Sulfur disproportionation is exergonic in the vicinity of marine hydrothermal vents

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Abstract :

Sulfur is abundant in different oxidation states in hydrothermal ecosystems, where it plays a central role in microbial energy production. The contribution of microbially catalyzed disproportionation of elemental sulfur (S0) to the energy fluxes of this ecosystem is unknown. Indeed, within the current knowledge it is impossible to study this process in a global way due to the lack of specific genetic markers and because of the difficulties in unraveling the isotopic signals from the different reactions of the sulfur cycle. In this context, calculations of the Gibbs energy (ΔGr) of sulfur disproportionation can identify whether this process is thermodynamically favorable and provides sufficient energy yields for growth at the temperatures, pressures, and chemical compositions found in the various niches of the hydrothermal ecosystem. Herein, free energy yield calculations were performed using internally consistent thermodynamic properties and geochemical data from four different hydrothermal systems. These calculations showed that S0-disproportionation is sufficiently exergonic to allow growth in most niches of the hydrothermal ecosystems, regardless of the geological and geochemical context, and depth; it is most favorable at elevated temperatures and alkaline pH, at low sulfide and sulfate concentrations, and in the presence of sulfide-chelating minerals, which are common in these environments.

Introduction

Sulfur compounds are ubiquitous in marine hydrothermal environments and several are central in catabolic reactions involved in primary production, recycling and terminal degradation of organic matter (Cao *et al.*, 2014). Sulfur is found in various oxidation states (H_2S, FeS, FeS_2, S^0, SO_3^{2-}, $S_2O_3^{2-}$, $S_nO_6^{2-}$, $S_nO_6^-$, SO_4^{2-}, etc.) in the chimney structures, vent fluids, surrounding seawater, and in pore spaces and fractures of the subseafloor, and consequently used as an electron donor or terminal electron acceptor in aerobic and anaerobic reactions. Sulfide/sulfur-oxidizing, sulfur-reducing and sulfate-reducing microbial taxa of hydrothermal habitats are well known (Godfroy *et al.*, in press; Zeng *et al.*, 2021), and models of the biogenic hydrothermal cycle of sulfur are based on these reactions. Another redox reaction involving sulfur species takes place in this unique ecosystem: microbial sulfur disproportionation (= dismutation) (MSD), most commonly represented as

$$4\ S^0 + 4\ H_2O = SO_4^{2-} + 3\ H_2S\ (aq) + 2\ H^+ \quad (Eq.\ 1)$$
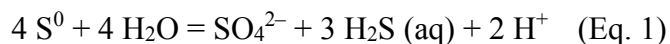
In this reaction, an intermediate valence inorganic sulfur compound is simultaneously reduced and oxidized into sulfide and sulfate, respectively (Bak and Cypionka, 1987; Finster, 2008; Slobodkin and Slobodkina, 2019). In addition to S^0, other studied substrates for MSD include thiosulfate ($S_2O_3^{2-}$) and sulfite (SO_3^{2-}) (Finster, 2008; Slobodkin and Slobodkina, 2019). MSD, also called inorganic fermentation, is poorly documented in hydrothermal ecosystems and the first hydrothermal microbial taxa able to disproportionate inorganic sulfur compounds under

anoxic conditions have been isolated quite recently (Slobodkin *et al*., 2012; Slobodkin *et al*., 2013; Slobodkina *et al*., 2016; Slobodkina *et al*., 2017; Frolova *et al*., 2018).

The metabolic pathways and enzymatic mechanisms of anaerobic sulfur disproportionation are only partially resolved (Finster, 2008; Mardanov *et al*., 2016; Slobodkin and Slobodkina, 2019; Allioux et al., 2020). A few archaea and bacteria, including one hydrothermal vent bacterium *Aquifex aeolicus*, are also capable of oxygen-dependent disproportionation of S^0 using the SOR (sulfur oxygenase reductase) enzymatic system (whose disproportionation sub-reaction is identical to Reaction (1) noted above) (Kletzin *et al*., 2004; Pelletier et al., 2008; Bertran *et al*., 2020). Although it has not been comprehensively investigated in hydrothermal environments, MSD is an important contributor to the sulfur cycle in other marine ecosystems and may prove to be integral to the hydrothermal sulfur cycle as well. Microorganisms capable of disproportionating intermediate sulfur species are widespread in marine surface sediments and are partly responsible for the isotopic signatures of sulfidic minerals in young and old sediments (Bak and Pfenning, 1987; Thamdrup *et al*., 1993; Finster, 2008; Jørgensen *et al*., 2019). Using radioisotope tracing of thiosulfate, it has even been shown that a significant proportion of the thiosulfate present in oxidized and reduced sediments is disproportionated (Bak and Cypionka, 1987; Fossing and Jørgensen, 1990; Jørgensen, 1990). However, the precise extent and distribution of the MSD process in natural environments is not known because (i) there is no specific genetic marker for this reaction, which prevents any application of genomic investigation methods to identify and quantify taxa implementing this process in natural habitats, because (ii) intermediate sulfur species are transient and generally short-lived *in situ* (Zopfi *et al*., 2004; Jørgensen *et al*., 2019), and (ii) because it is difficult to disentangle the relative magnitude of different sulfur compound-based redox metabolisms from the net isotopic signals (Jørgensen *et al*., 2019; Allioux *et al*., 2020).

