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Editorial: Why microbiologists are not barophiles

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1 **Editorial: On 115 years of sulfur microbiology.**

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6

7 *“Regions of sorrow, doleful shades, where peace*

8 *And rest can never dwell, hope never comes*

9 *That comes to all; but torture without end*

10 *Still urges, and a fiery deluge, fed*

11 *With ever-burning sulfur unconsum'd”*

12 John Milton (1608 – 1674), *Paradise Lost* (1667, I.65)

13

14 By coincidence alone, I find myself writing this Editorial 35 years to the day that the
15 discussion meeting on the sulfur bacteria was held at the Royal Society. The aims of this
16 article – which is sort of an Editorial and sort of a Review in a way – are to draw together the
17 contents of the *FEMS Microbiology Letters* Thematic Issue on *Sulfur Metabolism* and to
18 consider the state-of-the-art of our field, looking into the past and the future in equal measure,
19 much like Janus, the Roman god of doors and gates, transitions and time, passages and
20 endings, usually depicted as having two faces, looking in opposite directions at once (he is
21 also known as ‘Janus’, from the Medieval Latin). I do feel one has to have an eye on the past
22 and the future in equal measure as a scientist, and that one should not concentrate too much
23 on the present, thus this ‘state of the art’ article has both historical and perspective elements.

24 For my sins as Editor-In-Chief of this publication, I not only commissioned a Thematic Issue
25 on *Sulfur Metabolism*, but ended up editing every article therein, which became a very
26 interesting process of seeing the true breadth of our field, rather than just the little corner
27 (sulfur oxidation) in which I live. I’m pleased to say that only a small number of submissions

28 were rejected outright, and the quality was not compromised – there is a myth that Thematic
29 Issues are a way to get less-robust work published: not on my watch. Before I begin, I feel the
30 need to just make a small parish notice for the sake of completeness. In 2016 we lost one of
31 the great microbiologists – Professor Hans Georg Trüper (1936 – 2016) – known for his
32 highly respected contributions to the fields of taxonomy and systematics, and his work on
33 sulfur – and so I will urge the reader to read Professor Schink’s excellent obituary of
34 Professor Trüper, which can also be found in this publication (Schink, 2016).

35 Alexander Nathansohn (1878 – 1940) is not a name that is enormously widely known even
36 amongst sulfur microbiologists. Beijerinck – sure, Starkey – probably, Kelly – definitely,
37 Winogradsky – almost certainly, Trudinger – yes; Friedrich – yes...but Nathansohn has
38 somewhat faded – sadly – into obscurity. Nathansohn, was really where it all began. A little
39 bit about where he began, first of all: he was born in Brzeżany, East Prussia (now Berezhany,
40 Ukraine) in 1878 and in 1884, he moved to Leipzig with his family and eventually studied
41 natural history under the likes of Wilhelm Ostwald (chemist, one of the founders of what we
42 now call physical chemistry and winner of the Nobel Prize in Chemistry, 1909 for his work
43 on catalysis and reaction equilibria), Carl Chun (marine biologist responsible for naming one
44 of my favourite cephalopods – *Vampyroteuthis infernalis* Chun. ‘the vampire squid from
45 Hell’) and K. G. F. Rudolf Leuckart (parasitologist, particularly noted for his work on *Taenia*
46 L.). It is fair to say that they must have been a pretty inspiring bunch! Nathansohn obtained
47 his doctorate in 1900 on the amitotic nuclear division of plants and his 1902
48 Habilitationsschrift was on inorganic nutrient uptake in plant cells. From 1901 to 1903, he
49 worked on nitrogen and sulfur metabolism in the *Bacteria* and was based in Naples, and this
50 is where the beginnings of our story lie. He went on to become a Privatdozent in Leipzig
51 (1904) and Professor extraordinarius (1910 – 1917). What happened after that is a little more
52 murky, but worth noting: we know from 1918 – 1919 he was in Vienna and then in 1920 he

53 was living in Berlin (Lichterfelde-West). Into the 190s, he was an applied botanist at the
54 Kaiser-Wilhelm-Institute für physikalische Chemie und Elektrochemie (now the Fritz Haber
55 Institute of the Max Plank Society, FHI) in Dahlem, Berlin. In the 1930s he was working as
56 an industrial chemist in Turin, and tried repeatedly to move to Monaco to escape potential
57 oppression by the rising Nazi Party. After this, we know very little but we do know that he
58 died in Turin, early in World War II, in circumstances unknown (for those interested, I can
59 highly recommend Mills (2012), from which much of this biographical information was
60 obtained, and which details all of Nathansohn's research over his whole career).

61 Back to Naples. Back to microbiology. Nathansohn set up enrichment cultures of marine mud
62 supplemented with thiosulfate and upon which white pellicles of highly motile *Bacteria* and
63 particles of amorphous white sulfur were evident within 1-2 days. He went on to show that as
64 thiosulfate disappeared in the cultures, both sulfate and tetrathionate appeared (Nathansohn,
65 1902). It was Beijerinck, of course, who named one these organisms (Beijerinck, 1904a) as
66 *Thiobacillus thioparus* ('the sulfur-rod that produces sulfur', albeit grammatically incorrect –
67 it should be "*Thiobacillus thioparans*" but cannot be changed) and further studied them
68 (Beijerinck, 1904b). Parker then named an isolate from decomposing concrete in the sewers
69 of Melbourne, Australia '*Thiobacillus neapolitanus*' (now *Halothiobacillus neapolitanus*) on
70 the basis of it being very similar to Nathansohn's original description of his Bay of Naples
71 isolates (Parker, 1957) with their very rapid motility, so characteristic of that species.

72 Over the 115 years between Nathansohn and this Thematic Issue on sulfur metabolism, an
73 enormous amount has changed technologically, enabling vastly different areas of work than
74 Nathansohn could probably have ever dreamed. It's hard to put oneself in the mindset of a
75 scientist in 1902, observing for the first time the extremely vigorous motility of *H.*
76 *neapolitanus* down the microscope and not even considering the role of the recently
77 discovered 'nuclein' – which would not even be known to contain four bases, sugar and

