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Biogeosciences Discussions

Interactive comment on "Microbial conversion of inorganic carbon to dimethyl sulfide in anoxic lake sediment (Plußsee, Germany)" *by* Y.-S. Lin et al.

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First of all, we thank the referees for their thorough and constructive reviews. We sincerely appreciate their effort in helping us to improve this manuscript. Please find our responses to the general and specific comments below.

General comments

From Dr. Pohlman:

"Given the results were all obtained from incubations conducted at 55°C in freshwater sediments, it is not clear to this reviewer if the authors suggestion that this DMS-forming pathway is applicable to sulfate-methane transition zones that generally occur at relatively low temperatures (e.g. <5°C) in marine sediment. The most likely setting for

observing this process in the nature is within "hydrothermally influenced" systems."

We rephrase the text in the section "Implications for anoxic environments" to express our view more clearly. Although we obtained our results only in incubations at higher temperature, the possibility to detect this process at lower temperatures should not be excluded. We suggested that the high temperature during incubation facilitates this reaction by increasing the availability of substrates (MT, CO2 and H2) to methanogens. Accordingly we consider that there are low-temperature environments with similar chemical conditions that deserve further examination with sulfate-methane transition zones in marine sediment being one of them.

From Dr. Kiene:

"The abstract could use some work to make it clearer and more logical. It is understandable– after one has read the entire manuscript, but on its own, I don't think it has a logical flow. For example, on line 14 the sentence 'Labeling with NaH13CO3 showed that incorporation of bicarbonate into DMS occurred through methylation of MT (methanethiol)' does not make it clear that the bicarbonate was fixed via a reduction pathway of methanogenesis (thereby explaining the light 13C of the methyl of DMS) and it was methyl Co-M that was the methyl donor for methanethiol methylation. Likewise, the conclusions don't logically follow from preceding sentences. It would be better to say something like: BES inhibited DMS formation suggesting that methanogens were involved in production of DMS. Something like that."

We follow the suggestions and reorganize the abstract. We hope that the clarity of the revised has been improved.

"It might be worth pointing out somewhere in the manuscript that DMS consuming methanogens apparently use the reverse of the final methylation to generate methyl Co-M from Co-M, releasing MT in the process."

A sentence addressing this point is inserted into the third paragraph of Section 4.1

“Microbial DMS formation”.

“What were the sulfide concentrations in the lake sediment slurries? In the presence of sulfide, the inhibitor BES is converted slowly to Co-M (the compound BES mimics) – see Kiene, 1991. I doubt that this would have affected any of their results, but at the elevated temperatures used in this study perhaps it might have been important. In any case, it is something the authors should be aware of.”

1. Eller et al. (2005) reported that sulfide concentrations in the bottom lake water were about 50 μmol L-1. In our incubation experiments, Na2S was added either to a final concentration of 50 μmol L-1 as a reducing reagent or four times higher as a substrate. We only monitored sulfide concentrations during the H235S-labeling experiment; sulfide concentrations in the aqueous phase were not higher than 50 μmol L-1.

2. Kiene (1991) found that in the presence of 2 mmol L-1 sulfide, the inhibitor BES (1 and 10 mmol L-1) is converted slowly to Co-M abiotically. Unlike the experimental conditions of Kiene (1991), sulfide concentrations in our system were much lower while BES concentration was 20 mmol L-1. Thus a conversion of BES in large quantities was unlikely, though the higher incubation temperature may have accelerated this process. Without analytical data, it is hard to tell if and to which extent such a reaction is generating CoM and counteracting the effect of BES. We agree with the reviewer that for highly sulfidic and warm environments, this reaction should be monitored if inhibition of methanogenesis by BES is desired.

“Was the pH of the sediments measured? Flushing with N2 could remove CO2 and could change the pH and of course the addition of HCO3- could also affect (or buffer) the pH. How might this have affected the results?”

Unfortunately, we did not monitor the pH values over the course of experiments. There were neither published pH data for the Plußsee sediment or sediment slurries. The effect of flushing with N2 and hence removal of CO2 may have driven the slurries toward higher pH, whereas addition of bicarbonate should have buffered the system in

the range of 7 to 8. Based on Eq. (2), we expect that the DMS-forming process will become more favorable at a high proton concentration. Therefore, the DMS-forming process may have been even more prominent if the slurries were not buffered at neutral pH.

