

# Calculation of total free energy yield as an alternative approach for predicting the importance of potential chemolithotrophic reactions in geothermal springs

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## Abstract

To inform hypotheses regarding the relative importance of chemolithotrophic metabolisms in geothermal environments, we calculated free energy yields of 26 chemical reactions potentially supporting chemolithotrophy in two US Great Basin hot springs, taking into account the effects of changing reactant and product activities on the Gibbs free energy as each reaction progressed. Results ranged from  $1.2 \times 10^{-5}$  to  $3.6 \text{ J kg}^{-1}$  spring water, or  $3.7 \times 10^{-5}$  to  $11.5 \text{ J s}^{-1}$  based on measured flow rates, with aerobic oxidation of  $\text{CH}_4$  or  $\text{NH}_4^+$  giving the highest average yields. Energy yields calculated without constraining pH were similar to those at constant pH except for reactions where  $\text{H}^+$  was consumed, which often had significantly lower yields when pH was unconstrained. In contrast to the commonly used normalization of reaction chemical affinities per mole of electrons transferred, reaction energy yields for a given oxidant varied by several orders of magnitude and were more sensitive to differences in the activities of products and reactants. The high energy yield of aerobic ammonia oxidation is consistent with previous observations of significant ammonia oxidation rates and abundant ammonia-oxidizing archaea in sediments of these springs. This approach offers an additional lens through which to view the thermodynamic landscape of geothermal springs.

## Introduction

In terrestrial geothermal springs above the upper temperature limit for photosynthesis,  $\sim 73 \text{ }^\circ\text{C}$  at circumneutral pH (Brock, 1967), the energy driving primary production is supplied by chemolithotrophy. Because of the composition of geothermal fluids and their interaction with the surface environment (e.g. substantial mixing with atmospheric  $\text{O}_2$ ), these systems generally offer a wide array of electron ( $e^-$ ) donors and acceptors that are in thermodynamic disequilibrium and, when coupled in oxidation–reduction (redox) reactions, can potentially drive microbial chemolithotrophy. Cultured thermophiles and hyperthermophiles can collectively utilize a variety of redox couples (Bodrossy *et al.*, 1999; Huber *et al.*, 2000; Amend & Shock, 2001; de la Torre *et al.*, 2008). Study of these environments not only helps to enrich our understanding of the limits of life on Earth, but can also offer insights

into life in analogous systems earlier in Earth's history and the potential for life elsewhere in the solar system. It is therefore of considerable interest to determine which redox couples are responsible for primary production in hot springs and how physical and chemical parameters dictate, and might therefore be used to predict, the relative importance of different redox couples in geothermal environments.

It has been proposed that the most energetically favorable reactions are preferentially used by microorganisms (Amend & Shock, 2001) and that the relative thermodynamic yields of potential chemolithotrophic reactions, calculated based on a detailed geochemical analysis of the environment in question, might allow prediction of the most important redox couples. This approach has been applied to a variety of terrestrial and shallow marine geothermal systems (Amend *et al.*, 2003; Inskeep *et al.*, 2005; Meyer-Dombard *et al.*, 2005; Shock *et al.*, 2005, 2010;

Spear *et al.*, 2005; Hall *et al.*, 2008; Costa *et al.*, 2009; Vick *et al.*, 2010). In most of these studies, thermodynamic yields of potential reactions were quantitatively compared by their Gibbs free energy ( $\Delta G_{\text{rxn}}$ ) or chemical affinity (numerically equivalent, but in opposite sign, to  $\Delta G_{\text{rxn}}$ ), normalized to the number of moles of  $e^-$  transferred in the reaction (Amend *et al.*, 2003; Shock *et al.*, 2005). This allows a relatively straightforward comparison of the energetic yield of the reactions, and normalization per mol  $e^-$  transferred is fundamentally related to the molecular mechanism of energy conservation in respiration, that is,  $e^-$  transport coupled to generation of a proton-motive force (Amend *et al.*, 2003). Collectively, the above studies show that reactions involving  $O_2$  as an  $e^-$  acceptor generally have the highest chemical affinities, followed by those using  $NO_3^-$ ,  $NO_2^-$ , and sulfur, while sulfate, ferric oxides and  $CO_2$  have among the lowest, at least at neutral pH (Shock *et al.*, 2010). While chemical affinities of reactions involving production or consumption of protons can be affected dramatically by pH (Shock *et al.*, 2010), they are relatively insensitive to changes in the measured concentrations of other reactants and products (Inskeep *et al.*, 2005; Vick *et al.*, 2010). When considering reactions with a given  $e^-$  acceptor, such as  $O_2$ , coupling with various  $e^-$  donors known to be used by hyperthermophiles tends to yield rather similar chemical affinities. Two notable exceptions to this are reactions representing aerobic ammonia and nitrite oxidation, which consistently have less than half of the chemical affinity of aerobic oxidations of other  $e^-$  donors such as  $H_2$ ,  $CO$ ,  $CH_4$ ,  $H_2S$ , and  $Fe^{2+}$ ; even anaerobic respirations of these  $e^-$  donors coupled with  $NO_2^-$  or  $NO_3^-$  reduction have higher chemical affinities than ammonia or nitrite oxidation (Shock *et al.*, 2010). This leads to the prediction that ammonia oxidation and nitrite oxidation would not be important drivers of chemolithotrophy in geothermal systems.