78 phosphate until Levene (1919) – in the inheritance of that characteristic motility. In 2017, we
79 have a very different standpoint – with the genome sequence of *Thiobacillus thiooparus*
80 recently published by my group and the Joint Genome Institute (Hutt *et al.*, 2017 – it’s worth
81 noting that the type strain, DSM 505^T, is a soil isolate from New Jersey, USA, originating
82 from Starkey (1934)), and that of *H. neapolitanus* being closed in 2012 (NC_013422,
83 GenBankTM). These databases provide the blueprints for our archetypal organisms – our
84 workhorses – but even after 115 years of study, *T. thiooparus* still throws up surprises – *e.g.*
85 the presence of seemingly-complete operons for the denitrification pathway in the genome,
86 when *T. thiooparus* canonically does not denitrify, but the very closely related *Thiobacillus*
87 *denitrificans* does (also studied by and named by Beijerinck, 1904*a,b*, genome sequence
88 published by Beller *et al.*, (2006)). We are in a position of power that we have never been in
89 before, holding the very blueprints for autotrophic life – and, in theory, able to rebuild life
90 from them using synthetic life biotechnologies – yet we are still at a loss to understand them.
91 Of the 3,000 or so protein coding genes in *Thiobacillus* spp., how many do we truly know the
92 function of? How much of the physiology do we truly understand? Just as with understanding
93 a complex machine, one can stare at the blueprints for decades and still not fully understand it
94 without taking it apart and looking at how the pieces really fit together, and see it actually in
95 motion. The diversity of the sulfur community is now vast – we have groups all over the
96 world working on sulfate reduction and sulfur oxidation; on colourless, green and purple
97 sulfur *Bacteria*; on filamentous and unicellular *Bacteria*; on the mysterious ‘cable bacteria’;
98 on thermophiles, halophiles, psychrophiles and everything in between; on marine and
99 terrestrial and cave and mineral-associated *Bacteria*; on gut microflora and skin microflora;
100 on sulfur assimilation; on energetics, biochemistry and physiology; on genomics and
101 transcriptomics and gene regulation; on ecology and meta*omics; on taxonomy and
102 systematics; on biotechnologies and, importantly, on virtually every sulfur (chemical) species

103 that is known. By the 1960s, the inorganic sulfur biochemistry community had grown
104 sufficiently to justify Roy and Trudinger (1970) writing their book on the subject – which is
105 still very useful today. In February 1982, a two-day discussion meeting was held at the Royal
106 Society, which spawned a landmark thematic issue on *Sulphur Bacteria* in *Philosophical*
107 *Transactions of the Royal Society of London, Series B* (vol 298, issue 1093, September 1982),
108 edited by Postgate and Kelly, and numerous thematic issues of all manner of journal have
109 followed since. We have a thriving series of small but energetic conferences (‘Sulfur
110 Workshops’) that have been running for over a decade now (Münster, Germany, 2006; Tomar,
111 Portugal, 2009; Noordwijkerhout, Netherlands, 2012; Helsingør, Denmark, 2015, and
112 hopefully another one in 2018!). The author has presented at the Tomar and Helsingør
113 meetings and found them to be amongst the most fun but also thrilling conferences he has
114 attended in the last decade. If you’re not already *au fait* with them but work on sulfur in some
115 way, shape or form, look out for future meetings!

116 Whilst our understanding of *Thiobacillus* spp. and other ‘sulfur bacteria’ has been
117 enlightened by genome sequences and new technologies, it has also been hindered by a
118 number of misunderstandings. The taxonomy, systematics and nomenclature of this group of
119 the *Bacteria* was effectively wrong for almost 100 years, until Kelly and Wood (2000)
120 resolved it with the separation of the very large genus *Thiobacillus* into *Thiobacillus sensu*
121 *stricto* (*Betaproteobacteria*); *Acidithiobacillus* and *Thermithiobacillus* (originally subject to
122 much debate re: their position relative to the *Betaproteobacteria* and the
123 *Gammaproteobacteria*, but now are in the class *Acidithiobacillia*) and *Halothiobacillus*
124 (*Gammaproteobacteria*), with other former *Thiobacillus* spp. relegated to *Starkeya* and
125 *Paracoccus* (*Alphaproteobacteria*) and *Thiomicrospira* (*Gammaproteobacteria* – which has
126 just been extensively revised – Boden *et al.*, 2017). These ‘tidy ups’ may seem irksome to
127 some as they change the name ‘people are used to’ or ‘make it more complicated’, when

128 actually they make sense of things and often clarify the physiology and biochemistry
129 enormously when it is realised that organisms we try to comprehend relationships between
130 are actually quite unrelated – this must continue as the tree of life is a working document –
131 we need to continually move and change and circumscribe and reclassify to keep the tree
132 pruned and healthy and easy for the non-taxonomists to admire. It is worth noting that we’re
133 also compounded in our understanding by having to Google everything as “sulfur” and
134 “sulphur” because almost 27 years after IUPAC decided on the former, we still can’t make
135 our minds up either (*cf.* Kelly (1995) and/or Nature Chemistry (2009) for a ‘well worded rant’
136 on the subject – and a viewpoint to which I fully ascribe – ‘sulfur’ must be universally
137 adopted).

138 What else is new in our understanding, in the context of this Thematic Issue on *Sulfur*
139 *Metabolism*? Another archetypal genus, *Acidithiobacillus*, full of somewhat infuriating (I
140 speak from bitter experience...) sulfur and ferrous iron oxidising obligate acidophiles, has
141 revealed a little more of itself in the work of Christel *et al.* (2016), in which the sulfur-
142 oxidation pathways of the eurypsychrophile *Acidithiobacillus ferrivorans* SS3 (an isolate
143 from sediments in a copper-nickel mining area of Russia) were probed using transcript
144 studies, showing that the canonical *sox* genes of the Kelly-Friedrich pathway were expressed
145 along with those of the quinone-linked thiosulfate dehydrogenase (EC 1.8.5.2, *doxDA*),
146 heterodisulfide reductase (EC 1.8.98.1, *hdr*) and tetrathionate hydrolase (EC 3.12.1.B1, *tetH*),
147 indicating that DoxDA and Sox proteins oxidise thiosulfate to tetrathionate *in vivo*, passing
148 electrons onto cytochrome *c* and an *aa₃*-type terminal cytochrome *c* oxidase (EC 1.9.3.1) -
149 probably *via* the *bc₁* complex - (EC 1.10.2.2), translocating 2 protons, whereas electrons from
150 sulfide oxidation to elementary sulfur by sulfide-quinone reductase (EC 1.8.5.4, *Sqr*) enter
151 the quinone pool (mostly ubiquinone-8 in the *Acidithiobacillia*) along with electrons from the
152 oxidation of elementary sulfur by sulfur oxygenase-reductase (EC 1.13.11.55, *Sor*), which are

153 then either channelled to a *bo3*-type terminal ubiquinone oxidase (EC 1.10.3.10),
154 translocating 4 protons, or, to an NADH:ubiquinone reductase (EC 1.6.5.3), translocating 4
155 protons. To me, as a physiologist and biochemist, this is really rather exciting, and another
156 rung on our ladder to the Nirvana of understanding the sulfur oxidation pathways across the
157 *Bacteria* and their seemingly infinite variations and twists and turns.

