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Biogeosciences Discussions

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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Anonymous Referee #1

General comments:

Referee's Comment: The paper investigated the role of tetrathionate as an intermediate in the redox cycling of sulfur in sediments from the oxygen minimum zone from the Arabian Sea. Using metagenomics approach, the authors find the presence of tetrathionate generating, oxidizing or reducing genes and identify bacteria potentially responsible for such processes. Through slurry incubations, the authors show the in-

volvement of tetrathionate in the microbial sulfur cycle in these sediments. Tetrathionate itself was not detected in-situ most, likely due to its reactivity. The authors propose pyrite and/or thiosulfate as potential sources of tetrathionate, which is subsequently oxidized or reduced in the system.

Authors' Response: We thank the Reviewer for his appreciation of the phenomenon unearthed in this study.

Authors' Changes in Manuscript: Not applicable.

Referee's Comment: The sampling approach is rather unusual (subsampling of oxidation critical subsamples from split-cores, see comments below). In addition, the description of the different subsamples are not entirely clear to me, which is most likely a formulation issue (see details below). Description of the analytical methods are not precise (see detailed comments below).

Authors' Response: We agree that there were some deficiencies in our explanation of the sampling and analytical procedures, so in the revised manuscript we have now described these in a more detailed and scientifically explicit manner. Improvements in these regards can be identified through changes in the text shown in the Track Changes file of the manuscript and our responses to the similar points mentioned below in your line-by-line specific comments.

Authors' Changes in Manuscript: In the revised manuscript we have now described the sampling and analytical procedures in a more detailed and scientifically explicit manner.

Referee's Comment: Large parts of the text, especially in the results and discussion part should be rewritten and be more concise. The manuscript contains unnecessary text and phrases, which make reading complicated. Many sentences are too long and sometimes the grammar is not correct such that understanding is in parts not possible. Some examples (but not all) are pointed out/detailed below.

Authors' Response: We have now addressed these concerns by removing the extraneous details, making the language lucid and sentences simple. We believe all these have added to the quality of the writing and comprehensibility of the underlying science.

Authors' Changes in Manuscript: We have now removed the extraneous details, making the language lucid and sentences simple.

Referee's Comment: The figures should be better implemented and explained in the text where appropriate. Downhole analysis of chemical species could be visualized in a depth plot to provide a quicker overview for the reader. The data table can be part of the supplement. Results from the slurry incubations could be presented in an additional figure instead of (or in addition to) the tables. This would help understanding the complex results. From reading it seems very random when and in which samples e.g. tetrathionate is oxidized and at what rates. I strongly suggest splitting results and discussion, this would help to sort out the text and help the reader understanding the story. There is also almost no discussion of the results rather than a presentation, e.g. there is no discussion of the determined rates and what they indicate etc.

Authors' Response: We agree with these suggestions, and have now taken the following measures to ensure that the problems associated with the text and display items are all ameliorated.

Authors' Changes in Manuscript:

- More citations and explanations for the figures have been included in the text. - Analyses of chemical species along the sediment-cores have now been presented in the form of depth plots; accordingly, Table 1 is now moved from the main text to the Supplementary Information. - A new Figure (numbered as 4 in the revised manuscript) has now been incorporated for the results of the slurry incubation experiments. We believe that the visual impact of the data is now instrumental in an easier comprehension of the complex results pertaining to the formation and/or oxidation of tetrathionate, plus the reduction of tetrathionate. - Results and Discussion sections have now been split.

Implications of all the current findings (results/data) have been explained adequately in the new Discussion section.

Referee's Comment: Collectively, I think this manuscript needs a major overhaul with focus on precise description of the sampling and methods and separation of results and methods including a proper and streamlined discussion, before the scientific merit can be judged. The extend of required rewriting including methods, results and discussion extends what is justifiable as a revision. However, I would emphasis a re-submission as a new manuscript once rewritten.

Authors' Response: We have now overhauled the manuscript exactly as suggested.

Authors' Changes in Manuscript: Besides more streamlined description of the methods and sampling procedure, separation of Results and Discussion sections, and result-specific discussions, we have now included new lines of metagenomic, genomic and metatranscriptomic data (and their corresponding discussions) in the revised manuscript. New display items have also been provided for the existing data. Furthermore, we have now deleted a number of such geochemical data that were inexplicable under the current state of knowledge on the biogeochemical system explored in this study.