The recent cultivation of the thermophilic, ammonia-oxidizing archaeon '*Candidatus Nitrosocaldus yellowstonii*' (de la Torre *et al.*, 2008) and the detection of genes and transcripts predicted to encode the catalytic subunit of the archaeal ammonia monooxygenase (*amoA*) in a variety of geothermal systems (De La Torre *et al.*, 2008; Reigstad *et al.*, 2008; Zhang *et al.*, 2008; Jiang *et al.*, 2010) suggests that ammonia oxidation may nonetheless be an important energy source driving primary production in some of these environments. This is apparently the case in two hot springs in the US Great Basin, Great Boiling Spring (GBS) and Sandy's Spring West (SSW). Both of these springs are circumneutral, bicarbonate-buffered, and typically range from 75 to 87 °C at the source (Costa *et al.*, 2009; Hedlund *et al.*, 2011). Despite the relatively low chemical affinity of ammonia oxidation

based on measured spring water chemistry (Costa *et al.*, 2009), sediments of both springs exhibited significant ammonia oxidation rates, and close relatives of '*Ca. N. yellowstonii*' were prominent members of the sediment microbial communities (Dodsworth *et al.*, 2011).

Here, we explore an alternative approach for comparing potential chemolithotrophic reactions in GBS and SSW, calculation of the total energy released by each reaction, assuming that it proceeds fully to equilibrium. We hypothesized that this approach would give a view of the thermodynamic landscape that contrasts with that obtained by ranking reactions based on their chemical affinity per mol  $e^-$  transferred and that the overall energetic yield of aerobic ammonia oxidation would be high in GBS and SSW relative to other aerobic respirations. Calculation of overall energy yield to compare various catabolic reactions, specifically by comparing the total power made available, has been suggested previously (Shock & Holland, 2007). Using this approach, Inskeep *et al.* (2005) reported energetic yields, expressed as  $W\ m^{-2}$ , of reactions involving several  $e^-$  donors in a hot spring in Yellowstone National Park, although they did not report how the calculations were performed. We expand on this by calculating the total energetic yield of 26 reactions representing known or hypothesized chemolithotrophic strategies in GBS and SSW, taking into account the decrease in the  $\Delta G_{\text{rxn}}$  as the reaction progresses.

## Materials and methods

The geochemistry and microbiology of the hot springs GBS and SSW have been described previously (Costa *et al.*, 2009), including all physicochemical measurements of bulk spring water used for the calculations in this study. Activities ( $a_i$ ) of each aqueous species  $i$  were calculated using EQ3/6 (Supporting Information, Table S1) (Wolery, 1992). Values for the standard Gibbs free energy,  $\Delta G_{\text{rxn}}^\circ$ , for 26 known or theoretical chemolithotrophic reactions (Table S2), corrected for *in situ* temperature and pressure, were calculated using SUPCRT92 (Johnson *et al.*, 1992) using previously published data and parameters (Helgeson *et al.*, 1978; Shock *et al.*, 1989, 1997; Shock & Helgeson, 1990; Shock, 2009). The reactions used here are a subset of those for which chemical affinities (normalized to kJ per mol  $e^-$  transferred) were calculated in Costa *et al.* (2009) and were chosen to focus on reactions with aqueous  $e^-$  donors ( $H_2$ ,  $CH_4$ ,  $H_2S$ ,  $Fe^{2+}$ ,  $NO_2^-$ , and  $NH_4^+$ ) and acceptors ( $O_2$ ,  $NO_3^-$ ,  $NO_2^-$ , and  $SO_4^{2-}$ ) that were exothermic in both GBS and SSW.

The total energy released by each reaction as it progressed from initial conditions to equilibrium was cal-

culated using the Gibbs free energy ( $\Delta G_{\text{rxn}}$ ) equation,  $\Delta G_{\text{rxn}} = \Delta G_{\text{rxn}}^{\circ} + RT \ln(Q)$  (Amend & Shock, 2001). In this equation,  $R$  is the gas constant,  $T$  is the temperature in Kelvin, and  $Q$  is the reaction quotient, which is defined as the product of the activities of the reaction products divided by the activities of the reactants, each raised to the power of their stoichiometric coefficient ( $\nu$ ) and is calculated using the following equation:  $Q = \prod(a_i)^{\nu_i}$  for each chemical species  $i$ , where  $\nu$  is negative for reactants and positive for products. For initial conditions, activities in Table S1 were used for the calculation of  $Q$ . As the reaction progressed from these initial conditions to equilibrium ( $\Delta G_{\text{rxn}} = 0$ ), the activities of the reactants were decreased and products were increased stepwise (with step size  $x$ ) corresponding to their stoichiometry in the given reaction by replacing  $a_i$  with  $(a_i + \nu_i x)$ , where  $\nu$  is again negative for reactants and positive for products. At each step, the resulting modified  $Q$  and  $\Delta G_{\text{rxn}}$  were calculated. The total free energy released was then calculated by integration of the resulting curve of  $\Delta G_{\text{rxn}}$  from the initial conditions to equilibrium. This was achieved using a program written in Visual Basic for Applications (Microsoft Corp., Redmond, WA) incorporated as a macro in Microsoft Excel (Microsoft Corp.). The macro, which is available upon request, defined the boundary of the reaction at equilibrium using the secant method and approximated the integral using Simpson's rule with 10 000 equally spaced steps between the boundary conditions. Calculations were performed assuming either an infinitely buffered system, where the activity of  $\text{H}^+$  ( $a_{\text{H}^+}$ ) remained constant throughout the calculation, or an unbuffered system, where  $a_{\text{H}^+}$  was allowed to vary along with other reactants and products in the reaction. It should be noted that this method does not take into account changes in activity coefficients because of changes in ionic strength as the reaction progresses; this should be a good approximation for these springs, where the changes in ionic strength owing to the reactions considered here (with limiting reagent concentrations at or below the micromolar level) are insignificant when compared to the effects of the major solutes ( $\sim 55\text{--}70$  mM each  $\text{Na}^+$  and  $\text{Cl}^-$ ).

