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Biogeosciences Discussions

Interactive comment on "Dynamic mercury methylation and demethylation in oligotrophic marine water" *by* Kathleen M. Munson et al.

Anonymous Referee #2

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This paper studies mercury methylation and methylmercury demethylation in ocean water using isotope enriched mercury species. Using a variety of experimental approaches, the authors determine the magnitude of species transformation, including time series and amending water with other substrates such as organic carbon and cobalt. The use of isotope enriched species, combined with ICP/MS quantification allows the monitoring of both processes simultaneously in the same sample. The method is also sufficiently sensitive to work with spikes that are close to natural levels of mercury, providing as much environmental relevance as possible. I especially like the novel comparison of filtered with unfiltered waters, which required a meticulous experimental work to avoid contamination during handling of samples. Nevertheless, this study is extremely ambitious, and goes to the limit of what appears to be experimentally possible

today. One challenges of this type of study is the uncertainty regarding actual conversion rates that can be expected in previously untested environments, making the experimental design a bit of a guess work. The authors chose to incubate the majority of treatments for 24 hours, presumably to ensure detectable transformation results. However, as their time series suggest, it appears that these waters already showed measurable methylation and demethylation within 3-6 hours, after which the initially formed MMHg was again demethylated. Hence, I am concerned that the obtained data over the 24 hour incubation period cannot be considered "rates" of methylation or demethylation. In my opinion, the 24 hour data rather represent net MMHg formation. The time series suggest that there are multiple "rates" that are in play within 24 hours. An initial fast methylation, followed by a loss or at least a rapid decline in methylation activity. Instead, the demethylation process became more active at this point. I would interpret the data as if there was rapid initial methylation, but that the methylating agents or processes were exhausted with a few hours and the system never got into a (new) steady state. If this interpretation were correct, the calculation of rate constants is not possible using the data as suggested in the manuscript and the interpretation of [MMH]/[Hg] to Km/Kd ratios becomes questionable, though I agree that the results look intriguing, but maybe the similarity is generated for a different reason.

For clarification: it is not clear, how the 24 hour conversion rates were calculated. Is this obtained from the difference in MMHg concentrations between t(0) and t(24) or rather the difference between the nominal spike concentration and the concentration at t(24). It would be helpful, if the authors can provide the raw data in tabulated form in the appendix. From figure 3 it almost appears as if there frequently is no net change in concentration between t(0) and t(24), if this is the case, how can you calculate a methylation rate?

Specific comments: Title: What is supposed to be conveyed by adding "Dynamic" to the title? Is there also a "lethargic" methylation? Or are you referring to the "Dynamics" of mercury methylation . . .?

L 76: terminology: rather than referring to “enriched isotope spikes” use “isotope enriched spikes. It is the spike that is enriched with isotopes not the other way around.

L89: how exactly did you calculate ambient MMHg concentrations? What is meant by “correction for the added MM198Hg spike”?

L112: the concept of the “punches from McLane in situ pumps” requires more explanation. This appears to be lab lingo, which his incomprehensible to me at this point, though later on the authors shed a bit more light of what this likely means.

L116: given that incubations were not performed at in-situ T, I am missing a discussion how this might have affected the outcome, since T changes alone could alter bacterial activity, leading to changes in steady state MMHg levels.

L145: it is an interesting concept to determine Hg(II) through direct ethylation. However, to be convinced that this is actually a viable method, I would require more QA/QC data, especially ethylation blanks. I would assume that reagents used in the methods carry some inorganic Hg background (buffers, acids, the ethylation reagent . . .)

L150: How are you determining a first order decay constant from at best two data points? I assume that the two points (t(0) and t(24)) themselves carry considerable uncertainty. Given than the exponential relationship, this should translate in rather large uncertainty of the resulting linear relationship and rate constant. Even if this calculation was doable (which I somehow doubt), at the very least, you should provide an uncertainty estimate, which his suspiciously absent for Kd values of table 2, while on the other hand uncertainties for Km are provided. Equally concerning, I can’t find a single data point (in a table or on a graph) for measured MM198Hg levels before, during or after incubation. This needs to be provided in order to ascertain the conclusions drawn in this paper.

L158: as mentioned earlier, I think it is misleading to claim that this experiment determined “rates”. Instead, it determined the net methylation that occurred over a 24 hour

incubation period. In the absence of a time series showing a continuous change in concentration over time, I like challenge the idea that this dataset allows the calculation of rates, let alone rate constants. Here, the “rate” is obtained by drawing a straight line between two arbitrary points on the time axis. If the authors had chosen to incubate all samples for 6 hours, we would be facing very different “rates”.

L210: are you sure that MMHg was indeed demethylated prior to t(0)? Have you considered other loss mechanisms, e.g. adsorption to container walls? Did you try to determine the T198Hg concentration in these samples? If there was demethylation, leading to 198Hg(II), it should show up during a total Hg determination or in the diethylHg peak of the chromatogram. If absent, what does this say about the demethylation mechanism? Would that mean the product of the demethylation is 198Hg(0)? Is that possible? Where did the 198Hg isotopes go, if they are no longer detectable as MM198Hg?

L218. Be careful to not confuse “rates” with “rate constants”. Demethylation rates may be expressed as the % loss per day, but this is a rather unusual expression for a rate constant, which for first order processes, has the unit of d-1 (per day). Why do you add “%” at this point?

L223: I agree with this concept, but I disagree in that the data obtained here are indeed “rate constants” instead, they are more net conversions over 24 h of incubation.

L243: given the absence of any actual data on MM198Hg concentrations, it is difficult to validate this conclusion.

L259: this description of the “punch” should go to the methods section.

L285; this is an intriguing observation. I’d be curious if this an experimental artifact and artificial or if this indeed points to environmental relevance for the methylation process. Certainly worth exploring in more detail.

L294+296: “appears to be a dynamic process” what is “dynamic” in this process?

Seems to be an unnecessary filler. Please, omit “dynamic”.

L295: how did you calculate the rate of MM202Hg demethylation? Please, explain.

L297: I completely agree that the 24 hour incubations don’t offer the resolution which would allow rate estimates with any certainty. This is not a critique of the experiment, but merely an observation. As mentioned earlier, these experiments require an educated guess about appropriate incubation periods and one only discovers after the fact, how good the initial guess really was. But rather than risking an overinterpretation of the data, the authors should rephrase their conclusions accordingly. Take into account the inherent limitations of this type of study.

L343: this observation is indeed puzzling. Can it have something to do with the acidification that is used to stop incubations?

L352: another unnecessary filler: ”active” seems the wrong word here, unless there is also a “passive” methylation process.

L369-366: where is this discussion coming from? I fail to connect the body of this research to hgc genes.

L367: how do you know that cellular methylation is not important, when you only determined net 24 hour methylation, rather than studying what is going on in the first 6 hours, were cellular processes may very well be important. But after 6 hours cells die (for whatever reason) and only appear to be unimportant (in the artificial setting of a closed 250 mL bottle).

Figure 2: What is the difference between panel a+b and c+d? There is no legend for panels a+c. Was the concentration of the Hg(II) substrate determined (how?) or is this the nominal spike concentration?

Figure 3: the chosen presentation makes it very difficult, if not impossible for most treatments to decide if concentrations after 24 hours are smaller or larger compared to the t(0) starting point

Table 1: typo for THg of 17N CMX: 1:0.

Table2: Why are there no uncertainty estimates for Kd values?

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