In this context, calculations of the Gibbs energy of reaction (ΔG_r) at environmentally relevant conditions can identify whether MSD is thermodynamically favorable and consequently point to niches where sulfur disproportionators could be present and thriving. A common message in the literature is that MSD yields minimal energy or is endergonic (energy consuming) (Finster, 2008). It should be noted, however, that this claim is typically (and incorrectly) based on the value of the standard state Gibbs energy (ΔG_r^0) for Reaction (1) at 25°C and 1 bar, which is +120.5 kJ/mol (Amend and LaRowe, 2019). At low concentrations, and hence activities, of sulfate and sulfide, together with circumneutral to alkaline pHs, Reaction (1) can indeed be exergonic (Amend and LaRowe, 2019).

Deep-sea hydrothermal vents are seabed manifestations of hydrothermal convection of seawater through the permeable ocean floor, driven by heat from magma or subsurface rock (Humphris and Klein, 2018). These ecosystems occur in a range of tectonic settings, such as mid-ocean ridges, back-arc spreading basins, and volcanoes and seamounts at hotspots. They are characterized by a variety of fluid compositions, with acidic or alkaline pHs, and wide ranges of fluid temperatures (Orcutt *et al.*, 2011; Dick, 2019a). These singular habitats host a multitude of ecological niches with contrasting physico-chemical conditions along the mixing gradient between the venting fluid and seawater, allowing the settlement of luxuriant microbial and animal communities deriving their energy by chemosynthesis through oxidation-reduction reactions (Dick, 2019a). In addition, efficient sulfide scavenging manganese (IV) and iron (III) minerals can also increase the energy yield of MSD in these ecosystems (Slobodkin and Slobodkina, 2019).

In this paper, we use a thermodynamic approach and a range of physico-chemical parameters (*i.e.*, temperature, pressure, salinity, pH, sulfate, sulfide) to predict the Gibbs energy of reaction for S^0 disproportionation in seawater. We then calculate the free energy values of S^0 disproportionation from environmental data at four hydrothermal sites located in different

geological settings and characterized by contrasted fluid compositions. We show that S^0 disproportionation is energetically favorable in most, if not all, niches of the hydrothermal vent ecosystem, whatever the geological and geochemical context and depth.

Results and Discussion

In marine hydrothermal vents, particulate elemental sulfur is actively formed by oxidation of hydrogen sulfide during mixing of hydrothermal fluid with oxygenated seawater (Mc Collom, 2000), at interface of reduced free hydrogen sulfide with other oxidants (Fe^{3+} or NO_3^-) (Zopfi *et al*., 2004), and by weathering of sulfide minerals (https://www.mindat.org/). To date, only 6 sulfur disproportionating bacteria have been isolated from marine hydrothermal vents, with five of them isolated in the last decade (Zillig *et al*., 1986; Slobodkin *et al*., 2012; Slobodkin *et al*., 2013; Slobodkina *et al*., 2016; Slobodkina *et al*., 2017; Frolova *et al*., 2018; Bertran *et al*., 2020). At this point, we have limited knowledge about how much microbial disproportionation of sulfur contributes to the biotic sulfur cycle of hydrothermal ecosystems. In this work, we have tried to provide some clues to this question through the lens of thermodynamics, by performing two series of calculations which are described below.

