

## ***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.***

**Subhrangshu Mandal et al.**

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13A. Referee's Initial Comment: 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.

C1

13A. Authors' Initial Response: WE ARE EXTREMELY SORRY for this "copy-paste" goof-up committed in the haste of submitting multiple manuscripts within the same time-window! The actual reference that should have been used is Schippers, A., and Jørgensen, B. B.: Oxidation of pyrite and iron sulfide by manganese dioxide in marine sediments, *Geochim. Cosmochim. Acta.*, 65, 915-922, [https://doi.org/10.1016/S0016-7037\(00\)00589-5](https://doi.org/10.1016/S0016-7037(00)00589-5), 2001. (Please note that this was already included in the Reference list and cited in another context of Discussion). This paper should have also been cited in this particular context of tetrathionate production from pyrite, instead of the three irrelevant references that mistakenly got inserted. Please see Line numbers 4-8 of the Abstract itself of Schippers and Jørgensen, 2001, which clearly states that “FeS<sub>2</sub> and iron sulfide (FeS) were oxidized chemically at pH 8 by MnO<sub>2</sub> but not by nitrate or amorphous Fe(III) oxide. Elemental sulfur and sulfate were the only products of FeS oxidation, whereas FeS<sub>2</sub> was oxidized to a variety of sulfur compounds, mainly sulfate plus intermediates such as thiosulfate, trithionate, tetrathionate, and pentathionate. Thiosulfate was oxidized by MnO<sub>2</sub> to tetrathionate while other intermediates were oxidized to sulfate.”

13B. Referee's Subsequent Comment: Thank you for clarifying the correct references here, your thinking is far clearer now. Although this reference does describe pyrite oxidation via MnO<sub>2</sub>, I am still unconvinced that it is relevant to the sediments in the current study. If one reads beyond the abstract of that paper, one also finds that “Below 7.5 cm, where the content of Mn did not exceed 0.2% (w/w), a dissolution of FeS<sub>2</sub> was not detectable.” One also finds that the abiotic incubations produced intermediately only for days to weeks and not longer, decreasing rapidly with depth. Manganese concentrations in the current paper (71-172 ppm) are orders of magnitude lower than the threshold for activity reported before, and there are no depth trends in either pyrite or MnO<sub>2</sub> discussed, or porewater metal ion data, that might support this mechanism as active. Purported Mn driven oxidation also appears to increase, rather than decrease, with depth, in contrast with the prior report. I do not think pyrite is a source of dissolved S species in this system; stronger evidence is required.

C2

13B. Authors' Subsequent Response: We agree with your observation that the MnO<sub>2</sub>-FeS<sub>2</sub> interaction as a source of tetrathionate has been overstretched and indeed needs more experimentation to substantiate. We agree to curtail this particular discussion component and limit only to the observation and conclusion drawn from the microbial studies.

13A&B. Authors' Changes in Manuscript: All such portions of the previous text (data and discussions) which implicated "pyrite oxidation by MnO<sub>2</sub>" as a potential source of tetrathionate in the sediment horizons explored have now been removed together with all relevant display items.

14A. Referee's Initial Comment: 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.

14A. Authors' Initial Response: Chemolithotrophic conversion of thiosulfate to tetrathionate by members of the bacterial genera *Pseudomonas* and *Halomonas* (designated as A in Fig. 4) has been experimentally demonstrated - we have shown such isolates of both *Pseudomonas* and *Halomonas* which are capable of chemolithotrophically converting thiosulfate to tetrathionate *in vitro*. Furthermore, when metagenomic sequence data obtained for each of the 25 distinct sediment-samples of SSK42/5 and 6 were assembled and annotated individually, 23 out of the 25 contig-collections obtained were found to contain genes for tetrathionate formation (namely, genes encoding sub-units of the thiosulfate dehydrogenases *TsdA* that converts thiosulfate to tetrathionate; see Denkmann et al., 2012; Pyne et al., 2018) [Table S3]. Whole metatranscriptome

C3

sequencing and analysis for the 275 cmbf sediment-sample of SSK42/6 also revealed the gene-catalog obtained via annotation of the assembled contigs to encompass homologs of the thiosulfate dehydrogenase gene *tsdA* [Table S19]. These data clearly supported the potential *in situ* functionality (metabolically active state) of thiosulfate to tetrathionate converting bacteria.

14B. Referee's Subsequent Comment: I do not dispute that thiosulfate-to-tetrathionate conversion was demonstrated and is quite intriguing; the piece of your claim that has not been demonstrated is related to pyrite. Without showing any depth trends, pore-water metal ion data, or pyrite-specific (tracer) incubations, there is no data evidencing the involvement of pyrite.

14B. Authors' Subsequent Response: We agree with your observation that the MnO<sub>2</sub>-FeS<sub>2</sub> interaction as a source of tetrathionate has been overstretched and indeed needs more experimentation to substantiate. We agree to curtail this particular discussion component and limit only to the observation and conclusion drawn from the microbial studies.

14A&B. Authors' Changes in Manuscript: All such portions of the previous text (data and discussions) which implicated "pyrite oxidation by MnO<sub>2</sub>" as a potential source of tetrathionate in the sediment horizons explored have now been removed together with all relevant display items.

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C4