Specific comments

From Dr. Pohlman:

“Regarding the substrate addition experiment, the authors suggest there is no stoichiometric relationship between the production of DMS and MT. I mildly disagree with this point as the rate of the DMS production moving across the columns in Fig 2 increases continuously while that of MT production decreases continuously. To me, this inverse relationship implies increasing rates of DMS production are linked to a diminishing accumulation of MT, as would be expected from Eq. 2. I agree there is not a 1:1 stoichiometric ratio for the relationship, which is certainly related to the uncertainty in the source of the MT. This relationship is brought forward in the first paragraph of the discussion, which indicates an important experimental result. However, the point of discussion is based on the inhibition study. . .I believe this experiment also supports that important conclusion. Consequently, I feel this point should be emphasized now to prepare the reader for that discussion point.”

We agree with the reviewer on this observation. We modify our text in Section 3.1 “Addition of substrates” to address this point. Furthermore, this point is also added into the first paragraph of Section 4.1 “Microbial DMS formation” to support our conclusion.

“For the same section, I was also initially confounded by the suppression of methane production when H2 was added. Later, this trend was explained to be the result of enhanced production of fermentative products (e.g., propionate), which makes sense, but I had to wait a long time for that resolution. IF there is any data from these experiments that suggests this was the case here, it might be reported in closer proximity to the statement about H2 suppressing methanogenesis. What troubles me is that acetate

did not accumulate, which makes me wonder why propionate would be expected to do so. If acetate concentrations were measured, propionate would also have been."

We remove the discussion of fermentative products because, as already mentioned in earlier studies (Nüsslein and Conrad, 2000; Heuer et al., 2010), the reasons for the lack of stimulation of methanogenesis by high partial pressure of H2 remain unclear. Instead, we now describe the phenomenon in a separate paragraph in Section 4.1, cite the results of earlier studies, and end the paragraph with a sentence that links back to our observation of DMS formation.

"From the 13C-HCO3 labeling experiment, the authors conclude the results support the hypothesis that 13C-enrichement of DMS suggests direct incorporation of bicarbonate into DMS. I do not argue that conclusion as the ïĄď13C of the DMS was clearly affected by this process. However, from the reported results (maximum ïĄď13C of +119‰ for DMS) I cannot get a feel for how important this carbon source was for the accumulated DMS. This is simply a matter of how much the bicarbonate was enriched, but this is not included in the results. If it is a pure 13C source, +119 per mil enrichment is not the primary carbon source. . .or is it? A mass balance calculation would be interested and is advised. The natural abundance mass balance discussion on page 2583, I believe, assumes 50% of the carbon in the DMS comes from bicarbonate (as required by Eq. 2). . .doing the mass balance with the labeled substrates should actually provide a better estimate of the bicarbonate contribution than the natural abundance calculation."

We agree with the reviewer that such a calculation will improve our interpretation of the results. We first of all provide the rough estimate of the size and the 13C abundance of the spike bicarbonate pool (Section 2.4 "Stable isotope labeling"). We then briefly describe how the calculation was performed In Section 3.3 "Stable carbon isotopic compositions under non-labeling and 13C labeling conditions", followed by a summary of the result. We find that the newly fixed C in DMS had 13C abundance identical to that of the spiked bicarbonate pool, suggesting a precursor-product relationship.

"In the substrate addition experiments, it was clearly demonstrated that the addition of O-methyl compounds did not stimulate DMS or MT production. Given that, on page 2582, Lines 4-6, why would one expect MT pool in the 35S experiment to be derived from O-methyl pools? Is the reader expected to ignore the results from the preceding experiments?"

The concentration scale of substrate addition experiments differed from that of the 35S experiment. In the former case, effects of individual substrates were evaluated by changes in MT or DMS concentrations, which were monitored by GC-FID. With this technique, we were able to resolve differences in concentration only at the nanomolar scale. In the latter case, we were dealing with differences at the picomolar scale. Therefore, although the addition of O-methyl compounds did not stimulate DMS or MT production, we could not exclude the possibility that in the 35S study, sulfide methylation by O-methyl pools could still occur and contribute to our experimental signals.