Free-energy yield of sulfur disproportionation in seawater

In a first set of calculations, we determined the free energy change of sulfur disproportionation (Reaction 1) across a broad range of physicochemical parameters (Table 1) commonly observed in hydrothermal systems. In Fig. 1A-1E, we depict the energy yields as a function of pressure, salinity, pH, activity of sulfide and activity of sulfate, respectively, *versus* temperature; in Fig. 1F, energy yields are depicted as functions of activities of sulfide *versus* sulfate. Note that the other geochemical parameters (i.e., those not represented on the axes) are fixed at levels given

in Table 1. It can be seen in these six panels that $\Delta G_r < 0$ (exergonic) for Reaction (1) approaches and even exceeds energy yields of 100 kJ/mol at certain conditions. Pressure even up to 120 MPa (Fig. 1A) has little to no effect on ΔG_r of this reaction. Similarly, salinity (Fig. 1B), which affects the ionic strength of the system and hence the activity coefficients (see below), has only a tiny effect on the free energy values of the reaction, with ΔG_r slightly more exergonic at elevated salinities. In Fig. 1C it can be seen that pH has a large effect on ΔG_r of S^0 disproportionation, with energy yields increasing from <20 kJ/mol at low temperature and pH<2 to near 100 kJ/mol at temperatures above 100°C and pH in the 10-12 range. The effects of variable sulfide and sulfate activities (and concentrations) are demonstrated, respectively, in Figs. 1D and 1E. In both cases, values of ΔG_r change demonstrably, with relatively low energy yields at low temperature and high sulfide or sulfate levels, and ever higher energy yields as temperature increases and sulfide or sulfate levels drop. Lastly, the coupled effects of sulfide and sulfate activities on ΔG_r of S^0 disproportionation can be seen in Fig. 1F. Consistent with observations in panels D and E, the energy yields increase by ~20 kJ/mol as the activities of both of these aqueous sulfur species decrease by several orders of magnitude.

Overall, these careful but still rough predictions made on the basis of seawater ionic composition suggest that microorganisms can grow *via* sulfur disproportionation under almost any combination of physicochemical conditions tested here, that cover all conditions encountered in hydrothermal systems, except when sulfide concentration reaches 10 mM in a niche where the temperature is below 5°C. At very acidic pH (pH≦ 2) and low temperature (T°≦25°C), free-energy change values are close to the microbial Gibbs energy minimum amount for a catabolic reaction (ΔG_{min}, cost of minimal quantum of ATP synthesis- less than one third of ATP unit), somewhere in between −9 and −20 kJ per mole of reaction turnover (Schink, 1997; Hoehler, 2004). Conversely, at certain conditions (*e.g.* T°>100°C and pH>10, or T°>110°C and sulfate concentration <5mM), S^0-disproportionation free energy yields can be

significant and reach or exceed 100 kJ/mol. In summary, these predictions show that this catabolic reaction is thermodynamically more favorable at high temperature, high pH, and low sulfide and low sulfate concentrations, regardless of depth. Nevertheless, these predictions do not lead to the conclusion that the reaction will actually occur *in situ* under all these combinations of conditions, as microbial systems may face additional biological and physiological challenges to thrive in their natural extreme habitat. In the current state of knowledge, five thermophilic anaerobic S^0-disproportionating bacteria, and one hyperthermophilic microaerophilic S^0-disproportionating bacterium, all with optimal growth pH close to neutrality, have been isolated from marine hydrothermal environments (Huber and Stetter, 2001; Slobodkin *et al*., 2012; Slobodkin *et al*., 2013; Slobodkina *et al*., 2016; Slobodkina *et al*., 2017; Frolova *et al*., 2018) and three other anaerobic mesophilic ones are being characterized (Alain, personal communication). These strains have all been isolated and are all growing under conditions predicted here to be favorable for sulfur disproportionation, and their growth is enhanced in the presence of sulfide scavengers, predicted by thermodynamics to be more favorable. These thermodynamic calculations encourage the search for hydrothermal sulfur disproportionators at more acidic or more alkaline pHs than those tested to date, and to lower sulfate and sulfide concentrations in cultures.

To further refine these thermodynamic predictions, the free energy yields of S^0-disproportionation were calculated from real environmental data.

Free-energy yield of S^0 disproportionation along a dilution gradient from vent fluid to seawater: example of four contrasted vent sites

In a second set of calculations, we calculated the free energy change of elemental sulfur disproportionation (Reaction 1) at various points in the mixing zone between pure seawater and pure hydrothermal fluid for four well-characterized hydrothermal sites. These calculations were

carried out, at *in situ* pressure, from the compositions of hydrothermal fluids and seawater measured *in situ*. Given that there are 721 hydrothermal fields identified to date (confirmed or inferred, active and inactive fields) (InterRidge databaseVersion 3.4. PANGAEA, https://doi.org/10.1594/PANGAEA.917894), we selected active hydrothermal sites located in diverse marine geological and tectonic settings and characterized by contrasted fluid compositions to have an overview of the different types of existing hydrothermal systems.

