinky Waist	010624C	V	25°6.03'	57°35.90''
Acque Calde 1	010625C	V	24°59.33'	57°36.63''
Acque Calde 2	010625D	V	24°59.03'	57°36.69''
Pozzo Istmo	010622A	W	25°3.41'	57°31.42''
Pozzo Vasca	010622B	W	24°59.37'	57°34.28''
Pozzo Currò	010625B	W	24°45.2'	57°37.9''

S = seep from heated sediments; V = shallow submarine vent; W = geothermal well.

Metabolic strategies of moderate thermophiles and hyperthermophiles

Based on laboratory growth studies, it is known that thermophiles can use a vast array of catabolic processes, including CO₂-based and acetoclastic methanogenesis (Jones *et al.*, 1983; Nozhevnikova & Chudina, 1984; Kurr *et al.*, 1991); sulphate, sulphite and S⁰ reduction (Huber *et al.*, 1997; Stetter *et al.*, 1983; Stetter, 1988); aerobic and anaerobic sulphide oxidation (Brannan & Caldwell, 1980; Odinstova *et al.*, 1996); nitrate reduction (Huber *et al.*, 1996); Fe(III) reduction and Fe(II) oxidation (Ghauri & Johnson, 1991; Clark & Norris, 1996; Greene *et al.*, 1997; Slobodkin *et al.*, 2001); aerobic H₂ oxidation (Gotz *et al.*, 2002; Kawasumi *et al.*, 1984); aerobic and anaerobic oxidation of organic compounds (Burggraf *et al.*, 1990; Völkl *et al.*, 1993); and fermentation (Fiala & Stetter, 1986; Zillig *et al.*, 1990). A number of these processes have also been confirmed in sediments collected from marine hydrothermal systems and incubated in the laboratory. As an example, in heated deep-sea sediments of Guaymas Basin, Gulf of California, very high microbial sulphate reduction rates (up to 61 µM SO₄²⁻ per day) were measured at temperatures ≥ 80 °C, in fact as high as 110 °C (Jørgensen *et al.*, 1990, 1992; Elsgaard *et al.*, 1994; Weber & Jørgensen, 2002). In addition, a hyperthermophilic sulphate-reducing Archaeon, *Ag. profundus* (Burggraf *et al.*, 1990), was isolated from the sediments at Guaymas. Furthermore, in shallow marine hydrothermal sediments from the Baia di Levante, Vulcano, similarly high microbial sulphate reduction rates (72 µM SO₄²⁻ per day) were observed under strict anaerobic conditions at 90 °C (Tor *et al.*, 2003).

Our approach in this study builds on the concept that perhaps the most fundamental characteristic determining the progression and direction of a microbially mediated catabolic process, in fact any chemical reaction, is the amount of energy released or consumed. Thermodynamic modelling is a direct and quantitative approach to determine which of a plethora of possible catabolic strategies are exergonic (energy-yielding) or

endergonic (energy-consuming). In a recent review (Amend & Shock, 2001), standard Gibbs free energies (ΔG_r°) as a function of temperature to 200 °C were computed for over 350 reactions directly or indirectly linked to microbial metabolism. Of these, ~ 200 were redox reactions known to be mediated by micro organisms to obtain energy. Here, we evaluate the overall Gibbs free energy (ΔG_r) of 90 redox reactions at 10 Vulcano hydrothermal sites. Many of these reactions have been shown to support growth of thermophiles. However, we also include reactions for which microbial catalysts are unknown to investigate their energetic potential as possible modes of metabolism in Vulcano thermophiles.

METHODS

Sampling procedures

Hydrothermal fluids from 10 sites (four shallow submarine vents, three sediment seeps, three geothermal wells) were sampled and analysed immediately or stored for subsequent analysis. Samples for analysis of major cations were filtered consecutively through sterile 0.8-µm nylon, 0.45-µm MCE and 0.20-µm nylon filters using a sterile syringe rinsed with hydrothermal fluid and collected in 60-mL Nalgene bottles that had been washed in 10% nitric acid and rinsed in deionized water. Prior to sampling, 3 µL of 70% trace element-grade nitric acid (Fisher Scientific, PA, USA) was added to each bottle. Samples for anion analysis were filtered as described above and collected in clean 60-mL glass serum bottles, capped with sterile Teflon septa and Al caps. After collection, 0.5 mL of 24 mM HgCl₂ was added to each sample to minimize microbial degradation. An additional fluid sample for analysis of chloride concentration was collected in a 60-mL acid-washed Nalgene bottle that was first rinsed with hydrothermal fluid in the field. Anion and cation samples were refrigerated until analysis; Cl⁻ samples remained at room temperature. Gas samples were collected in glass flasks with two stopcocks connected to an inverted funnel placed directly on the sediment or in the water above the gas source.

Water analyses

Major cations (Na⁺, K⁺, Mg²⁺, Ca²⁺) and anions (Cl⁻, Br⁻, SO₄²⁻) were analysed by ion chromatography using a Dionex DX 500 system equipped with an electrochemical detector. Concentrations of the major anions were determined with an IonPac AS11 column, and concentrations of major cations were determined using the IonPac CS12A column. Prior to analysis, each sample was filtered through a sterile 0.20-µm nylon filter and diluted as necessary. Triplicate analyses were conducted on each sample. Data were optimized on a Dionex PeakNet Chromatography Workstation and reported as averages of the triplicate analyses. Method detection limits for these samples are generally < 1 ppm.

A number of redox-sensitive compounds (Fe^{2+} , NO_3^- , NO_2^- , NH_4^+ , H_2S , dissolved oxygen) as well as total phosphate and aqueous silica were analysed by spectrophotometry in the field seconds to minutes after sampling, or in the nearby geochemistry laboratory at the Marcello Carapezza Center on Vulcano within several hours of sampling. These analyses were performed on a HACH 2010 portable spectrophotometer (HACH Company, CO, USA). Each species was analysed with the method recommended by the manufacturer and samples were diluted as necessary. Briefly, with minimum detection limits (in ppm) given in parentheses, ferrous iron was measured using the 1,10 phenanthroline method (0.02); nitrate with the cadmium reduction method (0.1); nitrite using one of two variations of the diazotization chromotropic acid method (0.001); total ammonia (NH_3 and NH_4^+) by the salicylate method (0.02); dissolved oxygen with the indigo carmine method (0.006) or the 'High Range Dissolved Oxygen' method (0.3), depending on sample concentration; total phosphate using the orthophosphate ascorbic acid method (0.02); total sulphide with the methylene blue method (0.01); and aqueous silica by the silicomolybdate method (1.0).

In situ measurements of temperature, pH and conductivity were performed using hand-held meters and probes. Conductivity and temperature were measured on a YSI Model 30 Salinity, Conductivity and Temperature System (YSI Inc., OH, USA) with a temperature range of -5 to 95°C and a conductivity range of 0.1 – 200 mS/cm. The pH of each fluid was measured *in situ* using an Orion 290 portable pH meter (pH range 0 – 14 ; temperature range 0 – 100°C) equipped with a ROSS Sure-Flow Combination Electrode and an Automatic Temperature Compensation Probe (all from Orion Research Inc., MA, USA).

Gas analyses

The chemical composition of free gases was determined by gas chromatography. The gas chromatograph (Perkin-Elmer 8500) was equipped with a carboxisieve SII column, which separates He , H_2 , O_2 , N_2 , CO , CH_4 and CO_2 . A hot wire detector and a flame ionization detector with methanizer were placed in series. The detection limits are 1 ppmv for the C-bearing species, 5 ppmv for He and H_2 , and 100 ppmv for O_2 and N_2 .

Thermodynamic modelling

Composition data together with *in situ* temperatures and standard state^c thermodynamic properties for gases, aqueous species and minerals were used to calculate values of ΔG_r for the different redox reactions. The amount of energy liberated from a chemical reaction can be calculated from the expression

$$\Delta G_r = \Delta G_r^\circ + RT \ln Q_r, \quad (1)$$

where ΔG_r and ΔG_r° are as defined above, R and T represent the gas constant and temperature in Kelvin, respectively, and Q_r stands for the activity product. Values of Q_r can be computed from the relation

$$Q_r = \prod a_i^{v_{i,r}}, \quad (2)$$

where a_i denotes the activity of the i th species, and $v_{i,r}$ represents the stoichiometric reaction coefficient of the i th species in reaction r , which is negative for reactants and positive for products. The standard state term (ΔG_r°) can be calculated at any temperature and pressure by combining the apparent standard Gibbs free energies of formation (ΔG_i°) of the i th species in r at these conditions in accord with the expression

$$\Delta G_r^\circ = \sum v_{i,r} \Delta G_i^\circ. \quad (3)$$

The values of ΔG_i° at the temperature and pressure of interest for the aqueous species and minerals in the reactions given below can be calculated using established equations of state (Helgeson *et al.*, 1978, 1981; Shock & Helgeson, 1988, 1990; Tanger & Helgeson, 1988; Shock *et al.*, 1989, 1992).

Evaluating Q_r in Eq. (1) requires the activities or fugacities of the reactants and products at the environmental conditions of interest. The activities of pure minerals were set to unity, and the activities of the aqueous species were computed from partial pressures of gases (P_i) and concentrations of ions, aqueous H_2S and dissolved oxygen (Table 2) using the speciation program EQ3 (Wolery, 1992; Wolery & Daveler, 1992), which explicitly accounts for activity coefficients and complex formation. Values of P_i were computed from concentrations of the dry gases given in Table 2 assuming water/vapour equilibrium at 1 bar and *in situ* temperatures. In the speciation calculations, fugacity coefficients were taken to be unity, but activity coefficients were calculated according to the extended Debye–Hückel equation recommended by Helgeson (1969). Redox reactions among S-, C-, N-, Fe-, H- and O-bearing species were suppressed, thus maintaining the observed geochemical disequilibria in the hydrothermal systems. For the model calculations, a custom thermodynamic database was constructed using the program SUPCRT92 (Johnson *et al.*, 1992) and published data (Helgeson *et al.*, 1978; Shock & Helgeson, 1988, 1990; Shock *et al.*, 1989, 1992; Shock & Koretsky, 1993, 1995; McCollom & Shock, 1997). Activities of aqueous species computed in this manner are given in Table 3. Unless noted otherwise, we considered only the

^cStandard states used in the calculations are as follows: for solids and water, unit activity of the pure compound at any temperature and pressure; for gases, unit fugacity of the pure gas at any temperature and 1 bar; and for aqueous solutes, unit activity of a hypothetical 1 molal solution referenced to infinite dilution at any temperature and pressure.

