

Correspondence

Another chemolithotrophic metabolism missing in nature: sulfur comproportionation

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Summary

Chemotrophic microorganisms gain energy for cellular functions by catalyzing oxidation–reduction (redox) reactions that are out of equilibrium. Calculations of the Gibbs energy (ΔG_r) can identify whether a reaction is thermodynamically favourable and quantify the accompanying energy yield at the temperature, pressure and chemical composition in the system of interest. Based on carefully calculated values of ΔG_r , we predict a novel microbial metabolism – sulfur comproportionation ($3\text{H}_2\text{S} + \text{SO}_4^{2-} + 2\text{H}^+ \rightleftharpoons 4\text{S}^0 + 4\text{H}_2\text{O}$). We show that at elevated concentrations of sulfide and sulfate in acidic environments over a broad temperature range, this putative metabolism can be exergonic ($\Delta G_r < 0$), yielding $\sim 30\text{--}50\text{ kJ mol}^{-1}$. We suggest that this may be sufficient energy to support a chemolithotrophic metabolism currently missing from the literature. Other versions of this metabolism, comproportionation to thiosulfate ($\text{H}_2\text{S} + \text{SO}_4^{2-} \rightleftharpoons \text{S}_2\text{O}_3^{2-} + \text{H}_2\text{O}$) and to sulfite ($\text{H}_2\text{S} + 3\text{SO}_4^{2-} \rightleftharpoons 4\text{SO}_3^{2-} + 2\text{H}^+$), are only moderately exergonic or endergonic even at ideal geochemical conditions. Natural and impacted environments, including sulfidic karst systems, shallow-sea hydrothermal vents, sites of acid mine drainage, and acid–sulfate crater lakes, may be ideal hunting grounds for finding microbial sulfur comproportionators.

Introduction

Reaction energetics have been used, at least in part, to posit the existence of several previously undetected microbial metabolisms. The core argument reads that sufficiently exergonic redox reactions can drive cellular functions of hypothetical microorganisms. In a classic example, Broda calculated standard Gibbs energies to propose that anaerobic ammonia oxidation with nitrate or nitrite (later termed anammox) could fuel certain chemolithotrophs ‘missing in nature’ (Broda, 1977). It took nearly 20 years until microbial catalysis of this process was confirmed in a laboratory wastewater sludge reactor (Vandegraaf *et al.*, 1995), and several more years until it was documented in natural environments with nitrite as the oxidant (Kuypers *et al.*, 2003). It is now recognized that the activity of anammox bacteria may account for as much as half of the nitrogen turnover in marine sediments (Kuenen, 2008).

In another example, anaerobic oxidation of methane (AOM) by marine sediment microorganisms was hypothesized by Barnes and Goldberg on the strengths of methane and sulfate concentration profiles from the anoxic sediments in the Santa Barbara Basin (California, USA), and on a modest negative free energy change calculated for the in situ conditions (Barnes and Goldberg, 1976). Other geochemical evidence for AOM in methane–sulfate transition zones of marine sediments followed (Martens and Berner, 1977; Iversen and Jørgensen, 1985; Hoehler *et al.*, 1994). Lipid biomarkers, gene surveys, fluorescence microscopy and carbon isotopes then confirmed a structured symbiotic relationship between a methane-oxidizing archaeon and a sulfate-reducing bacterium (Hinrichs *et al.*, 1999; Boetius *et al.*, 2000; Orphan *et al.*, 2001).

A third example, which puzzled microbiologists for over a century, was the apparent lack in nature of complete ammonia oxidation (comammox). Based again in part on a thermodynamic argument, an organism with this putative metabolism should have growth advantages – but perhaps not kinetic advantages – over

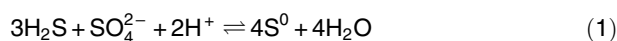
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incomplete ammonia oxidizers (Costa *et al.*, 2006). Analogous to the first two scenarios noted above, comammox bacteria were subsequently identified, cultivated and characterized from an elevated temperature biofilm in an oil exploration well (Daims *et al.*, 2015) and from a recirculating aquaculture system (van Kessel *et al.*, 2015). Their environmental distribution and ecological significance, however, are still largely speculative (Koch *et al.*, 2019).