For comparison, the total energy released for each reaction was also approximated as the product of the initial  $\Delta G_{\text{rxn}}$  and the activity of the limiting reagent divided by its stoichiometric coefficient; this simple approximation does not take into account the decrease in  $\Delta G_{\text{rxn}}$  as the reactants and products decrease and increase, respectively (constant  $\Delta G_{\text{rxn}}$ ). Results were expressed as either  $\text{J kg}^{-1}$  spring water or  $\text{J s}^{-1}$ , the latter of which was calculated using flow rates for GBS ( $1.1 \text{ L s}^{-1}$ ) and SSW ( $3.3 \text{ L s}^{-1}$ ) from Costa *et al.* (2009) and temperature-corrected densities for GBS ( $0.976 \text{ kg L}^{-1}$ ) and SSW ( $0.967 \text{ kg L}^{-1}$ )

spring water. For comparison of the total energy yield of aerobic ammonia oxidation in GBS with measured ammonia oxidation rates in this spring, it was assumed that rates measured in Dodsworth *et al.* (2011), performed on sediment slurries incubated without shaking in closed bottles with an air headspace, were representative of the top 1 cm of sediment throughout the entire surface area of the spring source pool ( $\sim 164 \text{ m}^2$ , calculated from dimensions in Costa *et al.* (2009)) and that  $1 \text{ cm}^3$  of this top 1 cm sediment layer contained  $\sim 0.22$  g dry weight of sediment (Dodsworth *et al.*, 2011).

## Results and discussion

### Free energy yields of reactions in GBS and SSW spring water at constant pH

The total free energy yields of 26 potential chemolithotrophic reactions, calculated for GBS and SSW spring water assuming a constant pH, ranged by over five orders of magnitude (Fig. 1). As expected, for a given  $e^-$  donor, reactions depicting aerobic respiration had the greatest energy yields, followed by nitrate reduction, nitrite ammonification (i.e. nitrite reduction to  $\text{NH}_4^+$ ), and finally sulfate reduction to either pyrite or sulfide. For a given  $e^-$  acceptor, yields for reactions with different  $e^-$  donors varied greatly, generally over three orders of magnitude. In both springs, aerobic respiration of  $\text{CH}_4$  and  $\text{NH}_4^+$  yielded the greatest energy, with  $\text{NO}_2^-$  oxidation also being high in GBS, while oxidation of  $\text{Fe}^{2+}$  and  $\text{H}_2$  yielded 100- to 1000-fold less energy. This was also the case for anaerobic respirations, with  $\text{CH}_4$  oxidation yielding the greatest energy, followed by oxidation of  $\text{H}_2\text{S}$ , with  $\text{Fe}^{2+}$  and  $\text{H}_2$  oxidation giving much lower yields. The wide range in total energy yields reported here is generally similar to that in Inskeep *et al.* (2005), where reaction energies were expressed as  $\text{kJ m}^{-2} \text{ min}^{-1}$ .

The wide range of energy yields, and the difference in yields of a given reaction between the two springs, was largely dependent on the activity, and thus concentration, of the limiting reagent in the reaction. Energy yields for reactions involving  $\text{H}_2$  and  $\text{Fe}^{2+}$  were always limited by the relatively low measured concentrations of these reactants (8.9 and 7.7 nM  $\text{H}_2$ , 0.23 and 0.22  $\mu\text{M}$  Fe in GBS and SSW, respectively) in comparison with those for  $\text{H}_2\text{S}$  (2.9  $\mu\text{M}$  in SSW),  $\text{CH}_4$  (0.48  $\mu\text{M}$  in GBS and 4.9  $\mu\text{M}$  in SSW),  $\text{NO}_2^-$  (35  $\mu\text{M}$  in GBS and 0.7  $\mu\text{M}$  in SSW), and  $\text{NH}_4^+$  (24  $\mu\text{M}$  in GBS and 61  $\mu\text{M}$  in SSW) (Costa *et al.*, 2009). Similarly, aerobic  $\text{NO}_2^-$  oxidation yielded  $\sim 50$ -fold more energy in GBS than in SSW because  $\text{NO}_2^-$  concentrations were proportionally higher in GBS, and  $\text{NO}_2^-$  was the limiting reagent for this reaction in both springs. In contrast, aerobic  $\text{CH}_4$  oxidation (reaction 2) yielded

## Potential chemolithotrophic reaction

Aerobic respiration

- 1]  $\text{NH}_4^+ + 3/2\text{O}_2(\text{aq}) \rightarrow \text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O}$
- 2]  $\text{CH}_4(\text{aq}) + 2\text{O}_2(\text{aq}) \rightarrow \text{CO}_2(\text{aq}) + 2\text{H}_2\text{O}$
- 3]  $\text{H}_2\text{S}(\text{aq}) + 2\text{O}_2(\text{aq}) \rightarrow \text{SO}_4^{2-} + 2\text{H}^+$
- 4]  $\text{NO}_2^- + 0.5\text{O}_2(\text{aq}) \rightarrow \text{NO}_3^-$
- 5]  $\text{H}_2\text{S}(\text{aq}) + 1/2\text{O}_2(\text{aq}) \rightarrow \text{Sulfur} + \text{H}_2\text{O}$
- 6]  $2\text{H}_2\text{S}(\text{aq}) + 1/2\text{O}_2(\text{aq}) + \text{Fe}^{2+} \rightarrow \text{Pyrite} + 2\text{H}^+ + \text{H}_2\text{O}$
- 7]  $\text{Fe}^{2+} + 1/4\text{O}_2(\text{aq}) + 3/2\text{H}_2\text{O} \rightarrow \text{Goethite} + 2\text{H}^+$
- 8]  $2\text{H}_2(\text{aq}) + \text{O}_2(\text{aq}) \rightarrow 2\text{H}_2\text{O}$

