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Energetics of potential heterotrophic metabolisms in the marine hydrothermal system of Vulcano Island, Italy

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Abstract

Values of overall Gibbs free energy of 144 organic oxidation (respiration) and disproportionation (fermentation) reactions are calculated at the temperatures and chemical compositions that exist in nine submarine vents, sediment seeps and geothermal wells in the hydrothermal system of Vulcano Island, Italy. The organic compounds considered here include four carboxylic acids (formic, acetic, propanoic and lactic), two C_5 aldoses (arabinose and xylose), three C_6 aldoses (galactose, glucose and mannose), and 15 protein-forming amino acids (Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Tyr, and Val). Oxidation of these compounds is coupled to five redox pairs: O_2/H_2O, SO_4^{2-}/H_2S, S^0/H_2S, NO_3^-/NH_4^+ and Fe_3O_4/Fe^{2+}. Energy yields from potential respiration reactions range from 6 to 118 kJ/mol of electrons transferred and show systematic behavior with respect to the terminal electron acceptor. Overall, respiration with O_2 yields the most energy (98–118 kJ/mol e^-), followed by reactions with NO_3^- (53– 86 kJ/mol e^-), magnetite (29–91 kJ/mol e^-), S^0 (11–33 kJ/mol e^-) and SO_4^{2-} (6–34 kJ/mol e^-). Energy yields show little correlation with organic compound family, but are correlated with fluid pH. Variability in energy yields across the nine sites is greatest for Fe(III) reduction and is primarily influenced by pH and the activity of Fe^{2+}. In addition to the potential respiration reactions, the energetics of 24 potential fermentation reactions are also calculated. As expected, fermentation reactions generally yield much less energy than respiration. Normalized to the number of moles of carbon transferred, fermentation yields −8 to 71 kJ/mol C, compared with 16 to 531 kJ/mol C for respiration reactions. All respiration and fermentation reactions, except for methionine (Met) fermentation, are exergonic under the *in situ* hydrothermal conditions and represent a plethora of potential metabolisms for Vulcano's diverse thermophilic heterotrophs.

1. Introduction

A majority of the thermophiles that have been isolated from hydrothermal systems are heterotrophs, using organic compounds to gain energy and synthesize biomass (Amend and Shock, 2001). In laboratory culturing studies, many of these organisms are grown on poorly defined, complex organic substrates, making interpretations of metabolic details difficult and computations of reaction energetics impossible. However, for some thermophilic heterotrophs, the specific compounds required for growth have been identified, including carboxylic acids, sugars and amino acids. For example, species of *Thermococcus*, *Pyrococcus* and *Desulfurococcus* can utilize mixtures of amino acids (Erauso et al., 1993; Hoaki et al., 1993, 1994; Watrin et al., 1995; Godfroy et al., 1996; Dirmeier et al., 1998; González et al., 1998). A number of thermophiles can also use sugars for growth (e.g., glucose, mannose and galactose), including *Bacillus aeolius*, *Caloramator proteoclasticus*, *Deinococcus* spp. *geothermalis* and *murrayi*, *Geobacillus* spp. *gargensis* and *vulcani*, *Sulfolobus yangmingensis*, *Thermanaerovibrio acidaminovorans*, and *Thermotoga maritima*, (Huber et al., 1986; Ferreira et al., 1997; Tarlera et al., 1997; Baena et al., 1999; Jan et al., 1999; Caccamo et al., 2000; Gugliandolo et al., 2003; Nazina et al., 2004). Additionally, some thermophiles grow on simple organic acid anions such as acetate, pyruvate, and

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lactate (e.g., *Anaeromusa acidaminophila*, *Archaeoglobus fulgidus*, *Ferroglobus placidus*, *Geobacillus gargensis*, *Geoglobus ahangari*, and *Thermoacetogenium phaeum*) (Nanninga et al., 1987; Stetter, 1988; Hafenbradl et al., 1996; Baena et al., 1999; Hattori et al., 2000; Tor et al., 2001; Kashefi et al., 2002; Nazina et al., 2004), and still others metabolize hydrocarbons (e.g. several species of *Geobacillus*) (Baena et al., 1999; Caccamo et al., 2000; Nazina et al., 2001; Nazina et al., 2004). Oxidation of these organic compounds is necessarily coupled to the reduction of terminal electron acceptors (TEAs). Elemental sulfur (S^0) is a common TEA, and in some cases also serves to detoxify metabolic products (Hoaki et al., 1994; Godfroy et al., 1996; Kengen et al., 1996; Magot et al., 1997; Dirmeier et al., 1998; González et al., 1998; Barbier et al., 1999). Other TEAs can also be utilized, including nitrate, ferric iron, oxygen, thiosulfate and sulfate (Magot et al., 1997; Jan et al., 1999; Caccamo et al., 2000; Hattori et al., 2000; Nicolaus et al., 2000; Tor et al., 2001; Kashefi et al., 2002; Nazina et al., 2004). Although metabolic reactants are often reported, only in rare cases have specific heterotrophic metabolisms been ascertained from culturing experiments (Tarlera et al., 1997; Plugge et al., 2000; Tor et al., 2001).

Nonetheless, the isolation of obligate heterotrophs from thermophilic environments is *de facto* evidence that heterotrophic respiration and/or fermentation reactions yield energy in hydrothermal systems. Furthermore, the correlation between amino acid consumption and cell density of hyperthermophilic heterotrophs in a shallow marine hydrothermal system (Hoaki et al., 1995) suggests that heterotrophic metabolisms are an important part of nutrient cycling in hydrothermal ecosystems. *In situ* heterotrophic reaction energetics may help to identify these net metabolisms. In order to calculate energy yields of putative metabolic reactions, accurate measurements of organic compounds must be coupled to complete inorganic geochemical characterizations of hydrothermal fluids and standard free energies at elevated temperatures and pressures. While some organic compounds have been identified in hydrothermal systems, measurements of their concentrations are sparse and rarely placed in sufficient geochemical context to allow for the evaluation of reaction energetics. As an example, several families of organic compounds have been identified in the hydrothermal sediments of Guaymas Basin (Gulf of California), including petroleum, amino acids, carboxylic acids and polycyclic aromatic hydrocarbons (Simoneit and Lonsdale, 1982; Haberstroh and Karl, 1989; Kawka and Simoneit, 1990; Martens, 1990). Amino acids have also been identified in the hydrothermal systems in the Bransfield Straight (Antarctic Peninsula), the Suiyo Seamount (Izu-Bonin island arc), the Juan de Fuca Ridge (East Pacific

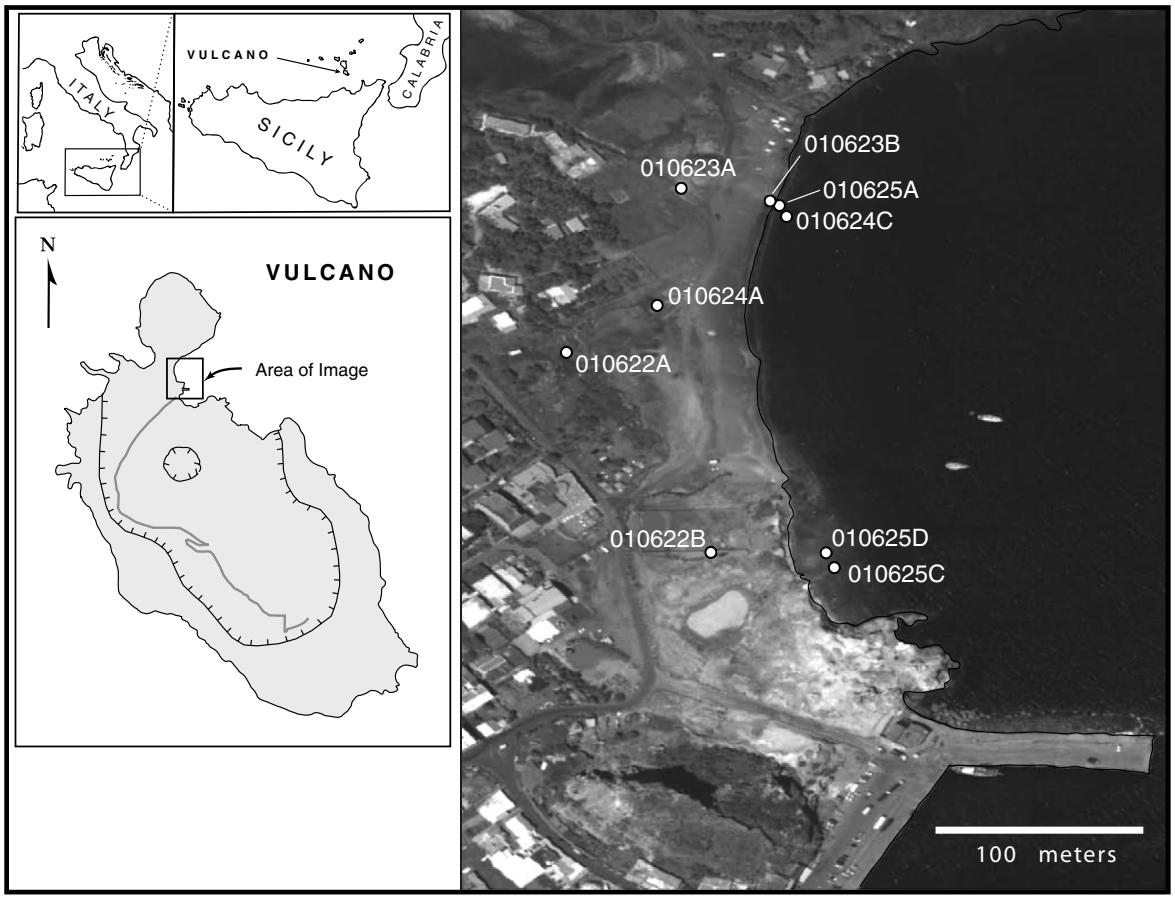

Fig. 1. Aerial photograph of the Baia di Levante, Vulcano Island, Italy. The nine sampling sites discussed in the text are indicated by circles and sample numbers (Table 1).

Table 1
Analytical and field data at nine sites in the Vulcano hydrothermal system

Sample name: Sample number:	Pozzo Istmo 010622a	Pozzo Vasca 010622b	Acque Calde 1 010625c	Acque Calde 2 010625d	Punto Uno 010623a	Punto Sette 010624a	Stinky Surf Rock 010623b	The Grip 010625a	Stinky Waist 010624c
Temperature	56.4	81.8	87.5	87.5	88.9	81.0	84.8	54.0	50.8
pH	5.840	1.976	5.518	5.650	3.561	2.717	3.673	5.205	5.207
O_2,aq	382	406	2900	2100	700	380	643		
H_2,g	8.1	2909		19813	1812	221	1068	2374	
SO_4^{2-}	3013	5976	2690	2624	1546	3646	2901	2268	2667
ΣS^{2-}	12.8	4.2	12.3	9.3	5.1	8.7	0.75	10.4	6
CO_2,g	99.1	99.16		98.84	98.9	70.66	98.83	98.64	
CH_4,g	1104	1215		1289	1228	5568	1253	1497	
NO_3^-	38	34	78	200	6	4	1.6	1.9	3.7
NH_4^+	3.31	30	11.4	20.6	55	5	12	10.6	11
Fe^{2+}	0.02	293	1.5	0.7	4	309	7.7	12.1	1.5
Organic acids									
Formic acid	0.07	0.18	0.06	0.06	0.14	1.14	0.18	0.05	0.06
Acetic acid	0.09	1.45	0.02	0.27	0.24	1.14	1.86	0.30	1.31
Propanoic acid	1.26	0.41	0.09		0.07	0.06	0.11	0.11	1.83
Lactic acid			0.05		0.29	0.99			
Aldoses									
Arabinose			18		178	2645			
Xylose		2349			2232	6689			
Galactose		55	95	22	875	8191			
Glucose		745	522	34	3933	23068			
Mannose	62	5248			2464	9955			
Amino acids[a]									
Alanine	0.21	11.74	0.46	0.24	2.81	6.3			
Arginine		4.78	0.23	0.16	0.39	2.01			
Aspartic acid	0.09	6.72	0.23	0.03	3.13	9.74			
Glutamic acid	0.14	18.18	0.32	0.14	3.75	7.01			
Glycine	0.11	27.53		0.02	2.72	7.18			
Histidine		3.93	0.05		0.09	0.66			
Isoleucine		3.13	0.09		1.32	2.4			
Leucine		4.37	0.15		1.69	2.87			
Lysine	0.86	6.16	0.78	1.27	3.62	3.31			
Methionine	0.17	0.61			0.12	0.27			
Phenylalanine		2.33	0.07		0.83	1.43			
Serine	0.20	4.57	0.03		1.58	4.02			
Threonine		3.01	0.14		1.58	3.93			
Tyrosine		4.52			0.37	0.60			
Valine		4.4	0.03		4.14	3.56			
Total	1.78	105.98	2.58	1.86	28.14	55.29			

Temperature reported in (°C) and pH in standard units ($-\log a_{H+}$). Aqueous inorganic species are reported in ppm, except for dissolved oxygen, which is in ppb; gas concentrations given in ppmv of the dry gas except CO_2, which is reported as vol % of the dry gas. Concentrations of organic acids in ppm, aldoses in nM and amino acids in μM.

[a] Combined values of DFAA (dissolved free amino acids) and DCAA (dissolved combined amino acids) from Svensson et al. (2004).

Rise), shallow marine vent sediments near Kodakara-Jima Island (Japan), and in a subsurface hydrothermal mine (Japan) (Silfer and Engel, 1990; Hoaki et al., 1995; Andersson et al., 2000; Takano et al., 2003a,b, 2004). Recently, the concentrations of amino acids, carboxylic acids and neutral aldoses were measured in the shallow marine hydrothermal system of Vulcano Island, Italy (Amend et al., 1998, 2003b, 2004; Svensson et al., 2004; Rogers, 2006). Because measurements at Vulcano were made in conjunction with extensive geochemical characterizations, *in situ* heterotrophic reaction energetics can be evaluated.

Here we combine these analyses with detailed *in situ* geochemical measurements to determine the energetics of 144 potential heterotrophic metabolisms at 9 sites in the Vulcano hydrothermal system. Respiration of carboxylic acids, neutral aldoses, and amino acids with 5 TEAs (O_2, SO_4^{2-}, S^0, NO^{3-} and Fe(III) in magnetite) is considered, along with examples of fermentation of each of these organic families. The effect of *in situ* geochemical parameters on the overall free energy of reaction (ΔG_r) is discussed. Energy yields from heterotrophic metabolisms are also compared to the *in situ* energetics of 90 lithotrophic metabolisms. For the first time a complete energy profile, including both heterotrophic and lithotrophic metabolisms, has been established for a hydrothermal system.