158 Also on the subject of acidophilic *Bacteria*, but this time those which reduce sulfate,
159 Nancuqueo *et al.* (2016) discussed various solid and liquid media for their cultivation. It is
160 seldom that I will let a ‘methods paper’ get into *FEMS Microbiol. Lett.* as they fall outwith
161 our scope, but I make exceptions where I can see true value to the community and in the case
162 of this work, it felt ‘right’ to put it into this Thematic Issue where the sulfur community could
163 benefit. The specific innovations herein were to use glycerol *in lieu* of a carboxylic acid as
164 the electron donor, since it remains uncharged even at low pH, and to include 7 mM zinc (II)
165 as a sink for the produced hydrogen sulfide, trapping it and keeping it away from life (the
166 organism or the operator!). Agar plates were produced with an underlayer seeded with
167 “*Acidocella aromatica*”, which can tolerate the low pH (*c.* pH 3 to pH 5) of the medium and
168 will, importantly, use acetic acid as a carbon source. As media pH drops below their pK_a ,
169 carboxylates present protonate and become carboxylic acids, which can cross a lipid bilayer
170 and, once in the cytoplasm, where the pH is higher than the pK_a , deprotonate, effectively
171 carrying protons into the cells, lowering the cytoplasmic pH and killing the cell. The presence
172 of “*Acc. aromatica*” in an agar underlay removes acetate (and potentially other carboxylates
173 from agarose hydrolysis) produced during the incomplete oxidation of glycerol by the target
174 organisms, preventing this toxic effect. These media had been used in the author’s laboratory
175 (the Johnson laboratory at Bangor University, UK) for many years, giving a robust case for
176 their efficacy in isolating organisms such as *Desulfosporosinus acididurans* and ‘*D.*
177 *acidavidus*’, noting that colonies grew well, in spite of often taking on a coating of sphalerite

178 (zinc sulfide), caused when sulfide ions leaving the cell contact zinc ions from the medium.
179 Something that struck me at the time as particularly strong about this study was simply that
180 they had *years* of road-testing experience with these media and could robustly demonstrate
181 their usefulness – rather than the myriad of “an improved medium for...” or “an optimal
182 medium for...” (“optimal” means “better than anything else” yet this is seldom actually
183 tested!) manuscripts that one rejects each month for not having performed any really robust
184 road-tests.

185 Mangiapia and Scott (2016) presented an interesting study on the costs of production of
186 metabolic intermediates *via* the Calvin-Benson-Bassham and Arnon-Buchanan cycles, using
187 metabolic maps derived from genome sequences to calculate the amount of ATP required to
188 yield 1g dry biomass from carbon dioxide. This is almost “Stouthamer for the 21st Century”
189 and it excited me greatly in terms of the possibilities that it presents as the first *in silico*
190 estimate of the relative costs of two metabolic ‘options’ – since I spend a lot of my time
191 determining such parameters for continuous cultures in organisms in which multiple
192 pathways can be used for carbon fixation or glycolysis and so on. The study showed that the
193 Arnon-Buchanan cycle (based on members of the *Epsilonproteobacteria*) required *c.* 0.195
194 mol ATP/g dry biomass, but the Calvin-Benson-Bassham cycle (based on members of the
195 *Gammaproteobacteria*) ‘cost’ more, at 0.238 mol ATP/g dry biomass. All such studies have
196 their limits, of course, and this was based on a rather small group of organisms for each
197 pathway – the Calvin-Benson-Bassham cycle organisms were all members of the genera
198 *Thiomicrospira*, *Hydrogenovibrio* or *Thiomicrothrix* (using the names following the
199 revision of Boden *et al.* 2017, for clarity) – all members of the *Piscirickettsiaceae* in the
200 *Thiotrichales*, and the Arnon-Buchanan cycle organisms were *Sulfurimonas*, *Sulfurovum*
201 (both members of the *Helicobacteraceae* in the *Campylobacterales* and *Nitratiruptor* in the
202 *Nautiliaceae* of the *Nautiliales*. I look forward to seeing expanded studies of this ilk now that

203 the concept has been proven, and it will be interesting to see just how much these metabolic
204 costs vary by family, order or even class as we move forward. This struck me as a really good
205 example of how cutting-edge genome sequencing and *in silico* technologies can interface
206 seamlessly with the “less fashionable” theoretical yields and growth physiology popularised
207 by the great physiologists Stouthamer, Pirt and Monod *et al.* Maybe this will lead to a
208 renaissance of growth physiology in the 21st century? I live in hope!

209 Shuman and Hanson (2016) contributed a kinetic study of a Type VI sulfide-quinone
210 reductase (EC 1.8.5.4) from *Chlorobaculum tepidum* that has been implicated in growth at
211 high sulfide concentrations. The recombinant protein in *Escherichia coli* was purified by
212 affinity chromatography and subjected to a number of detailed characterisations, revealing
213 flavin adenine dinucleotide (FAD) as a strongly bound co-factor. The enzyme had a very low
214 affinity for sulfide and a high turnover number, consistent with the previous implication of its
215 role. The specific activity of the enzyme became saturated at around 6 mM sulfide – the same
216 concentration above which *Cba. tepidum* does not grow. Importantly, this study showed that
217 one of the two conserved cysteines in the sulfide-binding site of the enzyme was not present
218 in *Cba. tepidum*, but the enzyme was still functional, which then cast doubt on the suggested
219 role of this residue by previous authors, in at least Type VI enzymes or possibly more widely.
220 Whilst this was a relatively ‘small’ study, it was an important one and shed clear light on the
221 role and function of this neglected class of enzyme.

222 An enzyme complex resembling heterodisulfide reductase (EC 1.8.98.1) was characterised in
223 “*Aquifex aeolicus*” VF5 by Boughanemi *et al.* (2016). This hyperthermophilic member of the
224 *Aquificae* was cultivated on thiosulfate or elementary sulfur as electron donors under air
225 supplemented with molecular hydrogen and a complex of heterodisulfide reductase-like
226 enzymes identified. A revised model of energy metabolism in “*Afx. aeolicus*” was proposed
227 on the basis of their data, in which thiosulfate is oxidised in the periplasm to an unknown

