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# *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.

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Dear Reviewer 2 and Dear Dr. Treude, We just felt the urgency of writing this intermittent mail so as to rectify and clarify the most serious issue that appeared in the argument regarding tetrathionate production from pyrite, and that which caused a serious misunderstanding about this process forcing Reviewer 2 to recommend rejection of the paper in its current form. While we keep working on an elaborate revision following all the other suggestions and criticisms offered by Reviewer 2 and others, below please find our responses to the key concern in hand, and kindly consider this amendment in your decision-making exercise. Yours, with regards Wriddhiman

Main comment: 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 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. Line 492 – The authors cite three papers to support a link between $MnO_2$ 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 $MnO_2$ 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.

Our 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, 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 “FeS2 and iron sulfide (FeS) were oxidized chemically at pH 8 by MnO2 but not by nitrate or amorphic Fe(III) oxide. Elemental sulfur and sulfate were the only products of FeS oxidation, whereas FeS2 was oxidized to a variety of sulfur compounds, mainly sulfate plus intermediates such as thiosulfate, trithionate, tetrathionate, and pentathionate. Thiosulfate was oxidized by MnO2 to tetrathionate while other intermediates were oxidized to sulfate.”

Allied key comment:

I would certainly not use this discussion to conclude that “Pyrites (via abiotic reaction with MnO2) 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.

Our 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 subunits of the thiosulfate dehydrogenases TsdA that converts thiosulfate to tetrathionate; see Denkmann et al., 2012; Pyne et al., 2018) [Table S3]. Whole metatranscriptome sequencing and analysis for the 275 cmbsf sediment-sample of SSK42/6 also revealed the gene-catalog obtained via annotation of the assembled contigs to encompass homologs of the thio-

sulfate dehydrogenase gene tsdA [Table S19]. These data clearly supported the potential in situ functionality (metabolically) of thiosulfate to tetrathionate converting bacteria.

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