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Bioenergetic constraints on microbial hydrogen utilization in

Precambrian deep crustal fracture fluids

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Abstract

Precambrian Shield rocks host the oldest fracture fluids on Earth, with residence times up to a billion years or more. Water-rock reactions in these fracture systems over geological time have produced highly saline fluids, which can contain mM concentrations of H_2 . Mixing of these ancient Precambrian fluids with meteoric or palaeometeoric water can occur through tectonic fracturing, providing microbial inocula and redox couples to fuel blooms of subsurface growth. Here, we present geochemical and microbiological data from a series of borehole fluids of varying ionic strength (0.6 M to 6.4 M) from Thompson Mine (Manitoba) within the Canadian Precambrian Shield. Thermodynamic calculations demonstrate sufficient energy for H_2 -based catabolic reactions across the entire range of ionic strengths during mixing of high ionic strength fracture fluids with meteoric water, although microbial H_2 consumption and cultivable H_2 -utilizing microbes were only detected in fluids of ≤ 1.9 M ionic strength. This pattern of microbial H_2 utilization can be explained by the greater potential bioenergetic cost of organic osmolyte synthesis at increasing ionic strengths. We propose that further research into the bioenergetics of osmolyte regulation in halophiles is warranted to better constrain the habitability zones of hydrogenotrophic ecosystems in both the terrestrial subsurface, including potential future radioactive waste disposal sites, and other planetary body crustal environments, including Mars.

Introduction

Precambrian Shield rocks host the oldest flowing fluids on Earth, with some bulk fracture fluids having residence times of a billion years, and components such as conservative noble gases (e.g. xenon) potentially as old as 2.64 billion years old (Holland et al. 2013). These ancient saline fluids can contain high concentrations of H_2 , CH_4 and other reduced gases formed by water-rock reaction such as serpentinization and radiolysis (Lin et al. 2006a; Sherwood Lollar et al. 1993; Sherwood Lollar et al. 2014; Sherwood Lollar et al. 2002). Precambrian rocks are exposed at or underlie over 70% of the Earth's continental lithosphere by surface area, and global fluxes of abiogenic H_2 from water-rock reaction rival that of marine hydrothermal systems (Sherwood Lollar et al. 2014). Both present day mining activity and tectonic fracturing of Precambrian Shield cratons over geological time periods can enable the mixing of previously isolated Precambrian fluids with more recent meteoric or palaeometeoric water depending on the locality, resulting in microbial colonization of the subsurface and potential blooms of subsurface life (Sherwood Lollar et al. 2007; Sleep and Zoback 2007). The growth of subsurface microorganisms could impact the safety of proposed schemes to dispose of radioactive waste in deep shafts drilled into Precambrian shield rocks, via the biocorrosion of metal storage containers and the alteration of radionuclide speciation via changes in pH and Eh (Libert et al. 2011; Stroes-Gascoyne and West 1997). Precambrian shield ecosystems also provide terrestrial analogues for potential extant ecosystems in the subsurface of the billions of year old crust of Mars (Boston et al. 1992; Michalski et al. 2013; Onstott et al. 2006; Sleep et al. 2004).

Abiogenic H_2 has been proposed as a key source of energy to support ecosystems in the deep subsurface, independent of photosynthesis, including fracture fluids in crystalline and marine settings (Kelley et al. 2005; Lin et al. 2005; Pedersen 1997; Pedersen 2012; Pedersen 2013; Sherwood Lollar et al. 2007; Spear et al. 2005; Stevens and McKinley 1995; Wu et al. 2017). Isotopic, molecular and microbiological evidence from fracture fluids in Precambrian Shield sites demonstrates the importance of microbial metabolisms that consume abiogenic H_2 (Chivian et al. 2008; Lin et al. 2006a; Pedersen 1997). Prior research suggests that the highest rates of microbial H_2 consumption occur within less saline fluids

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i.e. those with a substantial meteoric or palaeometeoric component (Kotelnikova and Pedersen 1998; Onstott et al. 2006; Sherwood Lollar et al. 2007; Ward et al. 2004). To date, there is less evidence of microbial H₂ utilization in highly saline brine end-members, despite the presence of abundant H₂ based redox couples. The reason(s) for this apparent paradox have not been elucidated, although it has been suggested that H₂ utilizing methanogens might be inhibited by high dissolved CH₄ concentrations (Moser et al. 2005).

In this study we propose an alternate hypothesis to explain the apparent lack of H₂ utilization at higher salinities: that the energetic cost of combating potential water loss via osmosis limits growth and activity. Microbial cell membranes are permeable to water, and if the water activity within the cell is higher than that of the external aqueous environment there will be rapid and catastrophic water loss from the cell. Microbes adapted to living at high ionic strengths prevent this osmotic loss of water by incorporating either inorganic (KCl) osmolytes or, more usually, organic osmolytes (compatible organic solutes) within their cytoplasm, to balance the osmotic forces (Oren 1999). Organic osmolytes are used by most halophilic prokaryotic microorganisms, with the synthesis of compounds such as glycine betaine (Oren 1999). The use of KCl requires the adaption of all intercellular machinery to high inorganic salt concentrations, and is used by two microbial orders (*Halobacteriales* and *Halanaerobiales*). The energetic cost of synthesizing organic osmolytes can be large at high ionic strengths, and can greatly exceed the energetic cost of replication (Head et al. 2014; Oren 1999).

To test this hypothesis, we sampled a suite of fracture fluids of varying ionic strength located in Precambrian crystalline rocks at Thompson Mine, Manitoba. We measured the aqueous and gaseous geochemistry of the fracture fluids, rates of microbial H₂ oxidation, and numbers of cultivable H₂-utilizing microorganisms grown at a range of different ionic strengths. We compared the rates and distributions of H₂-utilizing microbes to the potential energy available for the growth of microbial cells from the mixing of ancient brine and meteoric end members

94

95 **Materials and Methods**

96 *Study site*

97 A suite of five boreholes at the 1067 m level of Thompson Mine, Manitoba, were sampled in June 2006
98 (between two to ten months after drilling) to investigate their aqueous and gaseous geochemistry and
99 microbiology. Thompson is a nickel mine located within 2.1 Ga metasedimentary, metavolcanic and
100 ultramafic rocks that overlie Archean felsic and mafic gneisses, granulites and amphibolites of the Canadian
101 Shield (Brooks and Theyer 1981). The boreholes were specifically chosen to span a wide of conductivities.
102 All the boreholes had gases naturally exsolving from the fluids at the time of sampling. Drilling water
103 (derived from surface lakes, and circulated down to the mining operation levels) was also sampled.