The first sets of calculation were done for the TAG active mound located on the low spreading Mid-Atlantic Ridge. This massive sulfide deposit is located on 100,000 yr-old basaltic crust at 3620 m water depth (Thompson *et al*., 1988). Hydrothermal fluids at this site are emitted in the form of iron-rich acidic black smokers at a temperature of 369°C and a pH of 3.4 (at 25°C) (Humphris and Klein, 2018). The main dissolved gas is H_2S and the concentrations of CO_2, CH_4 and H_2 are low (Humphris and Klein, 2018). The second set of calculations focused on the Fenway felsic hydrothermal site, located at 1710 m depth in the PACMANUS hydrothermal system in the eastern part of the Manus back-arc basin (Western Pacific). Fluids of the large black smoker chimney complex at the summit of this system are characterized by a high emission temperature (358°C), a very acidic pH (2.7 at 25°C) and high concentrations of dissolved CO_2 and H_2S (Reeves *et al*., 2011; Humphris and Klein, 2018). The third round of calculations concerned the Rainbow vent field, located in ultramafic rocks, at 2300 m at the Mid-Atlantic Ridge. The Rainbow fluids are very acidic (pH 2.99 at 25°C), hot temperature (367°C), rich in dissolved H_2 and with very low Fe and Mn content (Douville *et al*., 2002; Humphris and Klein, 2018). The last calculations series focused on the Upper Cone of Brothers submarine volcano, located along the Kermadec arc, offshore New Zealand, at 1220 m depth. The end-member fluid of this white smoker is characterized by a mild temperature (122°C), an extremely low pH (1.9 at 25°C), a high concentration of Mg, presence of dissolved CO_2 and no detectable H_2S in the selected dataset (de Ronde *et al*., 2005; Humphris and Klein, 2018). The

presence of native sulfur was reported for the TAG, PACMANUS and Brothers volcano sites (https://www.mindat.org/). Unfortunately, no complete geochemical data set of ultrabasic end-member fluid was available to perform such calculations.

As seen in Fig. 2, Reaction 1 is exergonic at all four of these sites along mixing zones between pure seawater and pure hydrothermal fluid. It should be noted that at some of these points (*e.g.*, pure vent fluid; 75% vent fluid for all sites; 50% vent fluid at TAG, Fenway, and Rainbow) the temperatures are above the apparent limit for life. At present, microbial growth has not been demonstrated at temperature above 122°C (at 20 MPa, in the archaeon *Methanopyrus kandleri* strain 116) (Takai *et al*., 2008) but could exist a little beyond, up to the temperature that would allow the maintenance of the integrity of the genome and proteome, of the bonds governing the structure of macromolecules and of the precise 3D conformation of proteins with the degree of flexibility necessary to perform biological functions. On the outermost parts of the chimney edifices, where the temperature allows life, the S^0 disproportionation reaction is exergonic in the four examples studied here, providing ΔG_r ranging from ~ -6 to < -42 kJ mol^{-1} (Fig. 2). The lowest values, between -6 kJ mol^{-1} to -13 kJ mol^{-1}, are close to the critical free energy amount for a catabolic reaction (ΔG_{min}) reported so far for microbial metabolisms in field and culture studies (Hoehler, 2004), and well above the power minimum for microbial cells to remain viable or subsist at ultra-low energy fluxes (LaRowe and Amend, 2015; Bradley *et al*., 2020). Sulfur disproportionation is thus thermodynamically favorable in the vicinity of these four hydrothermal vents located in very different geological settings, and provides energy yields of up to 42 kJ mol^{-1}, based on calculations taking into account the *in situ* geochemical composition of the seawater and hydrothermal fluid, and a dilution model of the two solutions. These energy yields are rather low but sufficient to allow microbial growth. Nevertheless, given that chimney rocks contain various minerals that can strongly chelate sulfides, such as manganese (IV) and iron (III) minerals (Thamdrup *et al*., 1993; Ollivier *et al*., 2018, Saji, 2021),

and other less potent metal-based chelating minerals (containing Zn, Cu, Fe(II), Co, Ni, Mg…)(Saji, 2021), which were not included in our calculations, the amount of energy released by the reaction could be even higher inside the chimney walls, since the energy yield increases significantly as the sulfide concentration decreases. Indeed, chimneys at all four sites were reported to contain two to five iron(III) mineral species (Table 2), and numerous less powerful metal-based chelating minerals (https://www.mindat.org/), that could chelate the sulfide *in situ* and make the reaction even more energy-producing locally (Thamdrup *et al*., 1993; Ollivier *et al*., 2018). Because the composition of the chimney walls evolves with time and mineral precipitation and is uneven (e.g. Meng *et al*., 2019), it is difficult to make more accurate predictions without accurate mapping of the mineral composition of the chimney walls on a sub-millimeter scale at a given time. However, one can speculate that the reaction is likely much more exergonic locally in the chimney walls, in pores and fractures containing sulfide-chelating minerals, than calculated here, as the ΔG_r of sulfur disproportionation changes demonstrably with decreasing sulfide levels (Fig. 1D).

