Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2019-471-AC3, 2020

Biogeosciences Discussions
Open Access
EGU

Interactive comment on "Cryptic role of tetrathionate in the sulfur cycle: A study from Arabian Sea sediments" *by* Subhrangshu Mandal et al.

Subhrangshu Mandal et al.

wriman@jcbose.ac.in

Received and published: 24 March 2020

Anonymous Referee #3

Referee's Comment: Mandal and co-authors correctly point out tetrathionate, although seldom detected, may be an important intermediate in sulfur cycling in marine sediments. This is especially born out by the number of organisms that carry enzymes capable of reducing, oxidizing or even disproportionating tetrathionate. To examine potential tetrathionate sampling in marine sediments, the authors examined sulfur speciation in two long gravity cores, performed sediment slurry experiments, enriched tetrathionate reducingorganisms, and performed an extensive metagenomic analysis

Printer-friendly version

Discussion paper

on the two cores. My expertise does not lie in the field of metagenomic analysis, so I will limit my comments to the sulfur analyses and experimental set-ups.

Authors' Response: We thank the Reviewer for appreciating the overall objective and importance of the study. As for the details, following multiple reviewers' suggestions received previously, the entire set of results and discussions has now been streamlined in such a way as to be based, almost exclusively, on microbiological and omics data; so relative unawareness about these approaches may constraint specific appraisals.

Authors' Changes in Manuscript: Not applicable.

Referee's Comment: The authors took care to avoid oxidation artefacts by blowing inert dinitrogen gas over the cores and sample tubes. Was this done in a laminar flow hood? My experience is that blowing dinitrogen gas over samples in an open environment tends to entrain oxygen from the air and actually increases the flux of oxygen to surfaces. If the samples are taken out quickly and placed into vials containing inert atmospheres, this may not be much of a problem. However, the thiosulfate/sulfide data presented in Figure 3 shows that thiosulfate concentrations track those of sulfide with a 1:100 ratio. Could this be simply oxidation during sample handling?

Authors' Response: We thank the Reviewer for sharing important experiences with us. As for the present study, the existing Methods section already stated that samples were taken out immediately after cutting open only small C-halves of the PVC core-liners and placed into vials containing inert atmosphere, so O2-contamnation of samples was bare minimum (moreover, had O2-contamnation been significant we would not have detected the depth-trends for sulfide and methane in the different SSK42 cores; please see our previous paper Fernandes et al., 2018, Sci Rep: 8, 8665).

Sulfide is a potent source of thiosulfate in all marine sediments (Jørgensen, 1990). Sulfide, when present in sediment-cores, can abiotically reduce tetrathionate to thiosulfate and elemental sulfur (Rowe et al., 2015). Sulfide can also be produced alongside thiosulfate on account of microbial tetrathionate reduction (Barrett and Clark, 1987; Price-

Printer-friendly version

Discussion paper

Carter et al., 2001). Depth-trends of sulfide concentration, therefore, are expected to show certain degree of correlations with trends of thiosulfate concentration. Moreover, the pore-water thiosulfate concentrations detected in SSK42/5 and SSK42/6 were well within the range reported from physicochemically similar sediment horizons across the global ocean; for instance, Black sea sediments have 0 - 5.2 μM thiosulfate (Zopfi et al., 2004), Kysing Fjord (Denmark) sediments have <1-10 μM (Troelsen and Jorgensen., 1982).

Authors' Changes in Manuscript: These issues have now been discussed briefly in the revised manuscript.

Referee's Comment: The method for determining tetrathionate is highly unspecific. The thiocyanate resulting from the cyanolysis will include zerovalent sulfur contained not only in tetrathionate and polythionates, but also zerovalent sulfur contained in polysulfides and colloidal sulfur (See for instance Kamyshny et al., 2009, Geostandards and Geoanalytical Research, or Kamyshny, 2010, Marine Chemistry). The thiocyanate analysis is also problematic in saline solutions. There are far more compound specific methods for determining thiocyanate and tetrathionate (See for instance, Rong et al., 2005, Chromatographia; Bak et al., 1993, FEMS Microbiology Ecology).

