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Biogeosciences Discussions

Interactive comment on "The stable isotopic signature of biologically produced molecular hydrogen (H_2)" *by* S. Walter et al.

S. Walter et al.

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First we would like to thank both reviewers for their constructive comments, with which we mostly agree. A point-by-point reply to the points raised is given below.

Reviewer 1:

The authors present the first systematic study on the stable isotopic compositions of biologically produced H2. While the values have been already predicted by Bottinga (1969) in past studies to be highly D-depleted, this is the first systematic experimental evaluation on the values. They confirm the deuterium depletion of biologically produced H2 of biogas, and from microorganisms or green algae. Better estimates on the hydrogen isotopic composition are important for calculating the global isotopic mass

balance of atmospheric H2, especially for those with highly depleted in deuterium.

I recommend accepting this paper with minor revisions. However, there are some issues that need to be addressed prior to publication in Biogeosciences. General comments:

The way of calibration for the samples having highly D-depleted δD values (less than -535‰ has not been clear. To confirm the linearity of the IRMS system in such low δD range, they showed the relationship between reciprocal mixing ratios of H2 and δD values for those from –535‰ to +35 ‰ in Fig.1. However, they reported more D-depleted values, ranging from –758‰ to –556‰ for H2 from microorganisms. They should add further description to verify accurate determination on the highly depleted δD values of biologically produced H2 by presenting the linearity of their IRMS system in all the data range presented in this manuscript (from –758‰ to +35‰

Reply: We completely agree and are fully aware of the limitations of measurements at very low δD values, and believe that we acknowledge the potential errors carefully in our manuscript. The reviewer points out our main argumentation in the rebuttal: We apply a Keeling plot analysis for the biogas samples, which 1) avoids measurements at extremely low δD values and 2) shows no deviations from linearity down to -535‰.These samples are then used for the scientific interpretation. The headspace samples of the pure cultures showed extremely high concentrations and were diluted in a different way, which could produce additional errors. This is mentioned in the manuscript, and these data were not taken into account for the calculation of the εH2−H2O, but mentioned for completeness. We clearly state (p12531, l14 ff) "It was beyond the scope of this project to further investigate whether these differences are significant, but this would be an interesting task for the future. In the absence of further information, it may not be appropriate to simply average the results from this to some degree arbitrary selection of samples to obtain a representative mean." We think that this is a sufficient description of the limitations and of our approach.

The slope of 2.2‰ / °C for the relationship between εH 2−H 2O and temperature is larger than the theoretically predicted slope (1.4 ‰ / °C) in Figure 2b. Please discuss clearly whether this discrepancy is significant or not, by giving the uncertainty in the slope.

Reply: The statistical basis of our experimental estimate is small, since only one sample was measured at each temperature. The increasing differences at decreasing temperatures could be caused by random errors, by potential errors in the absolute isotope calibration or a non-linearity in the isotope scale at very low δD values (which is, however, not obvious from the Keeling plot). We state in the paper that the differences between the measurements and calculated values are within the accuracy of the measurements (see also the errors bars of the measurements in Fig. 2b). In the revise version we will use both slopes, the experimentally derived one and the calculated one, for extrapolation to 20°C.

For yielding the value of εH 2−H 2O (–728‰ at 20° C), they used the biogas data obtained under the temperature ranging from 45° C to 60° C by extrapolation the liner relationship between εH2−H2O and temperature. All the obtained εH2−H2O, including (biogas at 38° C and the cultures of microorganisms), however, almost corresponds to the theoretically predicted one within their errors. As a result, I guess the theoretically predicted εH2−H2O by Bottinga (1969) might be more preferable to obtain more accurate global average δD value for the biologically produced H2.