Table 2 Analytical data of 10 Vulcano hydrothermal fluids. Concentrations of aqueous species are in ppm, except for dissolved oxygen, which is in ppb H₂, CO and CH₄ are in ppmv of the dry gas; N₂ and CO₂ are in vol. % of the dry gas. Conductivity is in mS/cm

	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
T (°C)	88.9	81.0	84.8	54.0	50.8	60.7 ^a	87.5	56.4	81.8	42.1
pH	3.561	2.717	3.673	5.205	5.207	5.518	5.650	5.840	1.976	6.327
Cond.	6.32	17.6	118	53.4	54.7	88.8	86.9	96.0	24.3	6.85
Na ⁺	1365	1533	10 923	9363	11 047	11 008	10 483	12 209	1452	336
K ⁺	95	98	450	401	516	425	425	590	272	290
Mg ²⁺	227	258	1287	1063	1220	1266	1172	1414	200	153
Ca ²⁺	228	399	410	308	494	454	494	665	340	451
Fe ²⁺	4.0	309	7.7	12.1	1.5	1.5	0.7	0.02	293	1.8
NH ₄ ⁺	55	5	12	10.6	11	11.4	20.6	3.31	30	2.1
Cl ⁻	2259	2110	19 657	16 869	19 955	19 632	18 450	21 665	2184	284.6
Br ⁻	7.64	7.75	69.02	60.38	60.76	60.61	61.24	68.74	8.53	1.27
SO ₄ ²⁻	1546	3646	2901	2268	2667	2690	2624	3013	5976	1686
PO ₄ ³⁻	0.90	1.50	0.63	0.75	0.47	1.01	0.43	1.04	20.4	0.84
NO ₃ ⁻	6	4	1.6	1.9	3.7	78	200	38	34	bdl
NO ₂ ⁻	0.004	0.001	0.004	0.003	0.002	bdl	0.002	0.01	0.008	bdl
H ₂ S(aq)	5.1	8.7	0.75	10.4	6	12.3	9.3	12.8	4.2	0.11
SiO ₂ (aq)	337	466	199	143	169	150	260	193.8	419	124
O ₂ (aq)	700	380	643			2900	2100	382	406	739
H ₂ (g)	1812	221	1068	2374			19 813	8.1	2909	
N ₂ (g)	1.18	24.11	0.95	1.4			0.88	0.73	0.92	
CO(g)	6.4	97.9	bdl	1.9			4.35	2.5	1.43	
CH ₄ (g)	1228	5568	1253	1497			1289	1104	1215	
CO ₂ (g)	98.90	70.66	98.83	98.64			98.84	99.10	99.16	

^aRepresents a minimum temperature; the actual temperature is probably close to that reported for Acque Calde 2, which is the value used in the energy calculations below.

bdl: below detection limit, which for nitrite is 0.001 ppm, for nitrate is 0.1 ppm and for CO(g) is 5 ppmv.

Blank spaces indicate that these concentrations were not or could not be measured.

Table 3 Log activities of aqueous species used in energetic calculations (see text)

	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
H ⁺	-3.561	-2.717	-3.673	-5.205	-5.207	-5.518	-5.650	-5.840	-1.976	-6.327
H ₂ O(l) ^a	0.997	0.997	0.982	0.984	0.982	0.982	0.983	0.980	0.996	0.998
H ₂ (aq)	-6.21	-7.01	-6.37	-5.82	-5.81	-5.17	-5.14	-8.30	-5.89	
O ₂ (aq)	-4.66	-4.92	-4.68	-4.67 ^c	-4.66 ^c	-4.03	-4.17	-4.89	-4.89	-4.63
NO ₃ ⁻	-4.14	-4.33	-4.80	-4.70	-4.42	-3.11	-2.70	-3.41	-3.40	
NO ₂ ⁻	-7.18	-7.79	-7.24	-7.35	-7.53		-7.54	-6.83	-6.89	
N ₂ (aq)	-5.61	-5.57	-5.64	-5.22	-5.20	-5.74	-5.72	-5.51	-5.61	
NH ₄ ⁺	-2.66	-3.72	-3.42	-3.45	-3.44	-3.44	-3.18	-3.97	-2.95	-4.05
SO ₄ ²⁻	-2.52	-2.25 ^b	-2.69	-2.65	-2.62	-2.73	-2.73	-2.61	-2.33 ^b	-2.30
H ₂ S(aq)	-3.81	-3.58	-4.65	-3.52	-3.76	-3.49	-3.64	-3.50	-3.90	-5.63
CO ₂ (aq)	-2.29	-2.13	-2.20	-1.82	-1.79	-2.29	-2.26	-1.83	-2.15	
CO(aq)	-8.72	-7.41		-8.93	-8.91	-8.89	-8.86	-8.82	-9.26	
CH ₄ (aq)	-6.32	-5.53	-6.23	-5.89	-5.87	-6.29	-6.27	-6.04	-6.21	
Fe ²⁺	-4.63	-2.78	-4.62	-4.35	-5.28	-5.34	-5.66	-7.18	-2.81	-4.86

^aValues represent the activities, not log values.

^bHSO₄⁻ dominates, but in the energy calculations the activities of SO₄²⁻ were used throughout.

^cComputed assuming the same concentration as reported for Stinky Surf Rock.

activities of the dominant species in the calculations. For example, at the *in situ* temperatures and pHs, H₂S, NH₄⁺ and CO₂(aq) dominate over HS⁻, NH₃(aq) and HCO₃⁻, respectively.

Net metabolic redox reactions

The chemolithoautotrophic reactions discussed below are grouped by chemical system, starting with the H–O system

Table 4a Reaction in the system H–O

	Reaction	e [−]
A1	H ₂ + 0.5O ₂ → H ₂ O	2

Table 4b Values of $\Delta G_r/e^-$ (kJ/mol e[−]) for reactions in Table 4a at 10 Vulcano hydrothermal sites (see Table 1)

	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
A1	−100.07	−97.85	−100.00	−105.32	−105.71	−104.94	−104.79	−96.92	−101.58	

and followed by the H–O–N, H–O–S, H–O–C, H–O–Fe, H–O–N–S, H–O–N–C, H–O–S–C, H–O–N–Fe, H–O–S–Fe and H–O–C–Fe systems. Two tables are given for each system. The first table (denoted with an ‘a’) shows the reactions, in which compounds appear as aqueous species, liquid (H₂O), or minerals (S[°], Fe₃O₄).^d The second table (denoted with a ‘b’) lists values of ΔG_r , normalized per electron transferred ($\Delta G_r/e^-$) for each reaction in the system at each of the 10 hydrothermal sites. We tabulate normalized values of the Gibbs free energy ($\Delta G_r/e^-$) rather than ΔG_r , to permit a more straightforward comparison of the energetics of redox reactions. It is, after all, via the electron that net catabolic reactions, such as those considered here, are coupled to anabolic reactions, e.g. intracellular biosynthesis.

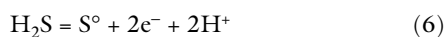
This normalization can perhaps best be illustrated with an example. Consider two chemotrophs; the first gains energy by catalysing the reaction



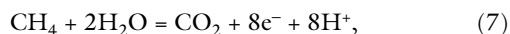
and the second catalyses the reaction



In reaction (4), two electrons are transferred from S in H₂S to O in O₂; in the process, the oxidation state of S changes from −2 in H₂S to 0 in S[°], and the oxidation state of O changes from 0 in O₂ to −2 in H₂O. In reaction (5), eight electrons are transferred from C in CH₄ to O in O₂; consequently, the oxidation state of C changes from −4 in CH₄ to +4 in CO₂, and that of O changes from 0 in O₂ to −2 in CO₂ and H₂O. Or, stated differently, the oxidation half-reactions are

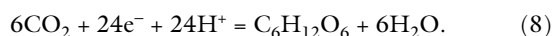


and



^dThe only minerals considered are S[°] and magnetite (Fe₃O₄), both of which are ubiquitous at the Vulcano hydrothermal sites (our unpublished data).

respectively. These catabolic reactions can be coupled, for example, to the anabolic process of glucose (C₆H₁₂O₆) synthesis. In autotrophs, the half-reaction for this process can be written as



Twelve moles of H₂S (see reaction 6), but only three moles of methane (see reaction 7) are required to couple to the glucose synthesis reaction (8). Methane oxidation as written in reaction (5) would have to yield four times as much energy as sulphide oxidation (reaction 4) to drive the same energy-consuming anabolic process. Thus, it is far more meaningful to know the energy yield per electron transferred, $\Delta G_r/e^-$, than the energy for the reaction as written, ΔG_r .

The H–O system

In this system, we consider only one reaction (Table 4a), the ‘knallgas’ reaction, which supports growth of the Vulcano hyperthermophilic bacterium *Aq. aeolicus*, among others. Values of $\Delta G_r/e^-$ at each of the 10 investigated sites, computed as described above, are given in Table 4b.

The H–O–N system

Sixteen redox reactions involving the four N-bearing species NH₄⁺, N₂, NO₂[−] and NO₃[−] are investigated in this system (Table 5a). Of these reactions, those most commonly associated with thermophile metabolism include the partial reduction of nitrate to nitrite (reaction B7) by the bacterium *Aq. pyrophilus* and the Vulcano archaeon *Eg. placidus*, as well as the complete reduction of nitrate to ammonium (reaction B9) by the archaeon *Pyrolobus fumarii*. By contrast, aerobic oxidation of reduced N-compounds (reactions B1–6) are unknown in thermophile metabolism. Values of $\Delta G_r/e^-$ for all reactions in Table 5a are shown in Table 5b.

The H–O–S system

Although only seven reactions with three S-bearing compounds (H₂S, S[°], SO₄^{2−}) are considered in this system (Table 6a), the oxidation and reduction of S are probably the most ubiquitous

Table 5a Reactions in the system H–O–N

	Reaction	e ⁻
B1	$2\text{NH}_4^+ + 1.5\text{O}_2 \rightarrow \text{N}_2 + 2\text{H}^+ + 3\text{H}_2\text{O}$	6
B2	$\text{NH}_4^+ + 1.5\text{O}_2 \rightarrow \text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O}$	6
B3	$\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O}$	8
B4	$\text{N}_2 + 1.5\text{O}_2 + \text{H}_2\text{O} \rightarrow 2\text{NO}_2^- + 2\text{H}^+$	6
B5	$\text{N}_2 + 2.5\text{O}_2 + \text{H}_2\text{O} \rightarrow 2\text{NO}_3^- + 2\text{H}^+$	10
B6	$\text{NO}_2^- + 0.5\text{O}_2 \rightarrow \text{NO}_3^-$	2
B7	$\text{NO}_3^- + \text{H}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$	2
B8	$2\text{NO}_3^- + 5\text{H}_2 + 2\text{H}^+ \rightarrow \text{N}_2 + 6\text{H}_2\text{O}$	10
B9	$\text{NO}_3^- + 4\text{H}_2 + 2\text{H}^+ \rightarrow \text{NH}_4^+ + 3\text{H}_2\text{O}$	8
B10	$2\text{NO}_2^- + 3\text{H}_2 + 2\text{H}^+ \rightarrow \text{N}_2 + 4\text{H}_2\text{O}$	6
B11	$\text{NO}_2^- + 3\text{H}_2 + 2\text{H}^+ \rightarrow \text{NH}_4^+ + 2\text{H}_2\text{O}$	6
B12	$\text{N}_2 + 3\text{H}_2 + 2\text{H}^+ \rightarrow 2\text{NH}_4^+$	6
B13	$4\text{N}_2 + 2\text{H}^+ + 9\text{H}_2\text{O} \rightarrow 3\text{NO}_3^- + 5\text{NH}_4^+$	15
B14	$\text{N}_2 + 2\text{H}_2\text{O} \rightarrow \text{NO}_2^- + \text{NH}_4^+$	3
B15	$4\text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O} \rightarrow 3\text{NO}_3^- + \text{NH}_4^+$	6
B16	$5\text{NO}_2^- + 2\text{H}^+ \rightarrow 3\text{NO}_3^- + \text{N}_2 + \text{H}_2\text{O}$	6