104 *Field measurements/sampling*

105 Conductivity, pH and temperature were measured *in situ* with standard field probes. O₂ was
106 measured *in situ* using a CHEMetrics colorimetric test kit (detection limit 0.1 ppm). All boreholes at
107 Thompson Mine were close to vertical, and completely full of water at the time of sampling, with water
108 naturally discharging from the boreholes. The gas flow rate was measured in triplicate by temporarily
109 sealing the borehole with a pre-sterilized (autoclaved) rubber stopper with a tube inserted through it, and
110 measuring the time taken for gas to displace a known volume of water. Water flow rates were measured by
111 removing 2 L of water from the borehole, and timing how long it took to refill.

112 Samples for aqueous geochemistry and microbiology were taken via a 50 cm long sterilized Tygon
113 tube attached to a sterile 50 mL plastic syringe. Sample bottles were pre-fixed with 50 µL saturated HgCl₂
114 solution to give a final sample HgCl₂ concentration of 270 µM to prevent any subsequent microbial activity.
115 Samples for ion analysis were filtered through 0.45 µm cellulose nitrate filters and collected in 30 mL
116 HDPE (High-Density Polyethylene) bottles. Water samples for measurement of stable isotopic

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composition of water were sampled in 30 mL HDPE bottles with no headspace, and analyzed at the University of Waterloo. Samples for H₂ oxidation rate experiments and Most Probable Number (MPN) analyses were collected in the field in 125 mL acid washed, 6 × MQ rinsed, and baked (450°C, overnight) borosilicate serum vials (Product #223748, Wheaton Industries Inc., NJ, USA) sealed with 14 mm thick butyl rubber stoppers (Product #2048-117800, Bellco Glass Inc., NJ, USA). Bottles for aerobic incubations initially contained ambient air, while bottles for anaerobic incubations had previously been flushed for 10 minutes with N₂, and a small amount of freshly synthesised FeS added to ensure reducing conditions. Water (50 mL) was injected through the stoppers into the bottles using sterile 50 ml syringes leaving 110 ml headspace. The samples were maintained at 4°C during transport, and inoculations occurred within 36 hours of sampling.

Laboratory chemistry measurements

Cation analyses were carried out at the University of Waterloo by coupled plasma atomic emission spectroscopy (ICP-AES). Precision was ± 5%. Reduced iron (Fe²⁺) was analysed by the Ferrozine method (Stookey 1970), with a precision of 8%. Anions were analysed by Dionex Ion Chromatography (IC). Acetate was analysed by IC in a separate run after removing Cl⁻ with Alltech Maxi-Clean™ IC-Ag cartridges (Product #30258, Alltech, Kentucky, US), using an AS11HC column and a gradient eluent program ramping from 50 mM NaOH to 1 mM NaOH. DIC was performed by acidification of samples to ≤ pH 2.5 and analysis of released CO₂ by infrared spectroscopy at the University of Arizona.

Compositional analyses of gas samples were performed at the Stable Isotope Laboratory at the University of Toronto. Concentrations of CH₄, C₂H₆, C₃H₈, i-C₄H₁₀ and n-C₄H₁₀ were quantified on a Varian 3400 GC equipped with a flame ionization detector (FID). The hydrocarbons were separated on a J&W Scientific GS-Q column (60 m x 0.32 mm ID) with a helium gas flow and the following temperature program: 32°C for 6 minutes, rising to 220°C at 20°C/minute. Concentrations of H₂, He, O₂ and N₂ were

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analysed on a Varian 3800 GC equipped with a micro-thermal conductivity detector (μ TCD). Gases were separated using a Varian Molecular Sieve 5A PLOT column (25 m x 0.53 mm ID) with an Ar gas flow and temperature program: 35°C for 6 minutes, then temperature increased to 220°C at 20°C min⁻¹. Reproducibility for triplicate analyses was better than $\pm 5\%$.

$\delta^2\text{H-H}_2$ analyses were performed at the University of Toronto using a Finnigan MAT Delta+XL isotope ratio mass spectrometer interfaced with an HP 6890 GC and a micropyrolysis furnace. Separation was achieved using a 60 m J&W Scientific GS-Q column (60 m x 0.32 mm ID) with the following temperature program: 35°C increasing to 120°C at 5°C min⁻¹, rising to 220°C at 10°C min⁻¹, holding at 220°C for 10 minutes. Total error incorporating both accuracy and reproducibility was $\pm 5\%$ with respect to V-SMOW (after Ward et al. 2000). The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of H_2O were analyzed at the University of Waterloo using offline preparatory methods and gas source stable isotope mass spectrometry. Precisions were 0.15 ‰ and 2.0 ‰ for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ respectively.

Laboratory microbiological measurements

H₂ oxidation rate experiments. Thirty mL of fluid from each of the Thompson boreholes and drilling fluid were added via a sterile syringe and needle to 2 x aerobic and 2 x anaerobic 60 mL borosilicate serum vials (Product #223746, Wheaton Industries Inc., NJ, USA), prepared as described above for the field sampling bottles. Half of the vials were autoclaved (121°C, 30 min) to act as killed controls. H_2 (5.0 grade) was added to each vial to give a starting headspace concentration of 0.8% v/v H_2 with a pressure of 150 kPa. The vials were shaken and left to equilibrate overnight. Vials were incubated at room temperature (~20-22°C, similar to borehole temperatures) and one mL aliquots of gas in the vial headspaces sampled and analysed by GC (H_2 , O_2 and CH_4 , as described above) on Day 0 (after overnight equilibration), and subsequently on Days 3, 7, 14, 23, 39 and 78.

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Most Probable Number analysis of H_2 -utilizing microorganisms. The cell numbers of different functional groups of cultivable H_2 utilizing microorganisms in the borehole fluids were estimated using a serial dilution Most Probable Number (MPN) method (Hurley and Roscoe 1983). Three growth media of three different ionic strengths were tested: brackish (0.06 M), saline (0.8 M) and supersaline (2.5 M). Note that due to the time intensive nature of preparing MPN media and our wish to inoculate as quickly as possible after sampling, the media solutions were prepared and dispensed prior to fieldwork, assuming from previous work that the maximum ionic strength that we would sample would be around 2.5 M. The composition of basal salts media was based on prior measurements of Precambrian Shield brines (Doig et al. 1995; Frape et al. 1984), as follows. Brackish medium (0.06 M): 21.7 mM NaCl, 13.4 mM $CaCl_2 \cdot 6H_2O$, 0.25 mM $MgCl_2 \cdot 6H_2O$, 7.5 mM NH_4Cl , 1.3 mM KCl, 20.5 mM $NaHCO_3$, pH 7.2. Saline medium (0.8 M): 141 mM NaCl, 215 mM $CaCl_2 \cdot 6H_2O$, 33.6 mM $MgCl_2 \cdot 6H_2O$, 2.7 mM KCl, 20.5 mM $NaHCO_3$, pH 6.8. Supersaline medium (2.5 M): 423 mM NaCl, 644 mM $CaCl_2 \cdot 6H_2O$, 101 mM $MgCl_2 \cdot 6H_2O$, 2.7 mM KCl, 20.5 mM $NaHCO_3$, pH 6.0. H_2 (as an electron donor) and varying electron acceptors were added to the various media to enumerate the following functional groups of cultivable H_2 utilizing microorganisms: aerobic oxidizers, Fe(III)-reducers, sulfate-reducers (SRBs), and methanogens/putative acetogens (Table 1). The pH of media was adjusted with 0.1 M HCl and NaOH after the addition of all nutrients, electron acceptors and trace elements.