Table 2
Values of parameters used in calculations of ΔG_r (kJ/mol e^-)

Sample name: Sample number:	Pozzo Istmo 010622a	Pozzo Vasca 010622b	Acque Calde 1 010625c	Acque Calde 2 010625d	Punto Uno 010623a	Punto Sette 010624a	Stinky Surf Rock 010623b	The Grip 010625a	Stinky Waist 010624c
Temperature	56.4	81.8	87.5	87.5	88.9	81.0	84.8	54.0	50.8
pH	5.840	1.976	5.518	5.650	3.561	2.717	3.673	5.205	5.207
H_2O	0.98	1.00	0.98	0.98	1.00	1.00	0.98	0.98	0.98
O_2,aq	−4.89	−4.89	−4.03	−4.17	−4.66	−4.92	−4.68	−4.67	−4.66
H_2,aq	−8.30	−5.89	−5.17	−5.14	−6.25	−7.01	−6.37	−5.82	−5.81
SO_4^{2-}	−2.61	−2.33[a]	−2.73	−2.73	−2.52	−2.25[a]	−2.69	−2.65	−2.62
H_2S,aq	−3.50	−3.90	−3.49	−3.64	−3.81	−3.58	−4.65	−3.52	−3.76
CO_2,aq	−1.83	−2.15	−2.29	−2.26	−2.29	−2.13	−2.20	−1.82	−1.79
CH_4,aq	−7.18	−2.81	−5.34	−5.66	−4.63	−2.78	−6.23	−4.35	−5.28
NO_3^-	−3.41	−3.40	−3.11	−2.70	−4.14	−4.33	−4.80	−4.70	−4.42
NH_4^+	−3.97	−2.95	−3.44	−3.18	−2.66	−3.72	−3.42	−3.45	−3.44
Fe^{2+}	−7.18	−2.81	−5.34	−5.66	−4.63	−2.78	−4.62	−4.35	−5.28
Organic acids									
Formic acid	−6.22[b]	−5.42	−6.28[b]	−6.22[b]	−5.75	−4.65	−5.78	−6.28[b]	−6.22[b]
Acetic acid	−6.16[b]	−4.62	−6.94[b]	−5.72[b]	−5.42	−4.73	−4.57	−5.68[b]	−5.05[b]
Propanoic acid	−5.10[b]	−5.26	−6.30[b]		−6.02	−6.11	−5.87	−6.21[b]	−4.99[b]
Lactic acid			−6.73[b]		−5.70	−5.00			
Aldoses									
Arabinose			−7.74		−6.75	−5.58			
Xylose		−5.63			−5.65	−5.17			
Galactose		−7.26	−7.02	−7.66	−6.06	−5.09			
Glucose		−6.13	−6.28	−7.47	−5.41	−4.64			
Mannose	−7.21	−5.28			−5.61	−5.00			
Amino acids									
Alanine	−6.68	−4.93	−6.34	−6.62	−5.55	−5.20			
Arginine$^+$		−5.47	−6.85	−7.01	−6.55	−5.85			
Aspartic acid	−7.22[b]	−5.18	−6.82[b]	−7.70[b]	−5.72	−5.05			
Glutamic acid	−7.03[b]	−4.74	−6.69[b]	−7.04[b]	−5.51	−5.17			
Glycine	−6.96	−4.56		−7.70	−5.57	−5.14			
Histidine$^+$		−5.56	−7.50[c]		−7.19	−6.33			
Isoleucine		−5.50	−7.05		−5.88	−5.62			
Leucine		−5.36	−6.82		−5.77	−5.54			
Lysine$^+$	−6.27	−5.36	−6.32	−6.11	−5.58	−5.63			
Methionine	−6.77	−6.21			−6.92	−6.57			
Phenylalanine		−5.63	−7.15		−6.08	−5.84			
Serine	−6.70	−5.34	−7.52		−5.80	−5.40			
Threonine		−5.52	−6.85		−5.80	−5.41			
Tyrosine		−5.34			−6.43	−6.22			
Valine		−5.36	−7.52		−5.38	−5.45			

All values in log activity except temperature (°C) and pH ($-\log a_{H+}$).
[a] HSO_4^- dominates; however equilibrium values of SO_4^{2-} (shown here) are used in calculations.
[b] Activity of the acid anion, which dominates at the *in situ* pH.
[c] Activity of neutral histidine, which dominates at the *in situ* pH.

1.1. The Vulcano hydrothermal system

Approximately 70 km north of Mount Etna is the Aeolian archipelago, which hosts some of the best-studied shallow marine hydrothermal systems in the world (Fig. 1). Vulcano, one of the seven volcanically active islands in this archipelago, has been the subject of numerous studies of hydrothermal geochemistry and microbiology. Hydrothermal activity is documented in many parts of the island, but is most extensive in the Baia di Levante on the eastern side of the isthmus that connects Vulcano with Vulcanello. The geochemistry of Vulcano hydrothermal fluids has received considerable attention, and the aqueous and gas phase compositions have been determined at nearly a dozen sites, including geothermal wells, onshore sediment seeps and shallow submarine vents (Capasso et al., 1997, 2001; Amend et al., 1998, 2003b, 2004; Nuccio et al., 1999). The complex interactions of magmatic fluids, seawater and freshwater produce a wide range of geochemical compositions, and consequently, an array of redox disequilibria, making Vulcano an ideal system in which to study the relationship between geochemical energetics and microbial diversity.

A majority of Vulcano's thermophiles[1] oxidize or ferment both complex organic substrates and simple organic

[1] Moderate thermophiles grow optimally between 45 and 80 °C, while hyperthermophiles have an optimum growth temperature ⩾ 80 °C. The general term thermophiles is used here to refer to both of these groups collectively.

Table 3
Organic acid oxidation reactions

Reaction		e^-
TEA = O_2		
A1	$HCOOH + 0.5O_2 \rightarrow CO_2 + H_2O$	2
A2	$CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O$	8
A3	$C_2H_5COOH + 3.5O_2 \rightarrow 3CO_2 + 3H_2O$	14
A4	$CH_3CH(OH)COOH + 3O_2 \rightarrow 3CO_2 + 3H_2O$	12
TEA = SO_4^{2-}		
A5	$HCOOH + 0.25SO_4^{2-} + 0.5H^+ \rightarrow CO_2 + 0.25H_2S + H_2O$	2
A6	$CH_3COOH + SO_4^{2-} + 2H^+ \rightarrow 2CO_2 + H_2S + 2H_2O$	8
A7	$C_2H_5COOH + 1.75SO_4^{2-} + 3.5H^+ \rightarrow 3CO_2 + 1.75H_2S + 3H_2O$	14
A8	$CH_3CH(OH)COOH + 1.5SO_4^{2-} + 3H^+ \rightarrow 3CO_2 + 1.5H_2S + 3H_2O$	12
TEA = S^0		
A9	$HCOOH + S^0 \rightarrow CO_2 + H_2S$	2
A10	$CH_3COOH + 4S^0 + 2H_2O \rightarrow 2CO_2 + 4H_2S$	8
A11	$C_2H_5COOH + 7S^0 + 4H_2O \rightarrow 3CO_2 + 7H_2S$	14
A12	$CH_3CH(OH)COOH + 6S^0 + 3H_2O \rightarrow 3CO_2 + 6H_2S$	12
TEA = NO_3^-		
A13	$HCOOH + 0.25NO_3^- + 0.5H^+ \rightarrow CO_2 + 0.25NH_4^+ + 0.75H_2O$	2
A14	$CH_3COOH + NO_3^- + 2H^+ \rightarrow 2CO_2 + NH_4^+ + H_2O$	8
A15	$C_2H_5COOH + 1.75NO_3^- + 3.5H^+ \rightarrow 3CO_2 + 1.75NH_4^+ + 1.25H_2O$	14
A16	$CH_3CH(OH)COOH + 1.5NO_3^- + 3H^+ \rightarrow 3CO_2 + 1.5NH_4^+ + 1.5H_2O$	12
TEA = Fe_3O_4		
A17	$HCOOH + Fe_3O_4 + 6H^+ \rightarrow CO_2 + 3Fe^{2+} + 4H_2O$	2
A18	$CH_3COOH + 4Fe_3O_4 + 24H^+ \rightarrow 2CO_2 + 12Fe^{2+} + 14H_2O$	8
A19	$C_2H_5COOH + 7Fe_3O_4 + 42H^+ \rightarrow 3CO_2 + 21Fe^{2+} + 24H_2O$	14
A20	$CH_3CH(OH)COOH + 6Fe_3O_4 + 36H^+ \rightarrow 3CO_2 + 18Fe^{2+} + 21H_2O$	12

Table 4
ΔG_r (kJ/mol e^-) of organic acid oxidation reactions

	Pozzo Istmo[a]	Pozzo Vasca	Acque Calde 1[a]	Acque Calde 2[a]	Punto Uno	Punto Sette	Stinky Surf Rock	The Grip[a]	Stinky Waist[a]
TEA = O_2									
A1	−101.18	−109.52	−102.57	−101.97	−108.81	−112.06	−108.65	−103.50	−103.16
A2	−99.79	−101.63	−100.67	−101.31	−101.38	−101.47	−102.05	−101.07	−101.61
A3	−101.73	−101.86	−102.66	−105.42	−101.92	−101.39	−101.93	−101.91	−102.50
A4			−107.76		−108.10	−107.93			
TEA = SO_4^{2-}									
A5	−8.12	−24.92	−10.11	−9.66	−21.37	−25.99	−21.35	−10.96	−11.35
A6	−6.73	−17.03	−8.21	−9.00	−13.94	−15.41	−14.75	−8.53	−9.11
A7	−8.68	−17.27	−10.20	−13.10	−14.47	−15.33	−14.63	−9.37	−10.00
A8			−15.30		−20.66	−21.86			
TEA = S^0									
A9	−12.52	−24.97	−15.76	−15.92	−24.37	−26.38	−26.63	−14.31	−15.06
A10	−11.12	−17.08	−13.87	−15.26	−16.94	−15.80	−20.03	−11.87	−12.83
A11	−13.07	−17.32	−15.86	−19.36	−17.48	−15.72	−19.92	−12.72	−13.71
A12			−20.95		−23.66	−22.25			
TEA = NO_3^-									
A13	−62.94	−77.46	−63.79	−63.34	−72.98	−78.63	−72.63	−64.45	−64.95
A14	−61.55	−69.57	−61.89	−62.68	−65.56	−68.05	−66.03	−62.02	−53.27
A15	−61.66	−69.81	−61.67	−64.58	−66.09	−67.97	−65.92	−61.07	−61.84
A16			−68.98		−72.27	−74.50			
TEA = Fe_3O_4									
A17	−44.77	−82.24	−26.08	−26.36	−66.39	−69.53	−64.49	−32.56	−42.27
A18	−44.82	−74.36	−26.16	−27.65	−58.96	−58.95	−57.89	−31.54	−41.42
A19	−45.33	−74.59	−26.17	−29.80	−59.50	−58.87	−57.78	−30.97	−40.93
A20			−31.28		−65.65	−65.37			

[a] Because the organic acid anion is the dominant species at these sites, the oxidation of the anion, rather than the neutral acid, was considered when calculating reaction properties. Values listed reflect organic acid anion oxidation. However, it should be noted that because the organic acid and organic acid anion are equilibrated with the *in situ* fluid pH, values of ΔG_r for either reaction are identical.

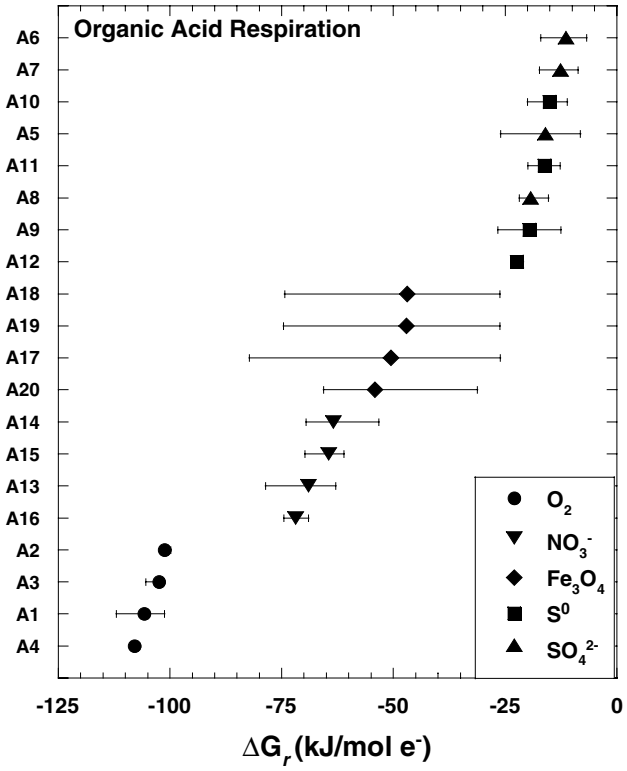

Fig. 2. Profile of ΔG_r (kJ/mol e^-) for respiration of four carboxylic acids (formic, acetic, propanoic and lactic), distinguished by terminal electron acceptor. Reactions are arranged in order of decreasing energy yield (bottom left to upper right) and reaction numbers on the *y*-axis correspond to those given in Table 3. Bars on each symbol represent the range of values observed across the sites investigated.

acids, sugars and amino acids. Reduction of elemental sulfur (S^0) is a common strategy, but aerobic respiration, as well as sulfate, iron and nitrate reduction are also utilized. Vulcano's thermophilic heterotrophs include *Archaeoglobus fulgidus*, *Geobacillus vulcani*, *Ferroglobus placidus*, *Palaeococcus helgesonii*, *Pyrococcus furiosus*, *Thermococcus acidaminovorans*, and *Thermotoga maritima* (Fiala and Stetter, 1986; Huber et al., 1986; Hafenbradl et al., 1996; Dirmeier et al., 1998; Caccamo et al., 2000; Amend et al., 2003a; Nazina et al., 2004). Additionally, molecular biology studies have shown that the microbial communities in heated sediments were dominated by thermophiles, including members of the Thermococcales, *Thermotoga*, *Thermosipho*, and thermophilic *Bacillus* (Rusch and Amend, 2004), all of which have representatives that use fermentative metabolisms, and Archaeoglobales (Rusch and Amend, 2004), which can oxidize organic acids and sugars with either sulfate or sulfite (Stetter et al., 1987, 1988). Furthermore, culture-independent surveys indicate that many Vulcano thermophiles remain uncultured (Simmons and Norris, 2002; Rusch and Amend, 2004; Rogers and Amend, 2005; Rusch et al., 2005). These uncultured organisms may be catalyzing any of the plethora of exergonic redox reactions that have been identified in the Vulcano hydrothermal system and calculation of energy yields for putative metabolic reactions can be used to inform isolation efforts.