Table 5b Values of $\Delta G_r/\text{e}^-$ (kJ/mol e⁻) for reactions in Table 5a at 10 Vulcano hydrothermal sites (see Table 1)

Reaction	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
B1	-92.83	-88.13	-91.43	-94.63	-94.69	-96.82	-97.44	-94.84	-88.28	
B2	-39.88	-37.11	-39.36	-42.93	-43.14		-45.38	-42.85	-35.32	
B3	-35.83	-33.42	-36.01	-39.05	-38.90	-38.78	-38.63	-38.25	-32.05	
B4	13.07	13.92	12.71	8.77	8.41		6.69	9.13	17.65	
B5	-1.62	-0.59	-2.76	-5.70	-5.42	-3.95	-3.34	-4.30	1.68	
B6	-23.66	-22.36	-25.97	-27.39	-26.18		-18.38	-24.45	-22.26	
B7	-76.42	-75.49	-74.03	-77.93	-79.53		-86.40	-72.47	-79.32	
B8	-98.45	-97.26	-97.23	-99.62	-100.29	-100.99	-101.45	-92.62	-103.26	
B9	-64.25	-64.43	-63.98	-66.27	-66.81	-66.16	-66.16	-58.67	-69.53	
B10	-113.14	-111.78	-112.70	-114.09	-114.12		-111.48	-106.05	-119.23	
B11	-60.19	-60.75	-60.64	-62.39	-62.57		-59.41	-54.06	-66.26	
B12	-7.24	-9.72	-8.57	-10.69	-11.02	-8.11	-7.34	-2.08	-13.30	
B13	91.21	87.54	88.67	88.94	89.26	92.87	94.11	90.54	89.97	
B14	105.90	102.06	104.14	103.40	103.10		104.14	103.97	105.93	
B15	16.22	14.75	13.39	15.54	16.96		26.99	18.41	13.06	
B16	-36.73	-36.28	-38.67	-36.16	-34.59		-25.08	-33.58	-39.90	

Table 6a Reactions in the system H–O–S

	Reaction	e ⁻
C1	$\text{H}_2\text{S} + 0.5\text{O}_2 \rightarrow \text{S}^0 + \text{H}_2\text{O}$	2
C2	$\text{H}_2\text{S} + 2\text{O}_2 \rightarrow \text{SO}_4^{2-} + 2\text{H}^+$	8
C3	$\text{S}^0 + 1.5\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 2\text{H}^+$	6
C4	$\text{SO}_4^{2-} + 3\text{H}_2 + 2\text{H}^+ \rightarrow \text{S}^0 + 4\text{H}_2\text{O}$	6
C5	$\text{SO}_4^{2-} + 4\text{H}_2 + 2\text{H}^+ \rightarrow \text{H}_2\text{S} + 4\text{H}_2\text{O}$	8
C6	$\text{S}^0 + \text{H}_2 \rightarrow \text{H}_2\text{S}$	2
C7	$4\text{S}^0 + 4\text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 3\text{H}_2\text{S} + 2\text{H}^+$	6

energy-yielding processes in chemolithoautotrophic thermophiles. As examples, sulphate reduction (reaction C5) is used by several thermophiles, including species of *Archaeoglobus* (Burggraf *et al.*, 1990; Stetter, 1988) and strains of *Thermodesulfobacterium* (Sonne-Hansen & Ahring, 1999; Jeanthon *et al.*, 2002); the aerobic oxidation of S⁰ (reaction C3) is mediated by

thermophilic members of *Acidianus*, *Aquifex*, *Sulphurococcus* and *Thermothrix*, among others; and the reduction of S⁰ to sulphide (reaction C6) provides metabolic energy to numerous thermophilic archaea and bacteria, including the Vulcano isolates *Pd. occultum* and *Td. maritimus*. Values of $\Delta G_r/\text{e}^-$ for reactions in Table 6a are given in Table 6b.

The H–O–C system

Carbon varies in oxidation state from +4 (CO₂) to -4 (CH₄). In organic compounds, carbon is in intermediate oxidation states, commonly between -2 and +1. Owing to the paucity of analytical data for aqueous organic compounds in hydrothermal fluids at Vulcano (Amend *et al.*, 1998), we consider only the energetics of redox reactions among inorganic compounds (CH₄, CO, CO₂) in this section. Of these reactions (Table 7a), aerobic CH₄ oxidation (reaction D2) and CO₂-based

Table 6b Values of $\Delta G_r/e^-$ (kJ/mol e^-) for reactions in Table 6a at 10 Vulcano hydrothermal sites (see Table 1)

Reaction	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
C1	-84.06	-85.66	-82.03	-89.18	-88.79	-86.80	-86.06	-88.66	-84.55	-84.06
C2	-93.00	-86.06	-87.31	-92.53	-92.50	-92.46	-92.32	-93.06	-84.59	-93.00
C3	-95.98	-86.19	-89.06	-93.65	-93.73	-94.35	-94.40	-94.53	-84.60	-95.98
C4		-11.66	-10.93	-11.67	-11.97	-10.59	-10.38	-2.39	-16.98	
C5		-11.79	-12.69	-12.79	-13.21	-12.48	-12.47	-3.86	-16.99	
C6		-12.19	-17.97	-16.14	-16.92	-18.13	-18.72	-8.26	-17.03	
C7	-11.92	-0.52	-7.04	-4.47	-4.95	-7.54	-8.34	-5.87	-0.05	-11.92

Table 7a Reactions in the system H–O–C

	Reaction	e^-
D1	$CH_4 + 1.5O_2 \rightarrow CO + 2H_2O$	6
D2	$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$	8
D3	$CO + 0.5O_2 \rightarrow CO_2$	2
D4	$CO_2 + H_2 \rightarrow CO + H_2O$	2
D5	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	8
D6	$CO + 3H_2 \rightarrow CH_4 + H_2O$	6
D7	$4CO + 2H_2O \rightarrow 3CO_2 + CH_4$	6

Table 7b Values of $\Delta G_r/e^-$ (kJ/mol e^-) for reactions in Table 7a at 10 Vulcano hydrothermal sites (see Table 1)

Reaction	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
D1	-91.87	-91.01		-93.25	-93.32	-93.23	-92.98	-92.60	-92.37	
D2	-95.18	-95.62	-95.33	-96.65	-96.79	-96.36	-96.12	-96.11	-95.07	
D3	-105.08	-109.46		-106.88	-107.21	-105.76	-105.52	-106.63	-103.20	
D4	5.01	11.61		1.56	1.50	0.82	0.73	9.72	1.62	
D5	-4.90	-2.23	-4.67	-8.66	-8.92	-8.57	-8.67	-0.81	-6.51	
D6	-8.20	-6.85		-12.07	-12.39	-11.71	-11.80	-4.31	-9.21	
D7	-13.21	-18.46		-13.63	-13.89	-12.53	-12.53	-14.03	-10.84	

methanogenesis (reaction D5) are the best known processes, which are mediated, for example, by *Methylococcus thermophilus* (reaction D2) and numerous thermophilic archaea, including members of *Methanocaldococcus*, *Methanobacterium* and *Methanothermus* (reaction D5). Values of $\Delta G_r/e^-$ for the seven reactions in Table 7a are shown in Table 7b.

The H–O–Fe system

It has been suggested (Fox, 1988; Lovley, 2000) that Fe(III) may have served as the terminal electron acceptor (TEA) for the earliest life on Earth. Several extant organisms are known that obtain metabolic energy from the aerobic oxidation of ferrous iron and the reduction with H_2 of ferric iron (Table 8a); they include *Geobacter sulfurreducens*, *Thermoterrabacterium ferrireducens*, *Geothermobacterium ferrireducens*, *Geoglobus abangari*, *Sulfobacillus thermosulfidooxidans* and *Sb. acidophilus*, *Acidianus brierleyi* and *Thiobacillus prosperus* (Brierley & Brierley, 1973; Golovacheva & Karavaiko, 1978; Segerer *et al.*, 1986; Huber & Stetter, 1989; Caccavo *et al.*, 1994; Norris

Table 8a Reactions in the system H–O–Fe

	Reaction	e^-
E1	$3Fe^{2+} + 0.5O_2 + 3H_2O \rightarrow Fe_3O_4 + 6H^+$	2
E2	$Fe_3O_4 + H_2 + 6H^+ \rightarrow 3Fe^{2+} + 4H_2O$	2

et al., 1996; Slobodkin *et al.*, 1997; Kashefi *et al.*, 2002a; Kashefi *et al.*, 2002b). Values of $\Delta G_r/e^-$ for reactions in Table 8a are given in Table 8b.

The H–O–N–S system

Reactions in which N- and S-bearing compounds serve as the TEAs and as e^- donors are given in Table 9a. Several of these reactions are known to be mediated by micro organisms, including thermophiles. Among these are the oxidation of S^0 (reverse of reaction F12) by the bacterium *Aq. pyrophilus* (Huber *et al.*, 1992) and of H_2S (reverse of reaction F18) by the archaeon *Eg. placidus* (Hafenbradl *et al.*, 1996) with NO_3^-

Table 8b Values of $\Delta G_r/e^-$ (kJ/mol e^-) for reactions in Table 8a at 10 Vulcano hydrothermal sites (see Table 1)

Reaction	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
E1	-42.43	-42.55	-44.18	-70.91	-61.58	-76.49	-75.62	-56.45	-27.23	-83.81
E2	-57.64	-55.30	-55.82	-34.41	-44.13	-28.45	-29.17	-40.47	-74.35	