Referee's Comment: In the following I provide many details, but this may not be complete. Specific comments (incl. few technical comments):

Abstract Line 25: introduce msbl, also: no dash between number and unit (msbl) here.

Authors' Response: We agree, and have now fixed this problem.

Authors' Changes in Manuscript: Mbsl has been defined and the 'dash' removed.

Referee's Comment: Lines 27/28: I suggest to be more precise and name the processes instead of generally speaking about "these metabolisms" or "these processes"

Authors' Response: We agree that the use of precise names for anything technical is

always preferable in scientific literature.

Authors' Changes in Manuscript: However, since in the present case the words "microbial formation, oxidation and reduction of tetrathionate" are appearing twice within the same sentence we prefer to mention the proper nouns initially and use the pronoun "these metabolism" in the second occasion.

Referee's Comment: Line 29: Provide conditions of the incubations under which you could observe tetrathionate generation or turnover. (What types of slurry-incubations, i.e. with amendment of tetrathionate or thiosulfate: : : etc.)

Authors' Response: We agree, and have now provided all the warranted information.

Authors' Changes in Manuscript: Here in the abstract, we have now mentioned the media compositions used in the slurry incubation experiments; more details are already there in the main text.

Referee's Comment: Lines 31/32: Can you calculate a molar concentration or g/sed for iron and manganese instead of giving ppm

Authors' Response: We agree that in any scientific literature molar concentrations are more preferable than ppm values.

Authors' Changes in Manuscript: Incidentally, however, all data and discussions pertaining to the in situ concentrations of iron and manganese have now been removed from the revised manuscript following the suggestions of Reviewer 2.

Referee's Comment: Line 34: instead of "converted" use "oxidized" here and throughout the manuscript (similarity use "reduced" if applicable)

Authors' Response: We agree, now fixed.

Authors' Changes in Manuscript: Instead of "converted" we have used "oxidized" here and throughout the manuscript.

Referee's Comment: Line 35: delete "back"

Authors' Response: Please note that the very preceding sentence reads as "Thiosulfate oxidation by chemolithotrophic bacteria prevalent in situ is the apparent source of tetrathionate in this ecosystem"; so, in this succeeding sentence, we think that it would be more appropriate to write "tetrathionate can be reduced back to thiosulfate" than simply say "tetrathionate can be reduced to thiosulfate".

Authors' Changes in Manuscript: Sentence has been restructured to make the above sense clearer.

Referee's Comment: Line 35: avoid "0" as a concentration, it reads odd. 0 means absence, so 0-2 mM present is wrong as 0 means not present. You could write e.g. up to 2 mM

Authors' Response: We agree.

Authors' Changes in Manuscript: Have now written "up to" wherever applicable.

Introduction

Referee's Comment: Line 45: delete "running"

Authors' Response: We have now done as suggested.

Authors' Changes in Manuscript: Deleted "running".

Referee's Comment: Line 48: delete "So"

Authors' Response: Done.

Authors' Changes in Manuscript: Deleted "so".

Referee's Comment: Line 54: Delete: "In this context" – unnecessary

Authors' Response: We agree.

Authors' Changes in Manuscript: "In this context" is now deleted.

Referee's Comment: Line 55: replace/reformulate "seldom appreciated" by rarely investigated or similar

Authors' Response: We agree.

Authors' Changes in Manuscript: We have now reworded this as suggested.

Material and Methods

Referee's Comment: Line 77: delete "the"

Authors' Response: Here we are referring to two specific cores out of the total 8 collected on board SSK42. Moreover, the first mention of two SSK42 cores constituting the raw material of this study had already happened only seven lines ahead of this line, so the article "the" should be used here ahead of the words "two gravity cores".

Authors' Changes in Manuscript: No change made.

Referee's Comment: Line 78: delete "on which the present study is based" (unnecessary text)

Authors' Response: Here we to introduce the readers to the core nomenclature and at the same time convey that out of the ten SSK42 cores, which are being studied and reported (from distinct biogeochemical perspectives) in a series of recent publications by our group, the 5th and 6th (already named in Fernandes et al., 2018 as SSK42/5 and SSK42/6) are the ones on which the present study is based.