228 species by a partial version of the thiosulfate-oxidising multienzyme system (TOMES) of the
229 Kelly-Friedrich pathway (often termed ‘Sox complex’). This unknown moiety is transported
230 into the cytoplasm where it has four fates i) it binds directly to TusA () and is then oxidised to
231 sulfite by the heterodisulfide reductase-like complex, with concomitant reduction of the
232 quinone pool; (ii) it binds to the sulfur carrier DsrE3 and is transferred onto the sulfur
233 carrier/transferase TusA prior to the same oxidation; (iii) it is directly oxidised by the
234 complex without a carrier protein; (iv) it is oxidised to hydrogen sulfide and sulfite by sulfur
235 oxygenase reductase (EC 1.13.11.55), the former is then oxidised to an unknown species by
236 sulfide:quinone reductase (EC 1.8.5.4), with reduction of the quinone pool. Sulfite is oxidised
237 to sulfate with reduction of the quinone pool by a membrane-bound sulfite dehydrogenase
238 (EC 1.8.2.1, Soe). Again, much like the *Acidithiobacillus ferrivorans* SS3 work of Christel *et al.*
239 *al.* (2016) in this issue, this provides significant insight into the complexities of sulfur
240 oxidation pathways, in this case in an ancient lineage within the *Bacteria*, and shows again
241 the promiscuity of the TOMES (Sox complex) – this could provide a basis for interesting
242 evolutionary studies that will further our understanding of the complex biochemistry of
243 thiosulfate.

244 From sulfur oxidation, we move to sulfur assimilation – a very much neglected topic in my
245 view – in *Escherichia coli*, in a study by Yamazaki *et al.* (2016). This study identified that
246 *ydjN* encodes an *S*-sulfocysteine transporter and is required for growth on this as a sulfur
247 source. YdjN was previously characterised as a CysB regulon for cystine (the dimer of
248 cysteine) uptake, however, this study shows that YdjN is regulated by a CysB master
249 regulator that controls most of the sulfur assimilation genes in *E. coli*. The authors examined
250 the *Gammaproteobacteria* for orthologs of *ydjN* and found highly similar genes in many
251 genome sequences, along with a less-similar gene in the class *Bacillales* – *tycP* in *Bacillus*
252 *subtilis*, for example. The importance of this finding and relation to the abundance of *S*-

253 sulfocysteine in the environment was noted, and thus the study of Yamazaki *et al.* is
254 particularly important in bridging the gaps of our understanding of the metabolism of this
255 amino acid.

256 A Commentary on the reduction of nitrate in sulfate-reducing bacteria was contributed by
257 Marietou (2016), in which the relative merits of these two terminal electron accepting
258 mechanisms was considered. *Desulfovibrio desulfuricans* 27774 from the
259 *Deltaproteobacteria* was discussed, which can use nitrate as a terminal electron acceptor
260 when sulfate is absent, reducing it to nitrite with the periplasmic nitrate reductase (EC
261 1.7.99.4, NapA), and then to ammonia with the membrane-bound nitrite reductase
262 (cytochrome) complex (EC 1.7.2.2, NrfHA), and compared to *Desulfosporosinus acididurans*
263 from the *Clostridia* which lacks the *napA* gene but can reduce nitrate *in vivo* – a putative
264 membrane-bound respiratory nitrate reductase (quinone) catalytic unit gene (*narG*, EC
265 1.7.5.1) has been identified in the genome sequence, and Marietou curated members of the
266 classes *Deltaproteobacteria*, “*Nitrospira*” and *Clostridia* containing further *narG* genes, and
267 considered the evidence that they can/not reduce nitrate, including, importantly, the long lag-
268 times observed when switching *Dsv. desulfuricans* from sulfate to nitrate, that may contribute
269 to a false-negative. This reminded me of a story that I’ve been told several times by Dr Ann P.
270 Wood – who was my undergraduate supervisor and later collaborator prior to her recent
271 retirement – regarding the growth of the then “*Thiobacillus A2*” (*Paracoccus versutus*) on
272 methanol, which was only discovered by accident. She had inoculated a basal medium,
273 supplemented it with methanol and incubated it in a shaking incubator, checking it frequently
274 and no growth was evident after 2 weeks, so she decided to terminate the experiment, just
275 before Christmas one year in the early 1980s. When she returned in January, she found she
276 had forgotten to dispose of the flasks and had left them in the incubator – and all of them
277 were now turbid with growth – thus it was established that *P. versutus* did indeed grow on

278 methanol, which was reported in this journal (Kelly and Wood, 1982). This and Marietou
279 mentioning it did make me wonder how many studies – and I’m thinking especially
280 taxonomic characterisations here – report “does not grow on…” but had only incubated it a
281 matter of days, when we know many organisms can have weeks of lag-phase when
282 transferring substrates or terminal electron acceptors. How much are we missing though
283 impatience? I feel another Editorial coming on! Marietou concluded by considering the many
284 missing aspects of metabolism that are seldom looked at in sulfate-reducing bacteria –
285 including oxygen respiration – let us hope some of these are answered in the coming years!

286 Sulfur and hydrogen stable-isotope fractionation in *Desulfovibrio vulgaris* was reported by
287 Leavitt *et al.* (2016), with demonstration that the expression of the sulfur carrier protein DsrC,
288 which links sulfite reduction by dissimilatory sulfite reductase (EC 1.8.99.5) in the cytoplasm
289 to energy metabolism on the cell membrane by the DsrMKJOP complex. did not influence
290 discrimination against sulfur-34 in sulfate. In a mutant (IPFG09), in which only a plasmid
291 copy of a Cys116Ala mutant *dsrC* is present, $\delta^{34}\text{S}$ in sulfide produced during growth does not
292 vary across the whole experiment, which was ascribed to the inability for this mutant to
293 produce the DsrC-trisulfide that is metabolised by the DsrMKJOP complex. This mutant also
294 showed a small decrease in the fractionation between water present in media and fatty acid
295 hydrogen isotopes, relative to the wildtype and other mutants, which was consistent with
296 some level of disruption to intracellular redox balance. The growth rate and yield were
297 obviously different in the mutant too, which probably also altered the fractionation, thus the
298 authors conclude that steady-state experiments in the chemostat are probably required to fully
299 ascertain the nature of this slight hydrogen fractionation. Anyone who knows me knows I do
300 love a steady-state. I review and edit a lot of papers in which shake-flask studies are used and
301 which report all manner of differences between mutant X and the wildtype, or when growing
302 in the presence of difference things, but even eyeballing the growth curves shows a difference

303 in growth rate, which means there is effectively an additional variable, which can't be
304 discounted. Use of the continuous culture may not be 'sexy' but it is still important useful
305 (and it also involves skills that are sadly not being handed down the generations in the era of
306 molecular biology and the Big Shiny Instrument, which require enormously complex skillsets
307 in themselves, but are prioritised over the 'classical' yet important techniques when training
308 the next generation – in my own laboratory, I'm ensuring people know how to use those older
309 methods alongside the new, and I know many of my collaborators do the same). It's
310 important to remember that so much of the kinetics and physiology and biochemistry of
311 sulfur oxidation were elucidated in continuous culture studies – for example the Kelly-
312 Trudinger pathway in *Thermithibacillus tepidarius* (previously '*Thiobacillus tepidarius*', cf.
313 Wood & Kelly, 1986, Boden *et al.* 2016) – if the reader is interested, I would suggest
314 Hoskisson & Hobbs (2005) as a 'primer', or, if feeling brave, Pirt (1975) – the infamous
315 'yellow book'.

