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1 **Sulfur disproportionation is exergonic in the vicinity of marine hydrothermal vents**

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4

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10 **Summary**

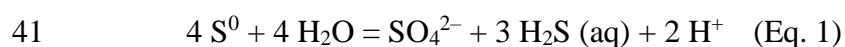
11 **Sulfur is abundant in different oxidation states in hydrothermal ecosystems, where it**
12 **plays a central role in microbial energy production. The contribution of microbially**
13 **catalyzed disproportionation of elemental sulfur (S⁰) to the energy fluxes of this ecosystem**
14 **is unknown. Indeed, within the current knowledge it is impossible to study this process in**
15 **a global way due to the lack of specific genetic markers and because of the difficulties in**
16 **unraveling the isotopic signals from the different reactions of the sulfur cycle. In this**
17 **context, calculations of the Gibbs energy (ΔGr) of sulfur disproportionation can identify**
18 **whether this process is thermodynamically favorable and provides sufficient energy yields**
19 **for growth at the temperatures, pressures, and chemical compositions found in the**
20 **various niches of the hydrothermal ecosystem. Herein, free energy yield calculations were**
21 **performed using internally consistent thermodynamic properties and geochemical data**
22 **from four different hydrothermal systems. These calculations showed that S⁰-**
23 **disproportionation is sufficiently exergonic to allow growth in most niches of the**

24 **hydrothermal ecosystems, regardless of the geological and geochemical context, and**
25 **depth; it is most favorable at elevated temperatures and alkaline pH, at low sulfide and**
26 **sulfate concentrations, and in the presence of sulfide-chelating minerals, which are**
27 **common in these environments.**

28

29 **Introduction**

30 Sulfur compounds are ubiquitous in marine hydrothermal environments and several are central
31 in catabolic reactions involved in primary production, recycling and terminal degradation of
32 organic matter (Cao *et al.*, 2014). Sulfur is found in various oxidation states (H_2S , FeS , FeS_2 ,
33 S^0 , SO_3^{2-} , $\text{S}_2\text{O}_3^{2-}$, $\text{S}_n\text{O}_6^{2-}$, S_nO_6^- , SO_4^{2-} , etc.) in the chimney structures, vent fluids, surrounding
34 seawater, and in pore spaces and fractures of the seafloor, and consequently used as an
35 electron donor or terminal electron acceptor in aerobic and anaerobic reactions. Sulfide/sulfur-
36 oxidizing, sulfur-reducing and sulfate-reducing microbial taxa of hydrothermal habitats are well
37 known (Godfroy *et al.*, in press; Zeng *et al.*, 2021), and models of the biogenic hydrothermal
38 cycle of sulfur are based on these reactions. Another redox reaction involving sulfur species
39 takes place in this unique ecosystem: microbial sulfur disproportionation (= dismutation)
40 (MSD), most commonly represented as



42 In this reaction, an intermediate valence inorganic sulfur compound is simultaneously reduced
43 and oxidized into sulfide and sulfate, respectively (Bak and Cypionka, 1987; Finster, 2008;
44 Slobodkin and Slobodkina, 2019). In addition to S^0 , other studied substrates for MSD include
45 thiosulfate ($\text{S}_2\text{O}_3^{2-}$) and sulfite (SO_3^{2-}) (Finster, 2008; Slobodkin and Slobodkina, 2019). MSD,
46 also called inorganic fermentation, is poorly documented in hydrothermal ecosystems and the
47 first hydrothermal microbial taxa able to disproportionate inorganic sulfur compounds under

48 anoxic conditions have been isolated quite recently (Slobodkin *et al.*, 2012; Slobodkin *et al.*,
49 2013; Slobodkina *et al.*, 2016; Slobodkina *et al.*, 2017; Frolova *et al.*, 2018).