Authors' Changes in Manuscript: We have now restructured this sentence to convey the above sense more appropriately.

Referee's Comment: Lines 81ff: The sampling strategy is unusual. Oxidation sensitive sample were collected after splitting the core into two halves. To prevent oxidation a shower of N2 was applied. How was this realized to ensure that no oxidation occurred? Usually smaller hole round core sections are subsamples inside an anaerobic camber (glove box) or subsamples are taken with cut-off syringes via small holes cut in the side

of a liner or alternative from fresh cuts during sectioning. Al halve split exposes large areas to air even though somehow a N2 shower was installed this sees quite unusual. Was this split done at the entire 3 m core? How was a N2 shower over the 3-m length maintained during the sampling of the 10 – 20 subsamples from each core?

Authors' Response: We have now overhauled the portion dealing with sampling details in such a way that all confusions emanating from the previous text are now resolved. This said, it is noteworthy that answers to these questions were already there in our previous publication Fernandes et al., 2018, which also dealt with these SSK42 cores (albeit form other perspectives) and was cited amply throughout the manuscript, including the sampling-related section. We had therefore thought that further repetition of the details would be unnecessary and also potent causes of unintended self-plagiarism. However, now we understand that as an independent paper this manuscript should carry its own sampling details and have therefore brought back many of those details taking sufficient care of literary repetitiveness.

Authors' Changes in Manuscript: The following has now been added to the revised manuscript.

- In order to protect the ASOMZ sediment-samples from aerial oxidation, the entire cores were not split open into two D-shaped halves directly; instead only one $\sim$15-cm-long C-shaped part of PVC core-liner was removed at a time, as shown in a new Supplementary Figure added to the revised manuscript. We have now restructured the old sentences and written additional new lines to explain this in an unambiguous way. - The 15 cm length exposed at a time for sampling was constantly and closely showered with high-purity N2 emitted from multiple nozzles fitted to multiple nitrogen-generators. This contrivance was sufficient to prevent atmospheric oxidation of the 15 cm exposed surface of the core. - Immediately after the C-shaped longitudinal part of the PVC core-liner was cut open, top one cm of the exposed surface was scrapped off along the core-circumference using sterile scalpels to eliminate potential contaminations from the core-liners' inner-surfaces and/or sea-waters through which the cores had passed.

- Subsequently, to sample a particular sediment-depth of the core for microbiological studies, an approximately 5-mm-thick sediment-slice (spanning equally on either side of the core-height marking) was scooped out with a sterile scalpel and put into a sterile polypropylene bottle. The head-space of every sample-containing bottle was flushed with high-pure N2, following which it was sealed with Parafilm - At the same time, for on board extraction of pore-waters, sediment-samples from a particular depth were taken out by inserting sterile 50 ml cut off syringes deep inside the core cross-section, multiple times along the circumference on the exposed 'C half'; the samples were immediately collected in sterile 50 ml centrifuge tubes; and all these were carried out under focused streams of high-pure N2.

Referee's Comment: What are the "adequate measures" to avoid contamination? Does this refer only to the use of sterile spatulas? Were the sample bottles autoclaved?

Authors' Response: It was already written in the previous manuscript and has now been made more informative. Also please note that ll the samples vials, spatulas and bottles used for sampling were sterile. They were either autoclaved on board or purchased as sealed packs of gamma irradiation sterilized laboratory wares.

Authors' Changes in Manuscript: The previous sentence (lines 84-86 of previous manuscript) reading "Adequate measures were taken to avoid post-sampling contamination of the native microbial communities and physicochemical alteration of the geochemical properties of the sediments" has now been removed. The previous articulation regarding the measure I question has been edited as "Immediately after the C-shaped longitudinal part of the PVC core-liner was cut open, top one cm of the exposed surface was scraped off along the core-circumference using sterile scalpels to eliminate potential contaminations from the core-liners' inner-surfaces and/or seawaters through which the cores had passed.

Referee's Comment: Line 92: fractions means subsamples?

Authors' Response: Yes, here fractions means subsamples.

Authors' Changes in Manuscript: The term "sample fraction" has now been replaced by "sample replicates".

Referee's Comment: Line 93: "while one fraction, each for chemistry : : :" you mean two subsamples, one for chemistry and one for microbiology?