316 Findlay (2016) presented an interesting Mini Review on the subject of microbe-polysulfide
317 interactions in the environment, considering the fundamental biological transformations of
318 polysulfide (into sulfate, sulfide or elementary sulfur, and the formation of polysulfide from
319 sulfide or elementary sulfur) and the many abiotic transformations (into sulfate, thiosulfate,
320 elementary sulfur, thiocyanate, carbonyl sulfide, or pyrite *etc*) that provide energy sources in
321 turn for autotrophic sulfur oxidisers. The review considered principally the metabolism of the
322 'green sulfur bacteria' and the 'purple sulfur bacteria', as well as *Beggiatoa* spp., *Spirillum*
323 spp., and in members of the classes '*Cyanobacteria*' and *Deltaproteobacteria* in the *Bacteria*
324 and the *Thermococci* (*Protoarchaea*) in the *Archaea* and so on. It is a thorough and engaging
325 review on a relatively forgotten suite of sulfur anions, drawing together data from both
326 metabolic studies and environmental chemistry – a pairing we will see more of as our field

327 progresses – it is becoming increasingly clear that one cannot and should not attempted to
328 separate the “bio” and “geo” in biogeochemistry.

329 If one mentions biotechnology to a sulfur microbiologist, our minds obviously jump to
330 biohydrometallurgy, and to great leach-heaps towering over mining sites in South America,
331 full of *Acidithiobacillus* spp. and allies, slowly releasing copper from low-grade ores.

332 Sánchez-Andrea *et al.* (2016) contributed a Commentary advocating a much wider
333 application of microbial biotechnologies to the mining sector. Obviously, I concur entirely,
334 since I’ve spent the last five or so years developing technologies for the mining and refinery
335 of f-block metals, *viz.* the rare-earth elements (though they aren’t based on sulfur or iron
336 metabolism). Examples were given including in-use biotechnologies for the biorecovery of
337 copper from mine waste, producing a copper sulfide-rich concentrate that can be smelted
338 economically, along with a consideration of socioeconomic factors.

339 We then moved into the realms of methylated sulfur compound metabolism – a subject close
340 to my heart as my Ph.D was on dimethylsulfide metabolism, principally. Firstly Ogawa *et al.*
341 (2016) contributed a paper on carbonyl sulfide metabolism in members of the *Actinomycetes*,
342 in which the presence of carbonyl sulfide hydrolase and a type of carbonic anhydrase (EC
343 4.2.1.1) that may have a role in carbonyl sulfide metabolism were demonstrated. High rates
344 of carbonyl sulfide oxidation were demonstrated in *Dietzia maris* NBRC 15801^T and in
345 *Mycobacterium* sp. THI405, which were comparable with the high rates of oxidation found in
346 *Thiobacillus thioparus* THI115 – understandable since it is using carbonyl sulfide to fuel
347 autotrophic growth – and the purpose of carbonyl sulfide degradation in the *Actinomycetes*
348 was discussed. As Ogawa *et al.* noted, *Mycobacterium* spp. have been shown to grow
349 chemolithoautotrophically at the expense of elementary sulfur – something that clearly
350 should lead to further work screening that whole genus for this ability – and that may explain
351 the oxidation rates observed (Kusimi *et al.*, 2011). A similar study in the *Eukarya* was also

352 contributed by Masaki *et al.* (2016), which considered both the degradation and emission of
353 carbonyl sulfide by fungi in forest soils, *viz.* *Trichoderma* sp. THIF08 and
354 *Umbelopsis/Mortierella* spp. THIF09 and THIF13, respectively. The role of fungal carbonic
355 anhydrases in carbonyl sulfide degradation was considered, and the strong need to fully
356 understand their sulfur metabolism was emphasised.

357 From the *Bacteria* and the *Eukarya*, we then moved into the *Archaea*, with a study on 3-
358 mercaptopropionate in *Methanocaldococcus jannaschii* by Allen and White (2016). The
359 production of this was considered to perhaps occur *via* the unexpected-yet-detected
360 metabolites 2-hydroxy-4-mercaptopropionate and 4-mercapto-2-oxopropionate, either of which
361 has been previously reported as natural products. This was the first report of 3-
362 mercaptopropionate in a methanogen, and it was felt this may explain the presence of this
363 compound in natural waters, as reported by Kiene and Taylor (1988). The authors noted that
364 they had previously suspected that 3-mercapto-2-oxopropionate was a precursor to coenzyme F430-
365 3, but that they had recently demonstrated that it was not. They finished by speculating that it
366 could have a role similar to that of coenzyme M (2-mercaptoethanesulfonate) – something I
367 hope to see further news of in the coming years as this is a potentially very big discovery!

368 A report of the presence of sulfidogenic *Bacteria* (*sensu* degradation of cysteine and
369 respiration of sulfite) in human feces was made by Feng *et al.* (2017). Whilst this was a
370 culture-based study – which are categorically not outmoded in ecology, providing the
371 conclusions drawn are appropriate, and the methods rigorous – it showed that *Bilophila* from
372 the *Deltaproteobacteria* and *Clostridium* spp. from Cluster XIVa (class *Clostridia*) were
373 dominant in the enrichment cultures. A sulfite-respiring strain (2C) was isolated that could
374 represent a novel genus near to *Sporomusa* in the class *Negativicutes*, which could represent a
375 whole new group of organisms important in gut health. A key ‘bottom line’ of this study has
376 been the emphasis that sulfate-reduction is not the only sulfidogenic process important in gut

377 health, and that sulfite respiration and amino acid degradation must also be considered. As I
378 have always maintained there is no such thing as “medical microbiology” (I’ll probably get
379 shot for saying that...!) – there’s only “organism-environment” interactions – not even
380 “organism-host”, as the “host” is just an environment, and the fact that it’s the human gut
381 doesn’t make it any different to a field or a lake. If we consider the fraction of the sulfide flux
382 within the gut that is sulfate reduction *versus* sulfite respiration *versus* amino acid
383 degradation, and “who does what” with regard to each of these three metabolic pathways, we
384 have a very basic biogeochemical pathway. This lead me to then consider just how much
385 ‘sulfate reduction’ is measured in sediments in terms of the amount or flux of sulfide, without
386 really probing how much of it originates from these other pathways, or, indeed, from purely
387 geochemical origins (which are obviously absent in the gut). As I’ve already said, one can’t
388 separate “bio” and “geo” and I think what we need to fully understand the gut microflora *etc*
389 are true geochemists to work with the microbiologists to really understand what’s going on in
390 there: treat it like any other environment and use the same disciplines and methods.