50 The metabolic pathways and enzymatic mechanisms of anaerobic sulfur disproportionation are
51 only partially resolved (Finster, 2008; Mardanov *et al.*, 2016; Slobodkin and Slobodkina, 2019;
52 Allieux *et al.*, 2020). A few archaea and bacteria, including one hydrothermal vent bacterium
53 *Aquifex aeolicus*, are also capable of oxygen-dependent disproportionation of S⁰ using the SOR
54 (sulfur oxygenase reductase) enzymatic system (whose disproportionation sub-reaction is
55 identical to Reaction (1) noted above) (Kletzin *et al.*, 2004; Pelletier *et al.*, 2008; Bertran *et al.*,
56 2020). Although it has not been comprehensively investigated in hydrothermal environments,
57 MSD is an important contributor to the sulfur cycle in other marine ecosystems and may prove
58 to be integral to the hydrothermal sulfur cycle as well. Microorganisms capable of
59 disproportionating intermediate sulfur species are widespread in marine surface sediments and
60 are partly responsible for the isotopic signatures of sulfidic minerals in young and old sediments
61 (Bak and Pfenning, 1987; Thamdrup *et al.*, 1993; Finster, 2008; Jørgensen *et al.*, 2019). Using
62 radioisotope tracing of thiosulfate, it has even been shown that a significant proportion of the
63 thiosulfate present in oxidized and reduced sediments is disproportionated (Bak and Cypionka,
64 1987; Fossing and Jørgensen, 1990; Jørgensen, 1990). However, the precise extent and
65 distribution of the MSD process in natural environments is not known because (i) there is no
66 specific genetic marker for this reaction, which prevents any application of genomic
67 investigation methods to identify and quantify taxa implementing this process in natural
68 habitats, because (ii) intermediate sulfur species are transient and generally short-lived *in situ*
69 (Zopfi *et al.*, 2004; Jørgensen *et al.*, 2019), and (ii) because it is difficult to disentangle the
70 relative magnitude of different sulfur compound-based redox metabolisms from the net isotopic
71 signals (Jørgensen *et al.*, 2019; Allieux *et al.*, 2020).

72 In this context, calculations of the Gibbs energy of reaction (ΔG_r) at environmentally relevant
73 conditions can identify whether MSD is thermodynamically favorable and consequently point
74 to niches where sulfur disproportionators could be present and thriving. A common message in
75 the literature is that MSD yields minimal energy or is endergonic (energy consuming) (Finster,
76 2008). It should be noted, however, that this claim is typically (and incorrectly) based on the
77 value of the standard state Gibbs energy (ΔG_r^0) for Reaction (1) at 25°C and 1 bar, which is
78 +120.5 kJ/mol (Amend and LaRowe, 2019). At low concentrations, and hence activities, of
79 sulfate and sulfide, together with circumneutral to alkaline pHs, Reaction (1) can indeed be
80 exergonic (Amend and LaRowe, 2019).

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

93 In this paper, we use a thermodynamic approach and a range of physico-chemical parameters
94 (*i.e.*, temperature, pressure, salinity, pH, sulfate, sulfide) to predict the Gibbs energy of reaction
95 for S⁰ disproportionation in seawater. We then calculate the free energy values of S⁰
96 disproportionation from environmental data at four hydrothermal sites located in different

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

100

101

102 **Results and Discussion**

103 In marine hydrothermal vents, particulate elemental sulfur is actively formed by oxidation of
104 hydrogen sulfide during mixing of hydrothermal fluid with oxygenated seawater (Mc Collom,
105 2000), at interface of reduced free hydrogen sulfide with other oxidants (Fe^{3+} or NO_3^-) (Zopfi
106 *et al.*, 2004), and by weathering of sulfide minerals (<https://www.mindat.org/>). To date, only 6
107 sulfur disproportionating bacteria have been isolated from marine hydrothermal vents, with five
108 of them isolated in the last decade (Zillig *et al.*, 1986; Slobodkin *et al.*, 2012; Slobodkin *et al.*,
109 2013; Slobodkin *et al.*, 2016; Slobodkina *et al.*, 2016; Slobodkina *et al.*, 2017; Frolova *et al.*,
110 2018; Bertran *et al.*, 2020). At this point, we have limited knowledge about how much microbial
111 disproportionation of sulfur contributes to the biotic sulfur cycle of hydrothermal ecosystems.
112 In this work, we have tried to provide some clues to this question through the lens of
113 thermodynamics, by performing two series of calculations which are described below.