Table 9a Reactions in the system H–O–N–S

	Reaction	e^-
F1	$2\text{NH}_4^+ + \text{SO}_4^{2-} \rightarrow \text{N}_2 + \text{S}^0 + 4\text{H}_2\text{O}$	6
F2	$8\text{NH}_4^+ + 3\text{SO}_4^{2-} \rightarrow 4\text{N}_2 + 3\text{H}_2\text{S} + 2\text{H}^+ + 12\text{H}_2\text{O}$	24
F3	$\text{NH}_4^+ + \text{SO}_4^{2-} \rightarrow \text{NO}_2^- + \text{S}^0 + 2\text{H}_2\text{O}$	6
F4	$4\text{NH}_4^+ + 3\text{SO}_4^{2-} \rightarrow 4\text{NO}_2^- + 3\text{H}_2\text{S} + 2\text{H}^+ + 4\text{H}_2\text{O}$	24
F5	$3\text{NH}_4^+ + 4\text{SO}_4^{2-} + 2\text{H}^+ \rightarrow 3\text{NO}_3^- + 4\text{S}^0 + 7\text{H}_2\text{O}$	24
F6	$\text{NH}_4^+ + \text{SO}_4^{2-} \rightarrow \text{NO}_3^- + \text{H}_2\text{S} + \text{H}_2\text{O}$	8
F7	$2\text{NH}_4^+ + 3\text{S}^0 \rightarrow \text{N}_2 + 3\text{H}_2\text{S} + 2\text{H}^+$	6
F8	$\text{NH}_4^+ + 3\text{S}^0 + 2\text{H}_2\text{O} \rightarrow \text{NO}_2^- + 3\text{H}_2\text{S} + 2\text{H}^+$	6
F9	$\text{NH}_4^+ + 4\text{S}^0 + 3\text{H}_2\text{O} \rightarrow \text{NO}_3^- + 4\text{H}_2\text{S} + 2\text{H}^+$	8
F10	$\text{N}_2 + \text{SO}_4^{2-} \rightarrow 2\text{NO}_2^- + \text{S}^0$	6
F11	$4\text{N}_2 + 3\text{SO}_4^{2-} + 4\text{H}_2\text{O} \rightarrow 8\text{NO}_2^- + 3\text{H}_2\text{S} + 2\text{H}^+$	24
F12	$3\text{N}_2 + 5\text{SO}_4^{2-} + 4\text{H}^+ \rightarrow 6\text{NO}_3^- + 5\text{S}^0 + 2\text{H}_2\text{O}$	30
F13	$4\text{N}_2 + 5\text{SO}_4^{2-} + 2\text{H}^+ + 4\text{H}_2\text{O} \rightarrow 8\text{NO}_3^- + 5\text{H}_2\text{S}$	40
F14	$\text{N}_2 + 3\text{S}^0 + 4\text{H}_2\text{O} \rightarrow 2\text{NO}_2^- + 3\text{H}_2\text{S} + 2\text{H}^+$	6
F15	$\text{N}_2 + 5\text{S}^0 + 6\text{H}_2\text{O} \rightarrow 2\text{NO}_3^- + 5\text{H}_2\text{S} + 2\text{H}^+$	10
F16	$3\text{NO}_2^- + \text{SO}_4^{2-} + 2\text{H}^+ \rightarrow 3\text{NO}_3^- + \text{S}^0 + \text{H}_2\text{O}$	6
F17	$4\text{NO}_2^- + \text{SO}_4^{2-} + 2\text{H}^+ \rightarrow 4\text{NO}_3^- + \text{H}_2\text{S}$	8
F18	$\text{NO}_2^- + \text{S}^0 + \text{H}_2\text{O} \rightarrow \text{NO}_3^- + \text{H}_2\text{S}$	2

Table 9b Values of $\Delta G_r/e^-$ (kJ/mol e^-) for reactions in Table 9a at 10 Vulcano hydrothermal sites (see Table 1)

Reaction	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
F1	-4.39	-1.94	-2.36	-0.98	-0.95	-2.48	-3.04	-0.31	-3.68	
F2	-5.40	-2.08	-4.12	-2.10	-2.19	-4.36	-5.13	-1.78	-3.69	
F3	48.56	49.08	49.71	50.72	50.60		49.03	51.68	49.28	
F4	47.55	48.95	47.95	49.58	49.36		46.94	50.21	49.27	
F5	52.61	52.77	53.05	54.60	54.84	55.57	55.77	56.28	52.55	
F6	51.61	52.64	51.29	53.49	53.60	53.68	53.69	54.81	52.54	
F7	-8.40	-2.47	-9.40	-5.46	-5.90	-10.02	-11.38	-6.18	-3.73	
F8	44.55	48.56	42.67	46.24	45.65		40.69	45.80	49.23	
F9	48.60	52.25	46.02	50.13	49.89	48.02	47.44	50.41	52.50	
F10	101.51	100.11	101.77	102.42	102.15		101.09	103.66	102.25	
F11	100.51	99.98	100.01	101.30	100.91		99.01	102.19	102.24	
F12	86.82	85.60	86.30	87.96	88.31	90.40	91.06	90.23	86.29	
F13	85.81	85.47	84.54	86.84	87.07	88.51	88.98	88.76	86.27	
F14	97.50	99.59	94.74	97.95	97.20		92.76	97.79	102.20	
F15	82.81	85.08	79.27	83.48	83.36	82.85	82.73	84.36	86.24	
F16	64.78	63.83	63.10	66.26	67.56		76.02	70.08	62.34	
F17	63.78	63.70	61.34	65.14	66.32		73.93	68.62	62.33	
F18	60.77	63.31	56.06	61.79	62.61		67.68	64.21	62.29	

as the TEA. Values of $\Delta G_r/e^-$ for all 18 reactions given in Table 9a are listed in Table 9b.

The H–O–N–C system

Table 10a lists 18 reactions in which inorganic N- and C-

bearing compounds are the electron acceptors and donors. Few, if any, of these reactions represent known net metabolic strategies for micro organisms. Nevertheless, they are included here to permit a quantitative assessment of both potential and known metabolic processes. Values of $\Delta G_r/e^-$ for reactions in Table 10a are given in Table 10b.

Table 10a Reactions in the system H–O–N–C

	Reaction	e ⁻
G1	$2\text{NH}_4^+ + 3\text{CO}_2 \rightarrow \text{N}_2 + 3\text{CO} + 2\text{H}^+ + 3\text{H}_2\text{O}$	6
G2	$8\text{NH}_4^+ + 3\text{CO}_2 \rightarrow 4\text{N}_2 + 3\text{CH}_4 + 8\text{H}^+ + 6\text{H}_2\text{O}$	24
G3	$\text{NH}_4^+ + 3\text{CO}_2 \rightarrow \text{NO}_2^- + 3\text{CO} + 2\text{H}^+ + \text{H}_2\text{O}$	6
G4	$4\text{NH}_4^+ + 3\text{CO}_2 + 2\text{H}_2\text{O} \rightarrow 4\text{NO}_2^- + 3\text{CH}_4 + 8\text{H}^+$	24
G5	$\text{NH}_4^+ + 4\text{CO}_2 \rightarrow \text{NO}_3^- + 4\text{CO} + 2\text{H}^+ + \text{H}_2\text{O}$	8
G6	$\text{NH}_4^+ + \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{NO}_3^- + \text{CH}_4 + 2\text{H}^+$	8
G7	$2\text{NH}_4^+ + \text{CO} \rightarrow \text{N}_2 + \text{CH}_4 + 2\text{H}^+ + \text{H}_2\text{O}$	6
G8	$\text{NH}_4^+ + \text{CO} + \text{H}_2\text{O} \rightarrow \text{NO}_2^- + \text{CH}_4 + 2\text{H}^+$	6
G9	$3\text{NH}_4^+ + 4\text{CO} + 5\text{H}_2\text{O} \rightarrow 3\text{NO}_3^- + 4\text{CH}_4 + 6\text{H}^+$	24
G10	$\text{N}_2 + 3\text{CO}_2 + \text{H}_2\text{O} \rightarrow 2\text{NO}_2^- + 3\text{CO} + 2\text{H}^+$	6
G11	$4\text{N}_2 + 3\text{CO}_2 + 10\text{H}_2\text{O} \rightarrow 8\text{NO}_2^- + 3\text{CH}_4 + 8\text{H}^+$	24
G12	$\text{N}_2 + 5\text{CO}_2 + \text{H}_2\text{O} \rightarrow 2\text{NO}_3^- + 5\text{CO} + 2\text{H}^+$	10
G13	$4\text{N}_2 + 5\text{CO}_2 + 14\text{H}_2\text{O} \rightarrow 8\text{NO}_3^- + 5\text{CH}_4 + 8\text{H}^+$	40
G14	$\text{N}_2 + \text{CO} + 3\text{H}_2\text{O} \rightarrow 2\text{NO}_2^- + \text{CH}_4 + 2\text{H}^+$	6
G15	$3\text{N}_2 + 5\text{CO} + 13\text{H}_2\text{O} \rightarrow 6\text{NO}_3^- + 5\text{CH}_4 + 6\text{H}^+$	30
G16	$\text{NO}_2^- + \text{CO}_2 \rightarrow \text{NO}_3^- + \text{CO}$	2
G17	$4\text{NO}_2^- + \text{CO}_2 + 2\text{H}_2\text{O} \rightarrow 4\text{NO}_3^- + \text{CH}_4$	8
G18	$3\text{NO}_2^- + \text{CO} + 2\text{H}_2\text{O} \rightarrow 3\text{NO}_3^- + \text{CH}_4$	6

Table 10b Values of $\Delta G_r/e^-$ (kJ/mol e⁻) for reactions in Table 10a at 10 Vulcano hydrothermal sites (see Table 1)

Reaction	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozz Currò
G1	12.25	21.33		12.24	12.52	8.94	8.07	11.79	14.92	
G2	2.34	7.49	3.90	2.02	2.11	-0.46	-1.33	1.27	6.79	
G3	65.20	72.36		63.94	64.07		60.14	63.78	67.88	
G4	55.29	58.51	55.97	53.72	53.66		50.74	53.26	59.76	
G5	69.26	76.04		67.83	68.31	66.98	66.89	62.63	71.15	
G6	59.35	62.20	59.32	57.61	57.90	57.59	57.49	57.86	63.02	
G7	-0.96	2.87		-1.39	-1.37	-3.59	-4.46	-2.24	4.08	
G8	51.99	53.90		50.31	50.18		47.61	49.75	57.05	
G9	56.05	57.59		54.20	54.42	54.45	54.36	54.35	60.31	
G10	118.15	123.38		115.65	115.62		112.21	115.77	120.85	
G11	108.24	109.54	108.04	105.42	105.21		102.81	105.24	112.72	
G12	103.46	108.87		101.18	101.79	101.81	102.18	102.34	104.89	
G13	93.55	95.03	92.57	90.96	91.37	92.41	92.78	91.81	96.76	
G14	104.94	104.93		102.01	101.73		99.68	101.74	110.01	
G15	90.25	90.42		87.55	87.90	89.28	89.65	88.30	94.05	
G16	81.43	87.11		79.49	81.03		87.13	82.19	80.94	
G17	71.52	73.26	69.36	69.27	70.61		77.73	71.67	72.82	
G18	68.21	68.65		65.86	67.14		74.60	68.16	70.11	

The H–O–N–Fe system

Ferroglobus placidus can obtain energy for growth from the oxidation of Fe²⁺ with NO₃⁻ (analogous to the reverse of reaction H6). This and five other redox reactions in the system H–O–N–Fe are given in Table 11a, together with values of $\Delta G_r/e^-$ listed in Table 11b.