391 From the gut to the field, we move into the realms of ecology, with Rossmassler *et al.* (2016)
392 contributing a comparison of the diversity of sulfur metabolisms detected in Lower Kane
393 Cave, Wyoming, and Glenwood Hot Springs, Colorado. I was quite excited to edit this
394 manuscript as I spent some time as a Research Fellow working on Movile Cave, Romania,
395 which caught a lot of press attention and I’m still being interviewed about it 7-8 years later.
396 Whilst I still love cave microbiology, I’m now limited by disability, ultimately exacerbated
397 by a back injury obtained whilst caving, so whilst I’m ‘grounded’, I do still enjoy keeping up
398 with what the cave ecologists are up to! The recombination protein A (previously
399 “recombinase A”, *recA*) gene was used for the phylogenetic analyses (a practice I fully
400 concur with – the 16S rRNA (*rrs*) gene has limitations), demonstrating that
401 *Epsilonproteobacteria* and *Gammaproteobacteria* dominated these sulfidic springs, *viz.* from

402 the former, *Sulfurovum* and *Sulfurospirillum* from the *Campylobacterales* and a third group
403 with no cultivated relatives, but somewhat closely related to *Nitratifractor* in the *Nautiliales*,
404 and, from the latter, *Thiothrix* from the *Thiotrichales*. The sulfide-quinone reductase (EC
405 1.8.5.4, *sqr*) gene was present in large copy-number in both sites, but distinct types and
406 taxonomic affiliations were present at each site. The *soxXABCDYZ* genes encoding the
407 TOMES of the Kelly-Friedrich pathway were not consistent across the sites, and they were
408 less abundant than the *sqr* genes. The sulfide-flavocytochrome *c* reductase (EC 1.8.2.3,
409 *fccBA*), polysulfide reductase (EC 1.12.98.4, *psr*), dissimilatory sulfite reductase (EC 1.8.7.1,
410 *dsr*), adenylylsulfate reductase (EC 1.8.4.8, *apr*) and dimethylsulfoxide reductase (EC 1.8.5.3,
411 *dms*) genes were mined within the metagenomic dataset. Sequences for *dsrA* and *dsrB* were
412 in low number, as were the *Deltaproteobacteria* in the *recA* libraries, and *dsrC* could only be
413 detected at Lower Kane and not at Glenwood, most of which actually affiliated to the sulfur
414 transfer protein gene (*tusE*) from *Thiothrix lacustris*-like organisms. Novel taxa within the
415 *Epsilonproteobacteria* were found at both sites and 25-88 % of the *Bacteria* found at each
416 site were from this class. Interestingly, in spite of thiosulfate being produced geochemically
417 in such environments, thiosulfate oxidation pathways were less abundant than sulfide
418 oxidation genes. This interested me since in the sulfidic spring at the Roman Baths in Bath,
419 UK, we find *Thiobacillus* spp. and *Thermithiobacillus* spp. in abundance, which grow on
420 thiosulfate – it must be stressed, however, that we still don't fully understand their thiosulfate
421 metabolism and thus the genes involved may not be the canonical *sox* operon used in the
422 *Alphaproteobacteria*, even though part of it is present (Boden *et al.*, 2016), it is interrupted
423 by a DUF302-family protein gene in *Thermithiobacillus*, which we have speculated may be
424 involved in thiosulfate or trithionate metabolism. As always, molecular ecology is only as
425 good as our understanding of the underpinning physiology and biochemistry, and until we are

426 confident of the gamut of thiosulfate oxidation pathways, all ecological studies will be
427 limited, which may under-estimate the contribution of this metabolism.

428 Another under-understood lineage was found in peatlands of the southern Appalachian
429 peatlands, this time representing novel members of the *Alphaproteobacteria*, in a study by
430 Harbison *et al.* (2016). An isolate (CS4) was obtained that could be assigned to the so-called
431 ‘candidate order’ Ellin 329, which was proposed to be potentially involved in sugar
432 fermentation and the decomposition of plant litter. Diversity amongst the phyla
433 ‘*Acidobacteria*’ and ‘*Chlorobi*’ and the class ‘*Cyanobacteria*’ in peatlands was discussed,
434 along with ‘rare lineages’ of organisms thus far only seen in molecular ecological studies
435 such as the Obsidian Pool hot spring lineage OP3, which is thought to be metabolically
436 similar to the *Deltaproteobacteria*.

437 Finally, a study of tetrathionate reduction in *Wolinella succinogenes* from the
438 *Epsilonproteobacteria* by Kurth *et al.* (2017). This concerned the diheme cytochromes *c*
439 referred to variously as thiosulfate dehydrogenase (EC 1.8.2.2) and tetrathionate reductase
440 (EC 1.8.99.B2) depending on their *in vivo* directionality, which catalyse the dimerisation of
441 thiosulfate to tetrathionate in one direction or the reduction of tetrathionate to yield two
442 thiosulfates in the opposite direction, the former being involved in the Kelly-Trudinger
443 pathway of thiosulfate metabolism in both chemolithoautotrophs and chemolithoheterotrophs
444 (though the latter usually contain only a partial pathway *viz.* this enzyme alone) and the latter
445 is a respiratory reductase. It is worth noting that multiple thiosulfate dehydrogenase families
446 probably exist – a subject I will review separately – and that a quinone-linked alternative has
447 long been understood in the *Acidithiobacillia* and in *Acidianus* spp. in the *Archaea* (EC
448 1.8.5.2). In this study, a gene (*tsdC*) was identified in *W. succinogenes* and was co-expressed
449 in *Escherichia coli* with the *tsdA* gene that encodes thiosulfate dehydrogenase, resulting in
450 the production of the latter and a lipoprotein (TsdC) of around 17 kDa. No TsdC homologs