MPN dilutions were carried out in triplicate in 5 mL borosilicate serum vials (Product #223738, Wheaton Industries Inc., NJ, USA) with 14 mm thick blue butyl rubber stoppers (Bellco, product #2048-117800, Bellco Glass Inc., NJ, USA) with an initial inoculum of 0.5 mL into 4 mL media, followed by four levels of $50 \times$ dilution. Triplicate $0.2 \mu m$ filtered samples were used as controls. The vials were inoculated at room temperature ($\sim 20^\circ C$; close to the *in situ* borehole temperatures, Table 2) for 6 months, and vials analyzed for positive growth as described in Table 1. The detection limit by this method was two viable cells mL^{-1} .

Thermodynamic modelling

Free energy of reactions. Thermodynamic modelling was performed in PHREEQ-C (Parkhurst 1995). Due to the high ionic strengths of the majority of borehole fluids, the aqueous activities of dissolved ions (with the exception of acetate) were calculated using the Pitzer database. The Pitzer model uses a specific interaction approach, rather than the Debye-Huckel approach, which breaks down at salinities much greater than seawater (Parkhurst 1995). As acetate was not included within the Pitzer database, we estimated this separately using activity coefficients from the equations of Hamer and Wu (1972), based on empirical data from 0 M (activity coefficient of 0.96) to 3.5 M (activity coefficient of 1.06). From an ionic strength of 3.5 M to 6.4 M we assumed the activity coefficient to be constant at 1.06. We modelled the mixing of the highest ionic strength end member at Thompson Mine (6.4 M) with the freshwater drilling fluid end member (0.002 M) in 10 equal increments, and calculated the aqueous activities of Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- , SO_4^{2-} , CO_2 , HCO_3^- , CO_3^{2-} , acetate, and H^+ . As dissolved sulfide was below detection in the endmembers, we assume a nominal H_2S activity of 1×10^{-9} M. Note that the Gibbs Free Energy of Fe(III)-reduction was not quantified due to uncertainties as to the availability of ferric iron.

As thermodynamic data for the gases H_2 , O_2 and CH_4 were not included in the Pitzer database, we used the ideal gas law ($PV = nRT$) to calculate gas molalities, and manually calculated the mixing of gases in Microsoft Excel. We calculated Gibbs Free Energy changes for a range of H_2 -utilizing catabolic microbial reactions (Table 2) for two end member scenarios. First, we corrected for partitioning to the gas phase at one atmospheric pressure using the equations of Wiesenburg and Guinasso (1979). This scenario approximated the condition of the fracture fluids at the point of sampling, when the sampled borehole fluids were at or close to atmospheric pressure. Second, we assumed that all gas was dissolved. This is an approximation to high pressure conditions within isolated deep crustal fracture systems (Sherwood Lollar et al. 1993).

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The free energy of reaction of the reactions in Table 1 were calculated from Equation (1):

$$\Delta G^r = \Delta G^\circ + RT \ln Q$$

where ΔG^r represents the Gibbs Free Energy available for a reaction, ΔG° represents the standard free energy (calculated using the dataset from Amend and Shock, 2001), R is the universal gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$), and Q is the activity product of the species involved in the reaction. The total amount of energy available for each reaction per litre of borehole fluid was calculated by multiplying the ΔG^r value by the activity of the limiting reactant, taking into account the stoichiometry of the reaction and using total gas molalities rather than solubility corrected molalities.

Estimating the impact of osmolyte synthesis on the % energy available for growth. The estimated percentage of ΔG^r required to combat osmosis during new cell growth in the fluids was calculated as follows. First, the mmoles cm^{-3} (M_O) of osmolytes required within the microbial cells at different fluid ionic strengths was calculated using Equation 2, based on the regression equation of intracellular compatible solute concentrations measured in halophilic growth cultures at a range of different ionic strengths (Brown 1990; Oren 1999) (Fig. 1)

$$M_O = 2.1 \times \text{Ionic strength} - 0.57$$

Next, the ATP required to synthesize M_O in one cm^3 of cells (ATP_M) was calculated from Equation (3):

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Equation (3)
$$\text{ATP}_M = M_O \times a$$

where a is the ATP_M molar equivalents required to autotrophically synthesize compatible solutes within a microbial cell (using $a = 30, 54, 58, 54, 85$ and 109 mmol of ATP mmol⁻¹ for glycerol, glycine betaine, ectoine, glucosyl glycerol, sucrose, and trehalose respectively, and $a = 0.59$ mmol of ATP mmol⁻¹ for the uptake of KCl by ion transporters; based on the theoretical calculations of Oren (1999) and Stouthamer (1973)). The relative percentage of ATP used for osmolyte synthesis or KCl uptake (% osmolyte) relative to normal cellular growth under non saline conditions (ATP_{cell}) was then calculated for the range of Thompson fluid ionic strengths using Equation 4:

Equation (4)
$$\% \text{ growth} = \text{ATP}_M / (\text{ATP}_{\text{cell}} + \text{ATP}_M) \times 100$$

where ATP_{cell} is assumed to be 30 mmol ATP cm⁻³ (Oren, 1999; Stouthamer, 1973).

Results

Aqueous geochemistry

The temperature of the borehole fluids ranged from 21.9°C to 22.7°C (Table 3). The conductivities of the borehole fluids ranged from 38.0 to 120 mS cm⁻¹, while the conductivity of drilling water was lower at 0.5 mS cm⁻¹ (Table 3). The calculated ionic strengths of the borehole fluids ranged from 0.6 M to 6.4 M. The aqueous geochemistry of the borehole fluids was dominated by Na⁺ (up to 1370 mM), Cl⁻ (up to 3510 mM) and Ca²⁺ (up to 1090 mM) (Table 3). Nitrate was detected in one of five of the borehole fluids (503 µM). Sulfate was detected in two of the five borehole fluids (105 and 409 µM). Dissolved O₂ was at or close to the detection limit in all the borehole fluids, but higher in the drilling water (0.16 mM) (Table 3). Total inorganic carbon (DIC) in the drilling water and two freshest boreholes ranged from 125 to 151 mM, with

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lower values (28 to 39 mM) in the three most saline borehole. Acetate concentrations ranged from 53 to 496 μM in the borehole fluids, and 5 μM in the drilling water (Table 3).