2. Methods

2.1. Sampling and geochemical analyses

Hydrothermal fluids were collected for geochemical analyses from 2 geothermal wells, 3 heated sediment seeps and 4 shallow submarine vents. Sample locations are shown in Fig. 1. Sampling and analysis procedures are described in detail elsewhere (Amend et al., 2003b, 2004; Svensson et al., 2004; Rogers, 2006). Briefly, temperature, pH and conductivity were measured *in situ* with handheld meters, and redox sensitive aqueous species (Fe^{2+}, NO_3^-, NO_2^-, NH_4^+, H_2S, O_2) were measured on site by spectro-

Table 5
C_5 and C_6 aldose oxidation reactions

Reaction		e^-
TEA = O_2		
B1	$C_5H_{10}O_5 + 5O_2 \rightarrow 5CO_2 + 5H_2O$	20
B2	$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$	24
TEA = SO_4^{2-}		
B3	$C_5H_{10}O_5 + 2.5SO_4^{2-} + 5H^+ \rightarrow 5CO_2 + 2.5H_2S + 5H_2O$	20
B4	$C_6H_{12}O_6 + 3SO_4^{2-} + 6H^+ \rightarrow 6CO_2 + 3H_2S + 6H_2O$	24
TEA = S^0		
B5	$C_5H_{10}O_5 + 10S^0 + 5H_2O \rightarrow 5CO_2 + 10H_2S$	20
B6	$C_6H_{12}O_6 + 12S^0 + 6H_2O \rightarrow 6CO_2 + 12H_2S$	24
TEA = NO_3^-		
B7	$C_5H_{10}O_5 + 2.5NO_3^- + 5H^+ \rightarrow 5CO_2 + 2.5NH_4^+ + 2.5H_2O$	20
B8	$C_6H_{12}O_6 + 3NO_3^- + 6H^+ \rightarrow 6CO_2 + 3NH_4^+ + 3H_2O$	24
TEA = Fe_3O_4		
B9	$C_5H_{10}O_5 + 10Fe_3O_4 + 60H^+ \rightarrow 5CO_2 + 30Fe^{2+} + 35H_2O$	20
B10	$C_6H_{12}O_6 + 12Fe_3O_4 + 72H^+ \rightarrow 6CO_2 + 36Fe^{2+} + 42H_2O$	24

Table 6
ΔG_r (kJ/mol e^-) of aldose oxidation reactions

	Pozzo Istmo	Pozzo Vasca	Acque Calde 1	Acque Calde 2	Punto Uno	Punto Sette
TEA = O_2						
B1-Arabinose			−116.11		−115.32	−115.11
B1-Xylose		−116.38			−116.95	−116.47
B2-Galactose		−114.13	−115.87	−115.39	−115.01	−114.68
B2-Glucose		−114.16	−115.80	−115.16	−114.92	−114.52
B2-Mannose	−113.99	−114.62			−115.08	−114.64
TEA = SO_4^{2-}						
B3-Arabinose			−25.36		−29.61	−30.74
B3-Xylose		−31.79			−29.52	−30.41
B4-Galactose		−31.23	−25.12	−24.79	−29.30	−30.31
B4-Glucose		−31.27	−25.05	−24.56	−29.21	−30.15
B4-Mannose	−22.48	−31.73			−29.37	−30.27
TEA = S^0						
B5-Arabinose			−31.02		−32.62	−31.14
B5-Xylose		−31.83			−32.52	−30.80
B6-Galactose		−31.27	−30.78	−31.04	−32.31	−30.71
B6-Glucose		−31.30	−30.71	−30.81	−32.21	−30.55
B6-Mannose	−26.89	−31.76			−32.38	−30.66
TEA = NO_3^-						
B7-Arabinose			−79.05		−81.23	−83.38
B7-Xylose		−84.33			−81.13	−83.05
B8-Galactose		−83.77	−78.80	−78.48	−80.91	−82.95
B8-Glucose		−83.80	−78.73	−78.25	−80.82	−82.80
B8-Mannose	−77.30	−84.26			−80.98	−82.91
TEA = Fe_3O_4						
B9-Arabinose			−42.13		−75.41	−74.98
B9-Xylose		−89.88			−75.31	−74.65
B10-Galactose		−88.60	−41.09	−41.49	−74.31	−73.83
B10-Glucose		−88.63	−41.02	−41.26	−74.21	−73.67
B10-Mannose	−59.10	−89.09			−74.37	−73.78

photometry (HACH Co., Colorado, USA). Concentrations of major cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) and major anions (Cl^-, Br^-, SO_4^{2-}) were measured by ion chromatography and gas compositions (He, H_2, N_2, CO, CO_2, and CH_4) were analyzed by gas chromatography.

At all nine sites, concentrations of four organic acids (formic, acetic, propanoic, and lactic) were measured and at six sites, five neutral aldoses (arabinose, xylose, galactose, glucose and mannose) and 15 amino acids (Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Tyr and Val) were analyzed. Organic acids (Amend et al., 2004) and neutral aldoses (Rogers, 2006) were determined by ion chromatography. Amino acid concentrations (both total dissolved amino acids (TDAA) and dissolved combined amino acids (DCAA)) were determined by HPLC, and dissolved free amino acid concentrations (DFAA) were determined by difference (Svensson et al., 2004). Analytical data and field measurements are given in Table 1.

2.2. Geochemical calculations

In hydrothermal systems, mixing of hot, reduced hydrothermal fluid with cold, oxidized seawater and/or meteoric water leads to chemical disequilibria, which may serve as potential energy sources for microorganisms. Values of overall Gibbs free energy (ΔG_r)—not standard free energy (ΔG_r^0), which is commonly incorrectly applied—can be used to quantify the energy yield from a reaction, regardless if it proceeds biotically or abiotically. To calculate values of ΔG_r, those of (ΔG_r^0) were combined with chemical activities according to the relation

$$\Delta G_r = \Delta G_r^0 + RT \ln Q, \quad (1)$$

in which R is the gas constant, T is the temperature (Kelvin), and Q, the activity product, is defined by

$$Q = \Pi a_i^{v_i}, \quad (2)$$

where a_i represents the activity of the ith species and v_i is its stoichiometric reaction coefficient, which is positive for products and negative for reactants. Values of (ΔG_r^0) were calculated at *in situ* temperatures using the geochemical software package SUPCRT92 (Johnson et al., 1992) and data from Shock (1992, 1995), Amend and Helgeson (1997), and Amend and Plyasunov (2001). Activities of aqueous species were calculated from fluid and gas composition data using the geochemical speciation program EQ3 (Wolery, 1992) and additional data (Shock, 1992, 1995; Shock and Koretsky, 1993, 1995; Amend and Helgeson, 1997; Prapaipong et al., 1999; Amend and Plyasunov, 2001) and applying the extended Debye–Hückle model to account for activity coefficients (Helgeson, 1969). Aqueous activities of volatile components (H_2, N_2, CO, CO_2, and

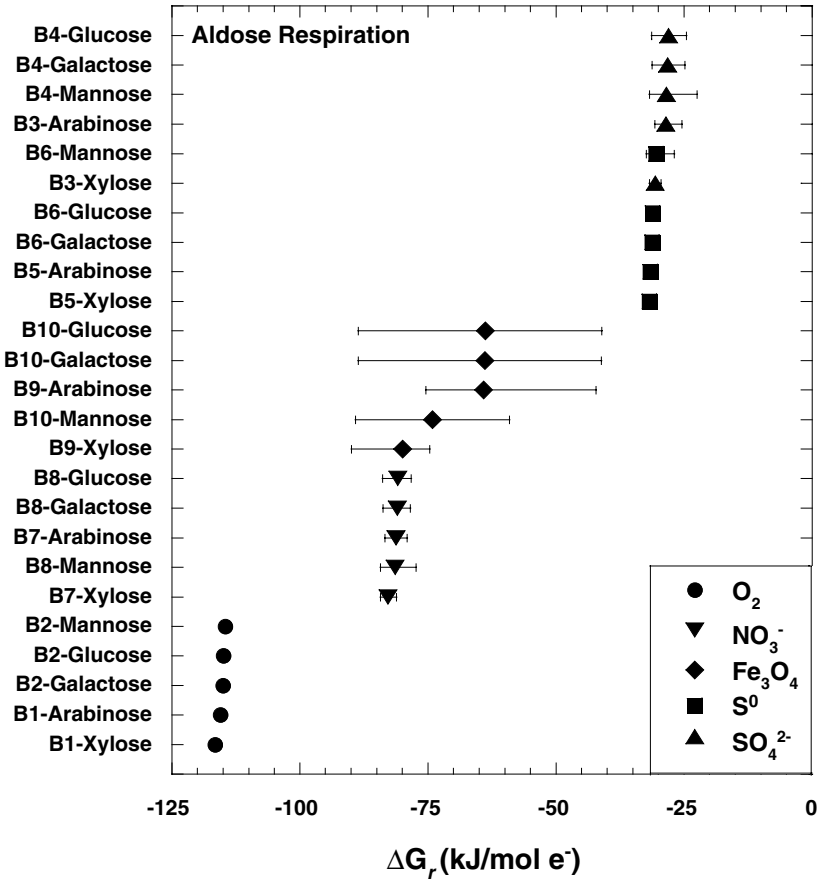

Fig. 3. Profile of ΔG_r (kJ/mol e^-) for respiration of five neutral aldoses (arabinose, xylose, galactose, glucose and mannose), distinguished by terminal electron acceptor. Reactions are arranged in order of decreasing energy yield (bottom left to upper right) and reaction numbers on the *y*-axis correspond to those given in Table 5. Bars on each symbol represent the range of values observed across the sites investigated.

CH_4) were calculated from gas composition data assuming equilibrium. The activity of pure minerals is assumed to be unity. Redox reactions among Fe, O, H, C, N, and S species were suppressed to preserve the redox disequilibria observed in these systems. Total dissolved amino acid concentrations were used to calculate amino acid activities. Activities of aqueous species are given in Table 2.

To permit ready comparison among different oxidation reactions, values of ΔG_r were normalized to the number of electrons transferred. This normalization is appropriate for the energetics of catabolic reactions, as it is through electron transfer that energy is utilized in the cell (Amend et al., 2003b). The number of electrons transferred in each reaction is given in Tables 3, 5 and 7. Electron transfer in fermentation reactions is less transparent and these reactions do not lend themselves easily to this normalization. Hence, these reactions were normalized to the number of moles of carbon transferred.

3. Results and discussion

3.1. Composition of hydrothermal fluids

Field and analytical data from 9 hydrothermal sites in the Baia di Levante are listed in Table 1. Large variations in temperature (54–89 °C) and pH (1.98–5.84) were observed. The activities of H_2(aq), CH_4(aq), NO_3^- and Fe^{2+} varied up to five orders of magnitude among sites, but those of O_2(aq), SO_4^{2-}, H_2S(aq), and CO_2(aq) were less variable, generally within one order of magnitude. Concentrations of dissolved organic carbon (DOC) vary by nearly 2 orders of magnitude (0.41–24.0 ppmC) across the Vulcano hydrothermal system (Svensson et al., 2004), and this variability is reflected in the concentrations of individual organic compounds. The concentrations of formic, acetic, propanoic and lactic acid varied by ~2 orders of magnitude, with the highest concentrations found at Punto Sette, Pozzo Vasca, and Stinky Waist (Amend et al., 2004). Total amino acid concentrations ranged from 1.78 to 106 μM, compared to 0.85 μM in local (Baia di Levante) seawater (Svensson et al., 2004). The highest concentration of a single amino acid was for glycine (27.53 μM) at Pozzo Vasca, the most acidic site. In general, amino acid concentrations in the hydrothermal fluid are positively correlated with the pH of the hydrothermal fluid, with the highest concentrations observed at Pozzo Vasca (pH 1.98) and the lowest concentrations at Acque Calde 1, Acque Calde 2 and Pozzo Istmo (pH 5.5–5.8) (Amend et al., 2003b; Svensson et al., 2004). The concentrations of neutral aldoses in the hydrothermal fluids ranged from 62 to ~23,000 nM, compared