Implications of an exergonic microbial sulfur disproportionation reaction

The thermodynamic approach implemented here indicates that MSD is sufficiently exergonic in most, if not all, marine hydrothermal niches at non-lethal temperatures, to drive cellular functions. This information is crucial because in the current absence of genetic markers of this process and given the difficulty of interpreting *in situ* isotopic data of sulfur species, it was not known whether this process could take place in this ecosystem. These results based on thermodynamics indicate that the reaction is possible and favorable but do not prove that it occurs *in situ*. Strains isolated from hydrothermal ecosystems capable of disproportionating S^0, among other things, could express this catabolism *in situ* to grow. In the future, it will be crucial to be able to document this process *in situ* and to determine what is the contribution of MSD to

the fluxes of sulfur species in hydrothermal habitats compared to those of sulfur-oxidation and sulfate-reduction, as MSD is confused with these pathways in global budgets, since it leads to the production of sulfates and sulfides. Sulfur-disproportionating taxa do not necessarily have the same ecophysiological properties as sulfur-oxidizers and sulfate-reducers, and this could have a significant impact on our understanding of the biotic cycle of sulfur. At present, with exception of *Dissulfurimicrobium hydrothermale* Sh68[T], sulfur disproportionating strains isolated from marine hydrothermal ecosystems are all capable of using other more energy-yielding catabolic pathways (sulfate reduction, or dissimilatory nitrate reduction to ammonium DNRA) to produce energy and to grow (Slobodkin and Slobodkina, 2019). It will be important to determine if MSD is used in the same way as the other catabolic pathways and confers a metabolic versatility to the taxa allowing them to adapt to the changing physico-chemical conditions of their natural habitat, or if it is simply an accessory metabolism used by the microorganisms to wait for more favorable conditions as this process is vastly less energetically favorable than sulfate reduction or DNRA.

Conclusion

Hydrothermal habitats are characterized by numerous geochemical and geochemical/lithological interfaces containing reduced free hydrogen sulfide on one side and strong oxidants on the other, where intermediate sulfide species (*i.e.*, substrates for sulfur disproportionation) are thus produced. Despite the popular belief that S^0 disproportionation is endergonic under standard conditions, the predictions made here from theoretical gradients and real data sets from a variety of hydrothermal geological settings indicate that this reaction is exergonic under the vast majority of physicochemical conditions tested. These calculations show that this reaction could produce enough energy to sustain growth in all hydrothermal niches of the four hydrothermal sites studied where the temperature is compatible with life.

This reaction could therefore be widespread in hydrothermal habitats, provided that the cellular systems are adapted to environmental constraints. Furthermore, as the first set of calculations of free energy changes in S^0 disproportionation were performed over very broad gradients of physical parameters and chemical species, these theoretical data could also inform the occurrence of this process in other ecosystems, such as subseafloor environments for example. The sulfur cycle is known to play a critical role in the redox balance of the ocean due to the multiple oxidation states of this element, and furthermore marine hydrothermal vents are widespread on the seafloor and the subseafloor ecosystem represents one of the largest biomes on Earth (Orcutt *et al*., 2011). By affecting the sulfur cycle, microbial activities in these ecosystems, including sulfur disproportionation, could influence the oxidation state of the global ocean and, by extension, the Earth's climate (Bowles *et al*., 2014; D'Hondt *et al*., 2019).

Experimental procedures

Thermodynamic modeling

The Gibbs energy yields (ΔG_r) for sulfur disproportionation (Reaction 1) was calculated with the relation

$$\Delta G_r = \Delta G_r^0 + RT\ln Q_r \qquad \text{(Eq. 2)}$$

where ΔG_r^0 is as defined above, R indicates the universal gas constant, T stands for the temperature in Kelvin, and Q_r refers to the reaction activity quotient. ΔG_r^0 values were calculated at the temperature and pressure of interest with the revised Helgeson-Kirkham-Flowers (HKF) equations of state (Helgeson *et al*., 1981; Tanger and Helgeson, 1988; Shock *et al*., 1992) using the "subcrt" command from the R software package CHNOSZ v1.4.1 (Dick,

