Energy sources for chemolithotrophs in an arsenic- and iron-rich shallow-sea hydrothermal system

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ABSTRACT

The hydrothermally influenced sediments of Tutum Bay, Ambitle Island, Papua New Guinea, are ideal for investigating the chemolithotrophic activities of micro-organisms involved in arsenic cycling because hydrothermal vents there expel fluids with arsenite (As^{III}) concentrations as high as 950 µg L^{-1}. These hot (99 °C), slightly acidic (pH ~6), chemically reduced, shallow-sea vent fluids mix with colder, oxidized seawater to create steep gradients in temperature, pH, and concentrations of As, N, Fe, and S redox species. Near the vents, iron oxyhydroxides precipitate with up to 6.2 wt% arsenate (As^V). Here, chemical analyses of sediment porewaters from 10 sites along a 300-m transect were combined with standard Gibbs energies to evaluate the energy yields ($-\Delta G_r$) from 19 potential chemolithotrophic metabolisms, including As^V reduction, As^{III} oxidation, Fe^{III} reduction, and Fe^{II} oxidation reactions. The 19 reactions yielded 2–94 kJ mol^{-1} e^-, with aerobic oxidation of sulphide and arsenite the two most exergonic reactions. Although anaerobic As^V reduction and Fe^{III} reduction were among the least exergonic reactions investigated, they are still potential net metabolisms. Gibbs energies of the arsenic redox reactions generally correlate linearly with pH, increasing with increasing pH for As^{III} oxidation and decreasing with increasing pH for As^V reduction. The calculated exergonic energy yields suggest that micro-organisms could exploit diverse energy sources in Tutum Bay, and examples of micro-organisms known to use these chemolithotrophic metabolic strategies are discussed. Energy modeling of redox reactions can help target sampling sites for future microbial collection and cultivation studies.

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INTRODUCTION

Chemolithotrophic micro-organisms can be key primary producers in geochemical environments where temperatures are high and light may be scarce (Inskeep *et al.*, 2005). A broad diversity of chemolithotrophic archaea and bacteria have been identified in deep-sea hydrothermal systems (e.g., Jones *et al.*, 1983; Blöchl *et al.*, 1997; Wirsen *et al.*, 1998; L'Haridon *et al.*, 2006; Nunoura *et al.*, 2008), shallow-sea hydrothermal systems (e.g., Huber & Stetter, 1989; Huber *et al.*, 1992; Hafenbradl *et al.*, 1996; Sievert *et al.*, 2000; Takai *et al.*, 2006), terrestrial hot springs (e.g., Huber *et al.*, 1998; Sokol-

ova *et al.*, 2002; Aguiar *et al.*, 2004; He *et al.*, 2004; Mori & Suzuki, 2008), and the deep subsurface (e.g., Kodama & Watanabe, 2003; Takai *et al.*, 2003; Hirayama *et al.*, 2005). Able to metabolize simple inorganic compounds, chemolithotrophs can play important roles in the biogeochemical cycling of numerous elements, including arsenic and iron (Nealson & Saffarini, 1994; Newman *et al.*, 1998; Oremland & Stolz, 2003; Lovley *et al.*, 2004; Kulp *et al.*, 2008).

Several recent studies in hydrothermal ecosystems calculated redox reaction energetics to establish a quantitative framework within which to investigate the potential roles of chemolithotrophs. In addition to energy sources, other factors such as the continued availability of trace elements, the potential buildup of toxic metabolic by-products, and a micro-organism's toxic response system can all influence the types of micro-organisms that can survive in a particular environment over time. Energy availability, however, is a key factor in understanding what types of microbial life can be

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supported in a particular environment. For example, in one study of acidic, circumneutral, and alkaline hot springs in Yellowstone National Park, USA, redox reactions yielded 0–150 kJ mol^{-1} e$^-$ transferred (Shock *et al.*, 2010). In four other acidic and circumneutral hot springs at Yellowstone, values of Gibbs energies (ΔG_r) of 33 inorganic redox reactions were −95 to +15 kJ mol^{-1} e$^-$ transferred (Inskeep *et al.*, 2005). These ranges in ΔG_r were similar to those documented for the shallow-sea hydrothermal vents, sediment seeps, and geothermal wells at Vulcano Island, Italy (Amend *et al.*, 2003; Rogers & Amend, 2006).

In contrast to the vent fluids at Vulcano, the shallow-sea hydrothermal system at Tutum Bay, Ambitle Island, eastern Papua New Guinea (Fig. 1) is rich in arsenic and iron, with concentrations of arsenite (As^{III}) and Fe^{2+} as high as 950 and 810 µg L^{-1}, respectively. Many of the rocks and corals near vent orifices are coated with Fe^{III}-oxyhydroxides and sorbed (coprecipitated) or adsorbed arsenate (As^V, up to 6.2 wt%) (Pichler & Dix, 1996; Pichler & Veizer, 1999). The co-occurrence of reduced and oxidized forms of As and Fe suggests that micro-organisms capable of oxidizing and reducing these elements can take advantage of the chemical disequilibria present in Tutum Bay for energy gain. In this study, we calculated values of ΔG_r for 19 chemolithotrophic reactions using a combination of *in situ* chemical analyses and thermodynamic calculations, and we explore the potential of these reactions to support microbial metabolic activity.