Here, we use a thermodynamic approach to predict the existence of another novel microbial metabolism – sulfur comproportionation. Based on geochemical parameters, we then identify a number of natural and impacted target environments where sulfur comproportionation is energetically favourable and where finding microorganisms capable of this metabolism may be possible.

Results and discussion

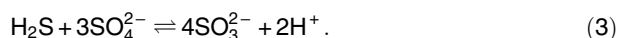
Sulfur comproportionation can be interpreted either as the anaerobic oxidation of sulfide with sulfate as the electron acceptor, or conversely, as the lithotrophic reduction of sulfate with sulfide as the electron donor. This process can be written to various intermediate oxidation state sulfur compounds, including to elemental sulfur as



to thiosulfate as



and to sulfite as



Note that the reverse of Reactions 1–3 describes sulfur disproportionation, a confirmed metabolism carried out by, among others, members of the *Deltaproteobacteria* and *Thermodesulfobacteria* in several natural environments, including marine sediments, shallow- and deep-sea hydrothermal systems and alkaline hot springs (Bak and Pfennig, 1987; Finster *et al.*, 1998; Slobodkin *et al.*, 2012; Kojima *et al.*, 2016; Slobodkina *et al.*, 2016; Slobodkin and Slobodkina, 2019). Amenabar and Boyd (2018) recently reported on the first archaeal S^0 disproportionator, the thermoacidophile *Acidianus* (strain DS80) that is widely distributed in hot springs of Yellowstone National Park (Wyoming, USA). Whether the forward (comproportionation) or reverse (disproportionation) reaction is thermodynamically favourable, depends predominantly on the chemical composition of the

environment under consideration; temperature and pressure play a far more limited role.

The direction in which a reaction can proceed and the associated energy yield are readily determined with the relation:

$$\Delta G_r = \Delta G_r^0 + RT \ln Q_r, \quad (4)$$

where ΔG_r and ΔG_r^0 stand for the overall and standard Gibbs energy of reaction r , respectively, R and T represent the gas constant and temperature (K), respectively, and Q_r denotes the activity product. Values of Q_r can be calculated with the expression

$$Q_r = \prod a_i^{\nu_{i,r}}, \quad (5)$$

where a_i represents the activity (unitless) of the i th species and $\nu_{i,r}$ stands for the corresponding stoichiometric reaction coefficient. The activities of liquid water and pure solids, including S^0 in Reaction 1, are typically set to unity (1.0), but those of aqueous solutes are computed with the equation

$$a_i = \gamma_i \frac{C_i}{C_i^0}, \quad (6)$$

where γ_i , C_i and C_i^0 stand for, respectively, the activity coefficient (unitless), concentration (usually in molality, m) and standard state concentration (usually $1m$). Values of γ_i can be calculated with an expression of the Debye–Hückel equation that takes into account the ionic strength of the solution, and the charge and other properties of the solute species. For recent discussions on this topic, the reader can consult Dick (2019), LaRowe and Amend (2019) and references therein.

Energy yields for Reaction 1 were calculated at elevated, but geochemically reasonable activities (and by extension, concentrations) of aqueous sulfide and sulfate and plotted in Fig. 1 as a function of temperature and pH. It can be seen in this figure that Reaction 1 is exergonic ($\Delta G_r < 0$) at acidic conditions over the entire temperature range (0–100°C) considered here. It is thermodynamically most favourable, with energy yields exceeding 30 kJ mol^{-1} , at $\text{pH} \leq 3$ and temperature $\leq 50^\circ\text{C}$; energy yields are greater than $\sim 50 \text{ kJ mol}^{-1}$ at $\text{pH} \leq 1$ and temperature $\leq 20^\circ\text{C}$. The same data are plotted in Fig. 2 as a function of sulfate and sulfide activities at four combinations of temperature and pH: 15°C and $\text{pH} 2$, 50°C and $\text{pH} 2$, 15°C and $\text{pH} 5$ and 50°C and $\text{pH} 5$. This figure demonstrates that Reaction 1 can be exergonic over wide ranges of elevated but reasonable sulfate and sulfide levels, but only at very low pH; to be

exergonic at pH 5 (or above), the required sulfate and sulfide activities would have to be extremely, perhaps unrealistically, high.