114

115 *Free-energy yield of sulfur disproportionation in seawater*

116 In a first set of calculations, we determined the free energy change of sulfur disproportionation
117 (Reaction 1) across a broad range of physicochemical parameters (Table 1) commonly observed
118 in hydrothermal systems. In Fig. 1A-1E, we depict the energy yields as a function of pressure,
119 salinity, pH, activity of sulfide and activity of sulfate, respectively, *versus* temperature; in Fig.
120 1F, energy yields are depicted as functions of activities of sulfide *versus* sulfate. Note that the
121 other geochemical parameters (i.e., those not represented on the axes) are fixed at levels given

122 in Table 1. It can be seen in these six panels that $\Delta G_r < 0$ (exergonic) for Reaction (1) approaches
123 and even exceeds energy yields of 100 kJ/mol at certain conditions. Pressure even up to 120
124 MPa (Fig. 1A) has little to no effect on ΔG_r of this reaction. Similarly, salinity (Fig. 1B), which
125 affects the ionic strength of the system and hence the activity coefficients (see below), has only
126 a tiny effect on the free energy values of the reaction, with ΔG_r slightly more exergonic at
127 elevated salinities. In Fig. 1C it can be seen that pH has a large effect on ΔG_r of S^0
128 disproportionation, with energy yields increasing from <20 kJ/mol at low temperature and
129 $\text{pH} < 2$ to near 100 kJ/mol at temperatures above 100°C and pH in the 10-12 range. The effects
130 of variable sulfide and sulfate activities (and concentrations) are demonstrated, respectively, in
131 Figs. 1D and 1E. In both cases, values of ΔG_r change demonstrably, with relatively low energy
132 yields at low temperature and high sulfide or sulfate levels, and ever higher energy yields as
133 temperature increases and sulfide or sulfate levels drop. Lastly, the coupled effects of sulfide
134 and sulfate activities on ΔG_r of S^0 disproportionation can be seen in Fig. 1F. Consistent with
135 observations in panels D and E, the energy yields increase by ~ 20 kJ/mol as the activities of
136 both of these aqueous sulfur species decrease by several orders of magnitude.

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

147 significant and reach or exceed 100 kJ/mol. In summary, these predictions show that this
148 catabolic reaction is thermodynamically more favorable at high temperature, high pH, and low
149 sulfide and low sulfate concentrations, regardless of depth. Nevertheless, these predictions do
150 not lead to the conclusion that the reaction will actually occur *in situ* under all these
151 combinations of conditions, as microbial systems may face additional biological and
152 physiological challenges to thrive in their natural extreme habitat. In the current state of
153 knowledge, five thermophilic anaerobic S⁰-disproportionating bacteria, and one
154 hyperthermophilic microaerophilic S⁰-disproportionating bacterium, all with optimal growth
155 pH close to neutrality, have been isolated from marine hydrothermal environments (Huber and
156 Stetter, 2001; Slobodkin *et al.*, 2012; Slobodkin *et al.*, 2013; Slobodkina *et al.*, 2016;
157 Slobodkina *et al.*, 2017; Frolova *et al.*, 2018) and three other anaerobic mesophilic ones are
158 being characterized (Alain, personal communication). These strains have all been isolated and
159 are all growing under conditions predicted here to be favorable for sulfur disproportionation,
160 and their growth is enhanced in the presence of sulfide scavengers, predicted by
161 thermodynamics to be more favorable. These thermodynamic calculations encourage the search
162 for hydrothermal sulfur disproportionators at more acidic or more alkaline pHs than those tested
163 to date, and to lower sulfate and sulfide concentrations in cultures.

164 To further refine these thermodynamic predictions, the free energy yields of S⁰-
165 disproportionation were calculated from real environmental data.

