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Editorial: Why microbiologists are not barophiles

Boden, Rich

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Editorial: On 115 years of sulfur microbiology.

Rich Boden[1,2]

Editor-In-Chief

1. School of Biological & Marine Sciences, University of Plymouth, Plymouth, UK.

2. Sustainable Earth Institute, University of Plymouth, Plymouth, UK.

"*Regions of sorrow, doleful shades, where peace*
And rest can never dwell, hope never comes
That comes to all; but torture without end
Still urges, and a fiery deluge, fed
With ever-burning sulfur unconsum'd"
John Milton (1608 – 1674), *Paradise Lost* (1667, I.65)

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

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

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

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

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

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

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

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

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

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

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

What else is new in our understanding, in the context of this Thematic Issue on *Sulfur Metabolism*? Another archetypal genus, *Acidithiobacillus*, full of somewhat infuriating (I speak from bitter experience…) sulfur and ferrous iron oxidising obligate acidophiles, has revealed a little more of itself in the work of Christel *et al.* (2016), in which the sulfur-oxidation pathways of the eurypsychrophile *Acidithiobacillus ferrivorans* SS3 (an isolate from sediments in a copper-nickel mining area of Russia) were probed using transcript studies, showing that the canonical *sox* genes of the Kelly-Friedrich pathway were expressed along with those of the quinone-linked thiosulfate dehydrogenase (EC 1.8.5.2, *doxDA*), heterodisulfide reductase (EC 1.8.98.1, *hdr*) and tetrathionate hydrolase (EC 3.12.1.B1, *tetH*), indicating that DoxDA and Sox proteins oxidise thiosulfate to tetrathionate *in vivo*, passing electrons onto cytochrome *c* and an aa_3-type terminal cytochrome *c* oxidase (EC 1.9.3.1) - probably *via* the bc_1 complex - (EC 1.10.2.2), translocating 2 protons, whereas electrons from sulfide oxidation to elementary sulfur by sulfide-quinone reductase (EC 1.8.5.4, Sqr) enter the quinone pool (mostly ubiquinone-8 in the *Acidithiobacillia*) along with electrons from the oxidation of elementary sulfur by sulfur oxygenase-reductase (EC 1.13.11.55, Sor), which are

153 then either channelled to a *bo*3-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 Ňancucheo *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

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

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

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

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

An enzyme complex resembling heterodisulfide reductase (EC 1.8.98.1) was characterised in "*Aquifex aeolicus*" VF5 by Boughanemi *et al.* (2016). This hyperthermophilic member of the *Aquificeae* was cultivated on thiosulfate or elementary sulfur as electron donors under air supplemented with molecular hydrogen and a complex of heterodisulfide reductase-like enzymes identified. A revised model of energy metabolism in "*Afx. aeolicus*" was proposed 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 oxidises 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*
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*-

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

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

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

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

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

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

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

If one mentions biotechnology to a sulfur microbiologist, our minds obviously jump to biohydrometallurgy, and to great leach-heaps towering over mining sites in South America, full of *Acidithiobacillus* spp. and allies, slowly releasing copper from low-grade ores. Sánchez-Andrea *et al.* (2016) contributed a Commentary advocating a much wider application of microbial biotechnologies to the mining sector. Obviously, I concur entirely, since I've spent the last five or so years developing technologies for the mining and refinery of f-block metals, *viz.* the rare-earth elements (though they aren't based on sulfur or iron metabolism). Examples were given including in-use biotechnologies for the biorecovery of copper from mine waste, producing a copper sulfide-rich concentrate that can be smelted economically, along with a consideration of socioeconomic factors.

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

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

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

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

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

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

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

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

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

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

were found in any protein databases and, on the basis of the *tsdC* gene, a signal peptide of 19 amino-acids previously shown to be associated to lipoproteins was encoded. The recombinant TsdC in *E. coli* was, indeed, membrane-associated and no prosthetic groups were evident from spectrophotometric studies. When TsdC was co-produced in *E. coli* with TsdA, the intracellular location of the latter moved to the membrane fraction, indicating that TsdC may serve to anchor TsdA against the membrane, with a dramatic increase in the enzyme activity of TsdA, though principally *sensu* tetrathionate reductase activity. Kurth *et al.* concluded that the *in vivo* role of TsdC anchored TsdA against the inner membrane, facing into the periplasm, close to the cytochrome *c*-linked formate dehydrogenase complex (FdhABC, EC 1.2.2.3). Electron flow during energy conservation was from FdhABC to either (i) the menaquinone pool and then to TsdA and the reduction of tetrathionate as terminal acceptor, or, (ii) the menaquinone pool and then the bc_1 complex and then to TsdA and the reduction of tetrathionate as terminal acceptor. It seemed that the role of TsdC was in both membrane anchoring the enzyme and ensuring proximity to the FdhABC complex. It is worth noting that this study also raised the question of the true medium composition of 'polysulfide' media that are made by combining sulfide and tetrathionate, as if these two species do not react completely, some tetrathionate would be present in the medium thus could act as a(n additional) terminal electron acceptor. It is not overly difficult to quantify tetrathionate in such media to see if (and how long) it persists, so no doubt this will be resolved in due course.

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

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

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

Alexander Nathansohn (1878 – 1940)

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

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