Authors' Response: The Reviewer's understanding of the sentence is exactly same as what was meant.

Authors' Changes in Manuscript: The term "sample fraction" however has now been replaced by "sample replicates".

Referee's Comment: Line 99: ion chromatography

Author Response: The typing error is now fixed.

Authors' Changes in Manuscript: The typing error is now fixed.

Referee's Comment: Line 101: what is the number in brackets? A catalog number? (should be removed).

Authors' Response: The number in the brackets are model numbers and we think that the IC detector's model number would be important here.

Authors' Changes in Manuscript: However, the column model number has now been deleted.

Referee's Comment: Line 106: "passed through : : :membranes" – the samples were probably "filtered" – with syringe filters?

Authors' Response: Yes, volumes of the samples were already mentioned to be in the order of μL, so it could be filtered only using a syringe.

Authors' Changes in Manuscript: Following your suggestion we have now added the word "filters" after the word "membrane", so this sentence in the revised manuscript now reads as "pore-water samples were ... passed through 0.22 μm hydrophilic

polyvinylidene fluoride membrane filters".

Referee's Comment: Line 108: Please more details on the method calibration: What standards were used for calibration, what calibration, how many points? External? What does "sample reproducibility," mean analytical precision? How was this determined, by how many replicate measurements of the same sample? The value should be given in molar concentration if for a specific sample or in RSD (%) if it refers to the precision of the method itself.

Authors' Response: We agree, and have now provided all details for the method of IC calibration.

Authors' Changes in Manuscript: We have now added the following here.

Analytical grade thiosulfate IC Standard [ICS024, Sigma Aldrich, (St. Louis, MO, USA)] was used to prepare the calibration curve for quantification of this anion. Three different concentrations of thiosulfate, 0.5 μM, 5 μM and 20 μM, were measured for the construction of calibration curve by plotting peak height against concentration. Based on triplicate analyses of the standards, deviations from actual concentrations were found to be less than 2.5%.

Referee's Comment: Line 129 ff: for the determination of AVS and CRS fractions, original literature should be cited. How was Ag2S quantified, gravimetrically?

Authors' Response: Yes, Ag2S was quantified gravimetrically following Canfield et al., (1986): Canfield, D.E., Raiswell, R., Westrich, J.T., Reaves, C.M., Berner, R.A. 1986. The use of chromium reduction in the analysis of reduced inorganic sulfur in sediments and shales. Chem. Geol. 54, 149-155.

Authors' Changes in Manuscript: However, in agreement with some of the comments of Reviewer 2 we have now removed those portions of the manuscript which envisaged in situ production of tetrathionate/thiosulfate from pyrite; accordingly, methods concerning the estimation of pyrite have also been removed from the revised text.

Referee's Comment: Line 261: 0% partial pressure? Pressure unit is not percent. Also 0 probably means anoxic?

Authors' Response: Yes, 0% partial pressure means anoxic; the workstation was set at this specification following the manufacturer's instruction.

Authors' Changes in Manuscript: Not applicable.

Referee's Comment: Line 268ff: This is the standard cline protocol, which is widely used and generally accepted - not necessary to describe the principle.

Authors' Response: We agree.

Authors' Changes in Manuscript: We have now removed the mention of the principle.

Referee's Comment: Line 286: what does serially diluted mean?

Authors' Response: Serial dilution as a basic microbiological practice is very much similar to that in chemistry. Here a microbial inoculum (cell suspension) is sequentially diluted by orders of magnitude so as to reduce the density of cells in the suspension to such levels which give rise to manually-countable colonies when the suspension is spread out on solid media plates.

Authors' Changes in Manuscript: Not applicable.

Referee's Comment: Line 288: here and elsewhere, please use until instead of till. As this is a scientific article, and till is considered to be informal which should be avoided.

Authors' Response: We agree and have now done so.

Authors' Changes in Manuscript: "Until" used instead of "till".

Referee's Comment: Line 289: "pure-plates"?

Authors' Response: Pure-plates refer to those microbial culture plates which have only one type of colonies, evidently representing one type of bacterium (strain).

Authors' Changes in Manuscript: Not applicable.

Results and discussion

Referee's Comment: Line 327 "relevant (microorganisms)" ?