2019b). Thermodynamic data in CHNOSZ are derived from the OrganoBioGeoTherm database (Helgeson *et al*., 2009), and the sources of these data are provided in the CHNOSZ package documentation. Values of Q_r were calculated with the relation

$$Q_r = \Pi a_i^{v_{i,r}} \qquad \text{(Eq. 3)}$$

where a_i represents the activity of the ith species raised to its stochiometric reaction coefficient $v_{i,r}$, in the rth reaction, which is positive for products and negative for reactants. Concentration data were converted to chemical activities using

$$a_i = m_i \gamma_i \qquad \text{(Eq. 4)}$$

where m_i and γ_i are the molality and activity coefficients of the ith species. Activity coefficients were calculated using the aqueous speciation package AqEquil v0.9.1 (Boyer *et al*., 2021), which interfaces with the geochemical speciation software EQ3/6 (Wolery, 1979). The activities of pure minerals (elemental sulfur (S^0)) and water were taken to be unity ($a_i = 1$). ΔG_r represents the Gibbs energy of reaction under *in situ* conditions and is expressed in units of kilojoules per mole of sulfur.

In situ data and gradient analyses

Gibbs energy yields for sulfur disproportionation were calculated using concentration and temperature data from seawater, Manus Basin (Fenway), Brothers Volcano Upper Cone, Mid-Atlantic Ridge Trans-Atlantic Geotraverse (TAG), and Mid-Atlantic Ridge Rainbow vents (Humphris and Klein, 2018). Seawater concentrations which were reported as below detection limit were assumed to be at the detection limit (i.e., 0.001 µmol/kg was used in place of <0.001

μmol/kg). Zero values were reported as 10^{-9} molal. Pressures were assumed to be 36 MPa for TAG vent field, 17 MPa for Fenway, 12 MPa for Brothers volcano, and 23 MPa for Rainbow, on the basis of the water depth at which these hydrothermal fields are located.

Gibbs energy yields for sulfur disproportionation were calculated along a linear dilution gradient from pure vent fluid to pure seawater at the four sites using endmember values as well as vent fluid:seawater mixing ratios of 75:25, 50:50, and 25:75. Calculated temperatures of the mixed fluids incorporated the specific heat capacity of water at that temperature, following the method described in Lu *et al.*, 2021. Activity coefficients from these dilution gradients were calculated after speciating with AqEquil. Energy yields for disproportionation in seawater were also calculated as functions of various geochemical parameters while holding other variables fixed (Table 1). Activity coefficients were recalculated using AqEquil v0.9.1 based on the parameters for each gradient. The CHNOSZ v1.4.1 "mosaic" function was used to speciate H_2S and SO_4^{2-} for energy yields that were calculated as a function of pH.

The mineral composition of the hydrothermal edifices is based on the open mineral database mindat.org. (https://www.mindat.org/).

K.A., S.Y. and M.A. designed the experiment; H.S.A. performed the calculations. J.P.A. supervised the calculations methods. K.A. and J.P.A supervised the project. All authors interpreted the data; K.A. wrote the manuscript. All authors reviewed the manuscript.

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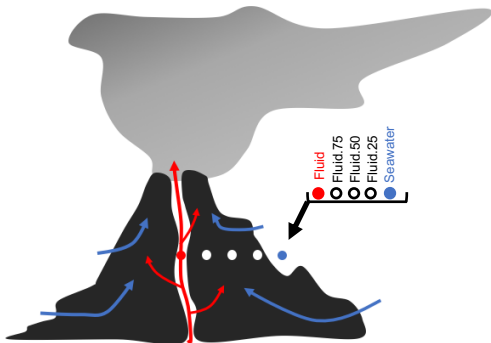Table 1. Gradients and fixed data used for Gibbs energy calculations for sulfur disproportionation in seawater.

Table 2. Iron (III) minerals present in the hydrothermal vent fields of the four sites studied (source: https://www.mindat.org/).

Fig. 1. Values of ΔG_r for elemental sulfur disproportionation (Reaction 1) as a function of pressure (A), salinity (B), pH (C), sulfide (D) and sulfate (E) versus temperature, and sulfide versus sulfate (F), calculated with the Equation $S^0 + H_2O = 0.25SO_4^{2-} + 0.75H_2S(aq) + 0.5H^+$ (Eq. 1bis) Geochemical parameters when not allowed to vary along the x- and y-axes are fixed as follows: T=25°C, pressure= 26MPa, salinity=545 mmol kg^{-1}, pH=7.0, sulfide concentration=0mM, sulfate concentration=28mM. Note that the color scale changes for each panel in this figure.