166

167 *Free-energy yield of S⁰ disproportionation along a dilution gradient from vent fluid to*
168 *seawater: example of four contrasted vent sites*

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

172 carried out, at *in situ* pressure, from the compositions of hydrothermal fluids and seawater
173 measured *in situ*. Given that there are 721 hydrothermal fields identified to date (confirmed or
174 inferred, active and inactive fields) (InterRidge databaseVersion 3.4. PANGAEA,
175 <https://doi.org/10.1594/PANGAEA.917894>), we selected active hydrothermal sites located in
176 diverse marine geological and tectonic settings and characterized by contrasted fluid
177 compositions to have an overview of the different types of existing hydrothermal systems.
178 The first sets of calculation were done for the TAG active mound located on the low spreading
179 Mid-Atlantic Ridge. This massive sulfide deposit is located on 100,000 yr-old basaltic crust at
180 3620 m water depth (Thompson *et al.*, 1988). Hydrothermal fluids at this site are emitted in the
181 form of iron-rich acidic black smokers at a temperature of 369°C and a pH of 3.4 (at 25°C)
182 (Humphris and Klein, 2018). The main dissolved gas is H₂S and the concentrations of CO₂,
183 CH₄ and H₂ are low (Humphris and Klein, 2018). The second set of calculations focused on the
184 Fenway felsic hydrothermal site, located at 1710 m depth in the PACMANUS hydrothermal
185 system in the eastern part of the Manus back-arc basin (Western Pacific). Fluids of the large
186 black smoker chimney complex at the summit of this system are characterized by a high
187 emission temperature (358°C), a very acidic pH (2.7 at 25°C) and high concentrations of
188 dissolved CO₂ and H₂S (Reeves *et al.*, 2011; Humphris and Klein, 2018). The third round of
189 calculations concerned the Rainbow vent field, located in ultramafic rocks, at 2300 m at the
190 Mid-Atlantic Ridge. The Rainbow fluids are very acidic (pH 2.99 at 25°C), hot temperature
191 (367°C), rich in dissolved H₂ and with very low Fe and Mn content (Douville *et al.*, 2002;
192 Humphris and Klein, 2018). The last calculations series focused on the Upper Cone of Brothers
193 submarine volcano, located along the Kermadec arc, offshore New Zealand, at 1220 m depth.
194 The end-member fluid of this white smoker is characterized by a mild temperature (122°C), an
195 extremely low pH (1.9 at 25°C), a high concentration of Mg, presence of dissolved CO₂ and no
196 detectable H₂S in the selected dataset (de Ronde *et al.*, 2005; Humphris and Klein, 2018). The

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

200 As seen in Fig. 2, Reaction 1 is exergonic at all four of these sites along mixing zones between
201 pure seawater and pure hydrothermal fluid. It should be noted that at some of these points (*e.g.*,
202 pure vent fluid; 75% vent fluid for all sites; 50% vent fluid at TAG, Fenway, and Rainbow) the
203 temperatures are above the apparent limit for life. At present, microbial growth has not been
204 demonstrated at temperature above 122°C (at 20 MPa, in the archaeon *Methanopyrus kandleri*
205 strain 116) (Takai *et al.*, 2008) but could exist a little beyond, up to the temperature that would
206 allow the maintenance of the integrity of the genome and proteome, of the bonds governing the
207 structure of macromolecules and of the precise 3D conformation of proteins with the degree of
208 flexibility necessary to perform biological functions. On the outermost parts of the chimney
209 edifices, where the temperature allows life, the S⁰ disproportionation reaction is exergonic in
210 the four examples studied here, providing ΔG_r ranging from ~ -6 to < -42 kJ mol⁻¹ (Fig. 2).
211 The lowest values, between -6 kJ mol⁻¹ to -13 kJ mol⁻¹, are close to the critical free energy
212 amount for a catabolic reaction (ΔG_{min}) reported so far for microbial metabolisms in field and
213 culture studies (Hoehler, 2004), and well above the power minimum for microbial cells to
214 remain viable or subsist at ultra-low energy fluxes (LaRowe and Amend, 2015; Bradley *et al.*,
215 2020). Sulfur disproportionation is thus thermodynamically favorable in the vicinity of these
216 four hydrothermal vents located in very different geological settings, and provides energy yields
217 of up to 42 kJ mol⁻¹, based on calculations taking into account the *in situ* geochemical
218 composition of the seawater and hydrothermal fluid, and a dilution model of the two solutions.
219 These energy yields are rather low but sufficient to allow microbial growth. Nevertheless, given
220 that chimney rocks contain various minerals that can strongly chelate sulfides, such as
221 manganese (IV) and iron (III) minerals (Thamdrup *et al.*, 1993; Ollivier *et al.*, 2018, Saji, 2021),