Nitrate reduction

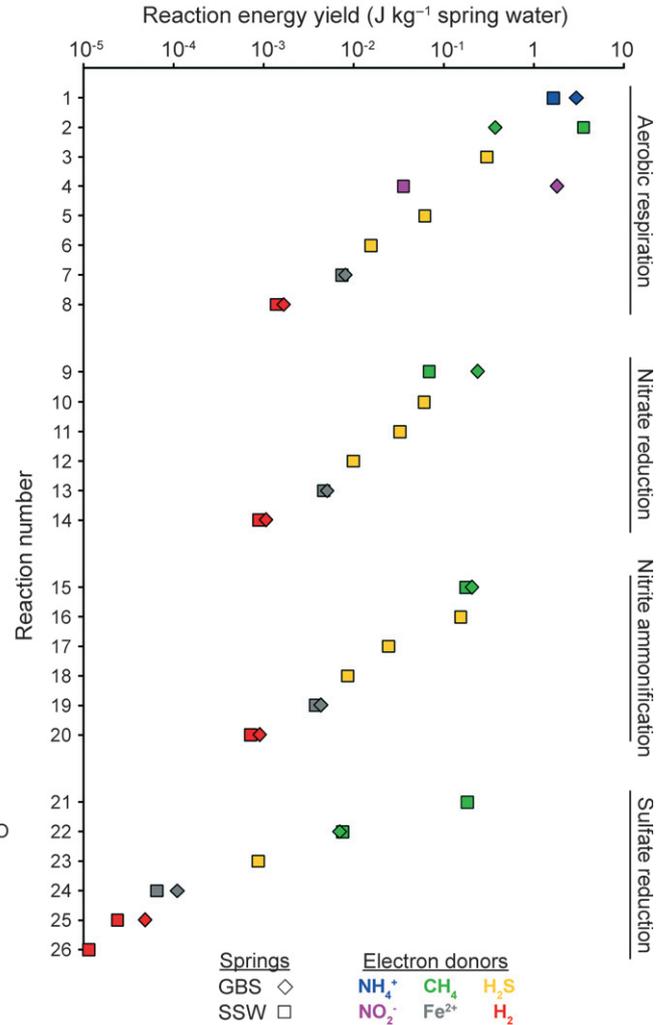
- 9]  $\text{CH}_4(\text{aq}) + 4\text{NO}_3^- \rightarrow \text{CO}_2(\text{aq}) + 4\text{NO}_2^- + 2\text{H}_2\text{O}$
- 10]  $\text{H}_2\text{S}(\text{aq}) + 4\text{NO}_3^- \rightarrow \text{SO}_4^{2-} + 4\text{NO}_2^- + 2\text{H}^+$
- 11]  $\text{H}_2\text{S}(\text{aq}) + \text{NO}_3^- \rightarrow \text{Sulfur} + \text{NO}_2^- + \text{H}_2\text{O}$
- 12]  $2\text{H}_2\text{S}(\text{aq}) + \text{NO}_3^- + \text{Fe}^{2+} \rightarrow \text{Pyrite} + \text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O}$
- 13]  $2\text{Fe}^{2+} + \text{NO}_3^- + 3\text{H}_2\text{O} \rightarrow 2\text{Goethite} + \text{NO}_2^- + 4\text{H}^+$
- 14]  $\text{H}_2(\text{aq}) + \text{NO}_3^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$

Nitrite ammonification

- 15]  $3\text{CH}_4(\text{aq}) + 4\text{NO}_2^- + 8\text{H}^+ \rightarrow 3\text{CO}_2(\text{aq}) + 4\text{NH}_4^+ + 2\text{H}_2\text{O}$
- 16]  $3\text{H}_2\text{S}(\text{aq}) + 4\text{NO}_2^- + 2\text{H}^+ + 4\text{H}_2\text{O} \rightarrow 3\text{SO}_4^{2-} + 4\text{NH}_4^+$
- 17]  $3\text{H}_2\text{S}(\text{aq}) + \text{NO}_2^- + 2\text{H}^+ \rightarrow 3\text{Sulfur} + \text{NH}_4^+ + 2\text{H}_2\text{O}$
- 18]  $6\text{H}_2\text{S}(\text{aq}) + \text{NO}_2^- + 3\text{Fe}^{2+} \rightarrow 3\text{Pyrite} + \text{NH}_4^+ + 4\text{H}^+ + 2\text{H}_2\text{O}$
- 19]  $6\text{Fe}^{2+} + \text{NO}_2^- + 10\text{H}_2\text{O} \rightarrow 6\text{Goethite} + \text{NH}_4^+ + 10\text{H}^+$
- 20]  $3\text{H}_2 + \text{NO}_2^- + 2\text{H}^+ \rightarrow \text{NH}_4^+ + 2\text{H}_2\text{O}$

Sulfate reduction

- 21]  $\text{CH}_4(\text{aq}) + \text{SO}_4^{2-} + 2\text{H}^+ \rightarrow \text{CO}_2(\text{aq}) + \text{H}_2\text{S}(\text{aq}) + 2\text{H}_2\text{O}$
- 22]  $7\text{CH}_4(\text{aq}) + 8\text{SO}_4^{2-} + 8\text{H}^+ + 4\text{Fe}^{2+} \rightarrow 4\text{Pyrite} + 7\text{CO}_2(\text{aq}) + 18\text{H}_2\text{O}$
- 23]  $7\text{H}_2\text{S}(\text{aq}) + \text{SO}_4^{2-} + 4\text{Fe}^{2+} \rightarrow 4\text{Pyrite} + 6\text{H}^+ + 4\text{H}_2\text{O}$
- 24]  $15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 20\text{H}_2\text{O} \rightarrow \text{Pyrite} + 14\text{Goethite} + 26\text{H}^+$
- 25]  $7\text{H}_2(\text{aq}) + 2\text{SO}_4^{2-} + \text{Fe}^{2+} + 2\text{H}^+ \rightarrow \text{Pyrite} + 8\text{H}_2\text{O}$
- 26]  $4\text{H}_2(\text{aq}) + \text{SO}_4^{2-} + 2\text{H}^+ \rightarrow \text{H}_2\text{S}(\text{aq}) + 4\text{H}_2\text{O}$



**Fig. 1.** Energetic yields of 26 potential chemolithotrophic reactions calculated assuming a constant pH. Diamonds and squares represent calculations based on spring water chemistry of GBS or SSW, respectively, and are colored by the reductant in the reaction as indicated. Calculations for reactions involving  $\text{H}_2\text{S}$  were only performed for SSW, because this analyte was not detected in GBS.