Fig. 2. Gibbs energies of sulfur disproportionation in the mixing zone between pure fluid and seawater, in various geological settings. (A) Schematic representation of the gradient points used to make the calculations from seawater and fluid compositions measured in situ. (B). Temperature, log activities of H^+, H_2S, and SO_4^{2-}, standard state Gibbs energies, and Gibbs energies of sulfur disproportionation (kJ mol-1 S) along a dilution gradient from seawater to pure hydrothermal vent fluid at TAG, Fenway Basin, Rainbow Vent, and Brothers Volcano Upper Cone. The suffixes after the vent names refer to the ratio of vent fluid to seawater (i.e., TAG.75 is a mixture of 75% vent fluid and 25% seawater). The standard state Gibbs energy and Gibbs energy of Fenway Basin hydrothermal vent fluid are listed as NA because it is below the minimum density for applicability of the revised HKF equations.

A $S + H_2O = 0.75H_2S + 0.25SO_4^{-2} + 0.5H^+$

B $S + H_2O = 0.75H_2S + 0.25SO_4^{-2} + 0.5H^+$

C $S + H_2O = 0.75H_2S + 0.25SO_4^{-2} + 0.5H^+$
$\log a_{H_2S} = -9.0$
$\log a_{SO_4^{-2}} = -2.4$
HS^-
H_2S
SO_4^{-2}
HSO_4^-

D $S + H_2O = 0.75H_2S + 0.25SO_4^{2-} + 0.5H^+$

E $S + H_2O = 0.75H_2S + 0.25SO_4^{-2} + 0.5H^+$

F $S + H_2O = 0.75H_2S + 0.25SO_4^{-2} + 0.5H^+$
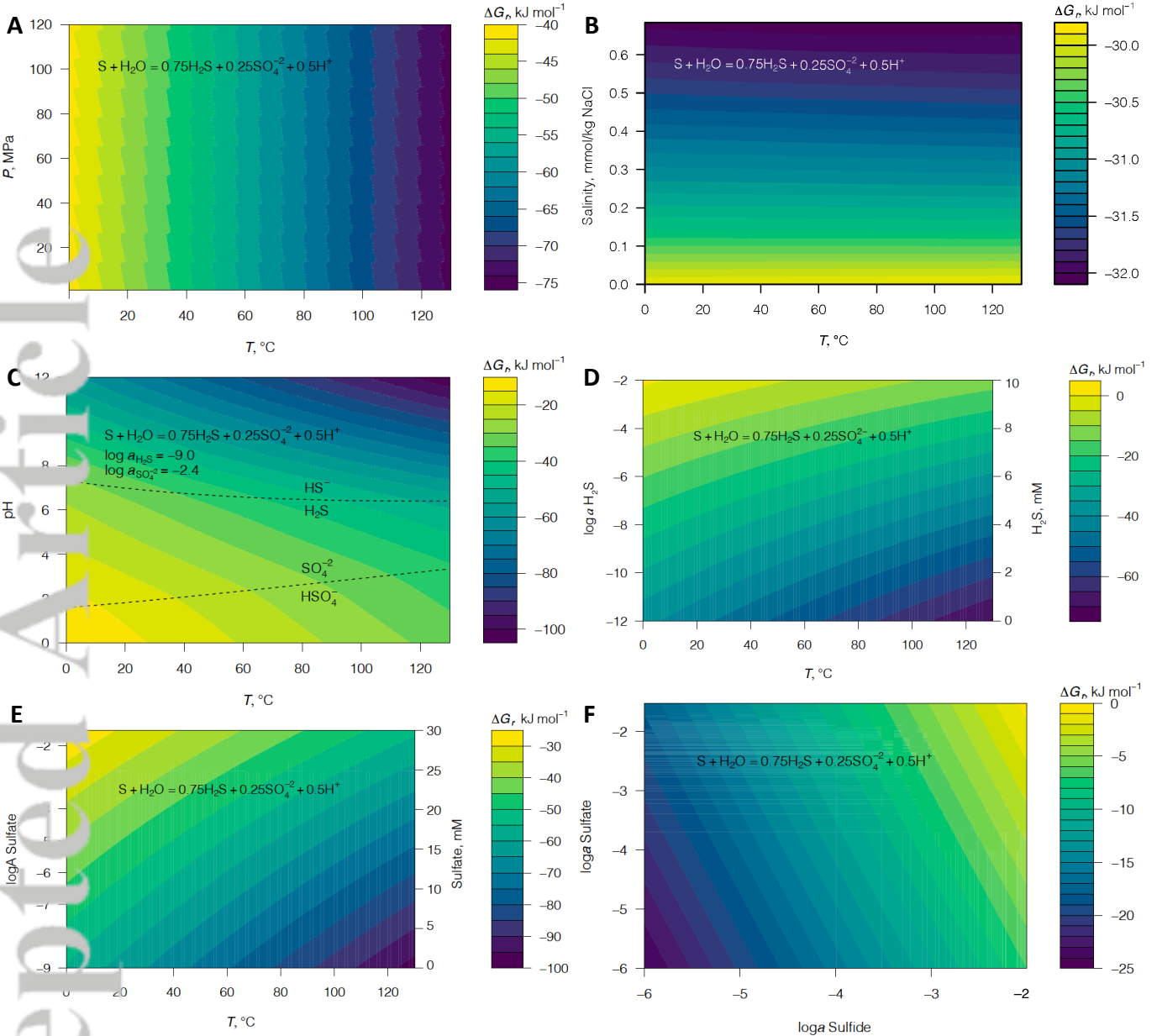
A