Gas and water flow rates, gas compositions and gas and water isotopic values

Gas flow rates in the Thompson Mine boreholes ranged from 0.2 to 45 mL min^{-1} (Table 4). Water flow emanating from the highest ionic strength borehole (6.4 M) was between 0.17 to 2.0 mL min^{-1} . Water flow in the remainder of boreholes was not measured. The gas:water ratio in the 6.4 M borehole was between 0.15 to 1.8. The $\delta^{18}\text{O}\text{-H}_2\text{O}$ and $\delta^2\text{H}\text{-H}_2\text{O}$ for the drilling water were -12.9 and -112 ‰ respectively, falling slightly below the meteoric water line (Fig. 2), consistent with their source in a local lake. All borehole fluids were more depleted in $\delta^{18}\text{O}\text{-H}_2\text{O}$ and more enriched in $\delta^2\text{H}\text{-H}_2\text{O}$ than drilling water (Table 5), with overall trends of $\delta^{18}\text{O}$ depletion and $\delta^2\text{H}$ enrichment with increasing salinity, and values elevated above the meteoric water line (Fig. 2).

The composition of the gases in borehole fluids was dominated by N_2 (49 to 62 % v/v) and CH_4 (35 to 46 % v/v). There were detectable concentrations of C_2+ alkanes (C_2H_6 , C_3H_8 , i- C_4H_{10} , n- C_4H_{10}), with a logarithmic decrease in concentration from CH_4 to C_4H_{10} (Table 4). H_2 was detected in all borehole fluids (0.03 to 2.7% v/v). Helium was detected in all boreholes, with concentrations ranging from 2.4 to 3.3 % v/v (Table 4). H_2 concentrations generally increased with Cl^- concentration, with a polynomial best fit rather than linear relationship ($R^2 = 0.99$; Figure 3). The $\delta^2\text{H}\text{-H}_2$ of Thompson borehole fluids were within a narrow range of -753 to -762‰ (Table 5) consistent with values typically observed in crystalline brines in Precambrian rocks (Sherwood Lollar et al. 2007).

Microbial H_2 oxidation microcosm experiments

Microcosm experiments demonstrated H_2 consumption in the two freshest borehole fluids (0.6 M and 1.9 M) (Fig. 4). Maximum H_2 consumption rates (the steepest gradient between two time points) in the freshest borehole were 0.66 $\mu\text{moles H}_2 \text{ L}^{-1} \text{ day}^{-1}$ under aerobic conditions and 0.45 $\mu\text{moles H}_2 \text{ L}^{-1} \text{ day}^{-1}$

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under anaerobic conditions. Maximum H_2 consumption rates in the second freshest borehole were 0.24 $\mu\text{moles H}_2 \text{ L}^{-1} \text{ day}^{-1}$ under aerobic conditions, and 0.12 $\mu\text{moles H}_2 \text{ L}^{-1} \text{ day}^{-1}$ under anaerobic conditions (Fig. 5). Maximum rates of H_2 consumption in the experiments conducted with the drilling fluid were 0.23 $\mu\text{moles H}_2 \text{ L}^{-1} \text{ day}^{-1}$ and 0.29 $\mu\text{moles H}_2 \text{ L}^{-1} \text{ day}^{-1}$ under aerobic and anaerobic conditions respectively (Fig. 5). There was no detectable H_2 consumption in the three most saline boreholes (2.6 M, 2.8 M and 6.4 M) (Fig. 4) nor in killed controls (not shown) in incubations that lasted 78 days.

H₂-utilizing MPN counts

Cultivable H_2 -utilizing microorganisms were detected in the 0.6 M borehole fluid, the 1.9 M borehole fluid and drilling water, but not in the 2.5 M, 2.7 M or 6.4 M borehole fluids (Table 6, Fig. 5). The largest mean estimated cultivable cell concentrations were in the 0.6 M borehole fluid, with an estimated $\sim 50,000 \text{ cells mL}^{-1}$ of SRBs and putative acetogens, nearly 9,800 cells mL^{-1} of aerobic H_2 oxidizers, and smaller numbers (6 cells mL^{-1}) of Fe(III) reducers (Table 6). All of these functional microbial groups were present in lower numbers in the 1.9 M borehole fracture fluid and drilling water (Table 6).

Cultivable aerobic H_2 oxidizers and Fe(III) reducers were only detected in brackish media (0.06 M in Table 6). SRBs were documented in both brackish and saline media (0.06 M and 0.8 M in Table 5, respectively) in the 0.6 M fluid, although with higher cell numbers in brackish media. Putative acetogens grew in brackish media in the drilling fluid and 0.6 M borehole, whereas low (6.5 cells mL^{-1}) but equal numbers of putative acetogens grew in media of all three salinities in the 1.9 M borehole (Table 6). Methanogens were only detected in one of the borehole fluids (1.9 M), and this was in supersaline media only (2.5 M in Table 6).

The only cultivable counts present in borehole fracture fluids but not in drilling fluids were saline SRBs, saline and supersaline putative acetogens, and supersaline methanogens (Table 6). Note that the ionic strength of the MPN media (2.5 M) was less than half the ionic strength of the most saline borehole fluid

(6.4 M), but within 11% and 4% of the ionic strengths of the next two most saline borehole fluids (Table 6).

Thermodynamic modeling results, and comparison to viable numbers of microbes

One atmosphere pressure scenario. Theoretical mixing of the Thompson saline end-member (6.4 M ionic strength) with the freshwater end-member (drilling water) indicated there was sufficient energy (ΔG^r) for H_2 -based catabolic reactions ($> 20 \text{ kJ mole}^{-1}$) at all ionic strengths, (Table 7), using the stoichiometry in Table 1. 20 kJ mol^{-1} is a commonly cited minimum energy threshold required to drive ATP synthesis (Scholten and Conrad 2000). Aerobic H_2 oxidation gave the highest free energy yield (up to $972 \pm 11 \text{ kJ mol}^{-1} H_2$) (Table 7). There was a gradual decrease in the free energy for aerobic H_2 oxidation with increasing ionic strength (Table 7, Fig. 6a), and opposing trends of gradually increasing free energy with greater ionic strength for the various anaerobic H_2 utilization reactions (Table 7; Fig. 6b).