~10-fold more energy in SSW, while  $\text{CH}_4$  oxidation coupled to  $\text{NO}_3^-$  reduction (reaction 9) yielded significantly more energy in GBS. This was due to the fact that  $\text{CH}_4$  (which is ~10-fold higher in SSW) was limiting in the aerobic respiration in GBS, while  $\text{NO}_3^-$  (which was higher in GBS) limited reaction 9 in SSW. These calculations thus directly reflect potentially meaningful differences in spring water chemistry within the context of each reaction. Because of the profound effect that variations in activities have on these calculations, the energy yields for GBS and SSW presented here cannot be generalized to other geothermal systems with even moderately different chemistries.

The energy yields calculated here may not apply *in situ* in a strictly quantitative sense, and the extent to which they do can depend on the environment in question. It is

likely that microbial communities, either in spring water or in sediments, can harvest only a fraction of the total energy theoretically available from each reaction as calculated by this method. This is due both to kinetic restraints (e.g. diffusion of reactants into sediments, differing substrate affinities for different microbial groups) and to the fact that the reactions, modeled here as proceeding completely to equilibrium in a closed system, do not behave this way in the open system *in situ*. In springs with a large source pool with long residence time like GBS [1–2 days (Costa *et al.*, 2009)], the chemistry of the system approaches a steady-state condition so reactions never proceed to equilibrium. At the very least, calculations such as these can be used to constrain the upper boundary of energy available for a given chemolithotrophic reaction. For example, ammonia oxidation rates

measured in GBS sediments by Dodsworth *et al.* (2011) would result in the consumption of  $0.67 \mu\text{mol NH}_4^+ \text{ s}^{-1}$ , approximately 2.6% of the  $25.9 \mu\text{mol NH}_4^+ \text{ s}^{-1}$  that would be necessary for the reaction to proceed to equilibrium (using yields expressed in  $\text{J s}^{-1}$  in Table 1). While this is only a small portion of the total theoretical energy yield, it is still more than 25-fold higher than the total amount of energy available from complete aerobic oxidation of  $\text{H}_2$  calculated for GBS. In fast-flowing springs with short residence time like SSW (5–15 min (Costa *et al.*, 2009)), some studies have documented near-stoichiometric oxidation of  $\text{e}^-$  donors by comparison of water chemistry at the spring source with multiple points in the outflow (Inskeep & McDermott, 2005; Nordstrom *et al.*, 2005). Thus, over the course of the spring outflow, the assumption that reactions approach equilibrium can actually be met, although any particular substrate-attached cell is not witness to the entirety of the reaction

progress because the reaction is spread over distances of meters to tens of meters in the outflow channel. In general, while the reaction yields calculated here likely do not quantitatively translate to energy utilized by microorganisms at any given point in space or time *in situ*, qualitatively they may still be useful for making testable predictions regarding the relative importance of different metabolisms; the wide range of total energy yields (>1000-fold) may have a significant impact on the relative importance of  $\text{e}^-$  donors in a given environment.

### Comparison of energy yields calculated using different assumptions and methods

To assess how energy yields for reactions where  $\text{H}^+$  is produced or consumed were affected by different buffering capacities of spring water, we compared calculations assuming constant pH (infinitely buffering capacity) with

**Table 1.** Total energy yields expressed in  $\text{J kg}^{-1}$  spring water and  $\text{J s}^{-1}$

Reaction	GBS				SSW			
	Total yield ( $\text{J kg}^{-1}$ )				Total yield ( $\text{J kg}^{-1}$ )			
	Constant pH	Unconstrained pH	Constant $\Delta G_{\text{rxn}}^*$	Total yield ( $\text{J s}^{-1}$ ) <sup>†</sup>	Constant pH	Unconstrained pH	Constant $\Delta G_{\text{rxn}}$	Total yield ( $\text{J s}^{-1}$ )
1	2.94E+0	2.58E+0	2.99E+0	3.16E+0	1.60E+0	1.44E+0	1.67E+0	5.12E+0
2	3.77E-1	No $\text{H}^+$ <sup>‡</sup>	3.78E-1	4.04E-1	3.60E+0	No $\text{H}^+$	3.64E+0	1.15E+1
3	nc <sup>§</sup>	nc	nc	nc	3.10E-1	3.05E-1	3.11E-1	9.88E-1
4	1.83E+0	No $\text{H}^+$	1.94E+0	1.96E+0	3.60E-2	No $\text{H}^+$	3.83E-2	1.15E-1
5	nc	nc	nc	nc	6.24E-2	No $\text{H}^+$	6.37E-2	1.99E-1
6	nc	nc	nc	nc	1.61E-2	1.57E-2	1.64E-2	5.13E-2
7	8.17E-3	7.60E-3	8.42E-3	8.77E-3	7.50E-3	7.11E-3	7.74E-3	2.39E-2
8	1.69E-3	No $\text{H}^+$	1.72E-3	1.82E-3	1.41E-3	No $\text{H}^+$	1.43E-3	4.50E-3
9	2.40E-1	No $\text{H}^+$	2.42E-1	2.57E-1	6.97E-2	No $\text{H}^+$	7.21E-2	2.23E-1
10	nc	nc	nc	nc	6.14E-2	6.05E-2	6.37E-2	1.96E-1
11	nc	nc	nc	nc	3.28E-2	No $\text{H}^+$	3.51E-2	1.05E-1
12	nc	nc	nc	nc	1.04E-2	1.00E-2	1.08E-2	3.33E-2
13	5.10E-3	4.52E-3	5.35E-3	5.47E-3	4.68E-3	4.29E-3	4.94E-3	1.49E-2
14	1.07E-3	No $\text{H}^+$	1.09E-3	1.15E-3	8.92E-4	No $\text{H}^+$	9.16E-4	2.85E-3
15	2.08E-1	5.83E-3	2.10E-1	2.23E-1	1.78E-1	9.77E-3	1.80E-1	5.68E-1
16	nc	nc	nc	nc	1.56E-1	3.53E-2	1.59E-1	4.98E-1
17	nc	nc	nc	nc	2.48E-2	5.61E-3	2.61E-2	7.90E-2
18	nc	nc	nc	nc	8.67E-3	8.48E-3	9.04E-3	2.77E-2
19	4.31E-3	3.88E-3	4.57E-3	4.63E-3	3.80E-3	3.51E-3	4.05E-3	1.21E-2
20	9.11E-4	9.09E-4	9.36E-4	9.78E-4	7.28E-4	7.27E-4	7.51E-4	2.32E-3
21	7.01E-3	1.40E-3	7.34E-3	7.53E-3	1.84E-1	1.25E-3	2.26E-1	5.86E-1
22	nc	nc	nc	nc	7.59E-3	2.76E-3	7.85E-3	2.42E-2
23	nc	nc	nc	nc	8.84E-4	6.59E-4	1.21E-3	2.82E-3
24	1.10E-4	2.17E-5	2.80E-4	1.18E-4	6.60E-5	1.87E-5	2.06E-4	2.11E-4
25	4.90E-5	4.88E-5	7.32E-5	5.26E-5	2.41E-5	2.41E-5	4.37E-5	7.70E-5
26	nc	nc	nc	nc	1.16E-5	1.14E-5	2.77E-5	3.69E-5

