

Akerman, Nancy H. ; Price, Roy E. ; Pichler, Thomas ; Amend, Jan Peter

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

Journal Article as: peer-reviewed accepted version (Postprint)

DOI of this document\* (secondary publication): <https://doi.org/10.26092/elib/3267>

Publication date of this document: 02/09/2024

\* for better findability or for reliable citation

Recommended Citation (primary publication/Version of Record) incl. DOI:

AKERMAN, N.H., PRICE, R.E., PICHLER, T. and AMEND, J.P. (2011), Energy sources for chemolithotrophs in an arsenic- and iron-rich shallow-sea hydrothermal system. *Geobiology*, 9: 436-445.  
<https://doi.org/10.1111/j.1472-4669.2011.00291.x>

Please note that the version of this document may differ from the final published version (Version of Record/primary publication) in terms of copy-editing, pagination, publication date and DOI. Please cite the version that you actually used. Before citing, you are also advised to check the publisher's website for any subsequent corrections or retractions (see also <https://retractionwatch.com/>).

This is the peer reviewed version of the article cited above, which has been published in final form at <https://doi.org/10.1111/j.1472-4669.2011.00291.x>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions. This article may not be enhanced, enriched or otherwise transformed into a derivative work, without express permission from Wiley or by statutory rights under applicable legislation. Copyright notices must not be removed, obscured or modified. The article must be linked to Wiley's version of record on Wiley Online Library and any embedding, framing or otherwise making available the article or pages thereof by third parties from platforms, services and websites other than Wiley Online Library must be prohibited.

This document is made available with all rights reserved.

Take down policy

If you believe that this document or any material on this site infringes copyright, please contact [publizieren@suub.uni-bremen.de](mailto:publizieren@suub.uni-bremen.de) with full details and we will remove access to the material.

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

N. H. AKERMAN,<sup>1,\*</sup> R. E. PRICE,<sup>1,2</sup> T. PICHLER<sup>2,†</sup> AND J. P. AMEND<sup>1,3</sup>

<sup>1</sup>*Department of Earth and Planetary Sciences, Washington University, St Louis, Missouri, USA*

<sup>2</sup>*Department of Geology, University of South Florida, Tampa, Florida, USA*

<sup>3</sup>*Division of Biology and Biomedical Sciences, Washington University, St Louis, Missouri, USA*

## 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 ( $\text{As}^{\text{III}}$ ) concentrations as high as  $950 \mu\text{g L}^{-1}$ . These hot ( $99^\circ\text{C}$ ), slightly acidic ( $\text{pH} \sim 6$ ), 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 ( $\text{As}^{\text{V}}$ ). 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  $\text{As}^{\text{V}}$  reduction,  $\text{As}^{\text{III}}$  oxidation,  $\text{Fe}^{\text{III}}$  reduction, and  $\text{Fe}^{\text{II}}$  oxidation reactions. The 19 reactions yielded 2–94  $\text{kJ mol}^{-1} \text{e}^-$ , with aerobic oxidation of sulphide and arsenite the two most exergonic reactions. Although anaerobic  $\text{As}^{\text{V}}$  reduction and  $\text{Fe}^{\text{III}}$  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  $\text{As}^{\text{III}}$  oxidation and decreasing with increasing pH for  $\text{As}^{\text{V}}$  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.

Corresponding author: N. H. Akerman. Tel.: +1 508 289 7659; fax: +1 508 457 4727; e-mail: nakerman@mbl.edu

## 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; Wirsén *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

\*Present address: Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA.

†Present address: Universität Bremen, Geowissenschaften, Postfach 330 440, D-28334 Bremen, Germany.