The H–O–S–C system

It was shown recently that the anaerobic oxidation of CH₄ (with SO₄²⁻ instead of O₂ as the TEA) can be mediated in marine sediments by an archaeal–bacterial consortium (Hinrichs *et al.*, 1999; Boetius *et al.*, 2000; Scholten *et al.*, 2003). This

Table 11a Reactions in the system H–O–N–Fe

	Reaction	e ⁻
H1	$2\text{NH}_4^+ + 3\text{Fe}_3\text{O}_4 + 16\text{H}^+ \rightarrow \text{N}_2 + 9\text{Fe}^{2+} + 12\text{H}_2\text{O}$	6
H2	$\text{NH}_4^+ + 3\text{Fe}_3\text{O}_4 + 16\text{H}^+ \rightarrow \text{NO}_2^- + 9\text{Fe}^{2+} + 10\text{H}_2\text{O}$	6
H3	$\text{NH}_4^+ + 4\text{Fe}_3\text{O}_4 + 22\text{H}^+ \rightarrow \text{NO}_3^- + 12\text{Fe}^{2+} + 13\text{H}_2\text{O}$	8
H4	$\text{N}_2 + 3\text{Fe}_3\text{O}_4 + 16\text{H}^+ \rightarrow 2\text{NO}_2^- + 9\text{Fe}^{2+} + 8\text{H}_2\text{O}$	6
H5	$\text{N}_2 + 5\text{Fe}_3\text{O}_4 + 28\text{H}^+ \rightarrow 2\text{NO}_3^- + 15\text{Fe}^{2+} + 14\text{H}_2\text{O}$	10
H6	$\text{NO}_2^- + \text{Fe}_3\text{O}_4 + 6\text{H}^+ \rightarrow \text{NO}_3^- + 3\text{Fe}^{2+} + 3\text{H}_2\text{O}$	2

process (reverse of reaction I4) and other reactions in which only S- and C-bearing compounds serve as oxidants and reductants are shown in Table 12a; corresponding values of $\Delta G_r/e^-$ are listed in Table 12b.

Table 11b Values of $\Delta G_r/e^-$ (kJ/mol e^-) for reactions in Table 11a at 10 Vulcano hydrothermal sites (see Table 1)

Reaction	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
H1	-50.40	-45.58	-47.25	-23.72	-33.11	-20.33	-21.83	-38.39	-61.05	
H2	2.55	5.44	4.82	27.98	18.44		30.24	13.59	-8.09	
H3	6.61	9.13	8.17	31.86	22.68	37.71	36.99	18.20	-4.83	
H4	55.50	56.47	56.89	79.68	69.99		82.31	65.58	45.25	
H5	-13.50	1.43	-13.97	-6.52	-14.90	-11.29	-13.56	-28.92	-0.63	
H6	18.78	20.19	18.21	43.52	35.40		57.25	32.00	4.97	

Table 12a Reactions in the system H–O–S–C

	Reaction	e^-
I1	$H_2S + CO_2 \rightarrow S^0 + CO + H_2O$	2
I2	$4H_2S + CO_2 \rightarrow 4S^0 + CH_4 + 2H_2O$	8
I3	$H_2S + 4CO_2 \rightarrow SO_4^{2-} + 4CO + 2H^+$	8
I4	$H_2S + CO_2 + 2H_2O \rightarrow SO_4^{2-} + CH_4 + 2H^+$	8
I5	$3H_2S + CO \rightarrow 3S^0 + CH_4 + H_2O$	6
I6	$3H_2S + 4CO + 8H_2O \rightarrow 3SO_4^{2-} + 4CH_4 + 6H^+$	24
I7	$S^0 + 3CO_2 + H_2O \rightarrow SO_4^{2-} + 3CO + 2H^+$	6
I8	$4S^0 + 3CO_2 + 10H_2O \rightarrow 4SO_4^{2-} + 3CH_4 + 8H^+$	24
I9	$S^0 + CO + 3H_2O \rightarrow SO_4^{2-} + CH_4 + 2H^+$	6

Table 12b Values of $\Delta G_r/e^-$ (kJ/mol e^-) for reactions in Table 12a at 10 Vulcano hydrothermal sites (see Table 1)

Reaction	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
I1	20.65	23.80		17.70	18.42	18.96	19.45	17.98	18.65	
I2	10.75	9.95	13.30	7.48	8.01	9.56	10.05	7.45	10.52	
I3	17.65	23.40		14.35	14.71	13.30	13.20	13.57	18.61	
I4	7.74	9.56	8.02	4.12	4.30	3.90	3.80	3.05	10.49	
I5	7.44	5.34		4.07	4.53	6.43	6.92	3.95	7.81	
I6	4.44	4.95		0.71	0.82	0.77	0.67	-0.46	7.78	
I7	16.64	23.27		13.23	13.47	11.41	11.12	12.10	18.60	
I8	6.74	9.43	6.26	3.00	3.06	2.02	1.72	1.58	10.47	
I9	3.43	4.82		-0.40	-0.41	-1.12	-1.42	-1.93	7.76	

The H–O–S–Fe system

Ferric iron, in the form of crystalline and amorphous solids, has been shown to serve as the TEA for a variety of anaerobic hyperthermophiles, including *Eg. placidus*, *Gg. ahangari*, *Pyrobaculum aerophilum*, *Pb. islandicum* and *Ag. profundus* (Tor *et al.*, 2001). The e^- donors include H_2 and acetate. In Table 13a, we list three reactions in which Fe(III) in magnetite (Fe_3O_4) serves as the TEA to oxidize reduced forms of S (S^0 and H_2S). Corresponding values of $\Delta G_r/e^-$ are given in Table 13b.

The H–O–C–Fe system

Analogous to the H–O–S–Fe system, we consider here the oxidation with magnetite of reduced C-bearing compounds (CO and CH_4). Three reactions and their

Table 13a Reactions in the system H–O–S–Fe

	Reaction	e^-
J1	$H_2S + Fe_3O_4 + 6H^+ \rightarrow S^0 + 3Fe^{2+} + 4H_2O$	2
J2	$H_2S + 4Fe_3O_4 + 22H^+ \rightarrow SO_4^{2-} + 12Fe^{2+} + 12H_2O$	8
J3	$S^0 + 3Fe_3O_4 + 16H^+ \rightarrow SO_4^{2-} + 9Fe^{2+} + 8H_2O$	6

corresponding values of $\Delta G_r/e^-$ are listed in Tables 14a and 14b, respectively.

RESULTS AND DISCUSSION

The amount of energy released or consumed by a reaction can be calculated from values of ΔG_r° and activities derived from *in situ* chemical compositions. In Table 2, we present analytical data of hydrothermal fluids from 10 Vulcano sites. Here, we highlight only some of the most pertinent facts. The

Table 13b Values of $\Delta G_r/e^-$ (kJ/mol e^-) for reactions in Table 13a at 10 Vulcano hydrothermal sites (see Table 1)

Reaction	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
J1	-41.99	-43.12	-37.85	-18.27	-27.21	-10.31	-10.45	-32.21	-57.32	-0.25
J2	-63.52	-57.33	-62.01	-46.08	-55.14	-44.55	-45.96	-64.25	-67.43	-37.83
J3	-46.01	-43.64	-44.89	-22.74	-32.16	-17.86	-18.78	-38.08	-57.37	-12.17

Table 14a Reactions in the system H–O–C–Fe

	Reaction	e^-
K1	$CH_4 + 3Fe_3O_4 + 18H^+ \rightarrow CO + 9Fe^{2+} + 11H_2O$	6
K2	$CH_4 + 4Fe_3O_4 + 24H^+ \rightarrow CO_2 + 12Fe^{2+} + 14H_2O$	8
K3	$CO + Fe_3O_4 + 6H^+ \rightarrow CO_2 + 3Fe^{2+} + 3H_2O$	2

Table 14b Values of $\Delta G_r/e^-$ (kJ/mol e^-) for reactions in Table 14a at 10 Vulcano hydrothermal sites (see Table 1)

Reaction	Punto 1	Punto 7	Stinky Surf Rock	Grip	Stinky Waist	Acque Calde 1	Acque Calde 2	Pozzo Istmo	Pozzo Vasca	Pozzo Currò
K1	-49.44	-48.46		-22.34	-31.74	-16.74	-17.37	-36.15	-65.14	
K2	-108.28	-94.52	-107.79	-99.10	-107.89	-105.61	-108.29	-122.58	-98.06	
K3	-62.65	-66.91		-35.97	-45.63	-29.27	-29.90	-50.19	-75.97	

sites range in temperature from 42.1 to 88.9 °C, in pH from 1.98 to 6.33 and in conductivity from 6.3 to 118 mS/cm. For comparison, local seawater values (data not included in Table 2) are 26.7 °C, 7.95 and 59 mS/cm, respectively. The array of sites covers the major environmental parameters required for moderate thermophiles and hyperthermophiles, acidophiles and neutrophiles, marine and non-marine organisms. The marine component can also be teased out of the Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- , Br^- and SO_4^{2-} concentrations. It is evident that waters at several sites (Punto 1, Punto 7, Pozzo Vasca, Pozzo Currò) consist of only a minor saline component, but the others are characteristic of marine hydrothermal fluids. Concentrations of Fe^{2+} , NH_4^+ , NO_3^- and $H_2S(aq)$ show very large variations; Fe^{2+} ranges from 0.02 ppm at Pozzo Istmo to 309 ppm at Punto 7, NH_4^+ from 2.1 ppm at Pozzo Currò to 55 ppm at Punto 1, NO_3^- is below detection limit (0.1 ppm) at Pozzo Currò, but 200 ppm at Acque Calde 2, and dissolved H_2S is between 0.11 ppm at Pozzo Currò and 12.8 ppm at Pozzo Istmo. Considerable variations are also observed in the dry gases. CO_2 is the dominant component at all sites investigated, but H_2 ranges from 8.1 ppmv at Pozzo Istmo to 19 813 ppmv at Acque Calde 2, and CO is below the detection limit (5 ppmv) at Stinky Surf Rock and 97.9 ppmv at Punto 7. These geochemical variations (and consequently the activities of all the species given in Table 3), when combined with standard Gibbs free energies of reaction (ΔG_r°) in Eq. (1), dictate the overall energetics of the reactions given in Tables 4a–14a.

Values of ΔG_r° at *in situ* temperatures for the 90 redox reactions are not explicitly tabulated here. These values can be

computed at temperatures from 0 to 200 °C using thermodynamic properties for compounds and reactions given in a review by Amend & Shock (2001). We combined values of ΔG_r° with analyses of chemical composition to compute values of ΔG_r . Note that the direction that each reaction is written was chosen, rather arbitrarily, by setting up and following a few simple guidelines. First, H_2 and O_2 never appear in the same reaction, except in reaction A1, the ‘knallgas’ reaction. Second, H_2 or O_2 , when present, always appears on the left-hand side of a reaction. Third, in the three-element systems (e.g. H–O–N), oxidation reactions with O_2 as the TEA are listed first, followed by reduction with H_2 , and then disproportionation. Fourth, in the four-element systems (e.g. H–O–N–S), compounds of the third and fourth elements (N and S in this example) serve both as TEAs and e^- donors; H and O do not undergo redox in these reactions, remaining in the oxidation states +1 and –2, respectively. Because all reactions are written only unidirectionally, the reader should be reminded that a reaction that is endergonic (energy-consuming) in one direction is, of course, exergonic (energy-yielding) in the opposite direction. For example, the oxidation of NH_4^+ to NO_2^- coupled with the reduction of S° to H_2S (reaction F8) is endergonic (positive values of ΔG_r) at all 10 sites, but the reduction of NO_2^- to NH_4^+ coupled with the oxidation of H_2S to S° (reverse of reaction F8) is exergonic (see Table 9b).