\*Calculated without taking into account changes in  $\Delta G_{\text{rxn}}$  as the reaction progressed; see text for details.

<sup>†</sup>Calculated from values for total yield ( $\text{J kg}^{-1}$ ) at constant pH.

<sup>‡</sup>No  $\text{H}^+$ , protons were not produced or consumed in the reaction.

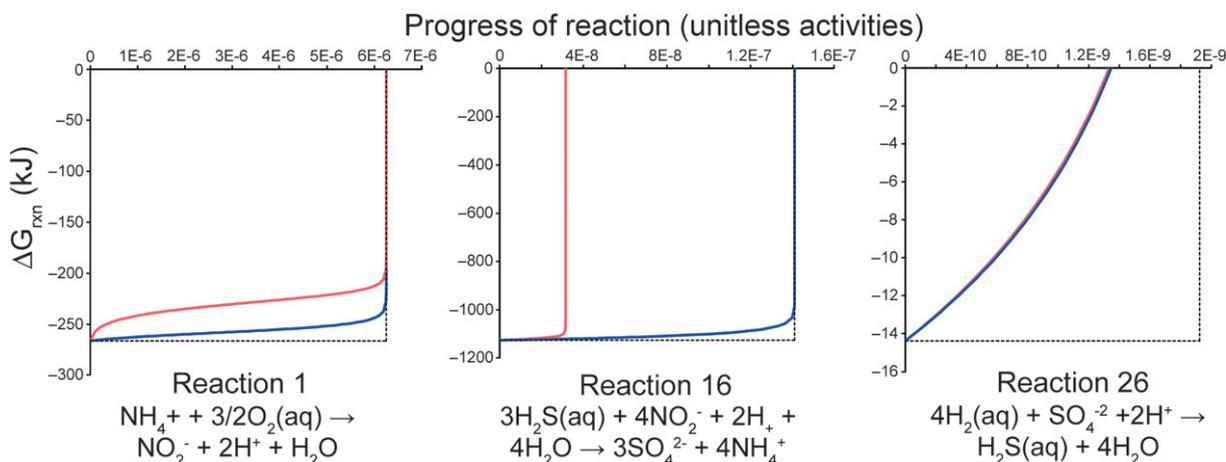
<sup>§</sup>Not calculated (nc) because  $\text{H}_2\text{S}$  was below the level of detection in GBS.

calculations performed where  $H^+$  was unconstrained (zero buffering capacity) and allowed to vary with the reaction products and reactants (Table 1). These two end-members constrain the possible reaction energy yield for any amount of buffering capacity present in the spring water. For reactions where  $H^+$  was produced, such as reaction 1 (Fig. 2), the difference between calculations using these two assumptions was relatively small, with the unbuffered reaction typically yielding 90% or more of the energy released in the infinitely buffered case. For these reactions at constant  $H^+$  (and reactions not involving  $H^+$ ), the  $\Delta G_{\text{rxn}}$  decreased gradually as the reaction progressed and rapidly dropped to zero only when the activity of the limiting reagent became vanishingly small (Fig. 2, reaction 1, blue line). When  $H^+$  was allowed to vary,  $\Delta G_{\text{rxn}}$  initially dropped as  $a_{H^+}$  increased, but otherwise behaved similarly to the infinitely buffered case as the reaction progressed further (Fig. 2, reaction 1, red line). The difference was much more pronounced, however, in reactions where protons were consumed. In these cases (reactions 15–17, 21, 22),  $H^+$  was the limiting reagent, and  $\Delta G_{\text{rxn}}$  dropped to zero much earlier during the reaction progression than when  $H^+$  was held constant (Fig. 2, reaction 16, red and blue lines). The exceptions to this were proton-consuming reactions where  $H_2$  was the  $e^-$  donor (reactions 20, 25–26), which were limited by  $H_2$  rather than  $H^+$  (Fig. 2, reaction 26). Because the predicted buffering capacity of GBS and SSW is rather high (assuming a carbonic acid–bicarbonate buffer system; total alkalinity of 680–840  $\mu\text{M}$ ) in comparison with activities of any given limiting reagent in the reactions considered here, the calculations assuming constant  $a_{H^+}$  are a more reasonable approximation for reaction energy yields in these springs. In fluids with considerably lower buffer-

ing capacity, but otherwise similar chemistry, energy yields for reactions where  $H^+$  is consumed might decrease substantially in comparison with those for GBS and SSW.