451 were found in any protein databases and, on the basis of the *tsdC* gene, a signal peptide of 19
452 amino-acids previously shown to be associated to lipoproteins was encoded. The recombinant
453 TsdC in *E. coli* was, indeed, membrane-associated and no prosthetic groups were evident
454 from spectrophotometric studies. When TsdC was co-produced in *E. coli* with TsdA, the
455 intracellular location of the latter moved to the membrane fraction, indicating that TsdC may
456 serve to anchor TsdA against the membrane, with a dramatic increase in the enzyme activity
457 of TsdA, though principally *sensu* tetrathionate reductase activity. Kurth *et al.* concluded that
458 the *in vivo* role of TsdC anchored TsdA against the inner membrane, facing into the
459 periplasm, close to the cytochrome *c*-linked formate dehydrogenase complex (FdhABC, EC
460 1.2.2.3). Electron flow during energy conservation was from FdhABC to either (i) the
461 menaquinone pool and then to TsdA and the reduction of tetrathionate as terminal acceptor,
462 or, (ii) the menaquinone pool and then the *bc*₁ complex and then to TsdA and the reduction of
463 tetrathionate as terminal acceptor. It seemed that the role of TsdC was in both membrane
464 anchoring the enzyme and ensuring proximity to the FdhABC complex. It is worth noting
465 that this study also raised the question of the true medium composition of ‘polysulfide’ media
466 that are made by combining sulfide and tetrathionate, as if these two species do not react
467 completely, some tetrathionate would be present in the medium thus could act as a(n
468 additional) terminal electron acceptor. It is not overly difficult to quantify tetrathionate in
469 such media to see if (and how long) it persists, so no doubt this will be resolved in due course.

470 Some pretty big findings, and some smaller but no less important findings. Overall, a very
471 interesting collection of papers that I am sure will be of interest to our now very diverse
472 community. If one were to compare what we are working on in 2017 *versus* at the 1982
473 Royal Society meeting, the gamut of what we now study is enormous and some of the core
474 questions from 1982 have still yet to be cracked.

475 What do I think comes next? Well, there'll be separate *Commentary* in due course from me
476 on that, so I won't answer it here, but I have to return to Nathansohn to finish where I started,
477 with a quote from his 1902 paper, which was later invoked by Kelly (1982) in his state-of-
478 the-art review of the biochemistry of chemolithotrophic sulfur oxidation, and again by myself
479 as the opening gambit of my Ph.D thesis – we are now 115 years after Nathansohn first said it,
480 and it is very much still the case – whilst we know an awful lot and have made leaps and
481 bounds, we still have an enormity to learn.

482 *“Es erhebt sich nun die Frage, was unter den natürlichen Lebensbedingungen dieser*
483 *Organismen die Ausgangs- und Endproducte ihres Stoffwechsels sind. Leider lässt sie sich*
484 *mit voller Bestimmtheit nicht beantworten.”*

485 Alexander Nathansohn (1878 – 1940)

486 (The question that now arises is what are the substrates and the end products of the
487 metabolism of these organisms in their natural conditions of life. Unfortunately, this cannot
488 be answered with absolute certainty.)

489

490 **References**

- 491 **Allen, K. D. and White, R. H. (2016)** Occurrence and biosynthesis of 3-mercaptopropionic
492 acid in *Methanocaldococcus jannaschii*. *FEMS Microbiol. Lett.* **363**, fnw217.
- 493 **Beijerinck, M. W. (1904a)** Phénomènes de réduction produits par les microbes. *Arch. Néerl.*
494 *Sci. Exact. Nat. (Section 2)*. **9**: 131-157.
- 495 **Beijerinck M. W. (1904b)** Ueber die Bakterien, welche sich im Dunkeln mit Kohlensäure als
496 Kohlenstoffquelle ernähren können. *Centralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt*
497 *II*. **11**: 592–599.
- 498 **Beller, H. R., Chain, P. S. G., Letain, T. E., Chakicherla, A., Larimer, F. W.,**
499 **Richardson, P. M., Coleman, M. A., Wood, A. P., Kelly, D. P. (2006)** The genome
500 sequence of the obligately chemolithoautotrophic, facultatively anaerobic bacterium
501 *Thiobacillus denitrificans*. *J. Bacteriol.* **188**, 1473-1488.
- 502 **Boden, R., Hutt, L. P., Huntemann, M., Clum, A., Pillay, M., Palaniappan, K., Varghese,**
503 **N., Mikhailova, N., Stamatis, D., Reddy, T., Ngan, C. Y., Daum, C., Shapiro, N.,**
504 **Markowitz, V., Ivanova, N., Woyke, T., Kyrpides, N. (2016)** Permanent draft genome of
505 *Thermithiobacillus tepidarius* DSM 3134^T, a moderately thermophilic, obligately
506 chemolithoautotrophic member of the *Acidithiobacillia*. *Stand. Genomic Sci.* **11**: 74.
- 507 **Boden, R., Scott, K. M., Williams, J. T., Russell, S., Antonen, K., Rae, A. W. and Hutt, L.**
508 **P. (2017)** An evaluation of *Thiomicrospira*, *Hydrogenovibrio* and *Thioalkalimicrobium*:
509 reclassification of 4 species of *Thiomicrospira* to each *Thiomicrothabodus* gen. nov. and
510 *Hydrogenovibrio*, and reclassification of all 4 species of *Thioalkalimicrobium* to
511 *Thiomicrospira*. *Int. J. Syst. Evol. Microbiol.*, **In Press**.

512 **Boughanemi, S., Lyonnet, J., Infossi, P., Bauzan, M., Kosta, A., Lignon, S., Guidici-**
513 **Ortoni, M-T., Guiral, M. (2016)** Microbial oxidative sulfur metabolism: biochemical
514 evidence of the membrane-bound heterodisulfide reductase-like complex of the bacterium
515 *Aquifex aeolicus*. *FEMS Microbiol. Lett.* **363**: fnw156.

516 **Christel, S., Fridlund, J., Buetti-Dinh, A., Buck, M., Watkin, E. L., Dopson, M. (2016)**
517 RNA transcript sequencing reveals inorganic sulfur compound oxidation pathways in the
518 acidophile *Acidithiobacillus ferrivorans*. *FEMS Microbiol. Lett.*, **363**: fnw057.

519 **Feng, Y., Stams, A. J. M., de Vos, W. M., Sánchez-Andrea, I. (2017)** Enrichment of
520 sulfidogenic bacteria from the human intestinal tract. *FEMS Microbiol. Lett.*, **In Press**.

521 **Findlay, A. J. (2016)** Microbial impact on polysulfide dynamics in the environment. *FEMS*
522 *Microbiol. Lett.*, **363**, fnw103.

523 **Harbisson, A. B., Carson, M. A., Lamit, L. J., Basiliko, N., Bräuer, S. L. (2016)** A novel
524 isolate and widespread abundance of the candidate alphaproteobacterial order (Ellin 329), in
525 southern Appalachian peatlands. *FEMS Microbiol. Lett.* **363**, fnw151.

526 **Hoskisson, P. A. and Hobbs, G. (2005)** Continuous culture – making a comeback?
527 *Microbiology (UK)*, **151**, 3153-3159.