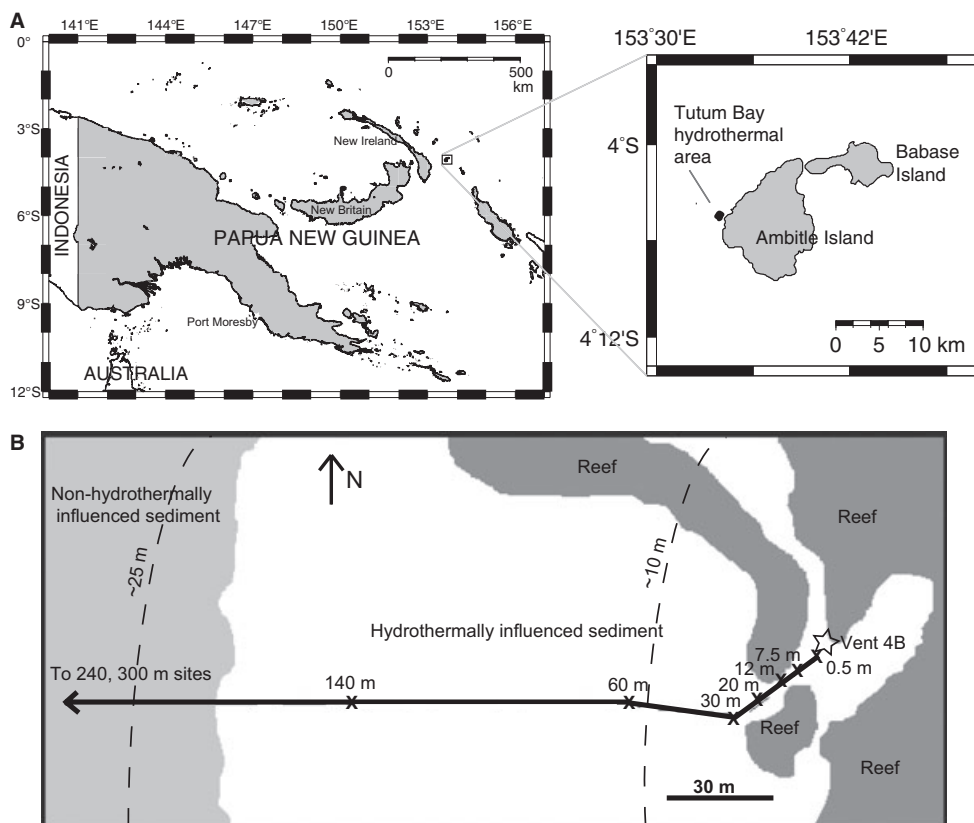
supported in a particular environment. For example, in one study of acidic, circumneutral, and alkaline hot springs in Yellowstone National Park, USA, redox reactions yielded 0–150 kJ mol<sup>-1</sup> e<sup>-</sup> transferred (Shock *et al.*, 2010). In four other acidic and circumneutral hot springs at Yellowstone, values of Gibbs energies ( $\Delta G_r$ ) of 33 inorganic redox reactions were -95 to +15 kJ mol<sup>-1</sup> e<sup>-</sup> transferred (Inskeep *et al.*, 2005). These ranges in  $\Delta G_r$  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<sup>III</sup>) and Fe<sup>2+</sup> as high as 950 and 810 µg L<sup>-1</sup>, respectively. Many of the rocks and corals near vent orifices are coated with Fe<sup>III</sup>-oxyhydroxides and sorbed (coprecipitated) or adsorbed arsenate (As<sup>V</sup>, 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  $\Delta G_r$  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<sub>2</sub>) 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<sub>3</sub><sup>-</sup> and depleted in Na, Cl, SO<sub>4</sub><sup>2-</sup>, and Mg (Pichler *et al.*, 1999; Price *et al.*, 2007). Arsenic is particularly elevated, with hydrothermal fluids containing up to ~950 µg L<sup>-1</sup> As<sup>III</sup>, compared with ~2 µg L<sup>-1</sup> 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<sup>III</sup>-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<sup>III</sup>-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  $\Sigma\text{PO}_4^{3-}$  and the redox-sensitive Fe<sup>2+</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>,  $\Sigma\text{S}^{2-}$ , 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<sup>-</sup>, Br<sup>-</sup>, and SO<sub>4</sub><sup>2-</sup> 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<sup>III</sup>, As<sup>V</sup>, 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  $\Delta G_r$ , which can be calculated with the equation

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

where  $\Delta G_r^o$  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  $\Delta G_r^o$  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										SW	Vent 4
	0.5	7.5	12	20	30	60	140	180	240	300		
$T$ (°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 <sub>3</sub> <sup>-</sup>	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 <sup>2+</sup>	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 <sup>-</sup>	3138	8831	2753	15 818	19 040	16 198	15 715	18 529	19 350	18 977	19 456	1182
Br <sup>-</sup>	12	26	7.5	46.7	55.5	49.7	46.4	54.1	59	55.7	67.2	7.6
SO <sub>4</sub> <sup>2-</sup>	879.5	1757	1174	2405.2	2734.4	2465.6	2401.3	2688	2785	2715.7	2666.3	974
PO <sub>4</sub> <sup>3-</sup>	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 <sup>(III)</sup>	22.64	40.93	395.72	5.83	2.31	1.97	1.8	4.89	5.07	2.01	1.3	944
As <sup>(V)</sup>	431.78	523.35	188	18.95	18.19	49.63	45.4	14.75	2.72	12.14	1.1	2
NO <sub>3</sub> <sup>-</sup>	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 <sub>2</sub> <sup>-</sup>	0.008	0.0155	0.004	0.016	0.022	0.02	0.025	0.4	0.015	0.023	n.a.	n.a.
$\Sigma\text{S}^{2-}$	8	3	n.a.	n.a.	5	4	3	n.a.	n.a.	2	n.a.	n.a.
NH <sub>3(aq)</sub>	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 <sub>2(aq)</sub>	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<sup>-1</sup> except for As<sup>(III)</sup>, As<sup>(V)</sup>, and  $\Sigma\text{S}^{2-}$ , which are in  $\mu\text{g L}^{-1}$ .

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

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

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  $\Delta G_r$ , 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  $\Delta G_r$  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  $\Delta G^\circ$  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  $\Delta G_r$  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  $\mu g L^{-1}$ . In the vent fluid, the total arsenic concentration was 946  $\mu g L^{-1}$ , almost entirely in the form of arsenite. Total arsenic in transect porewaters ranged from 7.8 to 583.7  $\mu g L^{-1}$ , with arsenite concentrations of 1.8–395.7  $\mu g L^{-1}$  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 <sup>†</sup>	-5.94	-8.25 <sup>†</sup>	-7.60 <sup>†</sup>	-6.74	-6.66	-8.57 <sup>†</sup>	-9.34 <sup>†</sup>	-7.93 <sup>†</sup>	-9.96 <sup>†</sup>	-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 <sup>‡</sup>	n.a.	n.a.	-7.21	-7.06	-7.13	n.a.	n.a.	-4.69 <sup>‡</sup>	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.