Table 7
Amino acid oxidation reactions

	Reaction	e^-
TEA = O_2		
C1	$C_3H_7NO_2 + 3O_2 + H^+ \rightarrow 3CO_2 + NH_4^+ + 2H_2O$	12
C2	$C_6H_{15}N_4O_2^+ + 5.5O_2 + 3H^+ \rightarrow 6CO_2 + 4NH_4^+ + H_2O$	22
C3	$C_4H_6NO_4^- + 3O_2 + 2H^+ \rightarrow 4CO_2 + NH_4^+ + 2H_2O$	12
C4	$C_5H_8NO_4^- + 4.5O_2 + 2H^+ \rightarrow 5CO_2 + NH_4^+ + 3H_2O$	18
C5	$C_2H_5NO_2 + 1.5O_2 + H^+ \rightarrow 2CO_2 + NH_4^+ + H_2O$	6
C6	$C_6H_{10}N_3O_2^+ + 5O_2 + 2H^+ \rightarrow 6CO_2 + 3NH_4^+$	20
C7	$C_6H_{13}NO_2 + 7.5O_2 + H^+ \rightarrow 6CO_2 + NH_4^+ + 5H_2O$	30
C8	$C_6H_{13}NO_2 + 7.5O_2 + H^+ \rightarrow 6CO_2 + NH_4^+ + 5H_2O$	30
C9	$C_6H_{15}N_2O_2^+ + 7O_2 + H^+ \rightarrow 6CO_2 + 2NH_4^+ + 4H_2O$	28
C10	$C_5H_{11}NO_2S + 5.5O_2 + H^+ \rightarrow 5CO_2 + NH_4^+ + H_2S + 3H_2O$	22
C11	$C_9H_{11}NO_2 + 10O_2 + H^+ \rightarrow 9CO_2 + NH_4^+ + 4H_2O$	40
C12	$C_3H_7NO_3 + 2.5O_2 + H^+ \rightarrow 3CO_2 + NH_4^+ + 2H_2O$	10
C13	$C_4H_9NO_3 + 4O_2 + H^+ \rightarrow 4CO_2 + NH_4^+ + 3H_2O$	16
C14	$C_9H_{11}NO_3 + 9.5O_2 + H^+ \rightarrow 9CO_2 + NH_4^+ + 4H_2O$	38
C15	$C_5H_{11}NO_2 + 6O_2 + H^+ \rightarrow 5CO_2 + NH_4^+ + 4H_2O$	24
TEA = SO_4^{2-}		
C16	$C_3H_7NO_2 + 1.5SO_4^{2-} + 4H^+ \rightarrow 3CO_2 + NH_4^+ + 1.5H_2S + 2H_2O$	12
C17	$C_6H_{15}N_4O_2^+ + 2.75SO_4^{2-} + 8.5H^+ \rightarrow 6CO_2 + 4NH_4^+ + 2.75H_2S + H_2O$	22
C18	$C_4H_6NO_4^- + 1.5SO_4^{2-} + 5H^+ \rightarrow 4CO_2 + NH_4^+ + 1.5H_2S + 2H_2O$	12
C19	$C_5H_8NO_4^- + 2.25SO_4^{2-} + 6.5H^+ \rightarrow 5CO_2 + NH_4^+ + 2.25H_2S + 3H_2O$	18
C20	$C_2H_5NO_2 + 0.75SO_4^{2-} + 2.5H^+ \rightarrow 2CO_2 + NH_4^+ + 0.75H_2S + H_2O$	6
C21	$C_6H_{10}N_3O_2^+ + 2.5SO_4^{2-} + 7H^+ \rightarrow 6CO_2 + 3NH_4^+ + 2.5H_2S$	20
C22	$C_6H_{13}NO_2 + 3.75SO_4^{2-} + 8.5H^+ \rightarrow 6CO_2 + NH_4^+ + 3.75H_2S + 5H_2O$	30
C23	$C_6H_{13}NO_2 + 3.75SO_4^{2-} + 8.5H^+ \rightarrow 6CO_2 + NH_4^+ + 3.75H_2S + 5H_2O$	30
C24	$C_6H_{15}N_2O_2^+ + 3.5SO_4^{2-} + 8H^+ \rightarrow 6CO_2 + 2NH_4^+ + 3.5H_2S + 4H_2O$	28
C25	$C_5H_{11}NO_2S + 2.75SO_4^{2-} + 6.5H^+ \rightarrow 5CO_2 + NH_4^+ + 3.75H_2S + 3H_2O$	22
C26	$C_9H_{11}NO_2 + 5SO_4^{2-} + 11H^+ \rightarrow 9CO_2 + NH_4^+ + 5H_2S + 4H_2O$	40
C27	$C_3H_7NO_3 + 1.25SO_4^{2-} + 3.5H^+ \rightarrow 3CO_2 + NH_4^+ + 1.25H_2S + 2H_2O$	10
C28	$C_4H_9NO_3 + 2SO_4^{2-} + 5H^+ \rightarrow 4CO_2 + NH_4^+ + 2H_2S + 3H_2O$	16
C29	$C_9H_{11}NO_3 + 4.75SO_4^{2-} + 10.5H^+ \rightarrow 9CO_2 + NH_4^+ + 4.75H_2S + 4H_2O$	38
C30	$C_5H_{11}NO_2 + 3SO_4^{2-} + 7H^+ \rightarrow 5CO_2 + NH_4^+ + 3H_2S + 4H_2O$	24
TEA = S^0		
C31	$C_3H_7NO_2 + 6S^0 + H^+ + 4H_2O \rightarrow 3CO_2 + NH_4^+ + 6H_2S$	12
C32	$C_6H_{15}N_4O_2^+ + 11S^0 + 3H^+ + 10H_2O \rightarrow 6CO_2 + 4NH_4^+ + 11H_2S$	22
C33	$C_4H_6NO_4^- + 6S^0 + 2H^+ + 4H_2O \rightarrow 4CO_2 + NH_4^+ + 6H_2S$	12
C34	$C_5H_8NO_4^- + 9S^0 + 2H^+ + 6H_2O \rightarrow 5CO_2 + NH_4^+ + 9H_2S$	18
C35	$C_2H_5NO_2 + 3S^0 + H^+ + 2H_2O \rightarrow 2CO_2 + NH_4^+ + 3H_2S$	6
C36	$C_6H_{10}N_3O_2^+ + 10S^0 + 2H^+ + 10H_2O \rightarrow 6CO_2 + 3NH_4^+ + 10H_2S$	20
C37	$C_6H_{13}NO_2 + 15S^0 + H^+ + 10H_2O \rightarrow 6CO_2 + NH_4^+ + 15H_2S$	30
C38	$C_6H_{13}NO_2 + 15S^0 + H^+ + 10H_2O \rightarrow 6CO_2 + NH_4^+ + 15H_2S$	30
C39	$C_6H_{15}N_2O_2^+ + 14S^0 + H^+ + 10H_2O \rightarrow 6CO_2 + 2NH_4^+ + 14H_2S$	28
C40	$C_5H_{11}NO_2S + 11S^0 + H^+ + 8H_2O \rightarrow 5CO_2 + NH_4^+ + 12H_2S$	22
C41	$C_9H_{11}NO_2 + 20S^0 + H^+ + 16H_2O \rightarrow 9CO_2 + NH_4^+ + 20H_2S$	40
C42	$C_3H_7NO_3 + 5S^0 + H^+ + 3H_2O \rightarrow 3CO_2 + NH_4^+ + 5H_2S$	10
C43	$C_4H_9NO_3 + 8S^0 + H^+ + 5H_2O \rightarrow 4CO_2 + NH_4^+ + 8H_2S$	16
C44	$C_9H_{11}NO_3 + 19S^0 + H^+ + 15H_2O \rightarrow 9CO_2 + NH_4^+ + 19H_2S$	38
C45	$C_5H_{11}NO_2 + 12S^0 + H^+ + 8H_2O \rightarrow 5CO_2 + NH_4^+ + 12H_2S$	24
TEA = NO_3^-		
C46	$C_3H_7NO_2 + 1.5NO_3^- + 4H^+ \rightarrow 3CO_2 + 2.5NH_4^+ + 0.5H_2O$	12
C47	$C_6H_{15}N_4O_2^+ + 2.75NO_3^- + 8.5H^+ + 1.75H_2O \rightarrow 6CO_2 + 6.75NH_4^+$	22
C48	$C_4H_6NO_4^- + 1.5NO_3^- + 5H^+ \rightarrow 4CO_2 + 2.5NH_4^+ + 0.5H_2O$	12
C49	$C_5H_8NO_4^- + 2.25NO_3^- + 6.5H^+ \rightarrow 5CO_2 + 3.25NH_4^+ + 0.75H_2O$	18
C50	$C_2H_5NO_2 + 0.75NO_3^- + 2.5H^+ \rightarrow 2CO_2 + 1.75NH_4^+ + 0.25H_2O$	6
C51	$C_6H_{10}N_3O_2^+ + 2.5NO_3^- + 7H^+ + 2.5H_2O \rightarrow 6CO_2 + 5.5NH_4^+$	20
C52	$C_6H_{13}NO_2 + 3.75NO_3^- + 8.5H^+ \rightarrow 6CO_2 + 4.75NH_4^+ + 1.25H_2O$	30
C53	$C_6H_{13}NO_2 + 3.75NO_3^- + 8.5H^+ \rightarrow 6CO_2 + 4.75NH_4^+ + 1.25H_2O$	30
C54	$C_6H_{15}N_2O_2^+ + 3.5NO_3^- + 8H^+ \rightarrow 6CO_2 + 5.5NH_4^+ + 0.5H_2O$	28
C55	$C_5H_{11}NO_2S + 2.75NO_3^- + 6.5H^+ \rightarrow 5CO_2 + 3.75NH_4^+ + H_2S + 0.25H_2O$	22
C56	$C_9H_{11}NO_2 + 5NO_3^- + 11H^+ + H_2O \rightarrow 9CO_2 + 6NH_4^+$	40
C57	$C_3H_7NO_3 + 1.25NO_3^- + 3.5H^+ \rightarrow 3CO_2 + 2.25NH_4^+ + 0.75H_2O$	10
C58	$C_4H_9NO_3 + 2NO_3^- + 5H^+ \rightarrow 4CO_2 + 3NH_4^+ + H_2O$	16

Table 7 (*continued*)

Reaction		e^-
C59	$C_9H_{11}NO_3 + 4.75NO_3^- + 10.5H^+ + 0.75H_2O \rightarrow 9CO_2 + 5.75NH_4^+$	38
C60	$C_5H_{11}NO_2 + 3NO_3^- + 7H^+ \rightarrow 5CO_2 + 4NH_4^+ + H_2O$	24
TEA = Fe_3O_4		
C61	$C_3H_7NO_2 + 6Fe_3O_4 + 37H^+ \rightarrow 3CO_2 + NH_4^+ + 18Fe^{2+} + 20H_2O$	12
C62	$C_6H_{15}N_4O_2^+ + 11Fe_3O_4 + 69H^+ \rightarrow 6CO_2 + 4NH_4^+ + 33Fe^{2+} + 34H_2O$	22
C63	$C_4H_6NO_4^- + 6Fe_3O_4 + 38H^+ \rightarrow 4CO_2 + NH_4^+ + 18Fe^{2+} + 20H_2O$	12
C64	$C_5H_8NO_4^- + 9Fe_3O_4 + 56H^+ \rightarrow 5CO_2 + NH_4^+ + 27Fe^{2+} + 30H_2O$	18
C65	$C_2H_5NO_2 + 3Fe_3O_4 + 19H^+ \rightarrow 2CO_2 + NH_4^+ + 9Fe^{2+} + 10H_2O$	6
C66	$C_6H_{10}N_3O_2^+ + 10Fe_3O_4 + 62H^+ \rightarrow 6CO_2 + 3NH_4^+ + 30Fe^{2+} + 30H_2O$	20
C67	$C_6H_{13}NO_2 + 15Fe_3O_4 + 91H^+ \rightarrow 6CO_2 + NH_4^+ + 45Fe^{2+} + 50H_2O$	30
C68	$C_6H_{13}NO_2 + 15Fe_3O_4 + 91H^+ \rightarrow 6CO_2 + NH_4^+ + 45Fe^{2+} + 50H_2O$	30
C69	$C_6H_{15}N_2O_2^+ + 14Fe_3O_4 + 85H^+ \rightarrow 6CO_2 + 2NH_4^+ + 42Fe^{2+} + 46H_2O$	28
C70	$C_5H_{11}NO_2S + 11Fe_3O_4 + 67H^+ \rightarrow 5CO_2 + NH_4^+ + 33Fe^{2+} + H_2S + 36H_2O$	22
C71	$C_9H_{11}NO_2 + 20Fe_3O_4 + 121H^+ \rightarrow 9CO_2 + NH_4^+ + 60Fe^{2+} + 64H_2O$	40
C72	$C_3H_7NO_3 + 5Fe_3O_4 + 31H^+ \rightarrow 3CO_2 + NH_4^+ + 15Fe^{2+} + 17H_2O$	10
C73	$C_4H_9NO_3 + 8Fe_3O_4 + 49H^+ \rightarrow 4CO_2 + NH_4^+ + 24Fe^{2+} + 27H_2O$	16
C74	$C_9H_{11}NO_3 + 19Fe_3O_4 + 115H^+ \rightarrow 9CO_2 + NH_4^+ + 57Fe^{2+} + 61H_2O$	38
C75	$C_5H_{11}NO_2 + 12Fe_3O_4 + 73H^+ \rightarrow 5CO_2 + NH_4^+ + 36Fe^{2+} + 40H_2O$	24

with 29–171 nM in Baia di Levante seawater (Rogers, 2006). As with the amino acids, the highest concentrations of the five neutral aldoses were found at the most acidic sites. In this and other shallow marine hydrothermal systems, the aqueous organic compounds are likely primarily derived from decomposition of primary producers and the influx of terrestrially derived organic compounds, as is evidenced by the positive correlation between aldose concentrations with plant-derived organic matter (Hoaki et al., 1995, A. Skoog, pers. comm.; Gugliandolo and Maugeri, 1998).

3.2. Respiration

Heterotrophic respiration is defined here as the oxidation of an organic compound coupled to the reduction of a TEA. We only consider the complete oxidation of carboxylic acids, sugars and amino acids to CO_2. Likewise, although some TEAs can be reduced to a number of different compounds, we considered here only the redox couples[2] O_2/H_2O, NO_3^-/NH_4^+, $Fe(III)/Fe^{2+}$, S^0/H_2S and SO_4^{2-}/H_2S.

3.2.1. Carboxylic acid respiration

Twenty potential metabolisms of carboxylic acid respiration, grouped by TEA, are shown in Table 3, and values of ΔG_r for these reactions at each of the nine sites investigated are listed in Table 4. Several of these reactions are known metabolisms of cultured thermophiles. For example, the hyperthermophile *Thermocrinus ruber* can oxidize formate with O_2 and S^0 (reactions A1 and A9) (Huber et al., 1998). The Vulcano hyperthermophile *Ferroglobus placidus* can respire acetate with nitrate and Fe(III) (reactions A14 and A18) (Hafenbradl et al., 1996) and *Geoglobus ahangari* also catalyzes acetate reduction with amorphous Fe(III) oxide (Tor et al., 2001; Kashefi et al., 2002). *Archaeoglobus fulgidus*, originally isolated from the Vulcano hydrothermal system, is known to couple sulfate reduction with the oxidation of formate (reaction A5), lactate (reaction A8), and pyruvate (Stetter, 1988). Furthermore, the combination of formic, acetic and propanoic acids is often used to grow thermophilic heterotrophs in laboratory culturing efforts.

Values of ΔG_r at the 9 sites are plotted in Fig. 2, arranged by increasing energy yield. Overall, values of ΔG_r for organic acid oxidation range from −6 to −112 kJ/mol e^-. The reaction energetics are remarkably systematic with respect to TEA, but much less so with respect to each organic acid. The oxidation of organic acids with O_2 yields the most energy (99–112 kJ/mol e^-), followed by reactions with NO_3^- (53–79 kJ/mol e^-), Fe_3O_4 (26–82 kJ/mol e^-), S^0 (11–27 kJ/mol e^-) and SO_4^{2-} (6–26 kJ/mol e^-). For each TEA more energy is yielded, in general, at the more acidic sites (Pozzo Vasca, Punto Uno, Punto Sette and Stinky Surf Rock) than at sites with circumneutral pH (Acque Calde 1, Acque Calde 2, The Grip and Stinky Waist). This follows from the fact that carboxylic acid concentrations are also higher at acidic sites. The variability in ΔG_r across all sites is relatively low for all TEAs (12–25 kJ/mol e^-) except magnetite (56 kJ/mol e^-). The wide range of ΔG_r values for iron reduction (Fig. 2, Table 4), is due in part to the activities of Fe^{2+}, which range from $10^{-7.18}$ to $10^{-2.81}$ across the 9 sites.

3.2.2. Aldose respiration

More than 20 species of hyperthermophiles have been shown to respire or ferment sugars (Kengen et al., 1996). Both polysaccharides and monomeric aldoses are common substrates for thermophilic heterotrophs, including *Deinococcus geothermalis*, *Sulfolobus yangmingensis* and *Pyrococcus glycovorans* (Ferreira et al., 1997; Barbier et al.,

[2] Fe(III) in magnetite (Fe_3O_4) is considered here. Magnetite and S^0 are both ubiquitous at Vulcano (our unpublished data).