Note that at the conditions considered here, H_2S is the dominant sulfide species, and HS^- (or S^{2-}) does not matter in the calculations. Furthermore, for neutral species (including H_2S) the numerical values of the activity and concentration are essentially equal; the activity coefficient (γ_i) is essentially 1.0 for most combinations of temperature and ionic strength. Therefore, $a_{\text{H}_2\text{S}} = 10^{-3}$ taken here corresponds to a concentration of 10^{-3} m for H_2S (aq) and, by extension, for total sulfide.

The case for sulfate is more complicated, because depending on pH, the dominant species can be HSO_4^- or SO_4^{2-} . However, since we stipulate equilibrium between HSO_4^- and SO_4^{2-} and partition the total sulfate activity between these two species, the thermodynamic calculations return the same value for ΔG_1 , regardless whether Reaction 1 is written with HSO_4^- or, as done here for convenience, with SO_4^{2-} . Contrary to the explanation for neutral sulfide above, the activity coefficient for SO_4^{2-} can be $\ll 1.0$, and therefore, the numerical values of its activity and its concentration can differ significantly. As an example, at 25°C in an aqueous solution whose ionic strength is that of seawater ($\sim 0.7 \text{ m}$), a total sulfate activity of 10^{-2} as taken here corresponds to a concentration of SO_4^{2-} of $\sim 10^{-1} \text{ m}$ (see Amend and LaRowe, 2019).

Sulfur comproportionation to thiosulfate (Reaction 2) can also be exergonic, but only moderately

so. Regardless of temperature, it requires high concentrations of sulfate and sulfide and very low concentrations of thiosulfate, but it is not limited to acidic pHs. As an example, values of ΔG_2 calculated with the same high activities for total sulfate (10^{-2}) and sulfide (10^{-3}) used above, and with a very low value for thiosulfate (10^{-9}) vary from approximately -9 kJ mol^{-1} near 0°C to approximately -15 kJ mol^{-1} near 100°C . Sulfur comproportionation to sulfite (Reaction 3) is an unlikely, perhaps impossible metabolism; energy calculations show that it is endergonic ($\Delta G_3 > 0$) even at geochemically optimal conditions (high temperature, alkaline pH, elevated sulfate and sulfide activities, and very low sulfite activities).

By comparison, S^0 disproportionation can also be exergonic. However, a thermodynamic drive would only exist at geochemical conditions where the concentrations (and by extension, the activities) of sulfate and sulfide are low. For example, the energy yield for the reverse of Reaction 1 is $\sim 96 \text{ kJ mol}^{-1}$ at 25°C , low levels of SO_4^{2-} and H_2S (activities set to 10^{-4} and 10^{-6} , respectively), in slightly alkaline aqueous solutions (pH 8). At these conditions and activity of thiosulfate equal 10^{-6} , thiosulfate disproportionation represented by the reverse of Reaction 2 yields $\sim 35 \text{ kJ mol}^{-1}$. Finally, at these conditions, activity of sulfite equal 10^{-6} , and neutral pH, sulfite disproportionation (reverse of Reaction 3), yields $\sim 200 \text{ kJ mol}^{-1}$. Recall that all three of these disproportionation reactions have been demonstrated to support the growth of numerous strains of Bacteria, and at least S^0 disproportionation has now been demonstrated in the Archaea. We suggest here that if an energy yield as low as $\sim 35 \text{ kJ mol}^{-1}$ is sufficient for sulfur disproportionators, then $\sim 30\text{--}50 \text{ kJ mol}^{-1}$ may similarly suffice to fuel the maintenance and growth of putative sulfur comproportionators.