It is apparent from values of $\Delta G_r/e^-$ given in Tables 4b–8b that aerobic oxidation reactions are generally the most exergonic. Reduction reactions in which H_2 serves as the e^- donor and disproportionation reactions commonly yield much less energy or are even endergonic. In other words, O_2 is a

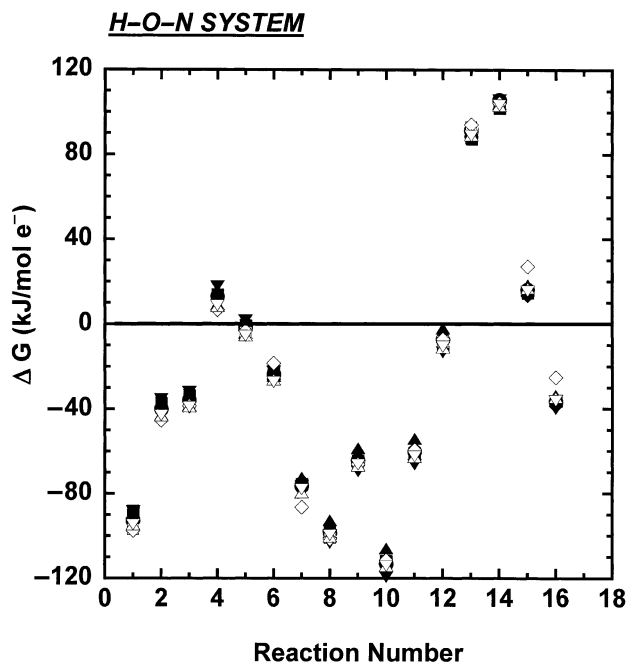


Fig. 2 ΔG_r (kJ/mol e^-) of the reactions in the H-O-N system listed in Table 5a (data shown in Table 5b) plotted against reaction number. In reactions B1–B6, O_2 is the terminal electron acceptor for oxidation of N-compounds; reactions B7–B12 describe the reduction of N-compounds in which H_2 is the electron donor, and reactions B13–B16 represent disproportionation reactions. Symbols correspond to the sampling sites as follows: ●, Punto 1; ■, Punto 7; ◆, Stinky Surf Rock; ▲, Pozzo Istmo; ▼, Pozzo Vasca; ○, Pozzo Currò; □, Acque Calde 1; ◇, Acque Calde 2; △, Stinky Waist; ▽, Grip. Equilibrium ($\Delta G_r = 0$) is indicated by a solid horizontal line.

powerful oxidant, followed by NO_3^- , Fe(III), SO_4^{2-} , CO_2 and other TEAs. This view is entirely consistent with vertical pore-water profiles of TEAs in typical freshwater, soil and marine systems (e.g. Nealson & Stahl, 1997) as well as the order of redox potentials for inorganic redox couples (commonly referred to as the electron tower or the pe or Eh scale). The detailed picture of the hydrothermal system at Vulcano, however, is quite a bit more complex, especially in the H-O-N and H-O-Fe systems. To depict the energetic variations among reactions and among sites clearly, values of $\Delta G_r/e^-$ are plotted against reaction number in Figs 2–10 for several of the three-element and four-element systems. The energetic variations among reactions in a chemical system are discussed first, followed by a discussion on the energy from particular reactions at the 10 different sites.

Variations in free energy among reactions

It can be seen in Fig. 2 that $\Delta G_r/e^-$ (taken from Table 5b) is negative for 11 reactions in this system, but positive or near 0 for five reactions. Note that O_2 is the TEA in reactions B1–B6; reactions B1–B3 and B6 are exergonic, but reactions B4 and B5 are endergonic or near equilibrium. In other words, the

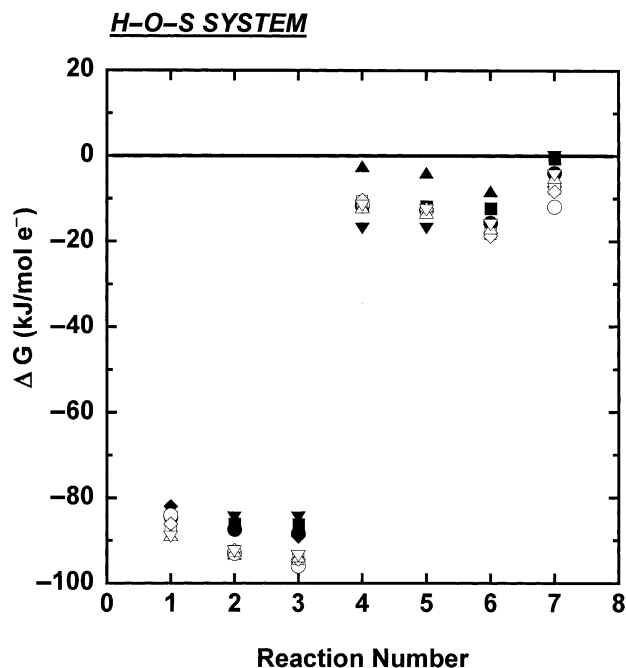


Fig. 3 ΔG_r (kJ/mol e^-) of the reactions in the H-O-S system listed in Table 6a (data shown in Table 6b) plotted against reaction number. In reactions C1–C3, O_2 is the terminal electron acceptor for oxidation of S-compounds; reactions C4–C6 describe the reduction of S-compounds in which H_2 is the electron donor, and reaction C7 represents a disproportionation reaction. Symbols are as in Fig. 2. Equilibrium ($\Delta G_r = 0$) is indicated by a solid horizontal line.

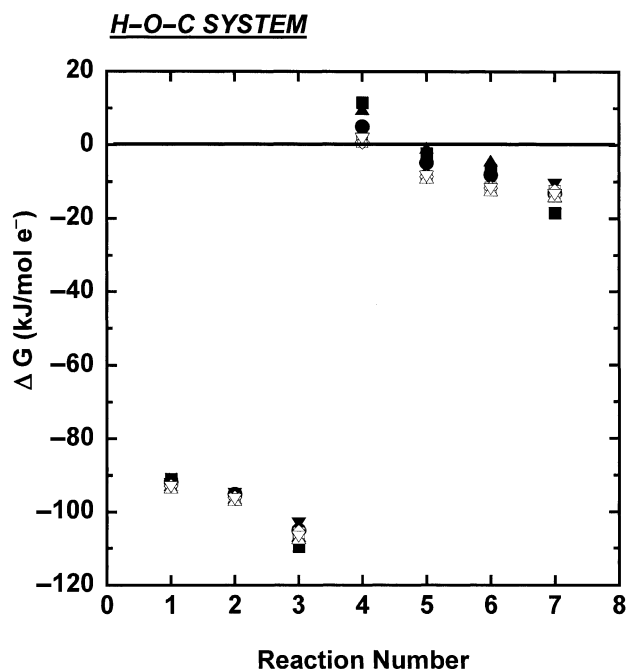


Fig. 4 ΔG_r (kJ/mol e^-) of the reactions in the H-O-C system listed in Table 7a (data shown in Table 7b) plotted against reaction number. In reactions D1–D3, O_2 is the terminal electron acceptor for oxidation of C-compounds; reactions D4–D6 describe the reduction of C-compounds in which H_2 is the electron donor, and reaction D7 represents a disproportionation reaction. Symbols are as in Fig. 2. Equilibrium ($\Delta G_r = 0$) is indicated by a solid horizontal line.

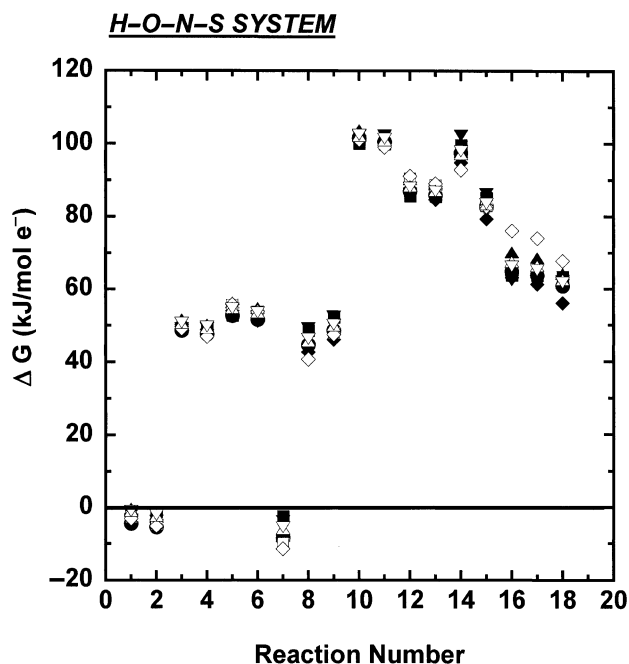


Fig. 5 ΔG_r (kJ/mol e^-) of the reactions in the H-O-N-S system listed in Table 9a (data shown in Table 9b) plotted against reaction number. Symbols are as in Fig. 2. Equilibrium ($\Delta G_r = 0$) is indicated by a solid horizontal line.

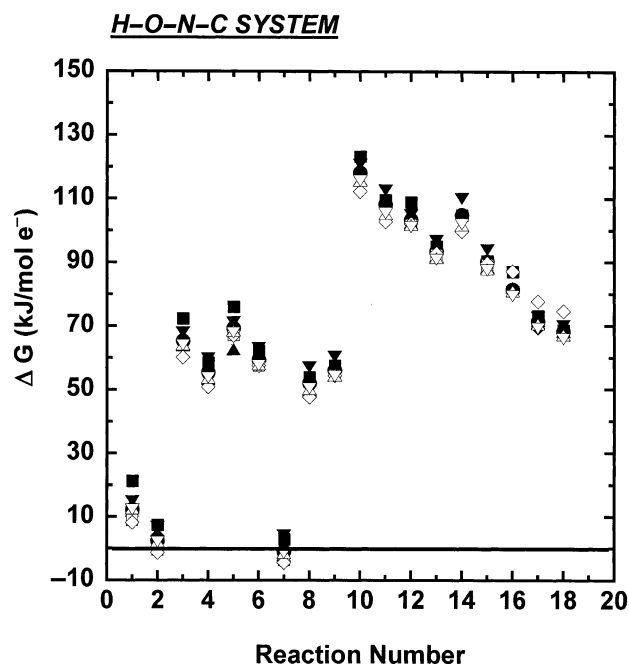


Fig. 6 ΔG_r (kJ/mol e^-) of the reactions in the H-O-N-C system listed in Table 10a (data shown in Table 10b) plotted against reaction number. Symbols are as in Fig. 2. Equilibrium ($\Delta G_r = 0$) is indicated by a solid horizontal line.