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

<sup>‡</sup> $HS^-$  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<sup>-</sup> transferred

	Reaction	e <sup>-</sup> transferred
1	$\text{H}_2\text{S}_{(\text{aq})} + 2\text{O}_{2(\text{aq})} \rightarrow \text{SO}_4^{2-} + 2\text{H}^+$	8
2	$\text{H}_3\text{AsO}_{3(\text{aq})} + 0.5\text{O}_{2(\text{aq})} \rightarrow \text{H}_2\text{AsO}_4^- + \text{H}^+$	2
3	$4\text{Fe}^{2+} + \text{O}_{2(\text{aq})} + 10\text{H}_2\text{O} \rightarrow 4\text{Fe}(\text{OH})_3 + 8\text{H}^+$	4
4	$2\text{NH}_4^+ + 3\text{O}_{2(\text{aq})} \rightarrow 2\text{NO}_2^- + 2\text{H}_2\text{O} + 4\text{H}^+$	12
5	$\text{NH}_4^+ + 2\text{O}_{2(\text{aq})} \rightarrow \text{NO}_3^- + \text{H}_2\text{O} + 2\text{H}^+$	8
6	$2\text{NO}_2^- + \text{O}_{2(\text{aq})} \rightarrow 2\text{NO}_3^-$	4
7	$\text{H}_2\text{S}_{(\text{aq})} + 4\text{NO}_3^- \rightarrow \text{SO}_4^{2-} + 4\text{NO}_2^- + 2\text{H}^+$	8
8	$\text{H}_2\text{S}_{(\text{aq})} + \text{NO}_3^- + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + \text{NH}_4^+$	8
9	$\text{H}_3\text{AsO}_{3(\text{aq})} + \text{NO}_3^- \rightarrow \text{H}_2\text{AsO}_4^- + \text{NO}_2^- + \text{H}^+$	2
10	$2\text{Fe}^{2+} + \text{NO}_3^- + 5\text{H}_2\text{O} \rightarrow 2\text{Fe}(\text{OH})_3 + \text{NO}_2^- + 4\text{H}^+$	2
11	$4\text{H}_3\text{AsO}_{3(\text{aq})} + \text{NO}_3^- + \text{H}_2\text{O} \rightarrow 4\text{H}_2\text{AsO}_4^- + \text{NH}_4^+ + 2\text{H}^+$	8
12	$8\text{Fe}^{2+} + \text{NO}_3^- + 21\text{H}_2\text{O} \rightarrow 8\text{Fe}(\text{OH})_3 + \text{NH}_4^+ + 14\text{H}^+$	8
13	$\text{NH}_4^+ + 3\text{NO}_3^- \rightarrow 4\text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O}$	6
14	$3\text{H}_2\text{S}_{(\text{aq})} + 4\text{NO}_2^- + 4\text{H}_2\text{O} + 2\text{H}^+ \rightarrow 3\text{SO}_4^{2-} + 4\text{NH}_4^+$	24
15	$3\text{H}_3\text{AsO}_{3(\text{aq})} + \text{NO}_2^- + \text{H}_2\text{O} \rightarrow 3\text{H}_2\text{AsO}_4^- + \text{NH}_4^+ + \text{H}^+$	6
16	$6\text{Fe}^{2+} + \text{NO}_2^- + 16\text{H}_2\text{O} \rightarrow 6\text{Fe}(\text{OH})_3 + \text{NH}_4^+ + 10\text{H}^+$	6
17	$\text{H}_2\text{S}_{(\text{aq})} + 8\text{Fe}(\text{OH})_3 + 14\text{H}^+ \rightarrow \text{SO}_4^{2-} + 8\text{Fe}^{2+} + 20\text{H}_2\text{O}$	8
18	$\text{H}_3\text{AsO}_{3(\text{aq})} + 2\text{Fe}(\text{OH})_3 + 3\text{H}^+ \rightarrow \text{H}_2\text{AsO}_4^- + 2\text{Fe}^{2+} + 5\text{H}_2\text{O}$	2
19	$\text{H}_2\text{S}_{(\text{aq})} + 4\text{H}_2\text{AsO}_4^- + 2\text{H}^+ \rightarrow \text{SO}_4^{2-} + 4\text{H}_3\text{AsO}_{3(\text{aq})}$	8

**Table 4** ΔG<sub>r</sub> of reactions in Tutum Bay (kJ mol<sup>-1</sup> e<sup>-</sup> transferred)

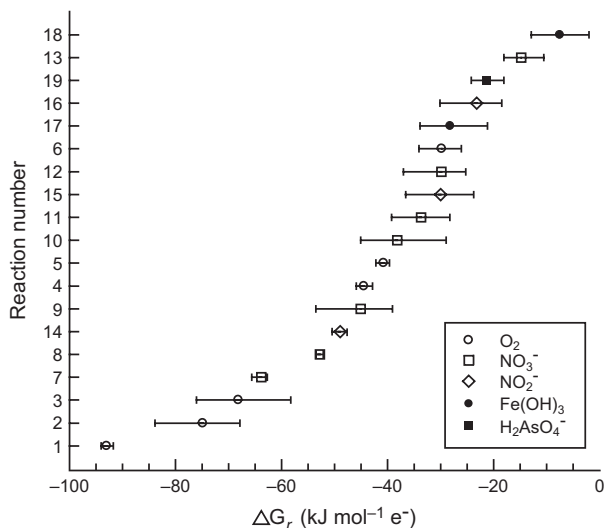
R'xn	Distance (in m) from Vent 4 along Transect 4B										Vent 4	Avg. ΔG <sub>r</sub>
	0.5	7.5	12	20	30	60	140	180	240	300		
1	-91.72	-92.61			-94.01	-92.28	-93.24				-94.04	-92.98
2	-67.88	-73.18	-72.92	-80.07	-74.66	-69.26	-69.57	-81.27	-83.90	-76.01		-74.87
3	-64.67		-62.93	-76.03	-72.65	-58.25		-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<sup>-1</sup>. Arsenate dominated arsenite at all sites except at 12 and 240 m. Concentrations of Fe<sup>2+</sup>, on the other hand, were relatively low at all sites (≤0.13 mg L<sup>-1</sup>), except at 30 m (0.81 mg L<sup>-1</sup>), 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 McColom & 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