Study site

Ambitle, a volcanic island in eastern Papua New Guinea (Fig. 1), is part of a Quaternary stratovolcano in the Tabar–Feni Island arc (Wallace *et al.*, 1983). Several hot mud pools, fumaroles, and springs with chloride or acid-sulphate waters dot the island (Wallace *et al.*, 1983), while Tutum Bay, off the west coast, features shallow-sea hydrothermal vents among coral-algal reefs in 5- to 10-m water depth, circa 150- to 200-m off shore (Pichler *et al.*, 1999). Two types of hydrothermal venting occur in Tutum Bay, focused discharge from 10- to 15-cm-diameter orifices, and diffuse discharge of gas bubbles (94–98% CO$_2$) through unconsolidated sediment on the seafloor (Pichler *et al.*, 1999; Price & Pichler, 2005). Compared to ambient seawater, the Tutum Bay hydrothermal fluids are enriched in As, B, Mn, Si, Fe, and HCO$_3^-$ and depleted in Na, Cl, SO$_4^{2-}$, and Mg (Pichler *et al.*, 1999; Price *et al.*, 2007). Arsenic is particularly elevated, with hydrothermal fluids containing up to ∼950 µg L^{-1} As^{III}, compared with ∼2 µg L^{-1} total arsenic in local seawater (this
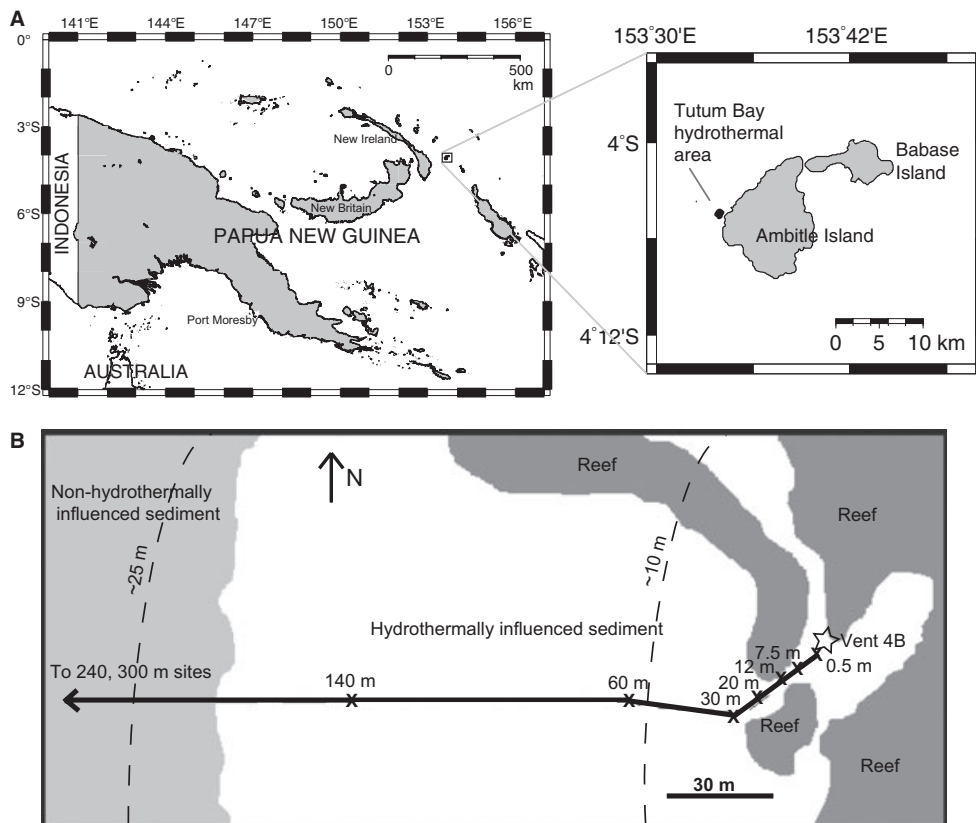
Fig. 1 (A) Papua New Guinea, with Feni Islands (Ambitle and Babase) enlarged. (B) Plan view of Tutum Bay hydrothermal area with sampling transect constructed from Vent 4B. Sampled sites are indicated by Xs. Water depth indicated by dashed lines.

study). Rocks and corals near vent orifices were coated in arsenate-rich Fe^{III}-oxyhydroxide precipitates. These were varied in appearance from soft orange and brown layers to hard greenish ones; they contained up to 6.2 wt% arsenic (Pichler & Veizer, 1999), which is two orders of magnitude higher than that found in other marine Fe^{III}-oxyhydroxide deposits in hot spots and seamounts in the southwest Pacific (Stoffers *et al.*, 1993; Hein *et al.*, 1994).

METHODS

Sampling and laboratory analyses

A sampling transect, 4B, was established beginning at one vent (designated Vent 4 in Pichler *et al.*, 1999) and extended southwest for 30 m, then continued west out to 300 m. The change in direction was necessary to avoid coral reef outcrops. A detailed discussion of sampling procedures and water analyses is given in Price *et al.* (2007). In brief, porewaters were collected by SCUBA divers at 10-cm intervals to 1-m depth with a specially designed sampler. Porewater temperature was measured *in situ* using a waterproof temperature probe. Porewater samples were brought on ship for rapid analyses of ΣPO_4^{3-} and the redox-sensitive Fe^{2+}, NO_3^-, NO_2^-, NH_4^+, ΣS^{2-}, and dissolved oxygen using portable spectrophotometers (HACH, Colorado), according to the manufacturer's instructions. The pH was measured on board using an Ultrameter II pH meter (Myron L, Carlsbad, CA) with tem-

perature compensation. Water samples were also collected and preserved for later analysis of major elements and arsenic concentration and speciation at the Center for Water and Environmental Analysis at the University of South Florida. Concentrations of Na, Ca, Mg, K, Si, Sr, Mn, and B were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES), and Cl^-, Br^-, and SO_4^{2-} were measured by ion chromatography. Hydride generation-atomic fluorescence spectrometry (HG-AFS) was used to measure concentrations of arsenic, and high pressure liquid chromatography was used to separate As^{III}, As^V, dimethylarsinic acid, and monomethylarsonic acid prior to detection by HG-AFS (see Price *et al.* (2007) and Price & Pichler (2005) for details). The porewater chemistries are given in Table 1.