Many natural and impacted environments exhibit geochemical conditions that are thermodynamically favourable for sulfur comproportionation, and hence may serve as ideal hunting grounds for microorganisms that catalyze this proposed metabolism. As noted, Reaction 3 is endergonic even at the most favourable conditions, but Reaction 2 might be a possibility, perhaps in marine oxygen minimum zones. There, near the sediment–water interface or in nitrate- and nitrite-depleted waters, sulfate and sulfide levels can be high enough for Reaction 2 to be exergonic (Canfield *et al.*, 2010; Wright *et al.*, 2012). As noted above and shown in Figs. 1 and 2, the best conditions for Reaction 1 from an energy standpoint are acidic (pH < 3), high sulfate ($> 50 \text{ mm}$), high sulfide ($> 1 \text{ mm}$), and low temperature ($< 20^\circ\text{C}$); the temperature dependence of the energy yield is rather moderate, however, and ΔG_1 can be reasonably exergonic even at temperatures approaching 100°C . It should be clear that

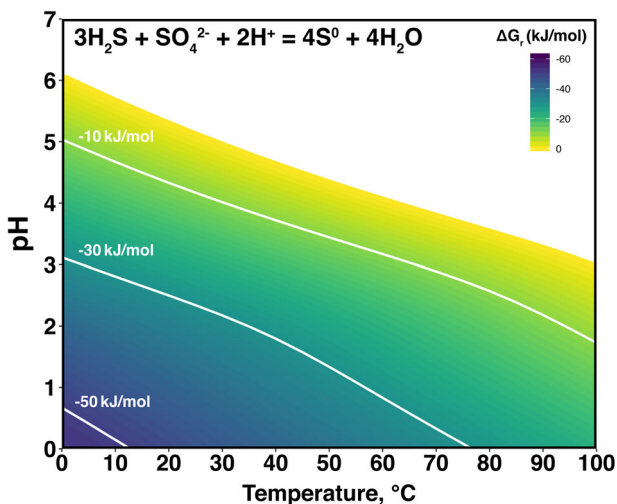


Fig. 1 Values of ΔG_r for Reaction 1 calculated with Equation 3 as a function of temperature and pH. Values of ΔG_r^0 were computed with the SUPCRT92 software package (Johnson *et al.* 1992). Activities of aqueous H_2S and SO_4^{2-} across the temperature and pH space represented here were calculated with equilibrium speciation among H_2S and HS^- given a total sulfide activity of 10^{-3} , and among HSO_4^- and SO_4^{2-} given a total sulfate activity of 10^{-2} . [Color figure can be viewed at wileyonlinelibrary.com]