Reaction energy yields calculated using the integration method were also compared to those calculated using a much simpler approximation where  $\Delta G_{\text{rxn}}$  held is constant throughout the progress of the reaction until the limiting reagent (excluding  $H^+$ ) is completely consumed (Table 1; Fig. 2, dotted lines). For most reactions, this approximation was rather robust, with energy yields being < 10% greater than those calculated using the integration method at constant pH. The exceptions to this were reactions that were already close to equilibrium (reactions 24–26). In these cases,  $\Delta G_{\text{rxn}}$  reached zero before the reaction had progressed sufficiently to consume the limiting reagent (Fig. 2, reaction 26, dotted line vs. blue and red lines). Therefore, this simple approximation is probably sufficient for a basic estimation of overall reaction energy yields, but may significantly overestimate yields in cases where either the reaction is already close to equilibrium or concentrations of one or more products are much lower than the concentration of the limiting reactant.

As an alternative to expressing energy yield per unit volume ( $\text{J kg}^{-1}$ ), units of  $\text{J s}^{-1}$  (i.e.  $\text{W}$ ) or  $\text{J s}^{-1} \text{m}^{-2}$  ( $\text{W m}^{-2}$ ) can be used (Table 1). Depending on the environment under consideration (e.g. a fast-flowing system with very short residence time such as SSW, or a large spring source pool with a relatively long residence time such as GBS), one set of units might be more appropriate than another. It should be noted that conversion from one set of units to another ultimately involves multiplying all reaction yields by the same scalar quantity, and therefore, reaction yields relative to one another for a given sample site will not be altered qualitatively or quan-



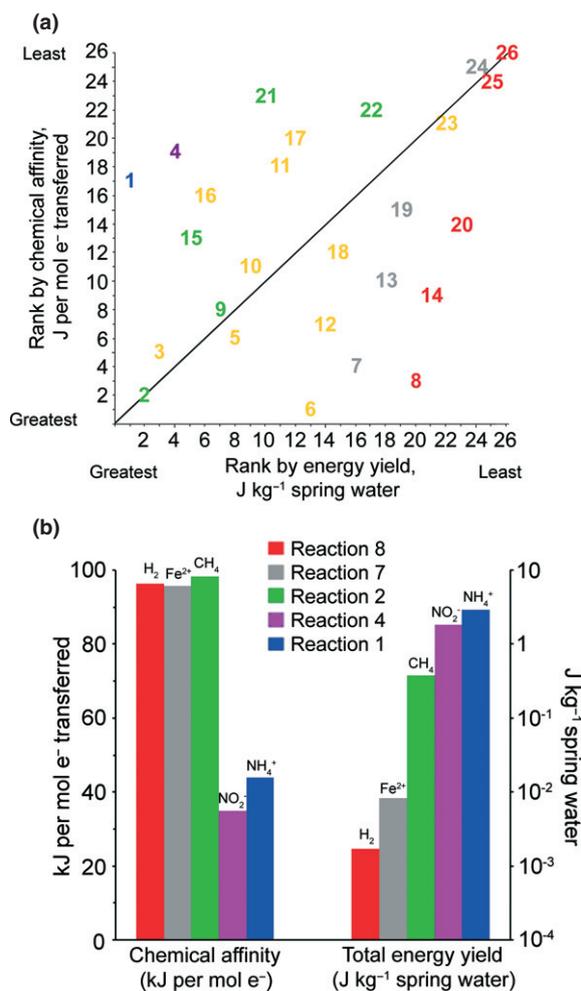
**Fig. 2.** Changes in  $\Delta G_{\text{rxn}}$  as a function of the progress of the reaction. Calculations are shown for SSW spring water assuming an unbuffered (unconstrained pH; red lines) or infinitely buffered (constant pH; blue lines) system in comparison with using a constant  $\Delta G_{\text{rxn}}$  (dotted lines). The area circumscribed by the curves and the x- and y-axes represents the total energy yield of the reaction.

tatively. This can provide a useful way of comparing different springs, however, which may have nearly identical aqueous chemistry but different flow rates.

### Comparison with reaction chemical affinities normalized per mol $e^-$ transferred

The total energy yield of a given reaction offers a contrasting view of the thermodynamic landscapes of GBS and SSW in comparison with normalization of reaction chemical affinities to the number of moles of  $e^-$  transferred, an approach that has been applied in several previous studies (Amend *et al.*, 2003; Inskeep *et al.*, 2005; Meyer-Dombard *et al.*, 2005; Shock *et al.*, 2005, 2010; Hall *et al.*, 2008; Costa *et al.*, 2009; Vick *et al.*, 2010). This is simply illustrated by comparing the ranks of the reactions (from highest to lowest) by energy yield to their ranks by chemical affinity (Fig. 3a). In comparison with ranking by chemical affinity, ranks of reactions involving oxidation of  $H_2$  and  $Fe^{2+}$  tended to be lower (Fig. 3a, below the 1 : 1 line), while reactions involving oxidation of  $CH_4$ ,  $NH_4^+$ , and  $NO_2^-$  tended to be ranked higher (Fig. 3a, above the 1 : 1 line). This is a direct reflection of the relative sensitivity of total reaction energy yields to the concentrations of products and reactants in comparison with reaction chemical affinities, which are generally rather insensitive to changes in reaction constituents, even changes of an order of magnitude or more (Inskeep *et al.*, 2005; Vick *et al.*, 2010).