Authors' Response: We agree that there are other specific methods available for quantifying tetrathionate and other polythionates. But the method described by Kelly and Wood (1994: Synthesis and determination of thiosulfate and polythionates. Methods in Enzymology 243, 475-501) is also a time-tested, sensitive and reliable method. Several seminal research papers on microbial sulfur-chemolithotrophy, including many from our group (Alam et al., 2013, Applied and Environemntal Microbiology: 79, 4455–4464; Pyne et al., 2018, Molecular Microbiology: 109, 169-191), have used this method to quantify tetrathionate reproducibly and precisely, within culture media containing high amount of total dissolved solids and mixtures of sulfur species such as thiosulfate, polysulfides, sulfur and sulfate.

Printer-friendly version

Discussion paper

We also agree that in Cyanolytic Method, thiosulfate as well as polythionates react with cyanide to form thiocyanate, which is subsequently measured spectrophotometrically as ferric thiocyanate after reacting with ferric nitrate. Notably, however, differences in the reactivity of the thionates with cyanide enable their discrimination and quantitative characterization within mixtures of such compounds. For instance, trithionate is stable at high pH and reacts with cyanide only at elevated temperatures; thiosulfate reacts with cyanide at room temperature, albeit only in the presence of copper(II) catalyst; in contrast, the higher polythionates (SnO62-, where n = 4 or more) react rapidly with cyanide at room temperature to form SCN–, S2O32–, SO42– and HCN. Furthermore, in the current context it is noteworthy that the importance of this paper lies in the multiple lines of evidences provided by culture-independent and culture-dependent microbiological data to point out the role of tetrathionate in the sulfur cycle. So, if subsequent geochemical explorations of these Arabian Sea sediment horizons indeed reveal the presence of tetrathionate in the pore-waters then it will only reinforce the possibilities already pointed out in this paper based on the process-detection power of molecular microbiological tools.

Authors' Changes in Manuscript: These issues have now been discussed in the revised manuscript.

Referee's Comment: In the slurry experiments, the authors used thioglycolate to reduce the media. Thioglycolate is a thiolic reducing agent that will reduce disulfide bonds. Did the authors test this on tetrathionate? I suspect that it may also react with zero valent sulfur in colloidal sulfur, polysulfides and polythionates to release thiosulfate.

Authors' Response: Sodium thioglycolate was used as an O2 scavenger only for the anaerobic RVTr medium. During RVTr preparation, inside a Whitley H35 Hypoxystation preset to 0% partial pressure of O2, pre-weighed amount of potassium tetrathionate salt was first dissolved in a premeasured volume of anoxic deionized water (degassed for several hours inside the H35 Hypoxystation till the resazurine indicator added in

Printer-friendly version

Discussion paper

the water became colorless). The anoxic tetrathionate solution was then added via filter-sterilization to a separate pre-autoclaved solution that contained the rest of the RVTr components in an appropriate volume and had cooled down to room temperature within the Hypoxystation. Thioglycolate that was there in the second solution had already reacted irreversibly, during autoclaving, with the dissolved O2 present in the mixed-salts solution to form dithiodiglycolate. Post-autoclave cooling of this second solution within the Hypoxystation, therefore, did not breakdown the S-S bonds of dithiodiglycolate to regenerate the SH–containing thioglycolate for a second round of action on the incoming tetrathionate solution. The one-time usability of thioglycolate (as a reducing agent) in the present set-up, is further evidenced by the following common laboratory experience: media solutions already rendered anoxic via autoclaving with thioglycolate get contaminated with dissolving O2 upon the slightest exposure to air (this is reflected in the stable regeneration of red color by resazurine indicator added to the media) because the thioglycolate already converted to dithiodiglycolate cannot reduce infiltrating O2 once again. Moreover, in this context, it is further reassuring that at neutral pH, thiol-group-containing reducing agents do not attack tetrathionate under non-enzymatic (abiological) conditions (Pyne et al. Molecular Microbiology: 109, 169–191).