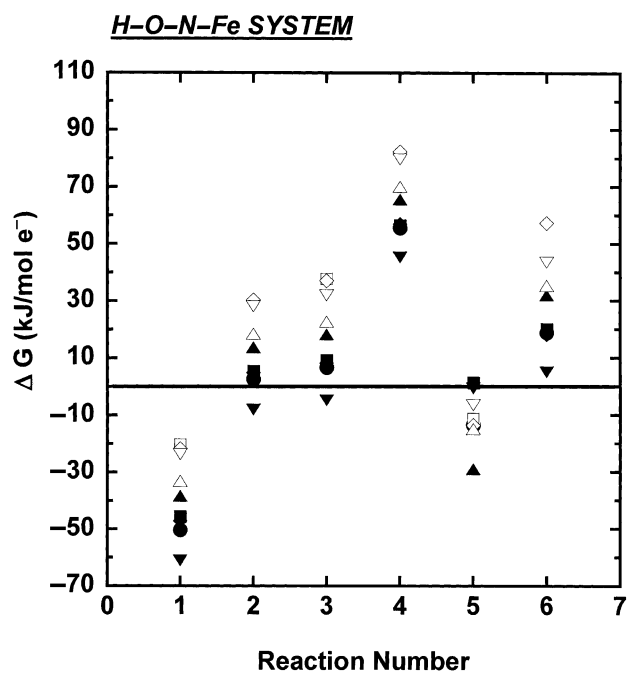


Fig. 7 ΔG_r (kJ/mol e^-) of the reactions in the H-O-N-Fe system listed in Table 11a (data shown in Table 11b) plotted against reaction number. Symbols are as in Fig. 2. Equilibrium ($\Delta G_r = 0$) is indicated by a solid horizontal line.

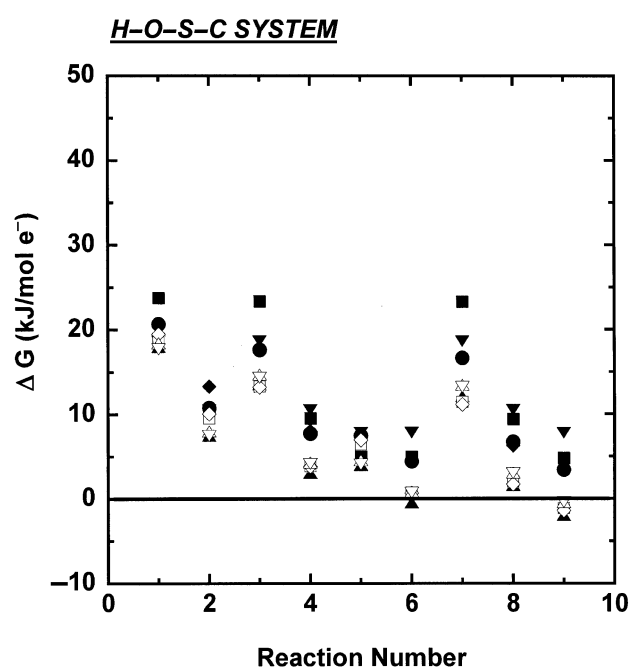


Fig. 8 ΔG_r (kJ/mol e^-) of the reactions in the H-O-S-C system listed in Table 12a (data shown in Table 12b) plotted against reaction number. Symbols are as in Fig. 2. Equilibrium ($\Delta G_r = 0$) is indicated by a solid horizontal line.

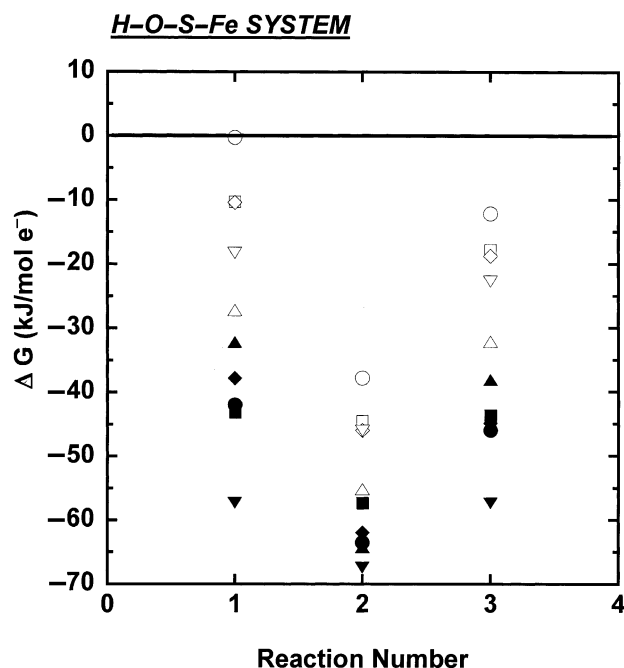


Fig. 9 ΔG_r (kJ/mol e^-) of the reactions in the H-O-S-Fe system listed in Table 13a (data shown in Table 13b) plotted against reaction number. Symbols are as in Fig. 2. Equilibrium ($\Delta G_r = 0$) is indicated by a solid horizontal line.

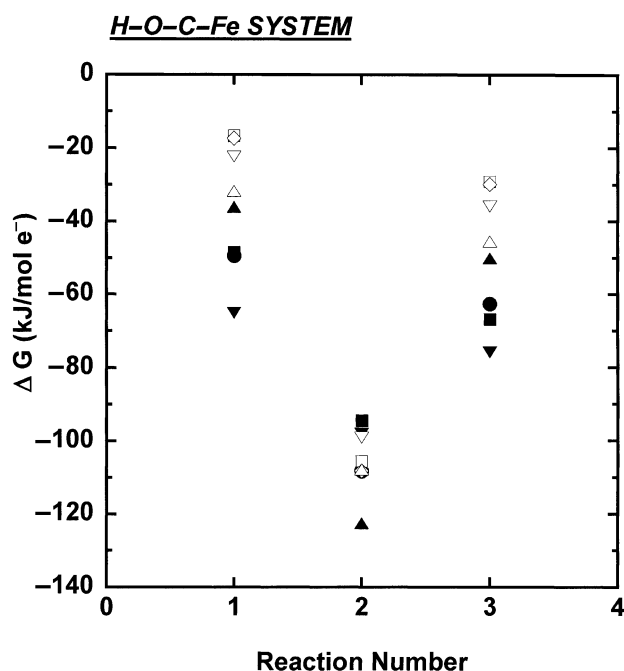


Fig. 10 ΔG_r (kJ/mol e^-) of the reactions in the H-O-C-Fe system listed in Table 14a (data shown in Table 14b) plotted against reaction number. Symbols are as in Fig. 2.

aerobic oxidation of N_2 represents a poor metabolic strategy for thermophiles at Vulcano, but the aerobic oxidation of NH_4^+ or NO_2^- could support thermophile growth. Note also that several reactions (B7–B11), in which NO_3^- or NO_2^- serves as the TEA, are more exergonic than aerobic oxidation of NH_4^+ to NO_3^- or to NO_2^- (reactions B2 and B3), but comparable with aerobic oxidation of NH_4^+ to N_2 (reaction B1). As examples, reaction B10, in which NO_2^- is the oxidant, yields between 106 and 120 kJ/mol e^- , but NH_4^+ oxidation with O_2 (reactions B1–B3) yields only 32–98 kJ/mol e^- . Furthermore, the anaerobic oxidation of NH_4^+ to N_2 (reverse of reactions B13 and B14) is strongly exergonic at Vulcano, releasing from 87 to 106 kJ/mol e^- .

The H-O-S and H-O-C systems, by contrast, show more familiar behaviour (Figs 3 and 4). Stated differently, the aerobic oxidation reactions (C1–C3 and D1–D3) are strongly exergonic, yielding between 82 and 96 kJ/mol e^- in the H-O-S system and between 91 and 110 kJ/mol e^- in the H-O-C system. In the H-O-S system (Fig. 3), the reactions with H_2 as the e^- donor (C4–C6) are slightly exergonic, with values of ΔG_r ranging from –2 to –19 kJ/mol e^- ; values of ΔG_r for the disproportionation of S^0 to SO_4^{2-} and H_2S (reaction C7) are between 0 and –12 kJ/mol e^- . In the H-O-C system (Fig. 4), CO_2 reduction to CO (reaction D4) is endergonic ($\Delta G_r/e^- = 0$ –12 kJ/mol e^-), but CO_2 or CO reduction to CH_4 (reactions D5 and D6) and the disproportionation of CO to CO_2 and CH_4 (reaction D7) are slightly exergonic ($\Delta G_r/e^- = 0$ to –19 kJ/mol e^-). Analogous to the H-O-S system, aerobic oxidation of CH_4 and CO (reactions D1–D3) yield the most energy ($\Delta G_r/e^- = -91$ to –110 kJ/mol e^-).

In the H-O-Fe system (values of ΔG_r not plotted), both the aerobic oxidation of Fe^{2+} (reaction E1) and the reduction of Fe(III) in magnetite with H_2 as the e^- donor (reaction E2) are strongly exergonic (Table 8b). Values of ΔG_{E1} are between –27 and –84 kJ/mol e^- and those of ΔG_{E2} between –28 and –75 kJ/mol e^- . At some sites (Grip, Stinky Waist, Acque Calde 1, Acque Calde 2, Pozzo Istmo), reaction E1 is more energy-yielding than reaction E2, but at others (Punto 1, Punto 7, Stinky Surf Rock, Pozzo Vasca), the opposite is true.

Values of $\Delta G_r/e^-$ as a function of reaction number in the four-element systems H-O-N-S, H-O-N-C, H-O-N-Fe, and H-O-S-C are plotted, respectively, in Figs. 5–8. It can be seen that, with few exceptions (reactions F7, H1, H5), all of the reactions as written are either endergonic or near equilibrium. Most of the reactions in the H-O-N-S (Fig. 5) and H-O-N-C (Fig. 6) systems consume 40–120 kJ/mol e^- , but those in the H-O-N-Fe (Fig. 7) and H-O-S-C (Fig. 8) systems are considerably less endergonic, with values of $\Delta G_r/e^-$ commonly less than 50 kJ/mol e^- . The most endergonic reactions in the H-O-N-S and H-O-N-C systems are the N_2 oxidation reactions (F10–F15 and G10–G15), in which SO_4^{2-} or S^0 and CO_2 or CO serve as the TEAs; values of $\Delta G_r/e^-$ for these reactions range from 79 to 104 kJ/mol e^- (Fig. 5) and from 87 to 124 kJ/mol e^- (Fig. 6). Similar to the examples of

aerobic oxidation of N_2 noted above, the anaerobic oxidation of N_2 represents an energetically costly mode of metabolism for Vulcano thermophiles. It should perhaps be reiterated, however, that endergonic reactions as written represent potential energy sources to micro organisms able to catalyse the reverse reactions. In these four four-element systems, the energetically best option for microbial metabolism is the reduction of Fe(III) in magnetite to Fe^{2+} coupled with the oxidation of NH_4^+ to N_2 (reaction H1). This reaction yields between 20 and 62 kJ/mol e^- , depending on site.