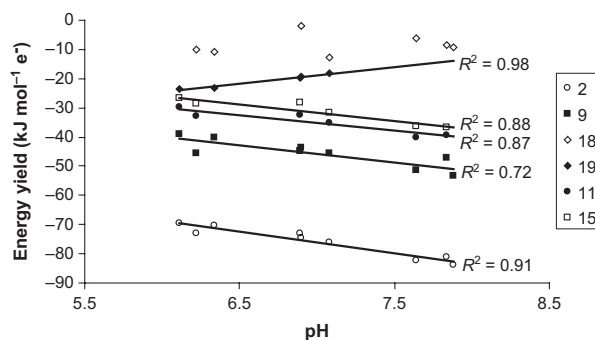


**Fig. 2** Average values of  $\Delta G_r$  ( $\text{kJ mol}^{-1} \text{e}^-$ ) of 19 chemolithotrophic reactions (listed in Table 3) in Tutum Bay porewaters. Horizontal lines represent range of values across up to 11 sites. Different symbols represent different terminal electron acceptors in the reactions.

produce  $698.9 \mu\text{g L}^{-1} \text{As}^{\text{III}}$ , while only  $395 \mu\text{g L}^{-1} \text{As}^{\text{III}}$  was observed. Similarly, for  $\text{As}^{\text{V}}$ ,  $1.8 \mu\text{g L}^{-1}$  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  $\Delta G_r$  are plotted from least exergonic at the top right to most exergonic at the bottom left, with a net range from  $-2.0$  to  $-94.0 \text{ kJ mol}^{-1} \text{e}^-$ . Aerobic sulphide oxidation yielded the highest average energy at  $-93.0 \text{ kJ mol}^{-1} \text{e}^-$ , followed by aerobic oxidation of  $\text{As}^{\text{III}}$  ( $-74.9 \text{ kJ mol}^{-1} \text{e}^-$ ) and aerobic ferrous iron oxidation ( $-68.1 \text{ kJ mol}^{-1} \text{e}^-$ ). The least exergonic reactions were anaerobic reduction of ferrihydrite with  $\text{As}^{\text{III}}$  ( $-7.5 \text{ kJ mol}^{-1} \text{e}^-$  on average) and ammonium oxidation to nitrite with nitrate as terminal electron acceptor (TEA) ( $-14.8 \text{ kJ mol}^{-1} \text{e}^-$  on average). The only  $\text{As}^{\text{V}}$  reduction reaction investigated, with  $\text{H}_2\text{S}$  as electron donor, also yielded minimal energy ( $-21.2 \text{ kJ mol}^{-1} \text{e}^-$  on average). Three anaerobic  $\text{As}^{\text{III}}$  oxidation reactions (9, 11, 15), with  $\text{NO}_3^-$  or  $\text{NO}_2^-$  as TEA, yielded moderate amounts of energy on average ( $-45.1$ ,  $-33.7$ , and



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

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

Values of  $\Delta G_r$  showed a moderately strong correlation with pH of the porefluid ( $R^2 = 0.72\text{--}0.98$ ) for all, but one (18) of the arsenic reactions (Fig. 3). It can be seen that energy yields ( $-\Delta G_r$ ) increase with increasing pH for four of the five  $\text{As}^{\text{III}}$  oxidation reactions (2, 9, 11, 15). The opposite trend is observed for the  $\text{As}^{\text{V}}$  reduction reaction (19), where energy yields decrease with increasing pH. There was no correlation between pH and  $\Delta G_r$  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  $\Delta G_r$  and temperature or  $\Delta G_r$  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  $\Delta G_r$  for inorganic redox reactions in the present study is  $92 \text{ kJ mol}^{-1} \text{e}^-$ , compared to  $\sim 120 \text{ kJ mol}^{-1} \text{e}^-$  in the shallow-sea hydrothermal system at Vulcano Island, Italy (Amend *et al.*, 2003) and  $\sim 150 \text{ kJ mol}^{-1} \text{e}^-$  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  $\text{H}_2$  and  $\text{CH}_4$  in the gas phase. Aerobic oxidation of  $\text{H}_2$  and  $\text{CH}_4$  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  $\Delta G_r$  for many redox reactions. Most of the reactions investigated at Yellowstone showed a correlation between  $\Delta G_r$  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  $\text{Fe}^{2+}$  varied over four orders of magnitude, and the  $\Delta G_r$  for any given Fe redox reaction could vary by as much as  $\sim 60 \text{ kJ mol}^{-1} \text{e}^-$  across the 10 sites investigated there.