We reword some sentences in the first paragraph of Section 3.4 "Labeling with H235S" to clarify the link between the preceding experiments and the 35S study.

"The discussion about the methanogens being stressed (not thermophilic) is interesting. But given that, how important would one expect this process to be in hydrothermally affected systems? Wouldn't one expect the thermophiles to competitively exclude the stressed methanogens in a hydrothermal system? I can see why this would not occur in the experiments conducted from lake sediments as thermophiles are likely absent. But in a hydrothermal environment this might not be the case. There are certainly other ways of looking at this (all speculative), but not addressed here. But in any case I would be careful about extrapolating the results from this study to hydrothermal field. . .at least use caution."

This is an interesting point. However, our intention was mainly to point out the potential implications of our findings and identify environments that appear suitable for further studies from the physicochemical point of view. The issue whether methanogens would

be stressed in hydrothermal environments is beyond the scope of our discussion.

From Dr. Kiene:

"P 2575, L2-4. This is somewhat unclear. Was the 10 mmol/L NaHCO3 13-C labeled? I don't think so, but this could be worded more clearly. What do you mean by background level? L 5-7. Did the storage at -20°C kill the samples?? And what about potential abiotic reactions for DMS and MT when samples were frozen and then heated at 60°C for 20 min? Can they be ruled out?"

1. In Section 2.4 "Stable isotope labeling" of the revised version, we provide the rough estimate of the size and the 13C abundance of the spiked bicarbonate pool.

2. It is not absolutely certain if -20°C will kill the samples. However, the process we were dealing with is a relatively slow reaction. Therefore, we assumed that this reaction was effectively retarded or stopped during the storage, and the potential bias introduced during the short-term heating was little compared to the main signals.

"P2575, L 23. It seems a pity that the first time point after H2S addition was made 2 h after the 35S-H2S was added. I understand that they did this to allow equilibration, but it would have been interesting to observe the equilibration kinetics."

Indeed, in hindsight, it would be interesting to follow the inorganic reduced S pools as they equilibrate, although we suspect that they equilibrate within minutes (based on work of Fossing and Joergensen in the early 90's). Nevertheless, the equilibration kinetics would represent another, difficult, study. Furthermore, the speciation within the dissolved H2S-HS–FeSx system is complicated (See the extensive literature on Fe-S chemistry by Luther, Rickard, and Morse).

"What is the rationale for assuming that only the dissolved phase compounds could be methylated to form DMS? Since most of the added 35S-H2S partitioned into solid phases, if this assumption is wrong, then it could lead to a large error."

We do not know, nor do we have any means at present, of knowing which of these

compounds are directly involved in the methylation reactions. Most likely, dissolved H2S and HS- that can readily cross cell membranes are the main player; thus, we exclude colloidal and solid forms of S(0) and FeSx species. Using the dissolved radioactivity and dissolved sulfide concentrations to calculate specific activity represents the most conservative approach.

"P2581. Top. The lack of effect of BES on acetate formation argues that there was no competition for H2 among methanogens and acetogens. Correct? But the Methanogens were CO2-limited? Is this common for Lake Plußsee – that Methanogens were CO2-limited?"

1. Yes, there seems to be no competition for H2 among methanogens and acetogens, as high partial pressure of H2 failed to stimulate methanogenesis.

2. We do not know if CO2-limitation is the reason for the lack of stimulation. Earlier studies with an incubation headspace of 80:20 H2/CO2 also failed to enhanced methanogenic activity. The related discussion is provided in a new paragraph in Section 4.1 "Microbial DMS formation".

"I doubt that demethylation of isotopically light DMS could explain the light methanethiol if methanethiol is the main precursor of DMS. That would imply a tight cycle, yet substantial MT is lost to other reactions (abiotic and biotic) so there must be some other major source."

We agree with the reviewer regarding the issue of tight cycling. We keep the discussion of a tight cycle of MT methylation and DMS demethylation in Section 4.2 "Microbial MT formation", but consider it now as an unlikely explanation for the 13C-depleted MT.