High pressure scenario (all gases dissolved). Under the scenario that all gases were dissolved, approximating high pressure conditions within closed fracture systems (Sherwood Lollar et al. 1993), the free energy available for all H_2 -based redox couples increased (Table 7). The increase in ΔG^r ranged from a minimum of $-23.5 \text{ kJ mole}^{-1}$ to a maximum of $-50.2 \text{ kJ mol}^{-1}$ depending on redox couple and ionic strength (Table 7).

When H_2 stoichiometries of the reactions in Table 2 were normalized to unity to better compare relative reaction yields, aerobic oxidation showed the greatest ΔG^r (Fig. 6a). Sulfate reduction showed the highest ΔG^r energy yields of the anaerobic H_2 oxidizing reactions (Fig. 6b), followed by methanogenesis (Fig. 6b). There was no apparent similarity between ΔG^r and the measured numbers of cultivable H_2 -utilizing microorganisms or rates of microbial H_2 oxidation (Fig. 5, Fig. 6).

The total energy available for microbial catabolic reactions of H_2 with O_2 , SO_4^{2-} , HCO_3^- and CO_2 with theoretical mixing of the end-member brine with the freshwater end-member is illustrated in Fig. 7. The largest total free energy yield was due to aerobic H_2 oxidation, with the largest potential energy yields

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at ~1 M ionic strength ($\sim 100 \text{ J L}^{-1}$) (Fig. 7). Smaller total energies were available from methanogenesis, acetogenesis and sulfate reduction, all with maximum yields at $\leq 2 \text{ M}$ ionic strength (Fig. 7).

Relative energetic cost of osmolyte synthesis relative to cell building during growth

The proportion of energy required for potentially combatting osmosis via the uptake of KCl did not exceed that required for normal cell growth over the range of borehole ionic strengths (0 M to 6.4 M), with a maximum of 30% at an ionic strength of 6.4 M (Fig. 8). The energy costs for potentially combating osmosis through the production of organic osmolytes within cells was far greater than for KCl (Fig. 8). At an ionic strength of 2.5 M organic osmolyte synthesis required an estimated 81% to 96% of the combined energetic cost of cell building and osmotic regulation (Fig. 8). At an ionic strength of 6.4 M these percentages rose to 91% to 98% of the combined energetic cost (Fig. 8).

Discussion

H₂ from ancient Precambrian fluids supports the growth of microorganisms

The trend of $\delta^{18}\text{O-H}_2\text{O}$ and $\delta^2\text{H-H}_2\text{O}$ values above the meteoric water line (Fig. 2) suggests mixing between a meteoric water derived end-member and a saline end-member whose $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values have been modified by water-rock interactions over geological time (Frape et al. 1984; Ward et al., 2004). The drilling water falls to the right of the meteoric water line, consistent with isotopic enrichment by evaporation in the surface lake from which the drilling water is derived. The $\delta^2\text{H-H}_2$ of Thompson borehole fluids were within a narrow range of -753 to -762‰, within the range of continental abiogenic H_2 described from a variety of Precambrian Shield terrains (Sherwood Lollar et al. 2007). These values are typical of abiogenic H_2 in ancient Precambrian Shield fracture fluids that have isotopically re-equilibrated with water over 10^3 to 10^5 years or more (Sherwood Lollar et al. 2007).

The non-linear relationship between the concentration of H₂ and Cl⁻ in the suite of borehole fluids from Thompson Mine (Fig. 3) cannot be explained by conservative mixing of a H₂-poor meteoric end-member and a H₂-rich saline end-member. The simplest explanation for the apparent preferential loss of H₂ in lower ionic strength borehole fluids (≤ 1.9 M) is microbial utilization of H₂ as proposed by Sherwood Lollar et al. (2006, 2007). This hypothesis is consistent with measured rates of potential microbial H₂ consumption with the utilization of H₂ under both aerobic and anaerobic conditions detected only in the two lowest ionic strength borehole fluids (0.6 M and 1.9 M; Fig. 4). Preferential microbial H₂ utilization in lower ionic strength borehole fluids was also consistent with the detection of cultivable H₂ utilizing microbes in the 0.6 M and 1.9 M borehole fluids, with none detected at ionic strengths ≥ 2.5 M (Fig. 5).

‘Palaeopickling’ of hydrogenotrophic deep crustal ecosystems?

End-member brines in isolated fractures can mix with meteoric derived water through mining activity or natural tectonic fracturing, providing potential blooms of subsurface microbial growth (Sherwood Lollar et al. 2007; Sleep and Zoback 2007). Our results show that where the resulting mixed fluids have an ionic strength ≤ 1.9 M, the utilization of abiogenic H₂ with available electron acceptors can potentially be rapid under both aerobic and anaerobic conditions, resulting in very brief (weeks, months) periods of subsurface growth (Fig. 4), followed by resource depletion. Following these subsurface blooms, much lower rates of microbial growth (below the detection limits of our methods) could potentially be sustained by the continual production of H₂ via radiolysis or low temperature serpentinization (Lin et al. 2005; Lin et al. 2006a; Sherwood Lollar et al. 2014).

In borehole fluids > 1.9 M, numbers of cultivable H₂-utilizing microorganisms were below the detection limit (Table 6). This by itself cannot be taken as conclusive evidence for a lack of hydrogenotrophs, due to the well documented difficulties of growing more than a small subsection of environmental microorganisms in the laboratory (Amann et al. 1995). For example, we cannot rule out the possibility that microbial growth in the MPN vials was limited by some essential trace nutrient. It is further

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possible that some hydrogenotrophic microorganisms at ionic strengths > 1.9 M were present and potentially cultivable, but due to slow growth rates would take longer than the 6 months used in this study to detect. Further studies could complement our culture-based approach with genetic culture-independent methods targeting both molecular phylogeny and functional genes within the borehole fluids, including functional genes for CO₂ reduction and hydrogenase activity, alongside more general measurements of microbial presence and activity including total cell counts, ATP concentration (Karl and Holm-Hansen 1978), and hydrogenase activity assays (Soffientino et al. 2006). In addition, more sensitive culture-based approaches such as concentrating microbial cells from borehole fluids prior to inoculation could be used.