Geochemical calculations

The maximum amount of energy available from any chemical reaction at the temperature, pressure, and chemical composition of interest is given by the Gibbs energy ΔG_r, which can be calculated with the equation

$$\Delta G_r = \Delta G_r^o + RT \ln Q_r, \tag{1}$$

where ΔG_r^o represents the standard Gibbs energy of reaction, R is the universal gas constant, T is the temperature in Kelvin, and Q_r denotes the reaction activity quotient. Values of ΔG_r^o were calculated at the temperatures and pressures of interest

Table 1 Composition of hydrothermal porewaters along Transect 4B in Tutum Bay, 2005

	Distance (in m) along Transect 4B from Vent 4											
	0.5	7.5	12	20	30	60	140	180	240	300	SW	Vent 4
T (°C)	69.9	73.94	81	29.69	33.22	33.9	34	32.8	29.72	29.69	30.2	99
pH	6.14	6.89	6.22	7.64	6.9	6.34	6.11	7.84	7.88	7.08	8.1	6.1
HCO_3^-	574.2	415	605	156	176	264	244	142	154	167	139.7	607.5
Na	1996	5360	2095	9340	10 283	8687	8975	9930	10 300	10 500	10 700	1080
K	151	234	147	356	394	351	365	403	387	401	394	117
Mg	157	636	136	1100	1257	1067	1075	1260	1260	1305	1289	49.7
Ca	219	121	222	372	406	377	369	355	395	411	416	189
Fe^{2+}	0.13	b.d.	0.04	0.11	0.81	0.05	b.d.	0.01	0.02	0.01	b.d.	0.947
Sr	5.7	3.5	6.4	7	7.2	6.9	6.7	10	6.9	7.2	7.35	5.6
Cl^-	3138	8831	2753	15 818	19 040	16 198	15 715	18 529	19 350	18 977	19 456	1182
Br^-	12	26	7.5	46.7	55.5	49.7	46.4	54.1	59	55.7	67.2	7.6
SO_4^{2-}	879.5	1757	1174	2405.2	2734.4	2465.6	2401.3	2688	2785	2715.7	2666.3	974
PO_4^{3-}	1.43	0.19	1.48	0.67	0.23	1.8	0.67	0.08	0.03	0.2	n.a.	n.a.
$As^{(III)}$	22.64	40.93	395.72	5.83	2.31	1.97	1.8	4.89	5.07	2.01	1.3	944
$As^{(V)}$	431.78	523.35	188	18.95	18.19	49.63	45.4	14.75	2.72	12.14	1.1	2
NO_3^-	11.8	8.3	1.3	4.1	4.3	6.4	8.5	7.5	7.1	8.4	n.a.	n.a.
NO_2^-	0.008	0.0155	0.004	0.016	0.022	0.02	0.025	0.4	0.015	0.023	n.a.	n.a.
ΣS^{2-}	8	3	n.a.	n.a.	5	4	3	n.a.	n.a.	2	n.a.	n.a.
$NH_{3(aq)}$	0.38	0.02	0.35	0.11	n.a.	n.a.	0.08	0.06	n.a.	0.07	n.a.	n.a.
B	1	1.3	0.75	5.2	4.8	5.6	5.9	b.d.	4.4	4.7	4.45	9.5
Si	94	56	91	13.6	2.8	15.6	16.4	2	0.4	1	0.6	91.6
$O_{2(aq)}$	3	6.9	2.4	2.8	2	1.8	3.5	2.6	3.7	1.9	n.a.	n.a.

n.a., not available; b.d., below detection; SW, ambient seawater.
All concentrations are in mg L^{-1} except for $As^{(III)}$, $As^{(V)}$, and ΣS^{2-}, which are in µg L^{-1}.

with the computer software package SUPCRT92 (Johnson *et al.*, 1992), and thermodynamic properties given in King & Weller, 1970; Helgeson *et al.*, 1978; Wagman *et al.*, 1982; Helgeson, 1985; Shock & Helgeson, 1988, 1989; Shock *et al.*, 1989; Diakonov *et al.*, 1994; Robie & Hemingway, 1995; and Shock *et al.*, 1997. Values of Q_r can be calculated from the equation

$$Q_r = \prod a_i^{v_i} \qquad (2)$$

where a_i is the activity of the *i*th species and v_i is the stoichiometric reaction coefficient, which is positive for products and negative for reactants. Activities were calculated from the measured porewater compositions (Table 1) using the REACT speciation module in THE GEOCHEMIST'S WORKBENCH software package (v.7.0, Rockware, University of Illinois; Bethke & Yeakel, 2008). Log activities of the aqueous species used in the energy calculations are given in Table 2. To calculate ΔG_r, activities of the dominant aqueous species were used, except in a few indicated cases where nondominant species were substituted to keep the reactions consistent as written. Activities of minerals were set to unity.