As a result of the above differences, these two methods contrast in the predictions that can be made regarding how spring water chemistry might influence the relative importance of various redox couples in driving microbial chemolithotrophy. For reactions representing aerobic respirations in GBS (Fig. 3b), oxidation of  $CH_4$ ,  $H_2$ , and  $Fe^{2+}$  all have very similar chemical affinities when normalized to mol  $e^-$  transferred (95.7–98.3 kJ per mol  $e^-$  transferred), while oxidation of  $NH_4^+$  and  $NO_2^-$  are much lower (34.9–43.8 kJ per mol  $e^-$  transferred), even in comparison with some anaerobic respirations of  $CH_4$ ,  $H_2$ , and  $Fe^{2+}$  involving  $NO_3^-$  and  $NO_2^-$  as oxidants (Costa *et al.*, 2009). This would predict that  $NH_4^+$  and  $NO_2^-$  oxidation are much less important than both aerobic and anaerobic  $CH_4$ ,  $H_2$ , and  $Fe^{2+}$  oxidation in driving chemolithotrophy in GBS, as well as in many other circumneutral terrestrial geothermal environments where chemical affinities for these reactions have been calculated (Inskeep *et al.*, 2005; Meyer-Dombard *et al.*, 2005; Hall *et al.*, 2008; Shock *et al.*, 2010; Vick *et al.*, 2010). In contrast, when these aerobic respirations are considered by using the total potential energy released,  $NH_4^+$  and  $NO_2^-$  oxidation are 100- to 1000-fold more energetic than  $H_2$  and  $Fe^{2+}$  oxidation (Fig. 3). In this view,  $NH_4^+$  and  $NO_2^-$  oxidation



**Fig. 3.** Comparison of energy yields calculated at constant pH as  $J kg^{-1}$  spring water vs. normalized to kJ per mol  $e^-$  transferred. All values for reaction chemical affinities normalized per mol  $e^-$  transferred are from Costa *et al.* (2009). (a) Mean energy yields and chemical affinities for reactions 1–26 in GBS and SSW, ranked from highest to lowest. For reactions calculated for both GBS and SSW, the geometric mean was used for ranking. The black diagonal line represents a 1 : 1 rank correspondence. Reactions are indicated by their number and colored by  $e^-$  donor as in Fig. 1. (b) Comparison of chemical affinity (kJ per mol  $e^-$  transferred, left y-axis) and energy yield ( $J kg^{-1}$  spring water, right y-axis) for reactions representing potential aerobic respirations in GBS, with electron donors indicated above the bars.

would be predicted to be prominent drivers of chemolithotrophy. In a recent study by Dodsworth *et al.* (2011), high ammonia oxidation rates were measured in both GBS and SSW sediments, where close relatives of the ammonia-oxidizing archaeon '*Ca. N. yellowstonii*' (de la Torre *et al.*, 2008) were prominent members of the microbial communities at  $3.5\text{--}3.9 \times 10^8$  *amoA* copies  $g^{-1}$  sediment. This is consistent with the importance of  $NH_4^+$

oxidation predicted by total energy yields in these springs. We acknowledge that the relative importance of various redox couples may ultimately be governed by any number of complex factors, such as enzyme kinetics, transport of reactants and products into and out of microenvironments in spring sediments, and upper temperature limits of microorganisms catalyzing these reactions, rather than simply by the abundance of potential energy available for microorganisms. Nonetheless, this example highlights the potential utility of considering total reaction energy yields, in concert with other methods, to generate predictable hypotheses regarding the relative importance of various redox couples as drivers of chemolithotrophy.

Intriguingly, both methods for assessing reaction energies predict that aerobic CH<sub>4</sub> oxidation is also important in SSW, and to a lesser extent in GBS. Although several thermophilic CH<sub>4</sub>-oxidizing microorganisms have been isolated (Bodrossy *et al.*, 1999; Tsubota *et al.*, 2005; Dunfield *et al.*, 2007), close relatives of these groups have not been detected in sediments of the source pools of GBS and SSW, despite multiple culture-independent censuses of microbial communities in these springs (Costa *et al.*, 2009; Dodsworth *et al.*, 2011). Measurements of CH<sub>4</sub> oxidation rates, however, in these and other terrestrial geothermal environments are lacking. Because of the presence, and sometimes prominence, of novel bacterial groups whose physiology is as yet unknown in these and other hot springs, this may be a fruitful area of future research.

## Conclusion

The calculation of the total energetic yield of potential chemolithotrophic reactions described in this study offers a view of the thermodynamics of chemolithotrophy that is complementary to other methods for evaluation of reaction energetics in geothermal environments (Amend *et al.*, 2003; Inskeep *et al.*, 2005; Meyer-Dombard *et al.*, 2005; Hall *et al.*, 2008; Costa *et al.*, 2009; Shock *et al.*, 2010; Vick *et al.*, 2010). A specific example of the utility of this approach is its prediction of the importance of aerobic ammonia oxidation, which has among the highest energy yields in GBS in SSW, despite being much less energetically favorable than aerobic oxidation of H<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>S, and Fe<sup>2+</sup> when reaction yields are normalized per mol e<sup>-</sup> transferred (Costa *et al.*, 2009; Shock *et al.*, 2010). The recent measurement of high ammonia oxidation rates and abundant ammonia-oxidizing archaea in these two hot springs (Dodsworth *et al.*, 2011) suggests that consideration of reactions by their total energy yield may have value as an additional tool for predicting which redox couples are important in geothermal environments. Because of their sensitivity to measured concentrations of

reactants and products, total energy yields for these reactions will likely vary considerably in different hot spring environments, either within a given system (e.g. water vs. sediment or in outflows) or between sites or springs with different chemistries or flow rates. Further insight might be gained by looking at how reaction energy yields change along an outflow channel or with increasing depth in spring sediment, and by coordination of measurement of the rates of processes predicted to be important and censuses of the resident microbial communities.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Activities for aqueous compounds in GBS and SSW.

**Table S2.** Potential chemolithotrophic reactions and their corresponding standard  $\Delta G_{\text{rxn}}^{\circ}$ .

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