Table 8
ΔG_r (kJ/mol e$^-$) of amino acid oxidation reactions

	Pozzo Istmo[a]	Pozzo Vasca	Acque Calde 1[a]	Acque Calde 2[a]	Punto Uno	Punto Sette
TEA = O_2						
C1	−106.83	−109.49	−108.62	−107.94	−108.63	−109.29
C2		−117.87	−116.50	−115.71	−116.36	−117.95
C3	−109.58	−113.82	−112.00	−110.89	−113.16	−113.83
C4	−107.22	−109.96	−109.33	−108.70	−109.58	−109.74
C5	−109.54	−115.65		−109.59	−113.04	−114.94
C6		−116.47	−115.50		−115.21	−116.42
C7		−98.71	−98.54		−98.29	−98.34
C8		−105.60	−106.18		−105.57	−105.51
C9	−106.57	−107.36	−108.15	−107.76	−107.32	−107.43
C10	−99.95	−101.29			−101.03	−101.03
C11		−105.84	−106.69		−105.96	−105.75
C12	−114.28	−117.42	−115.58		−116.48	−117.33
C13		−112.77	−112.58		−112.43	−112.76
C14		−107.11			−107.12	−106.90
C15		−106.29	−106.42		−106.25	−106.22
TEA = SO_4^{2-}						
C16	−13.76	−24.90	−16.16	−15.62	−21.19	−23.23
C17		−33.29	−24.05	−23.40	−28.93	−31.90
C18	−16.52	−29.23	−19.54	−18.57	−25.72	−27.77
C19	−14.16	−25.37	−16.87	−16.38	−22.14	−23.68
C20	−16.48	−31.06		−17.27	−25.61	−28.88
C21		−31.88	−23.04		−27.77	−30.36
C22		−21.30	−13.98		−18.42	−19.75
C23		−21.01	−13.72		−18.13	−19.45
C24	−13.51	−22.77	−15.69	−15.45	−19.88	−21.37
C25	−6.89	−16.70			−13.59	−14.97
C26		−21.25	−14.23		−18.52	−19.70
C27	−21.22	−32.83	−23.12		−29.04	−31.27
C28		−28.18	−20.12		−24.99	−26.71
C29		−22.52			−19.68	−20.84
C30		−21.70	−13.96		−18.81	−20.16
TEA = S^0						
C31	−18.17	−24.94	−21.82	−21.87	−24.20	−23.62
C32		−33.32	−29.70	−29.64	−31.93	−32.28
C33	−20.92	−29.26	−25.20	−24.82	−28.73	−28.16
C34	−18.56	−25.41	−22.53	−22.63	−25.15	−24.08
C35	−20.88	−31.09		−23.52	−28.61	−29.28
C36		−31.92	−28.70		−30.77	−30.75
C37		−21.33	−19.64		−21.43	−20.14
C38		−21.05	−19.37		−21.14	−19.85
C39	−17.91	−22.81	−21.35	−21.70	−22.89	−21.77
C40	−11.29	−16.74			−16.60	−15.37
C41		−21.29	−19.89		−21.53	−20.09
C42	−25.62	−32.87	−28.78		−32.04	−31.66
C43		−28.22	−25.78		−28.00	−27.10
C44		−22.56			−22.69	−21.23
C45		−21.74	−19.62		−21.82	−20.55
TEA = NO_3^-						
C46	−68.58	−77.44	−69.84	−69.31	−72.81	−75.87
C47		−85.82	−77.72	−77.08	−80.53	−84.53
C48	−71.33	−81.76	−73.22	−72.26	−77.33	−80.41
C49	−68.97	−77.91	−70.55	−70.07	−73.75	−76.33
C50	−71.29	−83.59		−70.96	−77.22	−81.52
C51		−84.42	−76.72		−79.38	−83.00
C52		−73.83	−67.67		−70.03	−72.39
C53		−73.55	−67.40		−69.74	−72.09
C54	−67.79	−73.93	−68.88	−68.67	−70.52	−72.82
C55	−61.70	−69.24			−65.20	−67.61
C56		−73.79	−67.91		−70.13	−72.34
C57	−77.67	−87.35	−78.19		−83.23	−86.67
C58		−80.71	−73.80		−76.60	−79.35

Table 8 (*continued*)

	Pozzo Istmo[a]	Pozzo Vasca	Acque Calde 1[a]	Acque Calde 2[a]	Punto Uno	Punto Sette
C59		−75.06			−71.29	−73.48
C60		−74.24	−67.64		−70.43	−72.80
TEA = Fe_3O_4						
C61	−50.38	−82.26	−32.14	−32.33	−66.20	−66.74
C62		−90.65	−40.01	−40.09	−73.93	−75.40
C63	−53.14	−86.59	−35.52	−35.27	−70.72	−71.28
C64	−50.78	−82.74	−32.85	−33.08	−67.15	−67.20
C65	−53.09	−88.42		−33.98	−70.61	−72.40
C66		−89.25	−39.02		−72.77	−73.87
C67		−78.66	−29.96		−63.43	−63.26
C68		−78.38	−29.69		−63.13	−62.97
C69	−49.60	−78.76	−31.17	−31.68	−63.91	−63.69
C70	−43.50	−74.07			−58.60	−58.49
C71		−78.62	−30.21		−63.53	−63.21
C72	−57.84	−90.19	−39.10		−74.04	−74.78
C73		−85.54	−36.10		−70.00	−70.22
C74		−79.88			−64.69	−64.35
C75		−79.07	−29.93		−63.82	−63.67

[a] Aspartate, glutamate and neutral histidine are the dominant species of these amino acids at these sites. Values reflect ΔG_r calculated with these species instead of those shown in Table 6.

1999; Jan et al., 1999). Members of the Sulfolobales, Archaeoglobales and Themoproteales can completely oxidize sugars to CO_2 with either O_2 or S^0 as the TEA (Grogan, 1989; Jan et al., 1999; Huber et al., 2001). Some species of *Geobacillus* and *Bacillus* that utilize neutral aldoses can also reduce nitrate, although this has not been directly linked to aldose oxidation (Nicolaus et al., 2000; Nazina et al., 2004). Finally, glucose oxidation to acetate via sulfate reduction was observed in enrichment studies of hot, anoxic sediments from Vulcano (Tor et al., 2003). Unfortunately, reaction products of heterotrophic metabolisms are rarely measured or reported, making it difficult to determine specific reaction stoichiometries. However, the widespread use of carbohydrates by thermophilic heterotrophs suggests that numerous metabolic strategies are possible. Below we discuss 25 potential metabolic reactions with neutral aldoses.

Here we consider the oxidation of two C_5 aldoses (arabinose and xylose) and three C_6 aldoses (galactose, glucose and mannose) with O_2, NO_3^-, Fe_3O_4, S^0 and SO_4^{2-} as TEAs (reactions B1–B10 in Table 5). Values of ΔG_r for these reactions are given in Table 6. The values of ΔG_r for the respiration of neutral aldoses range from −117 to −22 kJ/mol e^-, similar to values observed for organic acid oxidation. The average and range of values of ΔG_r, grouped by TEA, are shown in Fig. 3. The systematic behavior of reaction energetics with respect to TEA is even more pronounced for aldoses than for carboxylic acids, as discussed above. The most energy is available from aerobic respiration (114–117 kJ/mol e^-), followed by nitrate reduction (77–85 kJ/mol e^-), Fe(III) reduction (41–90 kJ/mol e^-) and finally S^0 and sulfate reduction (26–33 and 22–32 kJ/mol e^-, respectively). Even though aldose activities vary by ~3 orders of magnitude ($10^{-4.64}$ to $10^{-7.74}$), the range in ΔG_r is relatively small for each TEA (3–10 kJ/mol e^-), except for magnetite, for which values of ΔG_r vary by nearly 50 kJ/mol e^-. This large range in ΔG_r for ferric iron reduction reactions again can be attributed to the wide range of Fe^{2+} activities across the 6 sites investigated; however it should be noted that the highest energy yields from Fe(III) reduction are at the most acidic sites (Pozzo Vasca, Punto Uno and Punto Sette). The effects of pH and Fe^{2+} concentrations on overall reaction energetics are discussed in detail in Section 3.2.4.

3.2.3. Amino acid respiration

Amino acids are the building blocks of cellular proteins, and they either must be scavenged or synthesized for cells to survive. However, they can also serve as energy sources. As with sugars, amino acid metabolisms include partial and complete oxidation as well as fermentation. A number of thermophiles can oxidize amino acids for energy gain, including a number of *Thermococci* (e.g., *T. celer, T. fumicolans, T. acidaminovorans*), and *Pyrococci* (e.g., *P. furiosus* and *P. horikoshii*), as well as *Desulfurococcus* strain SY and *Sulfolobus yangmingensis*, which can grow on any of the 20 common amino acids (Hoaki et al., 1993, 1994; Godfroy et al., 1996; Dirmeier et al., 1998; González et al., 1998; Jan et al., 1999). In many cases mixtures of amino acids, but not individual amino acids, will support growth (e.g., Hoaki et al., 1993, 1994; Godfroy et al., 1996). As in sugar metabolism, growth on amino acids is often enhanced by, but not dependent on, S^0 (Hoaki et al., 1994; Godfroy et al., 1996; Dirmeier et al., 1998; González et al., 1998). Instead of S^0, *Dethiosulfovibrio peptidovorans* and *Pyrococcus abyssi* can use thiosulfate and cystine, respectively, as TEAs (Watrin et al., 1995; Magot et al., 1997), and *Geoglobus ahangari* can oxidize amino acids with Fe(III) (Kashefi et al., 2002). Additionally, *Thermanaerovibrio acidaminovorans*, which normally ferments amino acids, can oxidize amino acids when in syntrophy with a methanogen that scavenges H_2 (Baena et al., 1999). The ability of

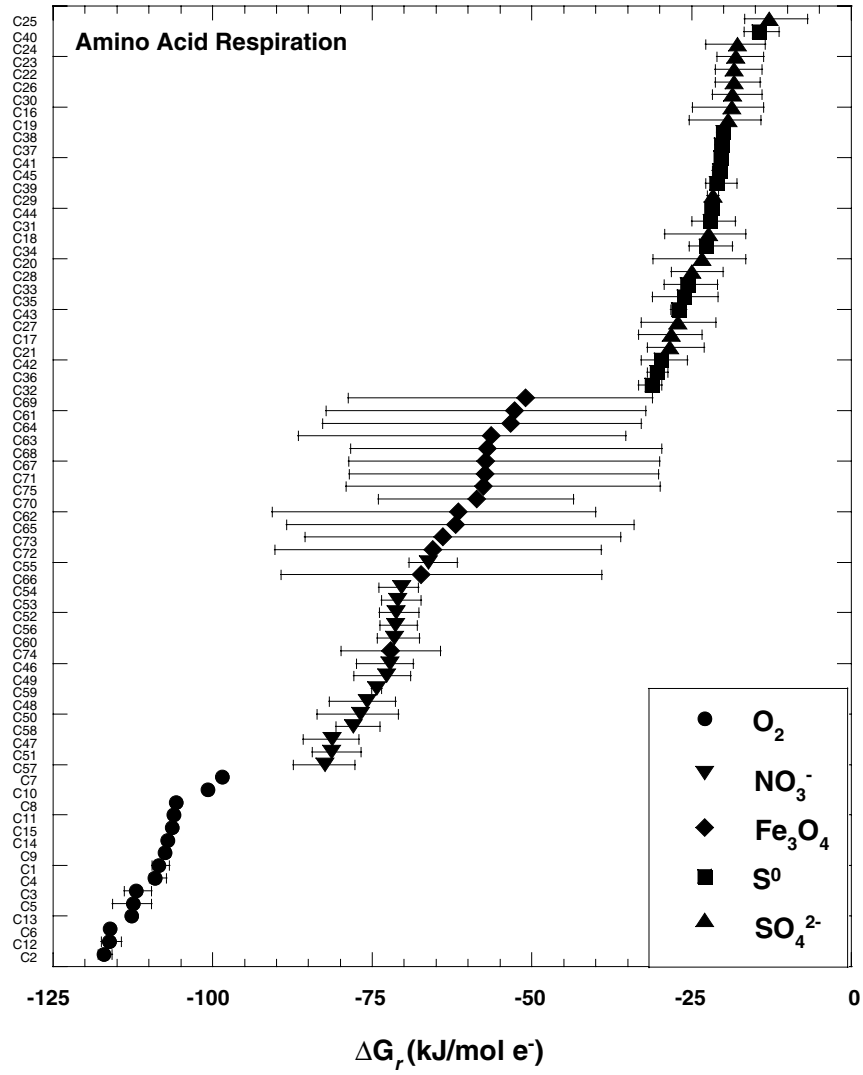

Fig. 4. Profile of ΔG_r (kJ/mol e^-) for respiration of 15 amino acids (see Table 2), distinguished by terminal electron acceptor. Reactions are arranged in order of decreasing energy yield (bottom left to upper right) and reaction numbers on the *y*-axis correspond to those given in Table 7. Bars on each symbol represent the range of values observed across the sites investigated.

thermophilic heterotrophs to exploit other TEAs in conjunction with amino acid oxidation has not been explored.

The concentrations of 15 amino acids were measured at 6 hydrothermal sites in the Baia di Levante, and their oxidation with 5 TEAs was considered as potential thermophile metabolisms (Table 7). The range of values (Table 8) for ΔG_r of amino acid respiration (−6 to −118 kJ/mol e^-) is similar to that of organic acids and neutral aldoses. As with the other organic compound families, the values of ΔG_r are ranked by TEA (Fig. 4), with O_2 yielding 98–118 kJ/mol e^-, followed by nitrate reduction (61–86 kJ/mol e^-), ferric iron reduction (29–91 kJ/mol e^-) and S^0 and SO_4^{2-} reduction (11–34 and 6–34 kJ/mol e^-, respectively). Pozzo Vasca featured the highest concentrations of all amino acids except for threonine, and the highest energy yields from all TEAs except O_2. Similarly, the minimum energy is yielded from anaerobic methionine oxidation at Pozzo Istmo. Overall, the range in ΔG_r across the sites is much larger for the amino acids than it is for both the organic acids and neutral aldoses. This is likely due to their greater structural complexity and range of oxidation states. Note that the highest variability in ΔG_r is seen for the iron reduction reactions, which vary from −30 kJ/mol e^- to −91 kJ/mol e^- across the sites and among different amino acids.

3.2.4. Variability of ΔG_r

Overall, the energetics of organic acid, aldose and amino acid respiration show similar patterns, with little variation in ΔG_r among these organic compound families. Rather, the choice of TEA seems to control ΔG_r of respiration reactions. From highest to lowest energy yield, the order of TEAs is $O_2 \rightarrow NO_3^- \rightarrow Fe_3O_4 \rightarrow S_0 \rightarrow SO_4^{2-}$. The same general pattern was observed for chemolithoautotrophic reactions in the Vulcano hydrothermal system (Amend et al., 2003b). In marine and freshwater sediments, pore water compositions reflect an order of TEAs that corresponds to the standard state properties of the redox couple,

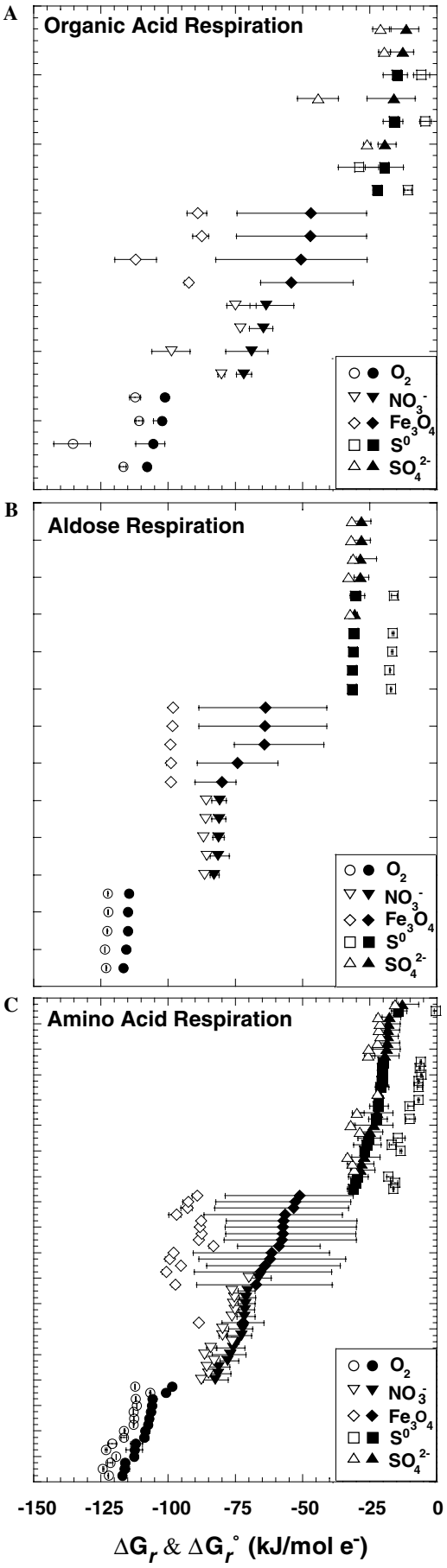

Table 9
Organic acid fermentation reactions

Reaction	
D1	$CH_3COOH \rightarrow CO_2 + CH_4$
D2	$C_2H_5COOH + 2H_2O \rightarrow 2CO_2 + CH_4 + 3H_2$
D3	$C_2H_5COOH + H_2 \rightarrow 2CH_4 + CO_2$
D4	$CH_3CH(OH)COOH + H_2O \rightarrow 2CO_2 + CH_4 + 2H_2$
D5	$CH_3CH(OH)COOH + H_2O \rightarrow CO_2 + 2CH_4$

often referred to as the electron tower (e.g., Nealson and Stahl, 1997). However, in the Vulcano hydrothermal system, the order of NO_3^- and Fe_3O_4 is inverted relative to the progression of TEAs predicted solely from ΔG_r^0 (Figs. 5A–C). It also should be noted that for all TEAs except S^0, the average value of ΔG_r^0 is more negative than that of ΔG_r, particularly with Fe(III), where the differences between ΔG_r and ΔG_r^0 are up to 61 kJ/mol e^- (Fig. 5). These results again emphasize the need to calculate values of ΔG_r and not just ΔG_r^0 when evaluating metabolic energetics.