Values of ΔG_r were calculated for 19 inorganic redox reactions that represent potential chemolithotrophic metabolisms. These reactions, listed in Table 3, involve the following aqueous species and one mineral: O_2/H_2O, $H_2AsO_4^-/H_3AsO_3$, $NO_3^-/NO_2^-/NH_4^+$, SO_4^{2-}/H_2S, and $Fe(OH)_3$(ferrihydrite)/Fe^{2+}. Micro-organisms conducting anoxygenic photosynthesis using As^{III}, Fe^{2+}, NO_2^-, and sulphide as electron donors are also known to exist (Castenholz *et al.*, 1990; Ehrenreich & Widdel, 1994; Griffin *et al.*, 2007; Kulp *et al.*, 2008), but these metabolisms are beyond the scope of this study. The solid-phase ferric iron in Tutum Bay is predominately present as 2-line ferrihydrite (Pichler & Veizer, 1999). The structure of ferrihydrite is still debated, and thermodynamic data at

elevated temperatures and pressures are not yet available; it has been noted that the thermodynamic properties of ferrihydrite are likely to be highly dependent on particle size (Navrotsky *et al.*, 2008). To permit energy calculations of reactions with ferrihydrite, we estimated the corresponding values of ΔG^o at elevated temperatures using the van't Hoff relation and thermodynamic properties at 25 °C and 1 bar from Navrotsky *et al.* (2008). To facilitate direct comparison of energy yields between potential metabolisms, values of ΔG_r for all redox reactions were normalized per mole of electrons transferred (Table 4), and all reactions were written in the direction in which they are exergonic.

RESULTS AND DISCUSSION

Composition of porewaters

Geochemical analyses of Tutum Bay porewaters are given in Table 1. Vent 4 hydrothermal fluids were 99 °C. Along the transect, the porewater temperatures ranged from 69.9 to 81 °C within the first 12 m and then decreased dramatically to ambient (~30 °C). The pH values were circumneutral at all sites including the vent fluid, varying between 6.1 and 7.88. The temperature, pH, and concentrations of several species (e.g., $SiO_{2(aq)}$, Mg^{2+}, SO_4^{2-}) demonstrate that porewaters at 0–12 m are dominated by hydrothermal fluid, while those further out are principally seawater. However, the elevated As concentrations point to a hydrothermal influence even at 300 m. All 10 transect sites are characterized by arsenic levels significantly higher than background total arsenic in local seawater of 2.4 μg L^{-1}. In the vent fluid, the total arsenic concentration was 946 μg L^{-1}, almost entirely in the form of arsenite. Total arsenic in transect porewaters ranged from 7.8 to 583.7 μg L^{-1}, with arsenite concentrations of 1.8–395.7 μg L^{-1} and arsenate

Table 2 Log activities of aqueous species used in the energy calculations

	Distance (in m) from Vent 4 along Transect 4B											
	0.5	7.5	12	20	30	60	140	180	240	300	SW	Vent 4
H^+	−6.14	−6.89	−6.22	−7.64	−6.9	−6.34	−6.11	−7.84	−7.88	−7.08	−8.1	−6.1
$H_2O^*_{(l)}$	0.997	0.992	0.997	0.985	0.983	0.986	0.986	0.984	0.984	0.983	0.983	0.999
$O_{2(aq)}$	−4.02	−3.64	−4.11	−4.00	−4.14	−4.82	−3.91	−4.03	−3.87	−4.16	n.a.	n.a.
$H_3AsO_{3(aq)}$	−6.52	−6.27	−5.28	−7.13	−7.51	−7.58	−7.62	−7.22	−7.20	−7.58	−7.81	−4.90
$H_2AsO_4^-$	−5.55	−6.03[†]	−5.94	−8.25[†]	−7.60[†]	−6.74	−6.66	−8.57[†]	−9.34[†]	−7.93[†]	−9.96[†]	−7.81
NO_3^-	−3.86	−4.06	−4.82	−4.38	−4.36	−4.18	−4.06	−4.12	−4.14	−4.07	n.a.	n.a.
NO_2^-	−6.90	−6.66	−7.20	−6.66	−6.52	−6.56	−6.46	−5.26	−6.69	−6.50	n.a.	n.a.
NH_4^+	−4.80	−6.15	−4.84	−5.41	n.a.	n.a.	−5.54	−5.69	n.a.	−5.61	n.a	n.a.
SO_4^{2-}	−2.74	−2.68	−2.63	−2.60	−2.58	−2.58	−2.60	−2.58	−2.56	−2.58	−2.59	−2.61
$H_2S_{(aq)}$	−6.77	−7.64[‡]	n.a.	n.a.	−7.21	−7.06	−7.13	n.a.	n.a.	−4.69[‡]	n.a.	n.a.
Fe^{2+}	−6.30	n.a.	−6.89	−6.47	−5.64	−6.82	n.a.	−7.54	−7.23	−7.53	n.a.	−5.47

n.a., not available; SW, ambient seawater.

*Represents activities, not log activities.

†$HAsO_4^{2-}$ dominates, but in the energy calculations, the log activities of $H_2AsO_4^-$ were used throughout.

‡HS^- dominates, but in the energy calculations, the log activities of $H_2S_{(aq)}$ were used throughout.