528 **Hutt, L. P., Huntemann, M., Clum, A., Pillay, M., Palaniappan, K., Varghese, N.,**
529 **Mikhailova, N., Stamatis, D., Reddy, T., Daum, C., Shapiro, N., Ivanova, N., Kyrpides,**
530 **N., Woyke, T., Boden, R. (2017)** Permanent draft genome sequence of *Thiobacillus*
531 *thioparus* DSM 505^T, an obligately chemolithoautotrophic member of the *Betaproteobacteria*.
532 *Stand. Genomic Sci.* **12**, 10.

533 **Kelly, D. P. (1982)** Biochemistry of the chemolithotrophic oxidation of inorganic sulphur.
534 *Phil. Trans. R. Soc. London. B.* **298**, 499-528.

535 **Kelly, D. P. (1995)** Sulfur and its Doppelgänger. *Arch. Microbiol.* **163**, 157-158.

536 **Kelly, D. P. and Wood, A. P. (1982)** Autotrophic growth of *Thiobacillus* A2 on methanol.
537 *FEMS Microbiol. Lett.* **15**: 229-233.

538 **Kelly, D. P. and Wood, A. P. (2000)** Reclassification of some species of *Thiobacillus* to the
539 newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov. and
540 *Thermithiobacillus* gen. nov. *Int. J. Syst. Evol. Microbiol.* **50**:511–516.

541 **Kurth, J. M., Schuster, A., Steel, W., Herresthal, S., Simon, J., Dahl, C. (2017)** TsdC, a
542 unique lipoprotein from *Wolinella succinogenes* that enhances tetrathionate reductase activity
543 of TsdA. *FEMS Microbiol. Lett.* **364**: fnx003.

544 **Kusimi, A., Li, X. S., Katayama, Y. (2011)** Mycobacteria isolated from Angkor monument
545 sandstones grow chemolithoautotrophically by oxidizing elemental sulfur. *Front. Microbiol.*,
546 **2**: 104.

547 **Leavitt, W. D., Venceslau, S. S., Pereira, I. A., Johnston, D. T., Bradley, A. S. (2016)**
548 Fractionation of sulfur and hydrogen isotopes in *Desulfovibrio vulgaris* with perturbed DsrC
549 expression. *FEMS Microbiol. Lett.* **363**: fnw226.

550 **Levene, P. A. (1919)** The structure of yeast nucleic acid. IV. Ammonia hydrolysis. *J. Biol.*
551 *Chem.* **40**, 415-424.

552 **Mangiapia, M. and Scott, K. M. (2016)** From CO₂ to cell: energetic expense of creating
553 biomass using the Calvin-Benson-Bassham and reductive citric acid cycles, based on genome
554 data. *FEMS Microbiol. Lett.* **363**, fnw054.

555 **Masaki, Y., Ozawa, R., Kageyama, K., Katayama, Y. (2016)** Degradation and emission of
556 carbonyl sulfide, an atmospheric trace gas, by fungi isolated from forest soil. *FEMS*
557 *Microbiol. Lett.* **363**: fnw197.

558 **Marietou, A. (2016)** Nitrate reduction in sulfate-reducing bacteria. *FEMS Microbiol. Lett.*
559 **363**, fnw155.

560 **Mills, E. I. (2012)** *Biological oceanography, an early history, 1870 – 1960*. University of
561 Toronto Press, Toronto, Canada.

562 **Ñancuqueo, I., Rowe, O. F., Hedrich, S., Johnson, D. B. (2016)** Solid and liquid media for
563 isolating and cultivating acidophilic and acid-tolerant sulfate-reducing bacteria. *FEMS*
564 *Microbiol. Lett.* **363**, fnw083.

565 **Nathansohn, A. (1902)** Über eine neue Gruppe von Schwefelbakterien und ihren
566 Stoffwechsel. *Mitt. Zool. Stn. Neapel.* **15**, 655–80.

567 **Nature Chemistry (2009)** So long sulphur. Why *Nature Chemistry* spells sulfur with an ‘f’.
568 *Nature Chemistry.* **1**: 333.

569 **Ogawa, T., Kato, H., Higashide, M., Nishimiya, M., Katayama, Y. (2016)** Degradation of
570 carbonyl sulfide by *Actinomycetes* and detection of clade D of β -class carbonic anhydrase.
571 *FEMS Microbiol. Lett.* **363**: fnw223.

572 **Parker, C. D. (1957)** Genus V. *Thiobacillus* Beijerinck 1904 In: Breed, R. S., Murray, E. G.
573 D. and Smith, N. R. (Eds) *Bergey’s Manual of Determinative Bacteriology*, 7th Edition, The
574 Williams & Wilkins Co, Baltimore, USA. pp. 83 – 88.

575 **Pirt, S. J. (1975)** Principles of microbe and cell cultivation. *Blackwell Scientific Publications*,
576 Oxford, UK.

577 **Rossmassler, K., Hanson, T. E., Campbell, B. J. (2016)** Diverse sulfur metabolisms from
578 two subterranean sulfidic spring systems. *FEMS Microbiol. Lett.* **363**: fnw162.

579 **Roy, A. B. and Trudinger, P. A. (1970)** The biochemistry of inorganic compounds of
580 sulphur. *Cambridge University Press, Cambridge, UK.*

581 **Sánchez-Andrea, I., Stams, A. J. M., Weijma, J., Gonzalez Contreras, P., Dijkman, H.,**
582 **Rozendal, R. A., Johnson, D. B. (2016)** A case in support of implementing innovative bio-
583 processes in the metal mining industry. *FEMS Microbiol. Lett.* **363**: fnw106.

584 **Schink, B. (2016)** Hans Georg Trüper (1936 – 2016). *FEMS Microbiol. Lett.* **363**: fnw085.

585 **Shuman, K. E. and Hanson, T. E. (2016)** A sulfide:quinone oxidoreductase from
586 *Chlorobaculum tepidum* displays unusual kinetic properties. *FEMS Microbiol. Lett.* **363**:
587 fnw100.

588 **Starkey, R. L. (1934)** Isolation of some bacteria which oxidise thiosulphate. *Soil Sci.* **39**,
589 197-219.

590 **Wood, A. P. and Kelly, D. P. (1986)** Chemolithotrophic metabolism of the newly-isolated
591 moderately thermophilic, obligately autotrophic *Thiobacillus tepidarius*. *Arch. Microbiol.*
592 **144**, 71-77.

593 **Yamazaki, S., Takei, K., Nonaka, G. (2016)** *ydjN* encodes an S-sulfocysteine transporter
594 required by *Escherichia coli* for growth on S-sulfocysteine as a sulfur source. *FEMS*
595 *Microbiol. Lett.* **363**, fnw185.