Authors' Response: The sentence here reads as "Tetrathionate-forming, oxidizing, or respiring genes and relevant microorganisms are abundant in ..."; so it is presumable that the microorganisms, like the genes identified, also refer to tetrathionate forming/oxidizing/reducing entities.

Authors' Changes in Manuscript: Not applicable..

Referee's Comment: Line 329-334: Very long sentence- almost not understandable: consider splitting and rewriting.

Authors' Response: We agree.

Authors' Changes in Manuscript: The entire paragraph spanning between the previous line numbers 329-343 have now been simplified; as a part of this restructuring, the sentence in question has also been split into two.

Referee's Comment: Line 334 "were found to contain" – shorter "contained"

Authors' Response: Done, as suggested.

Authors' Changes in Manuscript: Extra words deleted.

Referee's Comment: Line 341: Unpublished data should not be cited if not necessary. Here are 5 other references given. The reference to unpublished data is unnecessary.

Authors' Response: Done as suggested.

Authors' Changes in Manuscript: The unpublished data are not cited anymore.

Referee's Comment: Line 391-395: Long and unclear sentence.

Authors' Response: We agree.

Authors' Changes in Manuscript: We have now rewritten this sentence in a lucid way.

Referee's Comment: Line 406 – 411: Long and unclear sentence.

Authors' Response: We agree.

Authors' Changes in Manuscript: We have now rewritten this sentence in a lucid way.

Referee's Comment: Line 423: The discussion refers here to another unpublished paper. The suggestion/discussion here is based on unpublished data from the authors. Such data should either be included in this manuscript or published first. Alternatively, the results should be discussed in the light of other already published studies. Otherwise, this discussion is not solid.

Authors' Response: For these data illustrating the feasibility of aerobic metabolism in these sediment horizons, please note that the same constitute a completely separate paper of our group, under consideration elsewhere, and those data are too voluminous to be incorporated here. Anyway, in this paper we have now provided the key data as Supplementary Information so that the reader can make sense of what is there.

Authors' Changes in Manuscript: We have now mentioned in this revised manuscript that genes for aerobic respiration by aa3-/cbb3-type cytochrome-c oxidases (coxABCD / ccoNOPQ) and cytochrome-bd ubiquinol oxidase (cydABX / appX) were identified in the assembled metatranscriptome from 275 cmbsf of SSK42/6 in general, and the portions of the metatranscriptomic dataset in particular which matched with sequences from the tetrathionate-oxidizing isolates, thereby suggesting that potential activity of this aerobic metabolic process is possible in this environment (the relevant gene and transcript Tables have also been incorporated in the revised manuscript as Supplementary Materials).

Referee's Comment: Lines 425 ff. The results of the incubation appear very unsystematic or random. A figure could help for an overview. The writing is also not precise, i.e. the "samples" do not convert thiosulfate to tetrathionate : : : conversion was observed

in the samples or the organisms in the sample convert the species: : :

Authors' Response: We agree.

Authors' Changes in Manuscript: We have now made the presentation of all these results more systematic by adding new Figures and amending the language in the text in such a way that it is clear to the reader that the organisms in the samples converted the sulfur species. Furthermore, we have also amended the corresponding Methods section to put the principles of the slurry incubation experiments and the subsequent data in proper context.

Referee's Comment: Line 430ff: "In contrast, : : :" The sentence is very long. Also, there is no "contrast" obvious. "free and detectable" is unnecessary.

Authors' Response: We agree, but in this context it is also noteworthy that the data presented in the lines 430ff regarding the thiosulfate oxidation property of the 45, 60 and 295 cmbsf communities of SSK42/5 are distinct from those concerning 0, 15, 90 and 160 cmbsf presented just in the previous sentence; hence the succeeding sentence ought to start with "In contrast".

Authors' Changes in Manuscript: We have now simplified this sentence as well as the entire text in this Results section; accordingly, the phrase "free and detectable" has been removed.

Referee's Comment: Lines 434ff: the samples do not metabolize. Organisms have a metabolism but not a sediment samples. In this entire section is not clear how the rates were determined. A figure might help

Authors' Response: We agree.

Authors' Changes in Manuscript: Have now replaced the word "metabolize" by the word "oxidize" in the context of this sentence. In addition, a number of lines within this section have been restructured, while a more elaborate description of the rationale behind the determination of rates has been provided. The entire set of data has also

been presented graphically.

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