Table 3 Chemolithotrophic reactions evaluated for energy yield in Tutum Bay Reaction e⁻ transferred

	Reaction	e⁻ transferred
1	$H_2S_{(aq)} + 2O_{2(aq)} \rightarrow SO_4^{2-} + 2H^+$	8
2	$H_3AsO_{3(aq)} + 0.5O_{2(aq)} \rightarrow H_2AsO_4^- + H^+$	2
3	$4Fe^{2+} + O_{2(aq)} + 10H_2O \rightarrow 4Fe(OH)_3 + 8H^+$	4
4	$2NH_4^+ + 3O_{2(aq)} \rightarrow 2NO_2^- + 2H_2O + 4H^+$	12
5	$NH_4^+ + 2O_{2(aq)} \rightarrow NO_3^- + H_2O + 2H^+$	8
6	$2NO_2^- + O_{2(aq)} \rightarrow 2NO_3^-$	4
7	$H_2S_{(aq)} + 4NO_3^- \rightarrow SO_4^{2-} + 4NO_2^- + 2H^+$	8
8	$H_2S_{(aq)} + NO_3^- + H_2O \rightarrow SO_4^{2-} + NH_4^+$	8
9	$H_3AsO_{3(aq)} + NO_3^- \rightarrow H_2AsO_4^- + NO_2^- + H^+$	2
10	$2Fe^{2+} + NO_3^- + 5H_2O \rightarrow 2Fe(OH)_3 + NO_2^- + 4H^+$	2
11	$4H_3AsO_{3(aq)} + NO_3^- + H_2O \rightarrow 4H_2AsO_4^- + NH_4^+ + 2H^+$	8
12	$8Fe^{2+} + NO_3^- + 21H_2O \rightarrow 8Fe(OH)_3 + NH_4^+ + 14H^+$	8
13	$NH_4^+ + 3NO_3^- \rightarrow 4NO_2^- + 2H^+ + H_2O$	6
14	$3H_2S_{(aq)} + 4NO_2^- + 4H_2O + 2H^+ \rightarrow 3SO_4^{2-} + 4NH_4^+$	24
15	$3H_3AsO_{3(aq)} + NO_2^- + H_2O \rightarrow 3H_2AsO_4^- + NH_4^+ + H^+$	6
16	$6Fe^{2+} + NO_2^- + 16H_2O \rightarrow 6Fe(OH)_3 + NH_4^+ + 10H^+$	6
17	$H_2S_{(aq)} + 8Fe(OH)_3 + 14H^+ \rightarrow SO_4^{2-} + 8Fe^{2+} + 20H_2O$	8
18	$H_3AsO_{3(aq)} + 2Fe(OH)_3 + 3H^+ \rightarrow H_2AsO_4^- + 2Fe^{2+} + 5H_2O$	2
19	$H_2S_{(aq)} + 4H_2AsO_4^- + 2H^+ \rightarrow SO_4^{2-} + 4H_3AsO_{3(aq)}$	8

Table 4 ΔG_r of reactions in Tutum Bay (kJ mol^{-1} e⁻ transferred)

	Distance (in m) from Vent 4 along Transect 4B											
	0.5	7.5	12	20	30	60	140	180	240	300	Vent 4	Avg. ΔG_r
R'xn 1	−91.72	−92.61			−94.01	−92.28	−93.24			−94.04		−92.98
2	−67.88	−73.18	−72.92	−80.07	−74.66	−69.26	−69.57	−81.27	−83.90	−76.01		−74.87
3	−64.67		−62.93	−76.03	−72.65	−58.25		−72.62	−74.58	−63.12		−68.11
4	−44.12	−44.66	−44.46	−45.90			−42.80	−44.68		−44.25		−44.41
5	−39.61	−40.47	−40.19	−42.18			−39.74	−42.04		−40.77		−40.71
6	−26.09	−27.90	−27.38	−31.00	−30.94	−29.27	−30.53	−34.11	−30.41	−30.32		−29.79
7	−65.63	−64.71			−63.07	−63.00	−62.70			−63.72		−63.81
8	−52.11	−52.14					−53.50			−53.27		−52.75
9	−41.79	−45.28	−45.54	−49.07	−43.72	−39.99	−39.04	−47.17	−53.49	−45.69		−45.08
10	−38.59		−35.55	−45.03	−41.71	−28.97		−38.51	−44.17	−32.80		−38.17
11	−28.27	−32.71	−32.73	−37.89			−29.84	−39.24		−35.24		−33.70
12	−27.73		−25.28	−37.00				−33.68		−25.50		−29.84
13	−18.03	−16.76	−17.08	−14.90			−12.27	−10.57		−13.93		−14.79
14	−47.61	−47.94					−50.43			−49.79		−48.94
15	−23.76	−28.52	−28.45	−34.16			−26.77	−36.60		−31.76		−30.00
16	−20.56		−18.47	−30.13				−27.94		−18.87		−23.19
17	−27.00				−21.15	−33.85				−30.70		−28.17
18	−3.20		−9.99	−4.03	−2.01	−11.02		−8.66	−9.32	−12.89	−6.50	−7.51
19	−23.85	−19.43			−19.35	−23.01	−23.66			−18.03		−21.22

concentrations of 2.7–523.4 μg L^{-1}. Arsenate dominated arsenite at all sites except at 12 and 240 m. Concentrations of Fe^{2+}, on the other hand, were relatively low at all sites (≤ 0.13 mg L^{-1}), except at 30 m (0.81 mg L^{-1}), and concentrations of other redox species investigated were relatively constant at all sites.