Authors' Changes in Manuscript: That tetrathionate was added separately, via filter sterilization, to a pre-autoclaved solution containing rest of the components of the RVTr medium was already mentioned in the previous manuscript. Anyway, in the latest re-revised manuscript, we have now clarified the procedure more elaborately (including the points mentioned above) to remove any doubt that may be there.

We have also mentioned the following two points to prove that there was no possibility of thioglycolate attacking the tetrathionate of the RVTr medium. - Zero hour reading for all the slurry incubation sets in RVTr medium showed the intact presence of the 10 mM tetrathionate originally supplied in the medium. - Abiotic control incubations involving autoclaved sediment-samples showed that the 10 mM tetrathionate originally

Printer-friendly version

Discussion paper

supplied to the RVTr medium was almost intact after seven days of incubation. We had forgotten to include these routine, but potentially critical, negative data in the previous manuscript, so have now added them in this second round of revision.

Referee's Comment: While the genomic approach may be adequate to describe the distribution of potential organisms involved in S cycling, given the non-specificity of the analytical methods employed, I am afraid that the authors cannot draw any conclusions at all about the sulfur intermediate oxidation state cycling in the cores or in the experiments.

Authors' Response: We agree that concentrations and isotopic ratios of the various chemical constituents of sedimentary solid-phases and pore-fluids have long been central to the deciphering of in situ biogeochemical pathways. Significant information on the carbon-sulfur-iron cycles of modern marine and lacustrine sediments have been generated in this way; currently, however, there is an increasing consensus that several questions in biogeochemistry - such as those concerning sulfur compounds oxidation/dispropotionation, relative importance of simple fatty acids catabolism and anaerobic methane oxidation in sedimentary sulfate reduction, and biogeochemical processes within sulfate-methane transition zones - cannot be answered from preserved geochemical records alone. In recent times a lot of advancement has taken place in our overall understanding of carbon-sulfur cycling in marine systems by virtue of data obtained from metagenomic, metatranscriptomic, and in situ as well as in vitro geomicrobiological experiments. Forensic-level detection power of these approaches in unearthing such cryptic biogeochemical processes that do not get manifested, or leave their imprints, as detectable geological records, have been demonstrated in a number of recent papers that revealed such microbial community functions using meta-omics approaches which would have been considered improbable based on geochemical manifestations alone. Canfield et al., 2010, Science: 330, 1375-1378 (A Cryptic Sulfur Cycle in Oxygen-Minimum-Zone Waters off the Chilean Coast) and Garcia-Robledo et al., 2017, Proc Natl Acad Sci USA: 114, 8319-8324 (Cryptic oxygen cycling in anoxic

Printer-friendly version

Discussion paper

marine zones) are only a few examples to mention in this regard.

Authors' Changes in Manuscript: This key issue has now been discussed in the re-revised manuscript.

Referee's Comment: The experiments also lead to rates of tetrathionate reduction and oxidation that are spectacularly high (nearly a thousand fold greater than rates that one would expect for sulfate reduction in these deep sediments). Also something is wrong with the units in Figure 3 (umol/L*day*g?)

Authors' Response: The sole objective of the slurry incubation experiments was to check whether the tetrathionate-metabolizing bacteria were alive in situ (their active state in the sedimentary habitat was subsequently corroborated by pure-culture isolations and metatranscriptome analysis). The in vitro rates of tetrathionate formation, oxidation and reduction obtained in these experiments under specific media and culture conditions are not expected to have any correspondence with the actual rates of such processes potentially operating in situ. This could be explained as follows. When a natural sample is incubated in selective culture media certain specific microbial species present in the sample often outgrow all metabolic competitors by virtue of higher substrate affinity and culture-condition suitability. Consequently, the growth/substrate-utilization phenotype(s) manifested by such enriched consortia are actually contributed to by the selected few rather than the entire community of metabolic equivalents present in the sample (Roy et al., 2016).