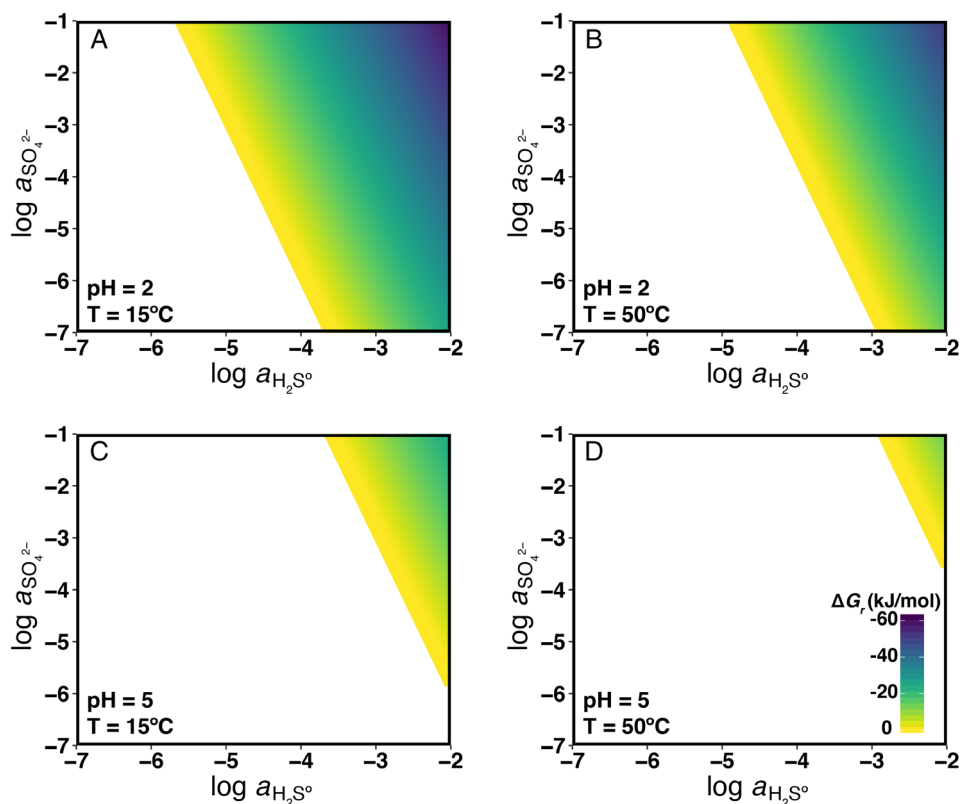


Fig. 2 Values of ΔG_r for Reaction 1 calculated as in Fig. 1 and plotted as a function of activities of H_2S and SO_4^{2-} .
 A. 15°C and pH 2.
 B. 50°C and pH 2.
 C. 15°C and pH 5.
 D. 50°C and pH 5.
 The scale for all four panels is shown in D. [Color figure can be viewed at wileyonlinelibrary.com]

different combinations of these geochemical parameters may also result in a thermodynamic drive for sulfur comproportionation, especially the version represented by Reaction 1.

In cool sulfidic karst systems formed by the dissolution of carbonate rock with sulfuric acid, aqueous solutions associated with biofilms on the walls ('snottites') can be extremely acidic (pH < 2.5) and exposed to cave air rich in hydrogen sulfide (~10–30 ppmv). High value targets include, but are certainly not limited to, the Frasassi cave system in Italy and La Cueva de Villa Luz in Mexico (Hose and Pisarowicz, 1999; Jones *et al.*, 2012). In shallow-sea and surf-zone hydrothermal systems, interfaces between acidic, sulfidic vent fluids and cooler, sulfate-rich seawater may be energy-yielding for sulfur comproportionators. For example, at Vulcano Island (Italy) mixed fluids (~40–90°C) have been described with pH as low as ~2, sulfate levels up to ~60 mM, and sulfide up to ~0.4 mM (Amend *et al.*, 2003; Rogers and Amend, 2006). At Milos Island (Greece) fluids are lower in sulfate (up to ~33 mM), but more sulfidic (up to 3 mM) (Price *et al.*, 2013; Gilhooly *et al.*, 2014). The dissolution and subsequent oxidation of sulfide minerals in acid mine drainage sites can also yield optimum conditions for sulfur comproportionators. For example, at the Richmond Mine (California, USA), temperatures are moderate (30–50°C), pH values can be negative and measured

sulfate concentrations are extremely high (up to 1161 mM) (Nordstrom *et al.*, 2000; Druschel *et al.*, 2004). Lastly, the search for sulfur comproportionation may be successful in acid-sulfate crater lakes. One such lake exists in Kawah Ijen Volcano (Indonesia): the lake temperature is moderate (37°C), pH is near zero (0.2–0.7), and sulfate levels are high (up to ~770 mM). Although measured sulfide levels are low (0.02 ppm), elevated concentrations of H_2S could exist where it is likely introduced to the system via fumarolic injections (Delmelle and Bernard, 1994; Delmelle *et al.*, 2000).

Conclusions

Phototrophs capture and convert the energy from solar radiation to drive all of their cellular processes, but chemotrophs must harness metabolic energy from redox disequilibria. Thermodynamic calculations at the temperature, pressure and chemical composition of interest can be used to propose new combinations of redox couples that may serve as new metabolisms. Here, we demonstrated the potential energy yields for sulfur comproportionation at geochemically reasonable conditions. It should be pointed out, however, that although a negative value of ΔG_r is essential for this (or any) proposed chemotrophic metabolism, it is not sufficient.

Physiological challenges may arise, especially at environmentally extreme conditions. For example, putative sulfur comproportionators may face toxic effects from high sulfide levels, which tend to increase as the solution pH decreases. The direct effects of low pH can also challenge cellular functions, but many microbial taxa have solved this problem, including sulfide-oxidizing bacteria that grow at pH 0–3 (Pokorna and Zabranska, 2015). The energetics approach described here can be expanded to speculate on other overlooked metabolic strategies, including, but certainly not limited to, nitrogen-redox, metal-redox and mineral-redox reactions. Such reaction energetics can be used as a hypothesis generator, taken up by geochemists to identify the most likely target environments and by microbiologists to design culturing strategies to find and grow chemotrophs on metabolisms currently missing from the literature.

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