The inability to culture hydrogenotrophs in higher ionic strength fluids was, however, consistent with the results of the H₂-amended microcosm experiments. The lack of any detectable microbial H₂ utilization in H₂ oxidation microcosm experiments after 78 days (Fig. 4) indicates that rates of microbial H₂ consumption in borehole fluids > 1.9 M were, if present, substantially slower than at weaker ionic strengths. This is seemingly at odds with the potential ΔG° yields, which predicted greater energy availability for anaerobic hydrogen utilizing reactions at higher ionic strengths (Fig. 7). While we cannot rule out that *in situ* hydrogenotrophic growth was inhibited by some essential trace nutrient or cofactor, our thermodynamic calculations (Fig. 7) suggest that bioenergetic factors alone can provide a potential explanation for this apparent paradox (Head et al. 2014; Oren 1999). Recently, it has been suggested that the high bioenergetic cost of forming osmolytes within microbial cells may retard the biodegradation of low temperature subsurface petroleum reservoirs, in a process termed ‘palaeopickling’ (Head et al. 2014). Our theoretical calculations for the Thompson Mine fracture fluids suggests that ‘pickling’ may also be an important factor in preventing the consumption of abiogenic H₂ gas in Precambrian Shield fractures. At ionic strengths > 1.9 M, the energetic cost of synthesizing organic osmolytes (Fig. 7) may become so high that microbial cell division in hydrogenotrophic microorganisms dramatically slows or stops (Fig. 5; Table 6). Few microbial groups are able to use the energetically much more favourable KCl as an osmolyte as it requires the adaption of all intracellular processes to high salt concentrations (Oren 1999).

The ‘pickling’ of hydrogenotrophic microorganisms within some Precambrian Shield rocks may have implications for evaluating this type of geologic setting for proposed nuclear waste repository sites. Deep (500 to 1000 m) shafts driven into stable plutons within Canadian Shield rocks were once proposed as sites for the long term disposal of nuclear fuel waste (Stroes-Gascoyne and West 1997). The excavation of such repositories could potentially mix ancient saline fluids with modern meteoric water, as documented in our Thompson Mine study. Within such settings, the radiolytic splitting of water by gamma radiation emitted from the high level radioactive waste can provide additional sources of both H_2 (Gales et al. 2004) and oxidants (Lin et al. 1996a; Li et al., 2016). Additional H_2 could be provided by the corrosion of metals used in some waste containers (Libert et al. 2011). In the short term (years to hundreds of years) after radioactive waste burial, high levels of gamma radiation and increased temperatures could inhibit microbial growth in zones up to 20 cm adjacent to the waste containers (Stroes-Gascoyne and West 1997). Over time (hundreds to many thousands of years) microbial colonization may become possible, depending on a range of additional factors including available space for colonization and growth, hydrogeological connectivity, and nutrient supply (Stroes-Gascoyne and West 1997; Sherwood Lollar, 2011). Our Thompson Mine data suggests that if the ionic strength of fluids in fracture systems adjacent to radioactive waste is < 1.9 M, and radiation levels sufficiently non-inhibitory, then hydrogenotrophic microbiological activity in the vicinity of stored nuclear waste may be promoted by H_2 and oxidants produced by continued radiolysis. Over time, it has been suggested that ongoing hydrogenotrophic activity could affect pH and Eh around waste containers, potentially altering the speciation of any radionuclides released from waste into adjacent fracture systems (Libert et al. 2011; Sherwood Lollar, 2011). One of the principal groups of microorganisms enumerated in our study using a culture based approach were sulfate-reducing bacteria, with up to an estimated 49,900 cells mL^{-1} grown in 0.6 M media, along with smaller populations of cultivable Fe(III)-reducers (Table 6). Importantly, both of these physiological groups can potentially accelerate the corrosion of various types of metal containers. The stimulation of sulfate-reducing bacteria by H_2 in nuclear waste repository settings in crystalline rock has been identified as something that could potentially enhance rates of biocorrosion of many types of metal containers via the production of corrosive H_2S (El Hajj et al. 2010).

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Fe(III)-reducing bacteria can potentially increase corrosion via the reduction and alteration of passive surface films containing ferric iron (Potekhina et al. 1999; Libert et al. 2011). One way of potentially limiting the growth of these and other hydrogenotrophic microorganism around radioactive waste sites might be to ensure that water in fracture systems immediately adjacent to future radioactive waste depositories is highly saline (> 1.9 M). For example, the use of high ionic strength drilling fluid could potentially help ensure that no pockets of fresher more habitable fluids are in close proximity to stored waste (Slater et al. 2013). However, most waste repositories rely on the use of bentonite clays as a barrier, and their swelling capacity, and hence effectiveness as a barrier, decreases with increasing ionic strength (Studds et al. 1998). The use of high ionic strength fluids is therefore not a viable option to limit rates of microbial corrosion.

Another key driver in terrestrial deep crustal ecosystem research is to gain insight into potential analogous crustal ecosystems in the subsurface of other planets such as Mars, and aid with future life detection planetary missions (McMahon et al. 2016; Michalski et al. 2013). It has been speculated that isolated crustal ecosystems could be present in the subsurface of Mars, isolated for the billions of years since Mars' surface became inhospitable to life. Subsurface crustal fluids on Mars are likely high ionic strength Ca-rich brines (Burt and Knauth 2003), comparable to those of our saline endmember Thompson Mine fracture fluid and similar ancient fluids isolated within Precambrian Shield terranes (Holland et al. 2013). Our study at Thompson Mine suggests that hydrogenotrophic microorganisms are unlikely to be present in similar highly saline brines in the subsurface of Mars unless they base their osmotic regulation around the accumulation of inorganic (e.g. K^+) rather than organic osmolytes (Fig. 7). This supports a recent assertion that ionic strength is an important deciding factor in the habitability of Martian brines (Fox-Powell et al. 2016).

Further research into the bioenergetics of osmolyte regulation in halophiles in Precambrian Shield fracture systems is required to better constrain the habitability zones of terrestrial and other planetary body crustal environments. Future studies could expand on our study by extending the focus to the ionic strength constraints on heterotrophic microorganisms within crystalline Precambrian Shield rocks, as well as using

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a greater variety of more sensitive methods culture and culture independent methods to target hydrogenotrophic activity in fracture fluids with ionic strengths > 1.9 M.