As for Figure 3, there is nothing wrong in the unit (μmol S day-1 g sediment-1) used to express the in vitro rates of the different tetrathionate-metabolizing processes. Since the individual panels of the figure involve different transformations between different sulfur species (having different valencies of sulfur) - for instance, conversion from thiosulfate to tetrathionate, tetrathionate to sulfate, and tetrathionate to thiosulfate/sulfide - for all the individual slurry incubation experiments, the substrate quantities depleted from the spent media have been expressed in equivalence of sulfur atom concentra-

tions.

Authors' Changes in Manuscript: The above explanations have now been more explicitly spelt out in the revised manuscript.

Referee's Comment: Finally, the authors have missed key earlier work on this topic that addresses specifically the distribution and cycling of tetrathionate, thiosulfate and sulfite in marine sediments: Bak et al., 1993; and in particular Zopfi et al., 2004, Distribution and fate of sulfur intermediates – sulfite, thiosulfate, tetrathionate and elemental sulfur – in marine sediments. Geol Soc America Sp. Paper 379, and more recently, Findlay, A. J., & Kamyshny, A. (2017) Turnover Rates of Intermediate Sulfur Species (Sx2-, S0, S2O32-, S4O62-, SO32-) in Anoxic Freshwater and Sediments. Frontiers in Microbiology, 8. https://doi.org/10.3389/fmicb.2017.02551

Authors' Response: We agree that these are important works in the context of distribution and cycling of intermediate sulfur species in diverse environments, including marine sediments, so have included them in our re-revised Discussion section, in proper perspective of the current findings.

These papers, based on data from geochemical experiments and preserved records, have revealed the occurrence and complex transformations of intermediate sulfur species, including tetrathionate, in diverse aquatic ecosystems, but none involved microbiological indicators for the role of tetrathionate as a key junction of sulfur cycling in marine sediments. Bak et al. (1993) had measured tetrathionate, trithionate and thiosulfate in diverse natural samples, while Zopfi et al. (2004) used different techniques of analytical geochemistry to track the fates and turnover times of sulfur cycle intermediates in non-sulfidic sediments of the Black Sea and North Sea. While the latter paper revealed the presence of tetrathionate in the sediments and delineated potential pathways for its transformation in situ, no microbiological corroboration of their findings was carried out. Findlay and Kamyshny (2017) has envisaged the potential fates and transformation rates of intermediate sulfur species in lacustrine water-columns and

Printer-friendly version

Discussion paper

sediments by introducing and tracking 35S-labeled sulfur compounds in the samples. In this context, our explorations based on the Arabian Sea sediments is unique in using microbiological findings to reveal tetrathionate as a key intermediate of the sulfur cycle, and identifying the potential biochemical pathways for its formation and transformation in situ.

In this context it is further noteworthy that both Zopfi et al. (2004), and Findlay and Kamyshny (2017), detected tetrathionate in non-sulfidic ecosystems, whereas the sediment horizons explored in the present study contained high concentrations of sulfide, which can readily react with tetrathionate to form thiosulfate, and elemental sulfur or polysulfides (Podgorsek and Imhoff, 1999; Schippers et al., 1999; Schippers and Jørgensen, 2001; Zopfi et al., 2004). This said, if future geochemical explorations of these territories, using more sensitive analytical techniques, reveal the presence of tetrathionate in the pore-waters, then such finding would not violate, but rather reinforce, our microbiology-based forecast of the key role of tetrathionate metabolisms in the sedimentary sulfur cycle.

Authors' Changes in Manuscript: The above mentioned points have now been included in the upgraded Discussion section of the re-revised manuscript.

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2019-471, 2019.

Printer-friendly version

Discussion paper