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

### Microbial metabolic strategies

Relatively few bacteria (and no archaea) are currently known to obtain metabolic energy from  $\text{As}^{\text{III}}$  oxidation (Oremland & Stolz, 2003; Oremland *et al.*, 2009). Examples include the heterotrophic *Hydrogenophaga* strain NT-14, the chemolithotrophic  $\alpha$ -Proteobacteria NT-26 and BEN-5 isolated from Australian gold mines, and the  $\beta$ -Proteobacterium CL-1 isolated from As-contaminated soil, all of which couple  $\text{As}^{\text{III}}$  oxidation to  $\text{O}_2$  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  $28^\circ\text{C}$ , 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  $80\text{--}84 \text{ kJ mol}^{-1} \text{ e}^-$ , these sites in particular could host microbial consortia dominated by aerobic  $\text{As}^{\text{III}}$  oxidizers. Reaction 9, oxidation of  $\text{As}^{\text{III}}$  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; Hoefl *et al.*, 2007). The arsenic concentration of Mono Lake ( $200 \mu\text{M}$  [ $0.015 \text{ g L}^{-1}$ ]) is approximately 15 times greater than that in Tutum Bay waters, but nevertheless, the energy yield from reaction 9 in Tutum Bay ( $39.0\text{--}53.5 \text{ kJ mol}^{-1} \text{ e}^-$ ) 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  $44.4 \text{ kJ mol}^{-1} \text{ e}^-$  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  $\text{NH}_3/\text{NH}_4^+$ -oxidizing reactions for metabolic growth.

In Tutum Bay, the reduction of ferric iron yielded on average  $28.2$  (reaction 17) and  $7.5 \text{ kJ mol}^{-1} \text{ e}^-$  (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,  $\text{H}_{2(\text{g})}$  was measured at  $<0.01 \text{ mmol of H}_{2(\text{g})}$  per total moles of dry gas in Tutum Bay (Pichler *et al.*, 1999), suggesting that reactions using  $\text{H}_{2(\text{g})}$  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  $23.2 \text{ kJ mol}^{-1} \text{ e}^-$  (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  $\Delta G_r$  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<sup>-1</sup> e<sup>-</sup> across the 10 sampling sites. The five most exergonic reactions used O<sub>2</sub> or NO<sub>3</sub><sup>-</sup> 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.

## ACKNOWLEDGMENTS

We thank the other members of the University of South Florida and Washington University Biocomplexity team for help-

ful discussions and assistance during field work: Jim Garey, Pam Hallock Muller, Bryan McCloskey, Dave Karlen, and D'Arcy Meyer-Dombard. We also thank David Heeszel for assistance with the figures. This work was funded by NSF grants BE/CBC 0221834 (to J.P.A. and T.P.) and EAR 0447231 (to J.P.A.) and by a McDonnell Center for the Space Sciences Graduate Fellowship and a NASA-Missouri Space Grant Consortium Graduate Fellowship (to N.H.A.).

## REFERENCES

- Aguiar P, Beveridge TJ, Reysenbach A-L (2004) *Sulfurhydrogenibium azorense*, sp. nov., a thermophilic hydrogen-oxidizing microaerophile from terrestrial hot springs in the Azores. *International Journal of Systematic and Evolutionary Microbiology* **54**, 33–39.
- Amend JP, Rogers KL, Shock EL, Gurrieri S, Inguaggiato S (2003) Energetics of chemolithoautotrophy in the hydrothermal system of Vulcano Island, southern Italy. *Geobiology* **1**, 37–58.
- Bethke CM, Yeakel S (2008) *The Geochemist's Workbench Release 7.0, GWB Essentials Guide*. University of Illinois, Urbana-Champaign, IL.
- Blöchl E, Rachel R, Burggraf S, Hafenbradl D, Jannasch HW, Stetter KO (1997) *Pyrolobus fumarii*, gen. and sp. nov., represents a novel group of archaea, extending the upper temperature limit for life to 113°C. *Extremophiles* **1**, 14–21.
- Brinkhoff T, Sievert SM, Kuever J, Muyzer G (1999) Distribution and diversity of sulfur-oxidizing *Thiomicrospira* spp. at a shallow-water hydrothermal vent in the Aegean Sea (Milos, Greece). *Applied and Environmental Microbiology* **65**, 3843–3849.
- Brinkhoff T, Kuever J, Muyzer G, Jannasch HW (2005) Genus VI. *Thiomicrospira* Kuenen and Veldkamp 1972, 253AL. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, 2nd edn (eds Brenner DJ, Krieg NR, Staley JT). Springer, New York, pp. 193–199.
- Castenholz RW, Bauld J, Jørgenson BB (1990) Anoxygenic microbial mats of hot springs: thermophilic *Chlorobium* sp. *FEMS Microbiology Letters* **74**, 325–336.
- Conrad R, Schink B, Phelps TJ (1986) Thermodynamics of H<sub>2</sub>-consuming and H<sub>2</sub>-producing metabolic reactions in diverse methanogenic environments under *in situ* conditions. *FEMS Microbiology and Ecology* **38**, 353–360.
- Diakonov I, Khodakovskiy I, Schott J, Sergeeva E (1994) Thermodynamic properties of iron oxides and hydroxides. I. Surface and bulk thermodynamic properties of goethite (alpha-FeOOH) up to 500 K. *European Journal of Mineralogy* **6**, 967–983.
- Ehrenreich A, Widdel F (1994) Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. *Applied and Environmental Microbiology* **60**, 4517–4526.
- García-Domínguez E, Mumford A, Rhine ED, Paschal A, Young LY (2008) Novel autotrophic arsenite-oxidizing bacteria isolated from soil and sediments. *FEMS Microbiology Ecology* **66**, 401–410.
- Griffin BM, Schott J, Schink B (2007) Nitrite, an electron donor for anoxygenic photosynthesis. *Science* **316**, 1870.
- Hafenbradl D, Keller M, Dirmeyer R, Rachel R, Roßnagel P, Burggraf S, Huber H, Stetter KO (1996) *Ferroplasma placidum* gen. nov., sp. nov., a novel hyperthermophilic archaeum that oxidizes Fe<sup>2+</sup> at neutral pH under anoxic conditions. *Archives of Microbiology* **166**, 308–314.
- He Z-G, Zhong H, Li Y (2004) *Acidianus tengchongensis* sp. nov., a new species of acidothermophilic archaeon isolated from an acidothermal spring. *Current Microbiology* **48**, 159–163.