Apart from the H_2 - and O_2 -consuming reactions discussed above (Tables 4b–8b, Figs 2–4), the most energy-yielding reactions are those in which Fe(III) in magnetite oxidizes the reduced S- or C-bearing compounds. In the H–O–S–Fe system (Fig. 9), values of $\Delta G_r/e^-$ are between 0 and 68 kJ/mol e^- ; especially exergonic is the magnetite/ Fe^{2+} – H_2S/SO_4^{2-} couple (reaction J2), which yields from 37 to 68 kJ/mol e^- . In the H–O–C–Fe system (Fig. 10), values of $\Delta G_r/e^-$ range from –16 to –123 kJ/mol e^- . The most energy-yielding reaction in this system is the magnetite/ Fe^{2+} – CH_4/CO_2 couple (reaction K2), with values of $\Delta G_r/e^-$ from –94 to –123 kJ/mol e^- . This anaerobic oxidation of CH_4 is, in fact, more energy-yielding per electron transferred than most of the aerobic oxidation reactions in the H–O–N, H–O–S, H–O–C or H–O–Fe systems discussed above.

Variations in free energy among sites

In the previous section, we discussed the range of free energy produced or consumed by 90 redox reactions at Vulcano. We now focus on the range of free energy produced or consumed by specific reactions at the 10 different sites investigated. It should be reiterated that most of the variation in ΔG_r as calculated with Eq. (1) arises from the $RT \ln Q_r$ term and not the ΔG_r° term. More specifically, the differences in the activities of reactants and products at the 10 sites affect the variations of ΔG_r far more than changes in ΔG_r° due to the differences in temperature.

For many of the reactions, values of ΔG_r among the sites tend to cluster, and differences range from relatively small (< 10 kJ/mol e^-) to intermediate (~ 10–20 kJ/mol e^-) for reactions in the H–O, H–O–N, H–O–S, H–O–C, H–O–N–S, H–O–N–C and H–O–S–C systems (see Tables 4b–7b, 9b, 10b, and 12b and Figs 2–6 and 8). As an example, the ‘knall-gas’ reaction (A1 in Table 4a) is strongly exergonic at all sites investigated (see Table 4b), but the range of values of $\Delta G_r/e^-$ is only 8.8 kJ/mol e^- . At Pozzo Istmo and Punto 7, thermophiles that catalyse this reaction (e.g. *Aq. aeolicus*) could gain up to ~ 97 kJ/mol e^- , and at Grip, Stinky Waist, Acque Calde 1 and Acque Calde 2, they could gain only slightly more energy, ~ 105 kJ/mol e^- .

In a second example, we consider reactions in the H–O–N system. Of all the sites, Pozzo Istmo yields the least energy for the reduction reactions (B7–B12), and Pozzo Vasca, with one

exception (reaction B7), yields the most. This is primarily due to the low activities of H^+ and H_2 at Pozzo Istmo and high activities of these species at Pozzo Vasca; note that H^+ and H_2 , if present, are reactants in these reactions. At Pozzo Vasca, however, four of the six aerobic oxidation reactions in the H–O–N system (B2–B5) have the highest values of ΔG_r . This is again largely due to the low pH at Pozzo Vasca, as H^+ is a product in these four reactions. The aerobic oxidation reactions (B1–B6) are the most exergonic (or least endergonic) at Grip and Acque Calde 2, where activities of O_2 are relatively high, pH is only slightly acidic and, in the case of Acque Calde 2, the activity of NH_4^+ is elevated compared with other sites.

In a third example, we investigate the H–O–S system, which includes some of the most ubiquitous reactions in thermophilic chemolithoautotrophy – sulphide oxidation, S° reduction and sulphate reduction. Partial and complete aerobic sulphide oxidation (reactions C1 and C2, respectively) yield the least amount of energy at Pozzo Vasca, Punto 7 and Stinky Surf Rock, where the pHs are low and activities of O_2 and H_2S are low to moderate. By contrast, these reactions are most exergonic at the sites where pH is slightly acidic^c and activities of O_2 and H_2S are moderate to high; these sites include Grip, Stinky Waist, Acque Calde 1, Acque Calde 2 and Pozzo Istmo. Sulphate reduction (reactions C4 and C5) is by far the most energy-yielding at Pozzo Vasca, where the pH is lowest and activities of H_2 and SO_4^{2-} are high. These reactions are only slightly exergonic at Pozzo Istmo, where the activity of H_2 (5.0×10^{-9}) is lowest among all the sites. Given that the reduction of S° to H_2S (reaction C6) is the preferred mode of metabolism for numerous hyperthermophiles at Vulcano and elsewhere, the energy yield is surprisingly low. This reaction releases between 8.26 kJ/mol e^- at Pozzo Istmo and 18.72 kJ/mol e^- at Acque Calde 2. The low a_{H_2} and high a_{H_2S} at Pozzo Istmo are responsible for the modest value of $\Delta G_{C6}/e^-$, and the highest a_{H_2} (7.2×10^{-6}), at Acque Calde 2, translates directly to the most negative value of $\Delta G_{C6}/e^-$.

The largest differences in $\Delta G_r/e^-$ (up to ~ 60 kJ/mol e^-) are observed in the four iron-bearing systems (H–O–Fe, H–O–N–Fe, H–O–S–Fe, H–O–C–Fe). These differences are due only partly to the large variations in Fe^{2+} concentrations (and hence activities) at the Vulcano sites, which range from 0.02 ppm at Pozzo Istmo to 309 ppm at Punto 7. Since each of the Fe-redox reactions as written also includes 1–2 mol of H^+ per mole of Fe^{2+} , the large variations in pH have perhaps the greatest effect on the ranges of $\Delta G_r/e^-$ in these systems. Correspondingly, the aerobic and anaerobic oxidation of Fe^{2+} to Fe(III) in magnetite is most exergonic (or least endergonic) at Pozzo Currò, Acque Calde 1, Acque Calde 2 and Grip, where, despite only modest concentrations of Fe^{2+} , the pH is

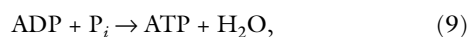
^cRecall that, owing to the temperature dependence of the equilibrium constant for the water dissociation reaction, neutral pH is 6.6 at 50 °C, 6.1 at 100 °C and 5.8 at 150 °C.

only slightly acidic. Conversely, at Pozzo Vasca and Punto 7, where the concentrations of Fe^{2+} are very high (~ 300 ppm) and the pH is low (2–3), values of $\Delta G_r/\text{e}^-$ for aerobic and anaerobic Fe^{2+} oxidation are the least exergonic or most endergonic. For example, the oxidation of Fe^{2+} to Fe(III) coupled to the reduction of NO_3^- to NH_4^+ (reverse of reaction H3), which can be used by the Vulcano hyperthermophile *Eg. placidus* to gain energy in laboratory growth studies, yields ~ 37 kJ/mol e^- at Acque Calde 1 and Acque Calde 2, but consumes ~ 5 kJ/mol e^- at Pozzo Vasca.

Minimum energy requirements for cells

In the two previous sections, we evaluated the *in situ* energy yields from numerous dissimilatory reactions. A logical next step would be to describe how these extracellular energy sources are converted to drive the intracellular synthesis of adenosine triphosphate (ATP), the metabolic energy currency in living cells. In a seminal paper, Thauer *et al.* (1977) presented a thermodynamic treatment of energy conservation in anaerobic chemotrophs. Extensive laboratory and theoretical investigations have further elaborated on the energetics of ATP formation and the minimum 'energy quantum' required in microbial metabolism (e.g. Maloney, 1983; Schink & Thauer, 1988; Schink, 1990). Several studies have also shown that at least some anaerobes can grow on energy yields close to the thermodynamic limit of equilibrium, i.e. $\Delta G_r = 0$ (Conrad *et al.*, 1986; Dwyer *et al.*, 1988; Smith & McCarty, 1989; Seitz *et al.*, 1990; Hickey & Switzenbaum, 1991; Wu *et al.*, 1994; Jackson & McInerney, 2002). All of these studies, however, were carried out at 25 °C. Because the thermodynamic properties of ATP have not been determined at elevated temperatures, the next step in deciphering the energy requirements of chemolithoautotrophs at Vulcano is currently intractable.

In fact, several factors preclude an assessment of the extracellular–intracellular energy conversion for life in hydrothermal systems, or any high-temperature environment. First, the value of ΔG_r° for the phosphorylation of adenosine diphosphate (ADP) to ATP, generally written as



where P_i denotes inorganic phosphate, is known only at 25 °C (32 kJ mol $^{-1}$). Second, even if ΔG_r° were known as a function of temperature, it would not suffice in linking the energy availability outside the cell with the energy requirement inside the cell. In a dynamic system, where cell numbers increase and decrease with time, the representation of ATP synthesis by phosphorylation of ADP, as shown in reaction (9), is insufficient. Biomass, including ATP, is synthesized from substrates available in the environment and not merely from direct precursor molecules such as ADP. In chemolithoautotrophs, this translates to synthesis from inorganic monomers,

including CO_2 , NO_3^- , NH_4^+ , H_2S and phosphate. As an example, ATP synthesis can be written as



To evaluate ΔG_{10}° at elevated temperatures, the standard Gibbs free energies of each reactant and product (ΔG_i°) need to be known at the temperature of interest. Values of ΔG_i° for CO_2 , NH_4^+ , PO_4^{3-} and hundreds of other aqueous, gaseous, liquid and solid compounds of biological and geological interest can be calculated to high temperatures and pressures (Amend & Shock, 2001), but ΔG_i° of ATP has neither been evaluated at standard conditions nor as a function of temperature. Third, the aforementioned studies on ATP synthesis at 25 °C almost exclusively considered fermentation and respiration of organic compounds; little is known with respect to energy minima and thermodynamic efficiency in chemolithoautotrophy. However, in an attempt to remedy this situation, group additivity approaches are being employed to estimate values of ΔG_i° for ATP and other nucleotides as a function of temperature and pressure (H. C. Helgeson, pers. comm.). In the interim, until the thermodynamic properties of intracellular catabolic and anabolic pathways are evaluated at a range of temperatures, assessments of potential *in situ* energy yields from myriad redox reactions, as tabulated in the present study, provide a framework for understanding the net bioenergetics of chemolithoautotrophy in hydrothermal systems.

CONCLUDING REMARKS

Many micro organisms, including moderate thermophiles and hyperthermophiles that thrive in hydrothermal systems, obtain chemical energy by catalysing thermodynamically favourable, but kinetically inhibited, redox reactions. Here, we evaluated the maximum amount of Gibbs free energy from 90 such reactions in 10 seeps, wells and vents at Vulcano. These sites serve as model systems of shallow marine and coastal hydrothermal environments and represent the 'type localities' of several thermophilic archaea and bacteria.

The present study is limited to the energetics of chemolithoautotrophy at Vulcano. The following will complement this study: investigations of the energetics of chemoorganoheterotrophy at Vulcano, the forms and abundances of geochemical energy in continental hot springs (e.g. Yellowstone National Park) and variations in free energy with time to incorporate aspects of fluid dynamics in active volcanic systems; several of these investigations are currently in progress (Shock *et al.*, in press; Skoog *et al.*, in press; Svensson *et al.*, in press). We hope that energetic calculations of the type discussed here prove useful in characterizing the microbiology/geochemistry interface in hydrothermal systems, designing growth media to culture and isolate novel thermophiles, understanding the geographical distribution of known organisms, and planning

and interpreting laboratory microbial growth experiments with isolates and consortia in batch and continuous culture.

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