The porefluids are a mixture of seawater and hydrothermal fluids. If we take the conservative approach that the porefluids have undergone mechanical mixing, as described in McCollom & Shock (1997), the temperature of the porefluid is determined by mixing of the seawater and hydrothermal fluid in discrete ratios. At the hydrothermally influenced 12-m site, for example, a mixture of 74% hydrothermal fluid (Vent 4) and 26% seawater produces the observed 81 °C porefluid temperature. However, simple mixing of the hydrothermal fluid and seawater at the same ratios would
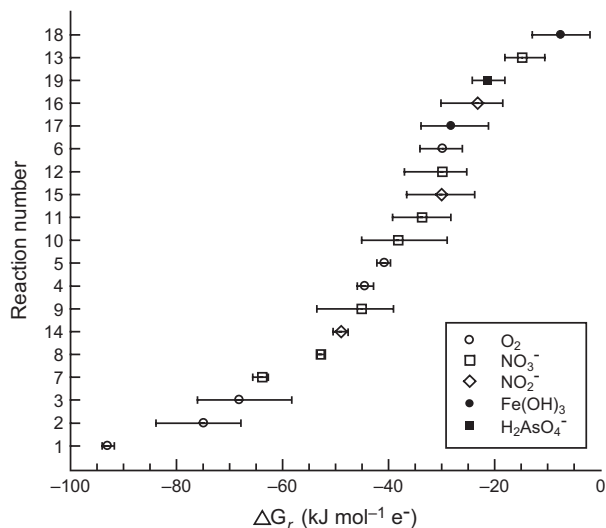
Fig. 2 Average values of ΔG_r (kJ mol^{-1} e$^-$) of 19 chemolithotrophic reactions (listed in Table 3) in Tutum Bay porewaters. Horizontal lines represent range of values across up to 11 sites. Different symbols represent different terminal electron acceptors in the reactions.
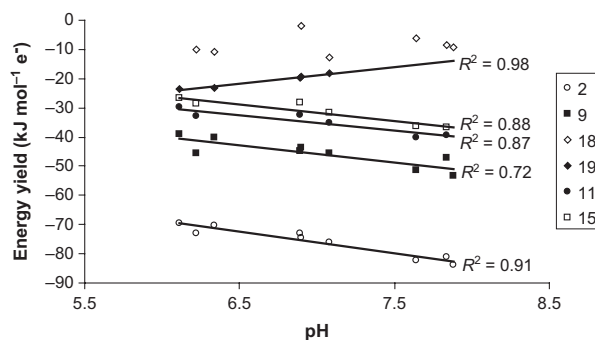

Fig. 3 ΔG_r (kJ mol^{-1} e$^-$) of arsenic redox reactions as a function of pH. Reaction numbers correspond to those in Table 3.

produce 698.9 µg L^{-1} As^{III}, while only 395 µg L^{-1} As^{III} was observed. Similarly, for As^{V}, 1.8 µg L^{-1} would be expected, but the observed concentration was two orders of magnitude higher. The observed arsenic concentrations are consistent with substantial arsenic oxidation, although it is not definitive that these differences in observed vs. expected arsenic concentrations are due solely to microbial processes. However, the nonconservative mixing behavior suggests that micro-organisms may be playing a role in catalyzing arsenic redox processes in Tutum Bay.

Gibbs free energy calculations

The Gibbs energies of the 19 redox reactions listed in Table 3 were calculated at up to 10 sites along the sampling transect. For each reaction, the average energy (symbol) and range across the sites (horizontal lines) were calculated (values given in Table 4) and plotted in Fig. 2. Values of ΔG_r are plotted from least exergonic at the top right to most exergonic at the bottom left, with a net range from −2.0 to −94.0 kJ mol^{-1} e$^-$. Aerobic sulphide oxidation yielded the highest average energy at −93.0 kJ mol^{-1} e$^-$, followed by aerobic oxidation of As^{III} (−74.9 kJ mol^{-1} e$^-$) and aerobic ferrous iron oxidation (−68.1 kJ mol^{-1} e$^-$). The least exergonic reactions were anaerobic reduction of ferrihydrite with As^{III} (−7.5 kJ mol^{-1} e$^-$ on average) and ammonium oxidation to nitrite with nitrate as terminal electron acceptor (TEA) (−14.8 kJ mol^{-1} e$^-$ on average). The only As^{V} reduction reaction investigated, with H$_2$S as electron donor, also yielded minimal energy (−21.2 kJ mol^{-1} e$^-$ on average). Three anaerobic As^{III} oxidation reactions (9, 11, 15), with NO$_3^-$ or NO$_2^-$ as TEA, yielded moderate amounts of energy on average (−45.1, −33.7, and

−30.0 kJ mol^{-1} e$^-$, respectively), as did the anaerobic oxidation of H$_2$S with ferrihydrite (−28.2 kJ mol^{-1} e$^-$).

Values of ΔG_r showed a moderately strong correlation with pH of the porefluid (R^2 = 0.72–0.98) for all, but one (18) of the arsenic reactions (Fig. 3). It can be seen that energy yields (−ΔG_r) increase with increasing pH for four of the five As^{III} oxidation reactions (2, 9, 11, 15). The opposite trend is observed for the As^{V} reduction reaction (19), where energy yields decrease with increasing pH. There was no correlation between pH and ΔG_r for reaction 18 (R^2 = 0). Aerobic ammonium oxidation to nitrate (5) was the only other reaction to show a strong correlation (R^2 = 0.93) between energy yield and pH. No correlations were seen between ΔG_r and temperature or ΔG_r and distance from the vent.