The broad range of ΔG_r values for iron reduction stands out relative to other TEAs and deserves further discussion. Variations due to temperature alone (reflected in values of ΔG_r^0) are relatively small (3–32 kJ/mol e^- for each organic compound family) compared to those observed in ΔG_r (up to 61 kJ/mol e^-), indicating that the activity product, (Q, Eq. (1)) accounts for much of the energy variability. Values of Q are affected by both activities of reaction constituents (a_i) and their stoichiometric reaction coefficients (v_i). Careful inspection of iron reduction reactions reveals that the stoichiometric reaction coefficients on H^+ and Fe^{2+} are quite large compared to other aqueous species. Additionally, activities of these compounds vary by up to 4 and 5 orders of magnitude, respectively. Together these factors produce significant variability in the value of Q, and therefore ΔG_r across the sites. For example, values of ΔG_r for organic acid oxidation coupled to Fe(III) reduction are 2-fold larger at Pozzo Vasca, which boasts the most acidic (pH 1.98) and most iron rich ($a_{Fe^{2+}} = 10^{-2.18}$) fluids, than they are at Pozzo Istmo, where the pH is 5.84 and Fe^{2+} concentrations are at their lowest ($a_{Fe^{2+}} = 10^{-7.18}$). Even at locations where Fe^{2+} concentrations are moderate, variations in pH can greatly affect values of ΔG_r for Fe(III) reduction reactions. For example, at Stinky Surf Rock and The Grip the activities of Fe^{2+} are nearly identical ($10^{-4.62}$ and $10^{-4.35}$, respectively), however the energy from iron reduction coupled to organic acid oxidation is nearly double at Stinky Surf Rock, where the pH is 3.67, compared to

Fig. 5. Profile of ΔG_r (closed symbols) and ΔG_r^0 (open symbols), in kJ/mol e^-, for respiration of (A) carboxylic acids, (B) neutral aldoses and (C) amino acids, distinguished by terminal electron acceptor. Reactions are arranged in order of decreasing overall energy yield (bottom left to upper right) and reaction numbers are the same as those shown in Figs. 2, 3 and 4. Bars on each symbol represent the range of values observed across the sites investigated. Values of ΔG_r^0 for formic acid respiration deviate from other carboxylic acids (A), which is consistent with standard state properties (Shock, 1995).

Table 10
ΔG_r (kJ/mol C) of organic acid fermentation reactions

	Pozzo Istmo	Pozzo Vasca	Acque Calde 1	Acque Calde 2	Punto Uno	Punto Sette	Stinky Surf Rock	The Grip	Stinky Waist
D1	−18.34	−14.64	−13.94	−18.70	−18.97	−14.07	−26.83	−12.82	−17.45
D2	−27.09	−10.91	−10.05		−18.05	−16.26	−21.47	−3.98	−7.57
D3	−31.62	−20.61	−30.71		−26.80	−15.97	−33.88	−23.88	−30.15
D4			−31.96		−41.46	−40.03			
D5			−52.62		−50.21	−39.74			

5.21 at The Grip. Thus, while the value of the activity product (Q) is a function of all reaction components, Fe^{2+} and pH are particularly important for determining the energetics of iron reduction in Vulcano hydrothermal fluids.

3.3. Fermentation

Fermentation is one of the most common metabolic strategies among the cultured thermophiles. However, its ubiquity may be an artifact of culturing methods, which often use mixtures of complex organic compounds as the basis for enrichments and isolation. While fermentation reactions are well understood in the food and beverage industry, fermentation in natural systems is less well defined. Some thermophiles maintain growth by fermenting simple organic compounds such as monosaccharides. For example, glucose is fermented by the hyperthermophiles *Desulfurococcus saccharovorans* and *Thermococcus zilligii* (Stetter, 1986; Ronimus et al., 1997), and two species of *Thermotoga*, *T. maritima* and *T. neapolitana* (Huber et al., 1986; Jannasch et al., 1988). The Vulcano thermophile *Geobacillus vulcani* ferments several sugars, including glucose, mannose, and galactose (Caccamo et al., 2000; Nazina et al., 2004). Amino acid fermentation is also a common strategy for the hyperthermophilic Thermococcales, including *Pyrococcus abyssi*, *P. horikoshii*, *P.* strain GB-D, *Thermococcus hydrothermalis*, and *T. acidaminovorans* (Erauso et al., 1993; Hoaki et al., 1993; Watrin et al., 1995; Godfroy et al., 1997; Dirmeier et al., 1998; González et al., 1998), the last of which was isolated from Vulcano.

The distinction between fermentation and partial oxidation pathways can be unclear (e.g., Kengen et al., 1996) and fermentation metabolisms are often poorly characterized because of the plethora of reaction products observed in many laboratory experiments (e.g. Erauso et al., 1993; Hoaki et al., 1993; Godfroy et al., 1996, 1997; Kengen et al., 1996; Tarlera et al., 1997). Given the nearly infinite reaction space that is occupied by fermentation it is unreasonable to consider all of the possible reactions. Here we limit our investigation to several examples to permit comparison with the respiration reactions discussed previously. In fermentation reactions, organic compounds are both oxidized and reduced, making the normalization to electrons transferred far more complicated. Therefore, we have normalized values of ΔG_r of potential fermentation reactions to the number of moles of carbon in the reactant molecule. To permit ready comparison between respiration and fermentation reactions we also renormalized values of ΔG_r of the oxidation reactions discussed previously.[3]

Carboxylic acid fermentation reactions are shown in Table 9, with corresponding values of ΔG_r given in Table 10. Values of ΔG_r for reactions D1–D5 range from −4 to −53 kJ/mol C across the sites. The highest energy yields are obtained for lactic acid fermentation (reactions D4 and D5) while propanoic acid fermentation (reaction D2) is the least exergonic. Unlike carboxylic acid respiration, fermentation energy yields are not correlated with pH. In Fig. 6A average values of ΔG_r for organic acid fermentation (filled squares) are compared to those for respiration (open circles). In general, fermentation yields less energy (4–53 kJ/mol C) than respiration (16 to 492 kJ/mol C), and often approaches equilibrium values.

Similar patterns are seen in both aldose and amino acid fermentation. Table 11 shows 14 examples of C_5 and C_6 aldose fermentation to organic acids, carbon dioxide, methane, and H_2, which are common products of thermophilic fermentation (Erauso et al., 1993; Hoaki et al., 1993; Godfroy et al., 1996, 1997; Kengen et al., 1996; Tarlera et al., 1997; Perevalova et al., 2005). Values of ΔG_r for aldose fermentation reactions in the Vulcano hydrothermal system are given in Table 12 and compared to aldose respiration energetics in Fig. 6B. There is very little variation in the energetics of fermentation reactions (−44 to −71 kJ/mol C), compared to the oxidation reactions (−89 to −468 kJ/mol C), and all fermentation reactions yield much less energy than respiration.

Fermentation reactions of four simple amino acids common in thermophile metabolisms (Ala, Glu, Gly, Met) are shown in Table 13 (e.g., Tarlera et al., 1997; Dirmeier et al., 1998; Plugge et al., 2000). Note that sulfur (in Met) and nitrogen do not undergo electron transfer, which is consistent with growth studies of *Caloramator coolhaasii* and *Eubacterium acidaminophilum*, in which NH_4^+ is observed as a product of amino acid metabolism (Baena et al., 1999; Plugge et al., 2000). The values of ΔG_r for these reactions at each site are given in Table 14 and range from 8 to −56 kJ/mol C. The fermentation of methionine is endergonic at all sites investigated. Compared to respiration

[3] To obtain values found in Fig. 6 for respiration reactions, multiply values of ΔG_r (kJ/mol e^-) in Tables 4, 6 and 8 by the number of electrons transferred (Tables 3, 5 and 7) and divide by the number of carbon atoms in the organic compound.

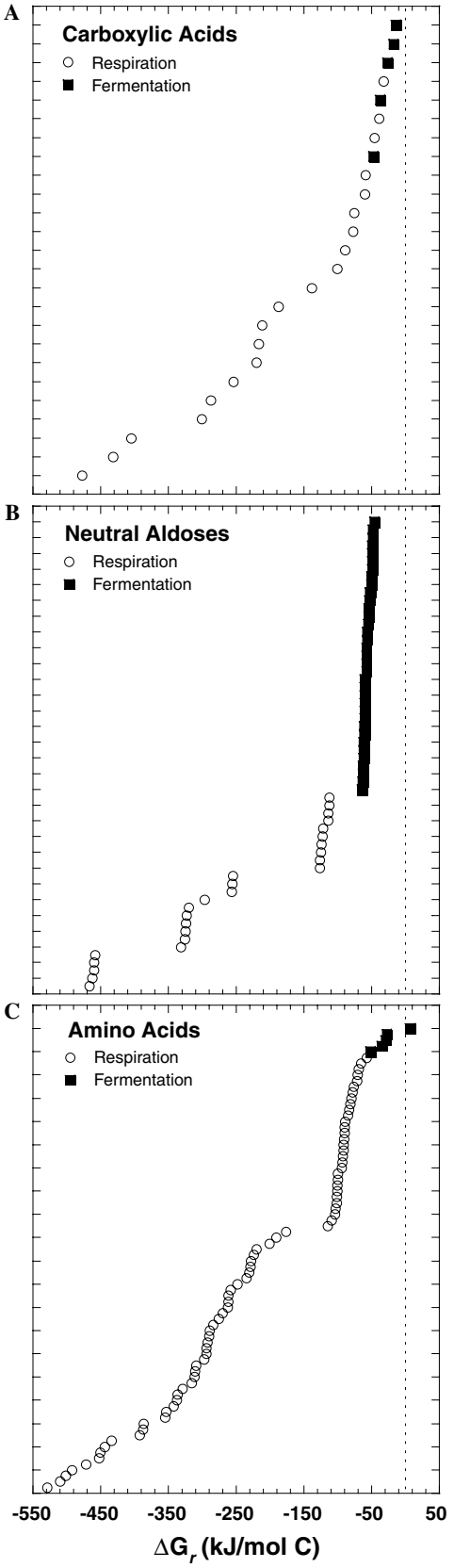

Fig. 6. Profile of ΔG_r (kJ/mol C) for respiration (open circles) and fermentation (filled squares) for (A) carboxylic acids, (B) neutral aldoses and (C) amino acids. Reactions are arranged in order of decreasing energy yield (bottom left to upper right).

Table 11
C_5 and C_6 aldose fermentation reactions

	Reaction
Fermentation of C_5 aldoses	
E1	$C_5H_{10}O_5 + H_2O \rightarrow 2CH_3COOH + CO_2 + 2H_2$
E2	$C_5H_{10}O_5 + 5H_2O \rightarrow 3HCOOH + 2CO_2 + 7H_2$
E3	$C_5H_{10}O_5 + H_2O \rightarrow C_2H_5COOH + 2CO_2 + 3H_2$
E4	$C_5H_{10}O_5 + H_2O \rightarrow C_2H_5COOH + 2HCOOH + H_2$
E5	$C_5H_{10}O_5 + H_2O \rightarrow 2CH_3COOH + HCOOH + H_2$
E6	$C_5H_{10}O_5 + 2H_2O \rightarrow CH_3CH(OH)COOH + 2CO_2 + 4H_2$
E7	$C_5H_{10}O_5 \rightarrow CH_3CH(OH)COOH + CH_3COOH$
Fermentation of C_6 aldoses	
E8	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$
E9	$C_6H_{12}O_6 + 6H_2O \rightarrow 3HCOOH + 3CO_2 + 9H_2$
E10	$C_6H_{12}O_6 + 2H_2O \rightarrow C_2H_5COOH + 3CO_2 + 5H_2$
E11	$C_6H_{12}O_6 \rightarrow C_2H_5COOH + CH_3COOH + HCOOH$
E12	$C_6H_{12}O_6 \rightarrow C_2H_5COOH + CH_3COOH + CO_2 + H_2$
E13	$C_6H_{12}O_6 + 3H_2O \rightarrow CH_3CH(OH)COOH + 3CO_2 + 6H_2$
E14	$C_6H_{12}O_6 + H_2O \rightarrow CH_3CH(OH)COOH + CH_3COOH + CO_2 + 2H_2$

reactions (Fig. 6C), fermentation of amino acids again yields much less energy than amino acid respiration (−30 to −530 kJ/mol C).