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

236

237 *Implications of an exergonic microbial sulfur disproportionation reaction*

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

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

261

262 **Conclusion**

263 Hydrothermal habitats are characterized by numerous geochemical and
264 geochemical/lithological interfaces containing reduced free hydrogen sulfide on one side and
265 strong oxidants on the other, where intermediate sulfide species (*i.e.*, substrates for sulfur
266 disproportionation) are thus produced. Despite the popular belief that S⁰ disproportionation is
267 endergonic under standard conditions, the predictions made here from theoretical gradients and
268 real data sets from a variety of hydrothermal geological settings indicate that this reaction is
269 exergonic under the vast majority of physicochemical conditions tested. These calculations
270 show that this reaction could produce enough energy to sustain growth in all hydrothermal
271 niches of the four hydrothermal sites studied where the temperature is compatible with life.

272 This reaction could therefore be widespread in hydrothermal habitats, provided that the cellular
273 systems are adapted to environmental constraints. Furthermore, as the first set of calculations
274 of free energy changes in S⁰ disproportionation were performed over very broad gradients of
275 physical parameters and chemical species, these theoretical data could also inform the
276 occurrence of this process in other ecosystems, such as seafloor environments for example.
277 The sulfur cycle is known to play a critical role in the redox balance of the ocean due to the
278 multiple oxidation states of this element, and furthermore marine hydrothermal vents are
279 widespread on the seafloor and the seafloor ecosystem represents one of the largest biomes
280 on Earth (Orcutt *et al.*, 2011). By affecting the sulfur cycle, microbial activities in these
281 ecosystems, including sulfur disproportionation, could influence the oxidation state of the
282 global ocean and, by extension, the Earth's climate (Bowles *et al.*, 2014; D'Hondt *et al.*, 2019).

283

284

285 **Experimental procedures**

286 *Thermodynamic modeling*

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

289

$$290 \Delta G_r = \Delta G_r^0 + RT \ln Q_r \quad (\text{Eq. 2})$$

291

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

297 2019b). Thermodynamic data in CHNOSZ are derived from the OrganoBioGeoTherm database
298 (Helgeson *et al.*, 2009), and the sources of these data are provided in the CHNOSZ package
299 documentation. Values of Q_r were calculated with the relation

300

$$301 \quad Q_r = \prod a_i^{v_{i,r}} \quad (\text{Eq. 3})$$

302

303 where a_i represents the activity of the i^{th} species raised to its stoichiometric reaction coefficient
304 $v_{i,r}$, in the r^{th} reaction, which is positive for products and negative for reactants. Concentration
305 data were converted to chemical activities using

306

$$307 \quad a_i = m_i \gamma_i \quad (\text{Eq. 4})$$

308

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

315

316 *In situ data and gradient analyses*

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

322 $\mu\text{mol/kg}$). Zero values were reported as 10^{-9} molal. Pressures were assumed to be 36 MPa for
323 TAG vent field, 17 MPa for Fenway, 12 MPa for Brothers volcano, and 23 MPa for Rainbow,
324 on the basis of the water depth at which these hydrothermal fields are located.
325 Gibbs energy yields for sulfur disproportionation were calculated along a linear dilution
326 gradient from pure vent fluid to pure seawater at the four sites using endmember values as well
327 as vent fluid:seawater mixing ratios of 75:25, 50:50, and 25:75. Calculated temperatures of the
328 mixed fluids incorporated the specific heat capacity of water at that temperature, following the
329 method described in Lu *et al.*, 2021. Activity coefficients from these dilution gradients were
330 calculated after speciating with AqEquil. Energy yields for disproportionation in seawater were
331 also calculated as functions of various geochemical parameters while holding other variables
332 fixed (Table 1). Activity coefficients were recalculated using AqEquil v0.9.1 based on the
333 parameters for each gradient. The CHNOSZ v1.4.1 “mosaic” function was used to speciate H_2S
334 and SO_4^{2-} for energy yields that were calculated as a function of pH.
335 The mineral composition of the hydrothermal edifices is based on the open mineral database
336 mindat.org. (<https://www.mindat.org/>).

337

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

349

350 The authors declare no competing financial interests.

351

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