B

	Temperature (°C)	logaH+	logaH₂S	logaSO₄²⁻	ΔG⁰ (kJ mol⁻¹)	ΔG (kJ mol⁻¹)
Basalt–hosted						
TAG	369	–3.40	–2.46	–16.33	164.39	–52.63
TAG.75	232.8	–4.55	–2.58	–3.90	133.96	–16.74
TAG.50	134.5	–5.70	–2.76	–3.06	123.84	–13.39
TAG.25	60.2	–6.85	–3.06	–2.61	120.70	–10.48
Seawater	2	–8.00	–12.00	–2.36	119.48	–41.72
Back–arc basin						
Fenway	358	–2.70	–1.73	–8.77	NA	NA
Fenway.75	223.7	–4.03	–1.85	–3.88	133.70	–8.15
Fenway.50	128.3	–5.35	–2.03	–3.03	123.76	–7.12
Fenway.25	57.2	–6.68	–2.33	–2.61	120.86	–6.05
Seawater	2	–8.00	–12.00	–2.38	119.93	–41.63
Ultramafic–hosted						
Rainbow	367	–2.99	–2.54	–17.22	169.46	–52.03
Rainbow.75	230.0	–4.24	–2.66	–3.97	134.28	–15.65
	132.2	–5.50	–2.84	–3.11	123.92	–12.89
Rainbow.25	58.9	–6.75	–3.14	–2.64	120.83	–10.41
Seawater	2	–8.00	–12.00	–2.38	119.79	–41.66
Submarine volcano						
Brothers	302	–3.10	–2.85	–6.76	150.81	–21.55
Brothers.75	196.1	–4.33	–2.98	–3.69	130.28	–15.22
Brothers.50	115.7	–5.55	–3.15	–3.00	123.03	–13.10
Brothers.25	52.7	–6.78	–3.46	–2.61	120.82	–11.17
Seawater	2	–8.00	–12.00	–2.39	120.06	–41.61

TAG	PACMANUS (Fenway)	Rainbow	Brothers volcano (Upper cone)
Goethite α-FeO(OH)	**Ferrihydrite** $Fe^{3+}_{10}O_{14}(OH)_2$	**Magnetite** $Fe^{2+}Fe^{3+}_2O_4$	**Celadonite** $K(Mg,Fe^{2+})(Fe^{3+},Al)[Si_4O_{10}](OH)_2$
Hematite α-Fe$_2$O$_3$	**Goethite** α-FeO(OH)	**Limonite** FeO(OH)$\cdot n$H$_2$O	**Goethite** α-FeO(OH)
Jarosite $KFe^{3+}_3(SO_4)_2(OH)_6$	**Hematite** α-Fe$_2$O$_3$		**Hematite** α-Fe$_2$O$_3$
Nontronite Na$_{0.3}$Fe$_2$((Si,Al)$_4$O$_{10}$)(OH)$_2 \cdot n$H$_2$O	**Nontronite** Na$_{0.3}$Fe$_2$((Si,Al)$_4$O$_{10}$)(OH)$_2 \cdot n$H$_2$O		**Jarosite** $KFe^{3+}_3(SO_4)_2(OH)_6$
			Nontronite Na$_{0.3}$Fe$_2$((Si,Al)$_4$O$_{10}$)(OH)$_2 \cdot n$H$_2$O

	Gradient analyses	**Fixed data**
Temperature (ºC)	0 – 130	25
Salinity (g L^{-1})	0 – 40	545 mmol kg^{-1}
Pressure (MPa)	0 – 120	26[#]
pH	0 – 12	7
Total sulfide concentration (mM)	0 – 10	0
Total sulfate concentration (mM)	0 – 30	28

[#]The average depth of mid-ocean ridges is 2600m; we set the pressure at 26 MPa because it corresponds to the hydrostatic pressure at this depth