3.4. Heterotrophy vs. lithotrophy

Here we have evaluated the energetics of 144 potential heterotrophic metabolisms at 9 sites in the Vulcano hydrothermal system. Given the ubiquity of thermophilic heterotrophs in this system, it seemed reasonable to hypothesize that heterotrophic metabolisms would yield more energy than most chemolithoautotrophic metabolisms. Surprisingly, the range in values of ΔG_r (per electron transferred) for heterotrophic and lithotrophic metabolisms is quite similar (Fig. 7). For example, maxima of ~120 kJ/mol e^- were obtained from both the heterotrophic reactions considered here and the 90 chemolithoautotrophic reactions considered in Amend et al. (2003b). The highest chemolithoautotrophic energy yields are generally with O_2 and NO_2^- as TEAs. As with heterotrophic reactions, nitrate and Fe(III) reduction reactions yield more energy than either S^0 or SO_4^{2-} reduction. The most striking difference between lithotrophic and heterotrophic reaction energetics at Vulcano is that many of the lithotrophic reactions are endergonic, whereas only methionine fermentation yields positive values of ΔG_r in the present study. However, the point has been made previously that the back-reactions of endergonic lithotrophic reactions are potential exergonic metabolisms (Amend et al., 2003b). The fermentation reactions evaluated here yield the lowest amount of energy for all of the reactions considered. Fermentation can be viewed as disproportionation of organic compounds and compared to inorganic disproportionation of S^0 to SO_4^{2-} plus H_2S and CO to CO_2 plus CH_4. The energy yields from these disproportionation reactions were also among the lowest of 90 chemolithoautotrophic reactions (Amend et al., 2003b). Overall, the main variations in ΔG_r are as a function of TEA, for both lithotrophic and heterotrophic

Table 12
ΔG_r (kJ/mol C) of aldose fermentation reactions

	Pozzo Istmo	Pozzo Vasca	Acque Calde 1	Acque Calde 2	Punto Uno	Punto Sette
Fermentation of C_5 aldoses						
E1-Arabinose			−65.21		−63.75	−64.21
E1-Xylose		−59.08			−63.36	−62.87
E2-Arabinose			−54.41		−57.48	−58.77
E2-Xylose		−49.72			−57.09	−57.44
E3-Arabinose			−57.93		−62.78	−65.89
E3-Xylose		−58.43			−62.39	−64.55
E4-Arabinose			−59.83		−55.81	−54.53
E4-Xylose		−52.10			−55.42	−53.19
E5-Arabinose			−66.15		−60.27	−58.53
E5-Xylose		−55.91			−59.88	−57.19
E6-Arabinose			−44.79		−48.68	−51.62
E6-Xylose					−48.29	−50.29
E7-Arabinose			−51.61		−46.59	−45.82
E7-Xylose					−46.20	−44.48
Fermentation of C_6 aldoses						
E8-Galactose		−56.87	−61.95	−58.54	−63.20	−64.42
E8-Glucose		−57.01	−61.67	−57.62	−62.82	−63.79
E8-Mannose	−66.83	−58.84			−63.47	−64.25
E9-Galactose		−49.07	−52.95	−52.11	−57.97	−59.89
E9-Glucose		−49.21	−52.67	−51.19	−57.59	−59.26
E9-Mannose	−70.22	−51.04			−58.23	−59.72
E10-Galactose		−56.33	−55.89		−62.39	−65.82
E10-Glucose		−56.48	−55.61		−62.01	−65.19
E10-Mannose	−63.25	−58.31			−62.66	−65.65
E11-Galactose		−53.63	−62.36		−57.75	−56.25
E11-Glucose		−53.77	−62.08		−57.37	−55.62
E11-Mannose	−57.98	−55.61			−58.01	−56.08
E12-Galactose		−56.27	−61.58		−60.65	−60.98
E12-Glucose		−56.41	−61.29		−60.27	−60.35
E12-Mannose	−59.41	−58.25			−60.92	−60.81
E13-Galactose			−44.94		−50.64	−53.93
E13-Glucose			−44.65		−50.26	−53.30
E13-Mannose					−50.90	−53.76
E14-Galactose			−50.62		−48.90	−49.10
E14-Glucose			−50.34		−48.52	−48.46
E14-Mannose					−49.16	−48.92

Table 13
Amino acid fermentation reactions

Reaction	
F1	$C_2H_5NO_2 + H_2O + H^+ \rightarrow 0.5CH_3COOH + CO_2 + NH_4^+ + H_2$
F2	$C_5H_8NO_4^- + 2H_2O + 2H^+ \rightarrow 2CH_3COOH + CO_2 + NH_4^+ + H_2$
F3	$C_5H_8NO_4^- + 2H_2O + 2H^+ \rightarrow C_2H_5COOH + 2CO_2 + NH_4^+ + 2H_2$
F4	$C_5H_{11}NO_2S + 4H_2O + H^+ \rightarrow C_2H_5COOH + 2HCOOH + NH_4^+ + H_2S + 2H_2$
F5	$C_3H_7NO_3 + H_2O + H^+ \rightarrow CH_3COOH + CO_2 + NH_4^+ + H_2$

Table 14
ΔG_r (kJ/mol C) of amino acid fermentation reactions

	Pozzo Istmo	Pozzo Vasca	Acque Calde 1	Acque Calde 2	Punto Uno	Punto Sette
F1	−32.14	−42.10		−21.37	−36.30	−44.01
F2	−27.88	−30.04	−29.47	−25.18	−30.07	−31.22
F3	−23.58	−29.39	−22.20		−29.10	−32.90
F4	3.60	8.37			7.88	7.27
F5	−50.21	−52.68	−46.87		−51.21	−55.26

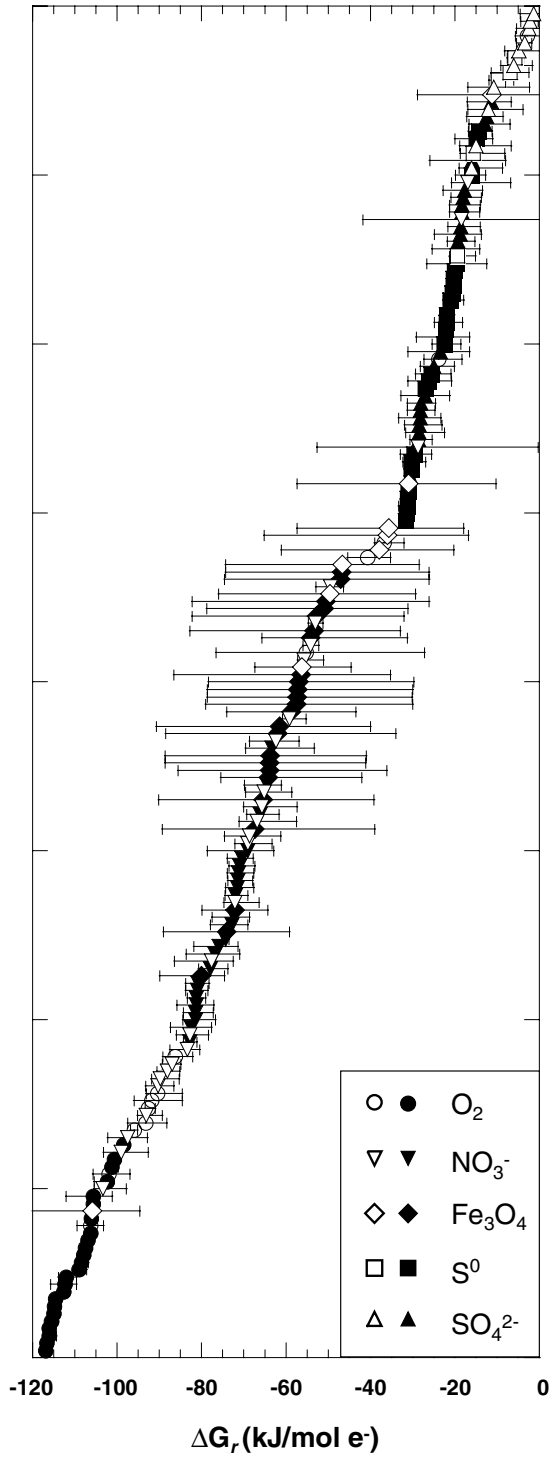

Fig. 7. Profile of ΔG_r (kJ/mol e^-) for heterotrophic respiration (filled symbols) and chemolithotrophic (open symbols) reactions in the Vulcano hydrothermal system. Reactions are arranged in order of decreasing energy yield (bottom left to upper right). Bars on each symbol represent the range of values observed across the sites investigated. Data for chemolithotrophic reactions are from Amend et al. (2003b). Sixty-three of the 90 chemolithotrophic reactions available included the same TEAs considered for chemoheterotrophy, and only these are plotted above. Complete chemolithotrophic reactions, which are not labeled here because of space constraints, are given in Amend et al. (2003b) and also can be found in similar plots in Rogers & Amend (2005, Figs. 3 and 4).

metabolisms, and in both cases disproportionation/fermentation reactions yield very little energy.

4. Concluding remarks

Energy yields of 144 potential heterotrophic metabolisms have been evaluated at nine sites in the Vulcano hydrothermal system. This is, to our knowledge, the first large scale assessment of *in situ* heterotrophic energetics in a shallow marine hydrothermal system. Respiration of carboxylic acids, neutral aldoses and amino acids with five TEAs yielded 6–118 kJ/mol e^- across the sites. Variability in ΔG_r for iron reduction across the sites can be attributed to the wide range in pH and Fe^{2+} concentrations found in this system. Fermentation reactions yielded much less energy than respiration (−8 to 71 kJ/mol C compared with 16 to 530 kJ/mol C). Methionine fermentation was the only endergonic reaction. Many of the reactions considered here can be carried out by cultivated thermophiles, however the vast majority of the reactions investigated have no known microbial catalysts. Nonetheless, energy yields indicate that these reactions could support significant microbial communities, and may well represent metabolic strategies for the as yet uncultured thermophiles in this system. Additionally, these data suggest that isolation of thermophilic heterotrophs need not rely on complex organic substrates, as the simple organic compounds that are found in hydrothermal fluids can support microbial growth, and likely do support heterotrophs in this system.

Values of ΔG_r of these potential metabolisms vary across the sites and show distinct systematic behavior with respect to TEA. Energy yields from respiration are greatest with O_2, followed by NO_3^-, Fe_3O_4, S^0 and SO_4^{2-}, and this order of TEAs could not be predicted from values of ΔG_r^0 alone. Despite the dominance of heterotrophic thermophiles in this system, energy yields from chemolithotrophic reactions are comparable to those from heterotrophic reactions. Furthermore, the order of TEAs that is evident for the heterotrophic reactions is mirrored by the lithotrophic reactions. Interestingly, a similar order of TEAs was observed for 182 chemolithotrophic reactions in Obsidian Pool, Yellowstone National Park (USA) (Shock et al., 2005). However, in that continental hot spring, where concentrations of sulfide, sulfate and Fe^{2+} were much lower, and the pH is near neutral, S^0 reduction yielded more energy than iron reduction. This comparison emphasizes the importance of *in situ* chemical compositions on reaction energetics in hydrothermal systems. Finally, comparable concentrations of the organic compounds discussed here have been measured in deep-sea and deep-subsurface hydrothermal systems (Haberstroh and Karl, 1989; Martens, 1990; Takano et al., 2003b), suggesting that similar reaction energetics may exist and could support a vast diversity of thermophilic heterotrophs in these systems as well.

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References

Amend, J.P., Amend, A.C., Valenza, M., 1998. Determination of volatile fatty acids in the hot springs of Vulcano, Aeolian Islands, Italy. *Organ. Geochem.* **28** (11), 699–705.

Amend, J.P., Helgeson, H.C., 1997. Calculation of the standard molal thermodynamic properties of aqueous biomolecules at elevated temperatures and pressures. I. L-α-amino acids. *J. Chem. Soc., Faraday Trans.* **93**, 1927–1941.

Amend, J.P., Meyer-Dombard, D.R., Sheth, S.N., Zolotova, N., 2003a. *Palaeococcus helgesonii* sp. nov., a facultatively anaerobic, hyperthermophilic Archaeon from a geothermal well on Vulcano Island, southern Italy. *Arch. Microbiol.* **179**, 394–401.

Amend, J.P., Plyasunov, A.V., 2001. Carbohydrates in thermophile metabolism: calculation of the standard molal thermodynamic properties of aqueous pentoses and hexoses at elevated temperatures and pressures. *Geochim. Cosmochim. Acta* **65**, 3901–3917.

Amend, J.P., Rogers, K.L., Meyer-Dombard, D.R., 2004. Microbially mediated sulfur-redox: energetics in marine hydrothermal vent systems. In: Amend, J.P., Edwards, K.J., Lyons, T.W. (Eds.), *Sulfur Biogeochemistry: Past and Present*, vol. 379, pp. 17–34.

Amend, J.P., Rogers, K.L., Shock, E.L., Inguaggiato, S., Gurrieri, S., 2003b. Energetics of chemolithoautotrophy in the hydrothermal system of Vulcano Island, southern Italy. *Geobiology* **1**, 37–58.

Amend, J.P., Shock, E.L., 2001. Energetics of overall metabolic reactions of thermophilic and hyperthermophilic Archaea and Bacteria. *FEMS Microbiol. Rev.* **25**, 175–243.

Andersson, E., Simoneit, B.R.T., Holm, N.G., 2000. Amino acid abundances and stereochemistry in hydrothermally altered sediments from the Juan de Fuca Ridge, northeastern Pacific Ocean. *Appl. Geochem.* **15**, 1169–1190.

Baena, S., Fardeau, M.-L., Woo, T.H.S., Ollivier, B., Labat, M., Patel, B.K.C., 1999. Phylogenetic relationships of three amino-acid-utilizing anaerobes, *Selenomonas acidaminovorans*, '*Selenomonas acidaminophila*' and *Eubacterium acidaminophilum*, as inferred from partial 16S rDNA nucleotide sequences and proposal of *Thermanaerovibrio acidaminovorans* gen. nov., comb. nov. and *Anaeromusa acidaminophila* gen. nov., comb. nov. *Int. J. Syst. Bacteriol.* **49**, 969–974.

Barbier, G., Godfroy, A., Meunier, J.-R., Quérellou, J., Cambon, M.-A., Lesongeur, F., Grimont, P.A.D., Raguénès, G., 1999. *Pyrococcus glycovorans* sp. nov., a hyperthermophilic archaeon isolated from the East Pacific Rise. *Int. J. Syst. Bacteriol.* **49**, 1829–1837.

Caccamo, D., Gugliandolo, C., Stackebrandt, E., Maugeri, T.L., 2000. *Bacillus vulcani* sp. nov., a novel thermophilic species isolated from a shallow marine hydrothermal vent. *Int. J. Syst. Evolut. Microbiol.* **50**, 2009–2012.

Capasso, G., D'Alessandro, W., Favara, R., Inguaggiato, S., Parello, F., 2001. Interaction between the deep fluids and the shallow groundwaters on Vulcano island (Italy). *J. Volcanol. Geothermal Res.* **108**, 187–198.

Capasso, G., Favara, R., Inguaggiato, S., 1997. Chemical features and isotopic composition of gaseous manifestations on Vulcano Island, Aeolian Islands, Italy: an interpretative model of fluid circulation. *Geochim. Cosmochim. Acta* **61** (16), 3425–3440.

Dirmeier, R., Keller, M., Hafenbradl, D., Braun, F.-J., Rachel, R., Burggraf, S., Stetter, K.O., 1998. *Thermococcus acidaminovorans* sp. nov., a new hyperthermophilic alkalophilic archaeon growing on amino acids. *Extremophiles* **2**, 109–114.

Erauso, G., Reysenbach, A.-L., Godfroy, A., Meunier, J.-R., Crump, B., Partensky, F., Baross, J.A., Marteinsson, V., Barbier, G., Pace, N.R., Prieur, D., 1993. *Pyrococcus abyssi* sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Arch. Microbiol.* **160**, 338–349.

Ferreira, A.C., Nobre, M.F., Rainey, F.A., Silva, M.T., Wait, R., Burghardt, J., Chung, A.P., Costa, M.S.D., 1997. *Deinococcus geothermalis* sp. nov. and *Deinococcus murrayi* sp. nov., two extremely radiation-resistant and slightly thermophilic species from hot springs. *Int. J. Syst. Bacteriol.* **47** (4), 939–947.

Fiala, G., Stetter, K.O., 1986. *Pyrococcus furiosus* sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100 °C. *Arch. Microbiol.* **145**, 56–61.

Godfroy, A., Lesongeur, F., Raguenes, G., Querellou, J., Antoine, E., Meunier, J., Guezennec, J., Barbier, G., 1997. *Thermococcus hydrothermalis* sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Int. J. Syst. Bacteriol.* **47** (3), 622–626.

Godfroy, A., Meunier, J.-R., Guezennec, J., Lesongeur, F., Raguénès, G., Rimbault, A., Barbier, G., 1996. *Thermococcus fumicolans* sp. nov., a new hyperthermophilic Archaeon isolated from a deep-sea hydrothermal vent in the North Fiji Basin. *Int. J. Syst. Bacteriol.* **46** (4), 1113–1119.

González, J.M., Masuchi, Y., Robb, F.T., Ammerman, J.W., Maeder, D.L., Yanagibayashi, M., Tamaoka, J., Kato, C., 1998. *Pyrococcus horikoshii* sp. nov., a hyperthermophilic archaeon isolated from a hydrothermal vent at the Okinawa Trough. *Extremophiles* **2**, 123–130.