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TABLES

Table 1. Details of media used for Most Probable Number (MPN) viable counts

Type	Additions to basal media*	Headspace gas	Assay for positive growth
1. Aerobic H ₂ oxidizers	7.5 mM NH ₄ Cl, 73 µM KH ₂ PO ₄ , vitamins (1 mL L ⁻¹), trace minerals (10 mL L ⁻¹), resazurin (0.1 mL L ⁻¹)	H ₂ , air (45:55 v/v) overpressurized to 180 kPa.	Visual – media turned from pink to colourless, confirmed by H ₂ loss by GC-µTCD.
2. Autotrophic Fe(III)-reducers	7.5 mM NH ₄ Cl, 73 µM KH ₂ PO ₄ , 50 mM ferric citrate, vitamins (1 mL L ⁻¹), trace elements (10 mL L ⁻¹), resazurin (0.1 mL L ⁻¹)	H ₂ , CO ₂ , N ₂ (60:4.5:35.5 v/v) overpressurized to 180 kPa.	Visual – positives turned from orange to green to colourless, confirmed by H ₂ loss by GC-µTCD.
3. Autotrophic SO ₄ ²⁻ reducers	7.5 mM NH ₄ Cl, 73 µM KH ₂ PO ₄ , 20 mM NaSO ₄ ²⁻ , 5x10 ⁻⁵ M FeS, iron nail, vitamins (1 mL L ⁻¹), trace elements (10 mL L ⁻¹), resazurin (0.1 mL L ⁻¹)	H ₂ , CO ₂ , N ₂ . (60:4.5:35.5 v/v) overpressurized to 180 kPa.	Visual – blackening of iron nail, confirmed by H ₂ loss by GC-µTCD.
4. Autotrophic methanogens/acetogens	7.5 mM NH ₄ Cl, 73 µM KH ₂ PO ₄ , Vitamins (1 mL L ⁻¹), trace elements (10 mL L ⁻¹), resazurin (0.1 mL L ⁻¹), 5x10 ⁻⁵ M FeS	H ₂ , CO ₂ , N ₂ (60:4.5:35.5 v/v) overpressurized to 180 kPa.	H ₂ loss by GC-µTCD. If CH ₄ detected by GC-FID then counted as methanogens, if there was no CH ₄ detected then counted as putative acetogens (as confirmative acetate was not measured)

* trace element and vitamin compositions as per Edwards et al. (1992).

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Table 2. Stoichiometry of H₂ based microbial catabolic reactions used for thermodynamic modelling

Microbial reaction	Balanced equation
1. Aerobic oxidation	$2\text{H}_2 + \text{O}_2 = 2\text{H}_2\text{O}$
2. Iron oxide reduction	$\text{H}_2 + \text{Fe}_2\text{O}_3 + 4\text{H}^+ = 2\text{Fe}^{2+} + 3\text{H}_2\text{O}^1$
3. Sulfate reduction	$4\text{H}_2 + \text{SO}_4^{2-} + 2\text{H}^+ = \text{H}_2\text{S} + 4\text{H}_2\text{O}$
4. Acetogenesis (using HCO_3^-)	$4\text{H}_2 + 2\text{HCO}_3^- + \text{H}^+ = \text{CH}_3\text{COO}^- + 4\text{H}_2\text{O}$
5. Acetogenesis (using CO_2)	$4\text{H}_2 + 2\text{CO}_2 = \text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2\text{O}$
6. Methanogenesis (using HCO_3^-)	$4\text{H}_2 + \text{HCO}_3^- + \text{H}^+ = \text{CH}_4 + 3\text{H}_2\text{O}$
7. Methanogenesis (using CO_2)	$4\text{H}_2 + \text{CO}_2 = \text{CH}_4 + 2\text{H}_2\text{O}$

¹Note that G^r of Fe₂O₃ reduction was not calculated due to uncertainties in the molarity of available Fe³⁺ in the boreholes. We include the equation for completeness as we assayed for viable numbers of Fe(III) reducers.

Table 3. Aqueous geochemistry of Thompson Mine borehole fluids and drilling water

Sample	Cond	Ionic strength	pH	Temp	O ₂ (aq)	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	SO ₄ ²⁻	NO ₃ ⁻	DIC*	Acetate
	mS cm ⁻¹	(M)		°C	mM	mM	mM	mM	mM	mM	μM	μM	μM	μM
1065750	120	6.4	6.3	22.3	< 0.03	1370	10.2	162	1090	3510	105	< 89	29.5	53.2
1065760	93.5	2.7	7.2	21.9	< 0.03	605	1.6	35.1	616	1680	< 73	< 89	27.9	56.6
1163930	89.4	2.5	6.4	22.1	< 0.03	618	2.2	6.7	544	1580	< 73	< 89	39.3	81.6
1163630	80.4	1.9	6.8	21.9	0.03	376	1.9	21.5	457	1160	409	503	125	66.6
1065800	38.0	0.6	6.8	22.7	0.03	117	< 0.01	8.6	138	454	< 73	< 89	164	496
Drilling water	0.5	0.002	7.1	24.5	0.16	0.18	0.03	0.19	0.42	0.21	88.5	< 3.2	151	5.0

*calculated as HCO_3^-

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Table 4. Gas compositions (in vol% of free gas phase) for Thompson Mine borehole fluids

Sample	Gas flow mL min ⁻¹	H ₂	He	O ₂	N ₂	CH ₄	C ₂ H ₆	C ₃ H ₈	i-C ₄ H ₁₀	n-C ₄ H ₁₀	Total %
1065750	0.3	2.7	2.4	6.5	52.5	34.7	1.7	0.01	0.004	0.008	100.6
1065760	0.2	0.8	2.7	0.9	62.2	36.5	2.8	0.23	0.021	0.036	106.1
1163930	6.7	0.9	3.3	0.7	52.9	43.8	2.7	0.26	0.016	0.022	104.7
1163630	45	0.3	3.0	0.9	59.2	36.6	2.9	0.29	0.022	0.042	103.3
1065800	0.3	0.03	2.8	2.3	49.0	45.5	1.9	0.15	0.009	0.015	101.7

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Table 5. $\delta^2\text{H}$ values for borehole fluid hydrogen gas, ($\pm 5 \text{ ‰}$), with $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values for borehole fluids and drilling fluid ($\pm 0.15 \text{ ‰}$ and 2.0 ‰ respectively)

Sample	$\delta^2\text{H}_2$	$\delta^{18}\text{O}-\text{H}_2\text{O}$	$\delta^2\text{H}-\text{H}_2\text{O}$
1065750	-755	-14.7	-82.8
1065760	-753	-14.5	-94.8
1163930	-762	-15.3	-98.5
1163630	BDL	-13.3	-97.4
1065800	BDL	-13.3	-104.2
Drilling water	ND	-12.9	-112.2

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Table 6. Mean number of viable H₂-utilizing cells in borehole fluids and drilling water, as enumerated by the Most Probable Number (MPN) method. Bold numbers indicate most probable numbers, italicized numbers indicate 95% confidence limits as calculated using the approach of (Hurley and Roscoe, 1983).