- Hein JR, Yeh H-W, Gunn SH, Gibbs AE, Wang C-H (1994) Composition and origin of hydrothermal ironstones from central Pacific seamounts. *Geochimica et Cosmochimica Acta* **58**, 179–189.
- Helgeson HC (1985) Errata II. Thermodynamics of minerals, reactions, and aqueous solutions at high pressures and temperatures. *American Journal of Science* **285**, 845–855.
- Helgeson HC, Delany JM, Nesbitt WH, Bird DK (1978) Summary and critique of the thermodynamic properties of rock-forming minerals. *American Journal of Science* **278A**, 1–229.
- Hirayama H, Takai K, Inagaki F, Nealson KH, Horikoshi K (2005) *Thiobacter subterraneus* gen. nov., sp. nov., an obligately chemolithoautotrophic, thermophilic, sulfur-oxidizing bacterium from a subsurface hot aquifer. *International Journal of Systematic and Evolutionary Microbiology* **55**, 467–472.
- Hoefl SE, Switzer Blum J, Stolz JF, Tabita FR, Witte B, King GM, Santini JM, Oremland RS (2007) *Alkalilimnicola ehrlichii* sp. nov., a novel, arsenite-oxidizing haloalkaliphilic gammaproteobacterium capable of chemoautotrophic or heterotrophic growth with nitrate or oxygen as the electron acceptor. *International Journal of Systematic and Evolutionary Microbiology* **57**, 504–512.
- vanden Hoven RN, Santini JM (2004) Arsenite oxidation by the heterotroph *Hydrogenophaga* sp. str. NT-14: the arsenite oxidase and its physiological electron acceptor. *Biochimica et Biophysica Acta* **1656**, 148–155.
- Huber H, Stetter KO (1989) *Thiobacillus prosperus* sp. nov., represents a new group of halotolerant metal-mobilizing bacteria isolated from a marine geothermal field. *Archives of Microbiology* **151**, 479–485.
- Huber R, Wilharm T, Huber D, Trincone A, Burggraf S, König H, Rachel R, Rockinger I, Fricke H, Stetter KO (1992) *Aquifex pyrophilus* gen. nov. spec. nov., represents a novel group of marine hyperthermophilic hydrogen-oxidizing bacteria. *Systematic and Applied Microbiology* **15**, 340–351.
- Huber R, Eder W, Heldwein S, Wanner G, Huber H, Rachel R, Stetter KO (1998) *Thermocrinis ruber* gen. nov., sp. nov., a pink-filament-forming hyperthermophilic bacterium isolated from Yellowstone National Park. *Applied and Environmental Microbiology* **64**, 3576–3583.
- Ilyaletdinov AN, Abdrashitova SA (1981) Autotrophic oxidation of arsenic by a culture of *Pseudomonas arsenitoxidans*. *Mikrobiologiya* **50**, 197–204.
- Inskip WP, Ackerman GG, Taylor WP, Kozubal M, Korf S, Macur RE (2005) On the energetics of chemolithotrophy in nonequilibrium systems: case studies of geothermal springs in Yellowstone National Park. *Geobiology* **3**, 297–317.
- Jackson BE, McInerney MJ (2002) Anaerobic microbial metabolism can proceed close to thermodynamic limits. *Nature* **415**, 454–456.
- Jannasch HW, Mottl MJ (1985) Geomicrobiology of deep sea hydrothermal vents. *Science* **229**, 717–725.
- Johnson JW, Oelkers EH, Helgeson HC (1992) SUPCRT92: a software package for calculating the standard molal thermodynamic properties of minerals, gases, aqueous species, and reactions from 1 to 5000 bar and 0 to 1000°C. *Computers and Geosciences* **18**, 899–947.
- Jones WJ, Leigh JA, Mayer F, Woese CR, Wolfe RS (1983) *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. *Archives of Microbiology* **136**, 254–261.
- Kashefi K, Holmes DE, Reysenbach A-L, Lovley DR (2002) Use of Fe(III) as an electron acceptor to recover previously uncultured hyperthermophiles: isolation and characterization of *Geothermobacterium ferrifeducens* gen. nov., sp. nov. *Applied and Environmental Microbiology* **68**, 1735–1742.