Grogan, D.W., 1989. Phenotypic characterization of the archaebacterial genus *Sulfolobus*: comparison of five wild-type strains. *J. Bacteriol.* **171** (12), 6710–6719.

Gugliandolo, C., Maugeri, T.L., 1998. Temporal variations in heterotrophic mesophilic bacteria from a marine shallow hydrothermal vent off the island of Vulcano (Eolian Islands, Italy). *Microb. Ecol.* **36**, 13–22.

Gugliandolo, C., Maugeri, T.L., Caccamo, D., Stackebrandt, E., 2003. *Bacillus aeolius* sp. nov. a novel thermophilic, halophilic marine *Bacillus* species from Eolian Islands (Italy). *Syst. Appl. Microbiol.* **26**, 172–176.

Haberstroh, P.R., Karl, D.M., 1989. Dissolved free amino acids in hydrothermal vent habitats of the Guaymas Basin. *Geochim. Cosmochim. Acta* **53**, 2837–2945.

Hafenbradl, D., Keller, M., Dirmeier, R., Rachel, R., Rossnagel, P., Burggraf, S., Huber, H., Stetter, K.O., 1996. *Ferroglobus placidus* gen. nov., sp. nov., a novel hyperthermophilic archaeum that oxidizes Fe^{2+} at neutral pH under anoxic conditions. *Arch. Microbiol.* **166**, 308–314.

Hattori, S., Kamagata, Y., Hanada, S., Shoun, H., 2000. *Thermoacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. *Int. J. Syst. Evolut. Microbiol.* **50**, 1601–1609.

Helgeson, H.C., 1969. Thermodynamics of hydrothermal systems at elevated temperatures and pressures. *Am. J. Sci.* **267**, 729–804.

Hoaki, T., Nishijima, M., Kato, M., Adachi, K., Mizobuchi, S., Hanzawa, N., Maruyama, T., 1994. Growth requirements of hyperthermophilic sulfur-dependent heterotrophic Archaea isolated from a shallow submarine geothermal system with reference to their essential amino acids. *Appl. Environ. Microbiol.* **60** (8), 2898–2904.

Hoaki, T., Nishijima, M., Miyashita, H., Maruyama, T., 1995. Dense community of hyperthermophilic sulfur-dependent heterotrophs in a geothermally heated shallow submarine biotope near Kodakara-Jima Island, Kagoshima, Japan. *Appl. Environ. Microbiol.* **61** (5), 1931–1937.

Hoaki, T., Wirsen, C.O., Hanzawa, S., Maruyama, T., Jannasch, H.W., 1993. Amino acid requirements of two hyperthermophilic archaeal isolates from deep-sea vents, *Desulfurococcus* strain SY and *Pyrococcus* strain GB-D. *Appl. Environ. Microbiol.* **59** (2), 610–613.

Huber, H., Huber, R., Stetter, K.O., 2001. Thermoproteales. In: Dworkin, M. (Ed.), *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*, vol. Release 3.10. http://link.springer-ny.com/link/service/books/10125/. Springer-Verlag.

Huber, R., Eder, W., Heldwein, S., Wanner, G., Huber, H., Rachel, R., Stetter, K.O., 1998. *Thermocrinis ruber* gen. nov., sp. nov., a Pink-Filament-Forming Hyperthermophilic Bacterium Isolated from Yellowstone National Park. *Appl. Environ. Microbiol.* **64** (10), 3576–3583.

Huber, R., Langworthy, T.A., Koning, H., Thomm, M., Woese, C.R., Sleytr, U.B., Stetter, K.O., 1986. *Thermotoga maritima* sp. nov. represents a new genus of unique extremely thermophilic eubacteria growing up to 90 °C. *Arch. Microbiol.* **144**, 324–333.

Jan, R.-L., Wu, J., Chaw, S.-M., Tsai, C.-W., Tsen, S.-D., 1999. A novel species of thermoacidophilic archaeon, *Sulfolobus yangmingensis* sp. nov. *Int. J. Syst. Bacteriol.* **49**, 1809–1816.

Jannasch, H.W., Huber, R., Belkins, S., Stetter, K.O., 1988. *Thermotoga neapolitana* sp. nov. of the extremely thermophilic, eubacterial genus *Thermotoga*. *Arch. Microbiol.* **150**, 103–104.

Johnson, J.W., Oelkers, E.H., Helgeson, H.C., 1992. SUPCRT92: A software package for calculating the standard molal thermodynamic properties of minerals, gases, aqueous species, and reactions among them as functions of temperature and pressure. *Comput. Geosci.* **18**, 899–947.

Kashefi, K., Tor, J.M., Holmes, D.E., Praagh, C.V.G.V., Reysenbach, A.L., Lovely, D.R., 2002. *Geoglobus ahangari* gen. nov., sp. nov., a novel hyperthermophilic archaeon capable of oxidizing organic acids and growing autotrophically on hydrogen with Fe(III) serving as the sole electron acceptor. *Int. J. Syst. Evolut. Microbiol.* **52**, 719–728.

Kawka, O.E., Simoneit, B.R.T., 1990. Polycyclic aromatic hydrocarbons in hydrothermal petroleum from the Guaymas Basin spreading center. *Appl. Geochem.* **5**, 17–27.

Kengen, S.W.M., Stams, A.J.M., Vos, W.M.D., 1996. Sugar metabolism of hyperthermophiles. *FEMS Microbiol. Rev.* **18**, 119–137.

Magot, M., Ravot, G., Campaignolle, X., Ollivier, B., Patel, B.K.C., Fardeau, M.-L., Thomas, P., Crolet, J.-L., Garcia, J.-L., 1997. *Dethiosulfovibrio peptidovorans* gen. nov., sp. nov., a new anaerobic, slightly halophilic, thiosulfate-reducing bacterium from corroding offshore oil wells. *Int. J. Syst. Bacteriol.* **47** (3), 818–824.

Martens, C.S., 1990. Generation of short chain organic acid anions in hydrothermally altered sediments of the Guaymas Basin, Gulf of California. *Appl. Geochem.* **5**, 71–76.

Nanninga, H.J., Drent, W.J., Gottschal, J.C., 1987. Fermentation of glutamate by *Selenomonas acidaminophila* sp. nov. *Arch. Microbiol.* **147**, 152–157.

Nazina, T.N., Lebedeva, E.V., Poltaraus, A.B., Tourova, T.P., Grigoryan, A.A., Sokolova, D.S., Lysenko, A.M., Osipov, G.A., 2004. *Geobacillus gargensis* sp. nov., a novel thermophile from a hot spring, and the reclassification of *Bacillus vulcani* as *Geobacillus vulcani* comb. nov. *Int. J. Syst. Evolut. Microbiol.* **54**, 2019–2024.

Nazina, T.N., Tourova, T.P., Poltaraus, A.B., Novikova, E.V., Grigoryan, A.A., Ivanova, A.E., Lysenko, A.M., Petrunyaka, V.V., Osipov, G.A., Belyaev, S.S., Ivanov, M.V., 2001. Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus*, *Bacillus thermoleovorans, Bacillus kaustophilus, Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus*, *G. thermocatenulatus*, *G. thermoleovorans*, *G. kaustophilus*, *G. thermoglucosidasius* and *G. thermodenitrificans*. *Int. J. Syst. Evolut. Microbiol.* **51**, 433–446.

Nealson, K.H., Stahl, D.A., 1997. Microorganisms and biogeochemical cycles: what can we learn from layered microbial communities? In: Banfield, J.F., Nealson, K.H. (Eds.), *Geomicrobiology: Interactions Between Microbes and Minerals*, vol. 35. The Mineralogical Society of America, pp. 5–34.

Nicolaus, B., Panico, A., Manca, M.C., Lama, L., Gambacorta, A., Maugeri, T., Gugliandolo, C., Caccamo, D., 2000. A thermophilic *Bacillus* isolated from an Eolian shallow hydrothermal vent, able to produce exopolysaccharides. *Syst. Appl. Microbiol.* **23**, 426–432.

Nuccio, P.M., Paonita, A., Sortino, F., 1999. Geochemical modeling of mixing between magmatic and hydrothermal gases: the case of Vulcano Island, Italy. *Earth Planet. Sci. Lett.* **167**, 321–333.

Perevalova, A.A., Svetlichne, V.A., Kublanov, I.V., Chernyh, N.A., Kostrikina, N.A., Tourova, T.P., Kuznetsov, B.B., Bonch-Osmolovskaya, E.A., 2005. *Desulfurococcus fermentans* sp. nov., a novel hyperthermophilic archaeon from a Kamchatka hot spring, and emended description of the genus *Desulfurococcus*. *Int. J. Syst. Evolut. Microbiol.* **55**, 995–999.

Plugge, C.M., Zoetendal, E.G., Stams, A.J.M., 2000. *Caloramator coolhaasii* sp. nov., a glutamate-degrading, moderately thermophilic anaerobe. *Int. J. Syst. Evolut. Microbiol.* **50**, 1155–1162.

Prapaipong, P., Shock, E.L., Koretsky, C.M., 1999. Metal-organic complexes in geochemical processes: temperature dependence of the standard thermodynamic properties of aqueous complexes between metal cations and dicarboxylate ligands. *Geochim. Cosmochim. Acta* **63** (17), 2547–2577.

Rogers, K.L. (2006). *Exploring the microbe-energy interface: Geochemical reaction energetics and microbial diversity in a shallow marine hydrothermal system*. Ph.D. thesis. Washington University in St. Louis.

Rogers, K.L., Amend, J.P., 2005. Archaeal diversity and geochemical energy yields in a geothermal well on Vulcano Island, Italy. *Geobiology* **3**, 319–332.

Ronimus, R.S., Reysenbach, A.-L., Musgrave, D.R., Morgan, H.W., 1997. The phylogenetic position of the *Thermococcus* isolate AN1 based on 16S rRNA gene sequence analysis: a proposal that AN1 represents a new species, *Thermococcus zilligii* sp. nov. *Arch. Microbiol.* **168** (3), 245–248.

Rusch, A., Amend, J.P., 2004. Order-specific 16S rRNA-targeted oligonucleotide probes for (hyper)thermophilic archaea and bacteria. *Extremophiles* **8**, 357–366.

Rusch, A., Walpersdorf, E., deBeer, D., Gurrieri, S., Amend, J.P., 2005. Microbial communities near the oxic/anoxic interface in the hydrothermal system of Vulcano Island, Italy. *Chem. Geol.* **224** (1-3), 169–182.

Shock, E.L., 1992. Stability of peptides in high temperature aqueous solutions. *Geochim. Cosmochim. Acta* **56**, 3481–3491.

Shock, E.L., 1995. Organic acids in hydrothermal solutions: standard molal thermodynamic properties of carboxylic acids and estimates of dissociation constants at high temperatures and pressures. *Am. J. Sci.* **295**, 496–580.

Shock, E.L., Holland, M., Meyer-Dombard, D.R., Amen, J.P., 2005. Geochemical sources of energy for microbial metabolism in hydrothermal ecosystems: Obsidian Pool, Yellowstone National Park. In: Inskeep, W.P., McDermott, T.R. (Eds.), *Geothermal Biology and Geochemistry in Yellowstone National Park*. Montana State University Publications, pp. 95–109.

Shock, E.L., Koretsky, C.M., 1993. Metal-organic complexes in geochemical processes: calculation of standard partial molal thermodynamic properties of aqueous acetate complexes at high pressures and temperatures. *Geochim. Cosmochim. Acta* **57**, 4899–4922.

Shock, E.L., Koretsky, C.M., 1995. Metal-organic complexes in geochemical processes: estimation of standard partial molal thermody-

namic properties of aqueous complexes between metal cations and monovalent organic acid ligands at high pressures and temperatures. *Geochim. Cosmochim. Acta* **59**, 1497–1532.

Silfer, J.A., Engel, M.H., 1990. The effect of hydrothermal processes on the distribution and stereochemistry of amino acids in Recent Antarctic sediments. *Appl. Geochem.* **5**, 159–167.

Simmons, S., Norris, P.R., 2002. Acidophiles of saline water at thermal vents of Vulcano, Italy. *Extremophiles* **6**, 201–207.

Simoneit, B.R.T., Lonsdale, P.F., 1982. Hydrothermal petroleum in mineralized mounds at the seabed of Guaymas Basin. *Nature* **295**, 198–202.

Stetter, K.O., 1986. Diversity of extremely thermophilic archaebacteria. In: Brock, T.D. (Ed.), *Thermophiles: General, Molecular and Applied Microbiology*. John Wiley, New York, pp. 40–74.

Stetter, K.O., 1988. *Archaeoglobus fulgidus* gen. nov., sp. nov.: a new taxon of extremely thermophilic Archaebacteria. *Syst. Appl. Microbiol.* **10**, 172–173.

Stetter, K.O., Lauerer, G., Thomm, M., Neuner, A., 1987. Isolation of extremely thermophilic sulfate reducers: evidence for a novel branch of Archaebacteria. *Science* **236**, 822–824.

Svensson, E., Skoog, A., Amend, J.P., 2004. Concentration and distribution of dissolved amino acids in a shallow hydrothermal vent system, Vulcano Island (Italy). *Org. Geochem.* **35** (9), 1001–1014.

Takano, Y., Horiuchi, T., Kobayashi, K., Marumo, K., Urabe, T., 2003a. Large enantiomeric excesses of L-form amino acids in deep-sea hydrothermal sub-vent of 156 °C fluids at the Suiyo Seamount, Izu-Bonin Arc, Pacific Ocean. *Chem. Lett.* **32**, 970–971.

Takano, Y., Kobayashi, K., Yamanaka, T., Marumo, K., Urabe, T., 2004. Amino acids in the 308 °C deep-sea hydrothermal system of the Suiyo Seamount, Izu-Bonin Arc, Pacific Ocean. *Earth Planet. Sci. Lett.* **219**, 147–153.

Takano, Y., Sato, R., Kaneko, T., Kobayashi, K., Marumo, K., 2003b. Biological origin for amino acids in a deep subterranean hydrothermal vent, Toyoha mine, Hokkaido, Japan. *Org. Geochem.* **34**, 1491–1496.

Tarlera, S., Muxí, L., Soubes, M., Stams, A.J.M., 1997. *Caloramator proteoclasticus* sp. nov., a new moderately thermophilic anaerobic proteolytic bacterium. *Int. J. Syst. Bacteriol.* **47** (3), 651–656.

Tor, J.M., Amend, J.P., Lovley, D.R., 2003. Metabolism of organic compounds in anaerobic, hydrothermal sulphate-reducing marine sediments. *Environ. Microbiol.* **5** (7), 583–591.

Tor, J.M., Kashefi, K., Lovely, D.R., 2001. Acetate oxidation coupled to Fe(III) reduction in hyperthermophilic microorganisms. *Appl. Environ. Microbiol.* **67** (3), 1363–1365.

Watrin, L., Martin-Jezequel, V., Prieur, D., 1995. Minimal amino acid requirements of the hyperthermophilic Archaeon *Pyrococcus abyssi*, isolated from deep-sea hydrothermal vents. *Appl. Environ. Microbiol.* **61** (3), 1138–1140.

Wolery, T.J., 1992. *EQ3/6: A Software Package for Geochemical Modeling of Aqueous Systems*. Lawrence Livermore National Laboratory.