Technical and editorial suggestions

From Dr. Pohlman:

"In the abstract, stating the 35S labeling studies demonstrated a 'slow' process is vague. By some measures, all of the rates from this study were 'slow'. Perhaps, this

could be rectified by simply saying the rates were slow relative to the others, or simply eliminate that part of the sentence as it must be slow if it accounted for <10% of the DMS."

We follow the suggestion and eliminate the term "slow".

"On pages 2571 and 2572, replace 'Firstly, Secondly, Thirdly and Lastly' with 'First, Second, Third and Last.' That way it is now written sounds a bit odd."

Done as suggested.

"Page 2575, First paragraph, Section 2.5: The statement 'x was not added. . .in order not to decrease. . .the specific activity' is very difficult to digest. Unless there is a reason for using that text that has escaped me, why not simplify this by writing 'x was not added. . .to maintain a low specific activity'. After all, to 'not decrease' is 'to maintain."'

We follow the suggestion and rephrase the sentence.

"Line 11, same paragraph: . . .was added with the redox indicator resazurin. . . (to let the reader know why resazurin was added.) Line 13, same paragraph: Consider amending end of sentence, . . .became colorless, which indicated. . ."

We rephrase the sentences to better explain the purpose of adding resazurin.

"Page 2577, line 27: To state that 'A (add this article) small amount of liquid was added' is vague. I imagine that the 'small amount' is roughly equivalent to the lowest amount reported in the study. To be clear, simply provide the amount that was injected into whatever volume."

We provide the information of the injected volume.

"Page 2580, line 9. What does 'these compounds were in the range that generated microcosm response in previous studies' mean? Please clarify this point."

We clarify this point by rephrasing the sentence, which now reads "experimental con-

centrations of each of these compounds were in the range that stimulated production of DMS and MT in previous studies of freshwater sediment".

"Page 2582, Line 1: delete 'a"'

Done as suggested.

"Page 2587, Line 13. To say methane starts to build up at the SMTZ is incorrect. Sulfate and methane from spatially separated sources are consumed there. It is a region of consumption, not initiation of production. That occurs immediately BELOW the SMTZ."

We follow the comment and correct our sentence.

From Dr. Kiene:

"P2571, L29. Add hyphen: DMSP-limited. . ."

Done as suggested.

"P2573, L10. Reword. Our study tested whether this reaction is. . ."

Done as suggested.

"P2579, L17. Indicate whether there was any time trend for DMS in the autoclaved control. L18. . . .AUTOCLAVED control. . ."

We follow the comment and reword our sentence.

"P2579, L24. . . .bicarbonate WHEN ADDED SEPARATELY. . ."

Done as suggested.

"L26. Add comma after In combination, . . ."

Done as suggested.

"P2580. L6. . . .formation WAS. . . (use past tense in these situations)"

Done as suggested.

“L12. Change improved to increased. . . .none of the OTHER four substrates significantly INCREASED. . .”

Done as suggested.

“L24. . . .was 60% inhibited by X mM BES”

The information is provided.

“L24. Indicated (past tense).”

Done as suggested.

“P2582. L 12-13. It’s not clear to me what you mean by conclusion #2 – that H2S vapor was incompletely fixed and trapped.”

We remove conclusion #2 because it is not an appropriately formulated statement and is not fully relevant to the discussion here.

“P2583. L 3. Make it clear that H2 alone did not stimulate DMS formation while bicarbonate alone did, and both TOGETHER stimulated DMS even more. As written this sentence is a bit misleading.”

We rewrite the sentence following the suggestion.

“P2585, L4. Are you implying here that Methanogens were growing (or at least conserving energy) with the process of DMS formation? If you don’t know that to be the case, then perhaps you should point out that growth by this mechanism remains to be determined, even if it is thermodynamically feasible.”

We add a sentence at the end of the fourth paragraph in Section 4.1 “Microbial DMS formation” to remind the reader that growth by this mechanism remains to be determined.

“L23. Re-order words. However, from these experiments we cannot yet identify. . .”

Done as suggested.

“P2586, L19. From what type of system was the ïĄď13C of S-adenosylmethionine methyl groups determined?”

The system was purine alkaloids in higher plants. The information is added into the text.

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