

## ***Interactive comment on “Cryptic role of tetrathionate in the sulfur cycle: A study from Arabian Sea oxygen minimum zone sediments” by Subhrangshu Mandal et al.***

**Anonymous Referee #2**

Received and published: 21 July 2019

Review of BG-2019-248 July 21, 2019

The authors investigate the capacity of microorganisms in sediments from the Arabian Sea to metabolize redox-intermediate sulfur species. This is an important question with significance for understanding carbon turnover in sediments as well as for interpreting sedimentary records, in which these rapid processes are often invisible. The study relies primarily on the successful culturing of organisms with at least the facultative capacity to metabolize tetrathionate, and on metagenomic and metatranscriptomic datasets for a series of depths in two cores. The data are interesting and would fill an important gap in the literature, if the authors can address possible methodological

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concerns below. A more serious issue, however, appears in the argument regarding tetrathionate production from pyrite. This idea is not present in any of the three papers cited, which evidences a serious misunderstanding about this process and causes me to recommend rejection of the paper in its current form. Additional comments and suggestions follow.

{Note – I am not able to speak to the appropriateness of the metagenomic methods described here, since this is outside my area of expertise.}

General comment – for reading clarity, there are many places where codes could be simplified. You only have two cores – they could be referenced as A and B, or by collection depth, much more readably than SSK42/5 and SSK42/6. The names of slurry media types are similarly difficult to read (e.g. was ‘T’ tetrathionate or thiosulfate?).

Bulk sediment porewater geochemistry – please consider converting information in Table 1 to a pair of depth profile figures, one for each site. Please provide an appropriate number of significant figures for your data (you are currently reporting sub-nM precision).

Thiosulfate and sulfide concentrations – No details are provided about how porewater sampling was conducted, or how porewater samples were chemically preserved. (Were zinc acetate, bromobimane, or other preservatives used? How much time elapsed between collection and IC analysis? What transformations might have occurred among your redox-sensitive dissolved species?)

Depth trends - Figure 1 seems to show that all five categories of relevant genera increase in their relative proportion smoothly with depth, which seems like it could be to first order a change in dilution of the microbial population with organisms that prefer the shallower sediments (aerobes or those that subsist on fresh, relatively shallow organic matter). The significance of this first-order depth trend needs to be discussed separately in the discussion. Standard depth profiles of the five genera groups would be much easier for the reader to interpret.

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A more thorough description of how cores 42/5 and 42/6 differ sedimentologically would improve the analysis, especially since the key correlations from Figs 1 and 2 are only strongly significant in one of the cases.

Line 267 – I read this statement to mean that some representatives of each of these groups have been observed to cycle tetrathionate. Clearly, though, that does not apply to all members of these broad taxonomic groups, which makes it very difficult to tell whether the organisms might generally or facultatively cycle tetrathionate or something else entirely. Are statistics available on what fraction of, say, *Salmonella* has the genetic machinery for tetrathionate conversion?

Line 423 – Unless the data is included here, your own unpublished conclusions can't be cited like this.

Much of section 3.3 is a reporting of results without context or discussion, which is challenging to parse for key points – consider separating out your results. Figures would be very helpful. How do these rates compare with other culture studies or with the size of the porewater pools? What are these depth patterns? What do you want your reader to gain from this information?

The title of section 3.3 isn't quite true – these experiments show the presence of living organisms that can at least facultatively do these metabolisms; it does not show that they are actively conducting these metabolisms in-situ. Can you integrate this conclusion with your RNA results or show actual in-situ abundances of your cultured organisms?

Line 385 – intracellular vs extracellular tetrathionate. Most of these examples of tetrathionate producers generate tetrathionate as an intracellular intermediate species during metabolism. Please discuss how you envision other members of the microbial community accessing these species.

Line 445-453 - How do you possibly get as many as six separate samples that have

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identical observed rates (e.g., of 141 $\mu$ mol S/d/g)?

Line 492 – The authors cite three papers to support a link between MnO<sub>2</sub> cycling and pyrite oxidation to tetrathionate and other dissolved species. I have no idea what the authors are referencing, which is troubling and forces me to recommend rejection. The Berner and Petsch paper from 1998 does not include the words manganese, thiosulfate, or tetrathionate. There is similarly no mention of MnO<sub>2</sub> in the Luther 1991 paper. And, although the Jørgensen and Bak paper discusses manganese, it is in the context of “manganese or iron oxides” which could similarly be used as electron acceptors, not anything about pyrite oxidation.

Line 498 – The entire argument for pyrite oxidation by MnO<sub>2</sub> appears to be that there is detectable Mn in the sediments. (Basically all sediments have this??) There must be more one could say on this topic... depth trends? Differences between the two cores? Comparison with typical sediment Mn concentrations? Otherwise I'd leave the Mn discussion out.

I would certainly not use this discussion to conclude that “Pyrites (via abiotic reaction with MnO<sub>2</sub>) and thiosulfate (via chemolithotrophic oxidation by members of the bacterial group designated as A in Fig. 4) are apparently the main sources of tetrathionate”. This has not been demonstrated.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2019-248>, 2019.

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