Viable H ₂ oxidizing group	Medium Ionic Strength	Viable numbers of microbial cells in fracture fluid samples and drilling water (Cells mL ⁻¹)					
		0.002 M (Drilling water)	0.6 M	1.9 M	2.6 M	2.8 M	6.4 M
Aerobic oxidizers	0.06 M	196 (26 to 1450)	9,800 (1,300 to 73,500)	20 (3 to 141)	< 2	< 2	< 2
	0.8 M	< 2	< 2	< 2	< 2	< 2	< 2
	2.5 M	< 2	< 2	< 2	< 2	< 2	< 2
FeIII reducers	0.06 M	2 (0.5 to 9)	7 (2 to 35)	2 (0.5 to 9)	< 2	< 2	< 2
	0.8 M	< 2	< 2	< 2	< 2	< 2	< 2
	2.5 M	< 2	< 2	< 2	< 2	< 2	< 2
SO ₄ ²⁻ reducers	0.06 M	7 (2 to 35)	49,900 (6850 to 360,000)	20 (3 to 141)	< 2	< 2	< 2
	0.8 M	< 2	7 (2 to 35)	< 2	< 2	< 2	< 2
	2.5 M	< 2	< 2	< 2	< 2	< 2	< 2
Methanogens	0.06 M	< 2	< 2	< 2	< 2	< 2	< 2
	0.8 M	< 2	< 2	< 2	< 2	< 2	< 2
	2.5 M	< 2	< 2	2 (0.5 to 9)	< 2	< 2	< 2
Acetogens	0.06 M	7 (2 to 35)	49,900 (6850 to 360,000)	7 (2 to 35)	< 2	< 2	< 2
	0.8 M	< 2	< 2	7 (2 to 35)	< 2	< 2	< 2
	2.5 M	< 2	< 2	7 (2 to 35)	< 2	< 2	< 2

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Table 7. ΔG^r , Gibbs Free Energy (kJ mol^{-1}) of H_2 -based catabolic reactions based on the theoretical mixing of drilling fluid with the highest ionic strength borehole fluid. First number shows mean ΔG^r assuming 1 atmosphere pressure, number in brackets assumes all gases are in dissolved phase in pressurized fractures.

M	Aerobic oxidation	SO_4^{2-} reduction	Methanogenesis - CO_2	Acetogenesis - CO_2	Methanogenesis - HCO_3^-	Acetogenesis - HCO_3^-
$\Delta G^r \text{ kJ mol}^{-1}$						
6.40	N.D.	-125 (-175)	-74 (-112)	-38 (-88)	-70 (-109)	-30 (-81)
5.76	-966 (-999)	-131 (-181)	-73 (-112)	-34 (-84)	-73 (-112)	-35 (-85)
5.12	-968 (-1000)	-130 (-179)	-73 (-111)	-34 (-84)	-73 (-111)	-35 (-84)
4.48	-969 (-1001)	-128 (-177)	-72 (-110)	-34 (-83)	-72 (-110)	-35 (-84)
3.84	-971 (-1001)	-127 (-175)	-71 (-108)	-34 (-82)	-71 (-109)	-34 (-83)
3.20	-971 (-1000)	-125 (-173)	-70 (-107)	-33 (-81)	-70 (-107)	-34 (-82)
2.56	-972 (-999)	-123 (-171)	-68 (-105)	-32 (-80)	-68 (-105)	-33 (-80)
1.92	-972 (-998)	-120 (-167)	-66 (-103)	-30 (-78)	-66 (-103)	-31 (-78)
1.28	-972 (-996)	-117 (-163)	-63 (-100)	-28 (-74)	-64 (-100)	-28 (-75)
0.64	-970 (-993)	-111 (-157)	-58 (-94)	-23 (-69)	-58 (-95)	-23 (-70)

Figures

Figure 1. Intracellular osmolyte concentrations of *Dunaliella salina* grown at different ionic strengths (data from experiments of Brown 1990).

Figure 2. The stable isotopic composition ($\delta^{18}\text{O}$ -H₂O and $\delta^2\text{H}$ -H₂O) of borehole fluids and drilling water at Thompson Mine from the current study, compared to prior data from the same mine. The dotted line shows the meteoric water line (Craig, 1961). Solid circles are borehole fluids and drilling water from this study. Remaining open symbols are Thompson Mine borehole fluids described in Frape et al. (1984). Open circles = fresh, open inverted triangles = brackish, open triangles = saline, and open squares = brines.

Figure 3. Chloride versus H₂ gas for the suite of borehole fluids sampled at Thompson Mine. The non-linear relationship suggests that H₂ concentrations are not controlled purely by the mixing of a H₂ poor freshwater end member with a H₂ rich saline end member, but that there may be microbial utilization of H₂ in fresher waters.

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Figure 4. Aerobic and anaerobic H_2 consumption microcosm experiments from Thompson Mine fracture fluids. There was significant H_2 consumption in the drilling water and two lowest ionic strength borehole fluids (0.6 M and 1.9 M) under both aerobic (a) and (b) anaerobic conditions. There was no detectable H_2 consumption in killed (autoclaved) controls (not shown). Solid square = 6.4 M, solid circle = 2.8 M, solid inverted triangles = 2.6 M, open triangle = 1.9 M, open diamonds = 0.6 M, and open circles = 0.002 M (drilling water).

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Figure 5. Evidence of microbial utilization of H₂ in Thompson Mine borehole fluids and drilling water a. Numbers of culturable H₂-utilizing microorganisms, based on Most Probable Number analysis. Solid circles = aerobic oxidizers, open hexagons = Fe(III)-reducers, open circle = SRBs, open squares = methanogens, open triangles = putative acetogens (acetogens are classed as presumptive, as confirmatory acetate production was not measured) b. Maximum rates of microbial H₂ utilization based on H₂ oxidation experiments. Solid circle = aerobic H₂ oxidation, open circle = anaerobic H₂ oxidation.

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Figure 6. Calculated Gibbs Free Energies of H_2 -based catabolic reaction (G^r), based on the theoretical mixing of the most saline Thompson borehole fluid (6.4 M) with freshwater drilling fluid. We assumed that all oxygen was derived from the palaeometeoric endmember, and that all H_2 was derived from the fracture fluids. a. G^r , all reactions. b. G^r , showing anaerobic reactions only. Closed circles = aerobic oxidation, open circles = sulfate reduction, open squares = methanogenesis (CO_2), closed squares = methanogenesis (HCO_3^-), open triangles = acetogenesis (CO_2), closed triangles = acetogenesis (HCO_3^-).

Figure 7. Total energy available from the mixing of the highest ionic strength Thompson Mine borehole fluid (6.4 M) with freshwater drilling water. See main text for detailed calculations. Closed circles = aerobic oxidation, open circles = sulfate reduction, open squares = methanogenesis (CO₂), closed squares = methanogenesis (HCO₃⁻), open triangles = acetogenesis (CO₂), closed triangles = acetogenesis (HCO₃⁻).

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Figure 8. Modelling the impact of organic osmolyte synthesis and KCl uptake on the % anabolic energy available for cell growth over the range of ionic strengths of the Thompson Mine borehole fluids. See main text for calculations. Solid circles = KCl, open circles = glycerol, inverted triangles = glycine betaine, open triangle = ectoine, solid square = glucosylglycerol, open square = sucrose, closed diamond = trehalose.