The energy yields calculated for the Tutum Bay system can be compared with those at other hydrothermal sites. For example, the net range in ΔG_r for inorganic redox reactions in the present study is 92 kJ mol^{-1} e$^-$, compared to ~120 kJ mol^{-1} e$^-$ in the shallow-sea hydrothermal system at Vulcano Island, Italy (Amend *et al.*, 2003) and ~150 kJ mol^{-1} e$^-$ in an array of terrestrial hot springs at Yellowstone National Park, USA (Shock *et al.*, 2010). It should be pointed out, however, that both the Vulcano study and the Yellowstone study were able to consider a far greater number of potential chemolithoautotrophic metabolisms—90 reactions at Vulcano and 150 at Yellowstone. This expanded coverage was possible in both locations because more redox-sensitive compounds were above measurable detection limits, including H$_2$ and CH$_4$ in the gas phase. Aerobic oxidation of H$_2$ and CH$_4$ were among the most exergonic inorganic redox reactions investigated in both Vulcano and Yellowstone. In addition, the ranges of pH are broader at Vulcano (2.0–6.3) and Yellowstone (<2 to >9) than at Tutum Bay (6.1–7.9), which translates to variations in ΔG_r for many redox reactions. Most of the reactions investigated at Yellowstone showed a correlation between ΔG_r and pH (Shock *et al.*, 2010), as seen for most of the arsenic redox reactions in Tutum Bay. Lastly, in the Vulcano system, concentrations of Fe^{2+} varied over four orders of magnitude, and the ΔG_r for any given Fe redox reaction could vary by as much as ~60 kJ mol^{-1} e$^-$ across the 10 sites investigated there.

By comparison, at Tutum Bay, the concentrations of Fe^{2+} differed by <1 order of magnitude across all sites, and the ΔG_r for each of the iron redox reactions (3, 10, 12, 16, 17, 18) ranged by ~13 kJ mol^{-1} e^{-} across all of the sites investigated. Arsenic concentrations, which varied by two orders of magnitude, affected the range of ΔG_r for a particular As redox reaction (2, 9, 11, 15, 18, 19) by as much as 16 kJ mol^{-1} e^{-}.

Microbial metabolic strategies

Relatively few bacteria (and no archaea) are currently known to obtain metabolic energy from As^{III} oxidation (Oremland & Stolz, 2003; Oremland *et al.*, 2009). Examples include the heterotrophic *Hydrogenophaga* strain NT-14, the chemolithotrophic α-Proteobacteria NT-26 and BEN-5 isolated from Australian gold mines, and the β-Proteobacterium CL-1 isolated from As-contaminated soil, all of which couple As^{III} oxidation to O$_2$ reduction (Santini *et al.*, 2000, 2002; vanden Hoven & Santini, 2004; Garcia-Dominguez *et al.*, 2008), and the Soviet gold mine isolate *Pseudomonas arsenitoxidans* (Ilyaletdinov & Abdrashitova, 1981). The latter species has been lost, preventing its further characterization, but the Australian isolates were grown at 28 °C, similar to many of the temperatures recorded in Tutum Bay. While there is no direct evidence of microbially mediated As oxidation in the Tutum Bay hydrothermal system, we can use the energy framework developed in this study to constrain where this metabolic strategy is most favorable. Reaction 2, aerobic arsenite oxidation, was consistently the second most exergonic reaction, or the most exergonic reaction at sites where geochemical data were not available to evaluate the energy yield of aerobic sulphide oxidation. This high energy yield at all sites investigated suggests that microbes could catalyze this reaction for energy gain across Tutum Bay, and at 20, 180, and 240 m where the energy yield was 80–84 kJ mol^{-1} e^{-}, these sites in particular could host microbial consortia dominated by aerobic As^{III} oxidizers. Reaction 9, oxidation of As^{III} with nitrate as the TEA, is catalyzed, for example, by the chemolithotrophic facultative anaerobe *Alkalilimnicola ehrlichii* strain MLHE-1, isolated from the arsenic-rich waters of Mono Lake, California (Oremland *et al.*, 2002; Hoeft *et al.*, 2007). The arsenic concentration of Mono Lake (200 μM [0.015 g L^{-1}]) is approximately 15 times greater than that in Tutum Bay waters, but nevertheless, the energy yield from reaction 9 in Tutum Bay (39.0–53.5 kJ mol^{-1} e^{-}) appears to be more than enough to support substantial biomass.

Many of the other reactions investigated here are utilized by micro-organisms known to thrive in other hydrothermal and geothermal sites and may support micro-organisms in Tutum Bay as well. Sulphur oxidation and reduction, for example, are widely utilized as metabolic strategies by chemolithotrophs, including thermophiles. Both shallow-sea and deep-sea hydrothermal systems are home to sulphide oxidizers. Strains of the sulphide-oxidizing *Thiomicrospira*, for

instance, have been documented off Milos, Greece and at deep-sea vents along the mid-Atlantic ridge, the Izu-Bonin arc in the Western Pacific, and the Galapagos Rift (Jannasch & Mottl, 1985; Muyzer *et al.*, 1995; Brinkhoff *et al.*, 1999, 2005; Kato *et al.*, 2009). Various *Thiobacillus* species are capable of sulphide oxidation coupled to oxygen and nitrate reduction (Robertson & Kuenen, 2006). The sulphide oxidation reactions (1, 7, 8, 14) that used oxygen, nitrate, and nitrite as TEAs all yielded similar amounts of energy across the sites investigated regardless of pH or temperature, and aerobic sulphide oxidation (reaction 1) was the most exergonic reaction at all sites evaluated, suggesting that aerobic and anaerobic sulphide oxidizers could thrive throughout Tutum Bay.