- Kato S, Hara K, Kasai H, Teramura T, Sunamura M, Ishibashi J-I, Kakegawa T, Yamanaka T, Kimura H, Marumo K, Urabe T, Yamagishi A (2009) Spatial distribution, diversity and composition of bacterial communities in sub-seafloor fluids at a deep-sea hydrothermal field of the Suiyo Seamount. *Deep Sea Research Part I: Oceanographic Research Papers* **56**, 1844–1855.
- King EG, Weller WW (1970) Low-temperature heat capacities and entropies at 298.15 K of goethite and pyrophyllite. *U. S. Bureau of Mines Report of Investigations* **6618**, 10.
- Kodama Y, Watanabe K (2003) Isolation and characterization of a sulfur-oxidizing chemolithotroph growing on crude oil under anaerobic conditions. *Applied and Environmental Microbiology* **69**, 107–112.
- Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**, 543–546.
- Kulp TR, Hoefl SE, Madigan MT, Hollibaugh JT, Fisher JC, Stolz JF, Culbertson CW, Miller LG, Oremland RS (2008) Arsenic(III) fuels anoxygenic photosynthesis in hot spring biofilms from Mono Lake, California. *Science* **321**, 967–970.
- L'Haridon S, Reysenbach A-L, Tindall BJ, Schönheit P, Banta A, Johnson U, Schumann P, Gambacorta A, Stackebrandt E, Jeanthon C (2006) *Desulfurobacterium atlanticum* sp. nov., *Desulfurobacterium pacificum* sp. nov. and *Thermovibrio guaymasensis* sp. nov., three thermophilic members of the *Desulfurobacteriaceae* fam. nov., a deep branching lineage within the *Bacteria*. *International Journal of Systematic and Evolutionary Microbiology* **56**, 2843–2852.
- Lovley DR, Holmes DE, Nevin KP (2004) Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology* **49**, 219–286.
- McCollom T, Shock EL (1997) Geochemical constraints on chemolithoautotrophic metabolism by microorganisms in seafloor hydrothermal systems. *Geochimica et Cosmochimica Acta* **61**, 4375–4391.
- Meyer-Dombard DR, Price RE, Pichler T, Amend JP (2012) Prokaryotic populations in arsenic-rich shallow-sea hydrothermal sediments of Ambitle Island, Papua New Guinea. *Geomicrobiology Journal*, in press.
- Mori K, Suzuki K-I (2008) *Thiofaba tepidiphila* gen. nov., sp. nov., a novel obligately chemolithoautotrophic, sulfur-oxidizing bacterium of the *Gammaproteobacteria* isolated from a hot spring. *International Journal of Systematic and Evolutionary Microbiology* **58**, 1885–1891.
- Muyzer G, Teske A, Wirsén CO, Jannasch HW (1995) Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Archives of Microbiology* **164**, 165–172.
- Navrotsky A, Mazeina L, Majzlan J (2008) Size-driven structural and thermodynamic complexity in iron oxides. *Science* **319**, 1635–1638.
- Nealson KH, Saffarini D (1994) Iron and manganese in anaerobic respiration: environmental significance, physiology, and regulation. *Annual Review of Microbiology* **48**, 311–343.
- Newman DK, Ahmann D, Morel FMM (1998) A brief review of microbial arsenate respiration. *Geomicrobiology Journal* **15**, 255–268.
- Norris PR, Clark DA, Owen JP, Waterhouse S (1996) Characteristics of *Sulfobacillus acidophilus* sp. nov. and other moderately thermophilic mineral-sulphide-oxidizing bacteria. *Microbiology* **142**, 775–783.
- Nunoura T, Miyazaki M, Suzuki Y, Takai K, Horikoshi K (2008) *Hydrogenivirga okinawensis* sp. nov., a thermophilic sulfur-oxidizing chemolithoautotroph isolated from a deep-sea hydrothermal field,