Aerobic oxidation of ammonium to nitrite (reaction 4) yields 44.4 kJ mol^{-1} e^{-} on average in the various Tutum Bay sites investigated. This reaction appears to support the ammonia-oxidizing archaeon, Candidatus *Nitrosopumilus maritimus* (Könneke *et al.*, 2005), isolated from a tropical water environment at similar temperature and pH to those found in Tutum Bay. Based on 16S ribosomal RNA gene surveys, organisms similar to *Nitrosopumilus maritimus* and the archaeal marine sponge symbiont *Cenarchaeum symbiosum,* which contains putative ammonia-oxidizing genes, have been identified in hydrothermally influenced Tutum Bay sediment samples (N. H. Akerman & J. P. Amend, unpubl. data). The energetics data in this study show that Tutum Bay could support microbial communities that rely on similar NH_3/NH_4^+-oxidizing reactions for metabolic growth.

In Tutum Bay, the reduction of ferric iron yielded on average 28.2 (reaction 17) and 7.5 kJ mol^{-1} e^{-} (reaction 18). Reaction 18, the reduction of ferrihydrite coupled to arsenite oxidation, was the least exergonic of the reactions investigated. Note, however, that this amount of energy is apparently sufficient to support microbial growth; several studies have shown that some anaerobes can grow at energy yields close to $\Delta G_r = 0$ (Conrad *et al.*, 1986; Wu *et al.*, 1994; Jackson & McInerney, 2002). The reduction of ferric iron has previously been noted as an ability of all hyperthermophilic micro-organisms, with many microbes able to harness energy from the reaction (Kashefi *et al.*, 2002), and the environment near the hydrothermal vent orifices in Tutum Bay may be conducive for similar thermophilic species to flourish. In addition, a number of microbial species are able to use hydrogen gas as an electron donor in the reduction of ferric iron; however, $H_{2(g)}$ was measured at <0.01 mmol of $H_{2(g)}$ per total moles of dry gas in Tutum Bay (Pichler *et al.*, 1999), suggesting that reactions using $H_{2(g)}$ in this environment would be less energy-yielding and subsequently microbial species would be less likely to gain energy from such reactions. The oxidation of ferrous iron, on the other hand, yielded a much wider range of energy, between 68.1 (reaction 3) and 23.2 kJ mol^{-1} e^{-} (reaction 16) on average. *Thiobacillus* species, in addition to oxidizing sulphur species, are also capable of oxidizing iron and although mainly found in highly acidic environments,

some species are capable of growing in neutral pH (Robertson & Kuenen, 2006). Other species capable of aerobic oxidation of ferrous iron include *Sulfobacillus thermosulfidooxidans* and *Sulfobacillus acidophilus* (Norris *et al.*, 1996). Combined, these energy data suggest the bay can support a variety of bacterial and archaeal species involved in the cycling of iron, arsenic, nitrogen, and sulphur.

SUMMARY

In Tutum Bay, values of ΔG_r were calculated for 19 different potential metabolic reactions using *in situ* chemical composition and physical parameters. The energy yields ranged from 2.0 to 94.0 kJ mol^{-1} e$^-$ across the 10 sampling sites. The five most exergonic reactions used O_2 or NO_3^- as TEAs. All reactions investigated yielded enough energy to potentially support microbial biomass at various temperatures and pHs. Although the hydrothermal system at Tutum Bay contains high amounts of arsenate, arsenite, and iron(III)-oxyhydroxide precipitates (predominately in the form of 2-line ferrihydrite), the most energy-yielding reaction was aerobic sulphide oxidation. Sulphur reduction and oxidation reactions are typically important and widespread in hydrothermal systems, and sulphur redox reactions were the most energetic group of reactions evaluated in Tutum Bay. Also of interest were the energy-yielding nitrogen redox reactions, which represent metabolic strategies for ammonia-oxidizing archaea and bacteria. The reactions involving arsenic and iron species are also known to support numerous microbial species, suggesting that Tutum Bay hydrothermal porewaters can support a variety of microbial communities that may play key roles in the redox cycling of As, Fe, N, and S. The energy calculations presented here provide a framework for identifying the most likely sampling sites for micro-organisms utilizing a particular metabolic strategy, opening the way for laboratory-based microbial studies. In the Tutum Bay field site, for instance, microbial community analyses focused on geochemical areas of interest are currently underway (N. H. Akerman & J. P. Amend, unpubl. data; Meyer-Dombard *et al.*, in press). Growth media that have been geochemically designed to target some of the metabolic strategies outlined above are also currently being used to cultivate enrichments from Tutum Bay with the goal of isolating micro-organisms capable of particular metabolic strategies, such as As oxidation with nitrate as the TEA. In general, energy modeling can be conducted in many other environments of interest, and the energetics can act as a practical guide for conducting further field- and laboratory-based microbiological studies.

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