- Southern Okinawa Trough. *International Journal of Systematic and Evolutionary Microbiology* **58**, 676–681.
- Oremland RS, Stolz JF (2003) The ecology of arsenic. *Science* **300**, 939–944.
- Oremland RS, Hoefl SE, Santini JM, Bano N, Hollibaugh RA, Hollibaugh JT (2002) Anaerobic oxidation of arsenite in Mono Lake water and by a facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1. *Applied and Environmental Microbiology* **68**, 4795–4802.
- Oremland RS, Saltikov CW, Wolfe-Simon F, Stolz JF (2009) Arsenic in the evolution of Earth and extraterrestrial ecosystems. *Geomicrobiology Journal* **26**, 522–536.
- Pichler T, Dix GR (1996) Hydrothermal venting within a coral reef ecosystem, Ambitle Island, Papua New Guinea. *Geology* **24**, 435–438.
- Pichler T, Veizer J (1999) Precipitation of Fe(III) oxyhydroxide deposits from shallow-water hydrothermal fluids in Tutum Bay, Ambitle Island, Papua New Guinea. *Chemical Geology* **162**, 15–31.
- Pichler T, Veizer J, Hall GEM (1999) The chemical composition of shallow-water hydrothermal fluids in Tutum Bay, Ambitle Island, Papua New Guinea and their effect on ambient seawater. *Marine Chemistry* **64**, 229–252.
- Price RE, Pichler T (2005) Distribution, speciation and bioavailability of arsenic in a shallow-water submarine hydrothermal system, Tutum Bay, Ambitle Island, PNG. *Chemical Geology* **224**, 122–135.
- Price RE, Amend JP, Pichler T (2007) Enhanced geochemical gradients in a marine shallow-water hydrothermal system: unusual arsenic speciation in horizontal and vertical pore water profiles. *Applied Geochemistry* **22**, 2595–2605.
- Robertson LA, Kuenen JG (2006) The genus *Thiobacillus*. In *The Prokaryotes* (eds Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E). Springer, Berlin, pp. 812–827.
- Robie RA, Hemingway BS (1995) Thermodynamic properties of minerals and related substances at 298.15 K and 1 bar (105 pascals) and at higher temperatures. In: *US Geological Survey Bulletin*, p. 461.
- Rogers KL, Amend JP (2006) Energetics of potential heterotrophic metabolisms in the marine hydrothermal system of Vulcano Island, Italy. *Geochimica et Cosmochimica Acta* **70**, 6180–6200.
- Santini JM, Sly LI, Schnagl RD, Macy JM (2000) A new chemolithoautotrophic arsenite-oxidizing bacterium isolated from a gold mine: phylogenetic, physiological, and preliminary biochemical studies. *Applied and Environmental Microbiology* **66**, 92–97.
- Santini JM, Sly LI, Wen A, Comrie D, de Wulf-Durand P, Macy JM (2002) New arsenite-oxidizing bacteria isolated from Australian gold mining environments – phylogenetic relationships. *Geomicrobiology Journal* **19**, 67–76.
- Shock EL, Helgeson HC (1988) Calculation of the thermodynamic and transport properties of aqueous species at high pressures and temperatures: correlation algorithms for ionic species and equation of state predictions to 5 kb and 1000°C. *Geochimica et Cosmochimica Acta* **52**, 2009–2036.
- Shock EL, Helgeson HC (1989) Corrections to Shock and Helgeson (1988). *Geochimica et Cosmochimica Acta* **53**, 215.
- Shock EL, Helgeson HC, Sverjensky DA (1989) Calculation of the thermodynamic and transport properties of aqueous species at high pressures and temperatures: standard partial molal properties of inorganic neutral species. *Geochimica et Cosmochimica Acta* **53**, 2157–2183.
- Shock EL, Sassani DC, Willis M, Sverjensky DA (1997) Inorganic species in geologic fluids: correlations among standard molal thermodynamic properties of aqueous ions and hydroxide complexes. *Geochimica et Cosmochimica Acta* **61**, 907–950.
- Shock EL, Holland M, Meyer-Dombard DR, Amend JP, Osburn GR, Fischer TP (2010) Quantifying inorganic sources of geochemical energy in hydrothermal ecosystems, Yellowstone National Park, USA. *Geochimica et Cosmochimica Acta* **74**, 4005–4043.
- Sievert SM, Heidorn T, Kuever J (2000) *Halothiobacillus kellyi* sp. nov., a mesophilic, obligately chemolithoautotrophic, sulfur-oxidizing bacterium isolated from a shallow-water hydrothermal vent in the Aegean Sea, and emended description of the genus *Halothiobacillus*. *International Journal of Systematic and Evolutionary Microbiology* **50**, 1229–1237.
- Sokolova TG, Kostrikina NA, Chernykh NA, Tourova TP, Kolganova TV, Bonch-Osmolovskaya EA (2002) *Carboxydocella thermautotrophica* gen. nov., sp. nov., a novel anaerobic, CO-utilizing thermophile from a Kamchatkan hot spring. *International Journal of Systematic and Evolutionary Microbiology* **52**, 1961–1967.
- Stoffers P, Glasby GP, Stuben D, Renner RM, Pierre TG, Webb J, Cardile CM (1993) Comparative mineralogy and geochemistry of hydrothermal iron-rich crusts from the Pitcairn, Teahitia-meahitia, and Macdonald hot spot areas of the S.W. Pacific. *Marine Georesources and Geotechnology* **11**, 45–86.
- Takai K, Kobayashi H, Neelson KH, Horikoshi K (2003) *Sulfurivibrium subterraneum* gen. nov., sp. nov., from a subsurface hot aquifer. *International Journal of Systematic and Evolutionary Microbiology* **53**, 823–827.
- Takai K, Miyazaki M, Nunoura T, Hirayama H, Oida H, Furushima Y, Yamamoto H, Horikoshi K (2006) *Sulfurivirga caldicurallii* gen. nov., sp. nov., a novel microaerobic, thermophilic, thiosulfate-oxidizing chemolithoautotroph, isolated from a shallow marine hydrothermal system occurring in a coral reef, Japan. *International Journal of Systematic and Evolutionary Microbiology* **56**, 1921–1929.
- Wagman DD, Evans WH, Parker VB, Schumm RH, Halow I (1982) *The NBS Tables of Chemical Thermodynamic Properties. Selected Values for Inorganic and C1 and C2 Organic Substances in SI Units*. American Chemical Society and the American Institute of Physics for the National Bureau of Standards, Washington, DC.
- Wallace DA, Johnson RW, Chappell BW, Arculus RJ, Perfit MR, Crick IH (1983) *Cainozoic Volcanism of the Tabar, Libir, Tanga, and Feni Islands, Papua New Guinea: Geology, Whole-Rock Analyses, and Rock-Forming Mineral Compositions*. Bureau of Mineral Resources, Geology and Geophysics, Sydney, Australia.
- Wirsen CO, Brinkhoff T, Kuever J, Muyzer G, Molyneux S, Jannasch HW (1998) Comparison of a new *Thiomicrospira* strain from the Mid-Atlantic Ridge with known hydrothermal vent isolates. *Applied and Environmental Microbiology* **64**, 4057–4059.
- Wu WM, Jain MK, Zeikus JG (1994) Anaerobic degradation of normal- and branched-chain fatty acids with four or more carbons to methane by a syntrophic methanogenic triculture. *Applied and Environmental Microbiology* **60**, 2220–2226.