Reply: We think that this is a misunderstanding and apologize if our approach was not clear. For the calculation of εH2−H2O, we used only the biogas data at 38°C, which is our most robust estimate in this study, excluding measurements from other temperatures and pure cultures. The predicted value at 38°C by Bottinga is -695‰ and from our measurements at this temperature we obtain a value of 689 $\pm$ 20‰ from the Keeling plot. This is in very good agreement with Bottinga. We then used our measured T dependence to extrapolate to 20C and came up with a value of -731‰ (value adjusted to a slope of 2.3, values will be corrected in the revised version). Using

the calculated T dependence from Bottinga, the value is -715‰Ḃoth values are very close to the predicted value of Bottinga, which is -722‰İn the revised version, we will use both the calculated and measured T dependence for this extrapolation.

Please add a new figure to facilitate comparison of the relationship between the obtained εH2−H2O and the theoretically predicted εH2−H2O for all data.

Reply: Given the additional uncertainties for the other data points as discussed above and in the paper, we refrain from adding another picture where these less robust data are compared to the theoretical calculations. We stress that the good reproduction of the theoretical results with the approach outlined above (i.e. using only our most robust values) in this extremely low δD range is a significant achievement. In past global model studies, the Bottinga results were even ignored and a poorly constrained value of -628‰ (not yet in the reviewed literature) was used instead. This approach is clearly refuted by our new data.

Specific comments: p.12524 L.21 Highly D-depleted δD values on biological H2 production in soils C5946 have also been pointed out recently (Komatsu et al., RCM 2011). This recent result should be referred.

Reply: The authors determined the temporal variations in the δD values of H2 in a static flux chamber. They found indeed very low δD values and concluded therefore, that biological production is probably responsible for this result. We completely agree with their conclusion, however here we wanted to point out the lack of individual measurements for biologically produced H2. The authors were not investigating the biological production per se and biological production is a conclusion of them for their findings based on previous measurements of other authors, and for this reason it was not taken into account at this point.

p.12525 L.9 “highly depleted H2” should be “highly depleted in deuterium of H2”

Reply: will be changed to “highly deuterium-depleted H2”

p.12531 L.20 Is this a typo of 60âŮęC ? or is the temperature really 65âŮęC ? The temperature of biogas in second line in Table 2 is also typo? If the temperature is really 65âŮęC, please give the δDH2 in 65âŮęC together with its theoretically predicted εH2−H2O in Table 2 and Figure 2.

Reply: Typo, must be 60°C for both

Table 1. Please add the uncertainties in measured δD and corrected δD. Table 1. Please also give each temperature for pure microorganisms cultures as was described in text.

Reply: will be changed

Table 2. Please add the uncertainties in δDH2.

Reply: Will be changed.

Table 2. Please add the theoretically predicted εH2−H2O by Bottinga (1969) in biogas (38âŮęC) and each microorganism culture.

Reply: We will include the predicted value for the biogas (38°C). For the microorganism cultures we do not think that these values are useful, because these data were not taken into account for the calculation of the εH2−H2O, but mentioned for completeness.

Figure 1. The each corrected δD value for a temperature range of 45âŮęC to 60âŮęC was different source signature ranged from –743‰ to –703 ‰ as was described in p.12532 L.6. To confirm the linearity of the IRMS system in the low δD range, the Keeling plot using different source signatures is not adequate. Please plot symbols for samples at a treatment temperature of 38âŮęC.

Reply: We agree with the reviewer that using all source signatures in one Keeling plot is not adequate. The figure will be changed.

Technical corrections: p.12532 L.5 There is contradiction between the slope in Fig. 2b

(2.3 ‰ŮęC) and C5947 the slope stated here and abstract (2.2 ‰ŮęC). Please check.

Reply: Typo, must be 2.3‰Ẇill be changed. Calculated values will be adjusted.

Tables 1 and 2. There is contradiction between the corrected δD at 45âŮęC in Ta- ble 1 (–734‰ and that in Table 2 (–743‰Ṗlease check.

Reply: Typo, must be -734 ‰Ẇill be changed.

Reviewer 2:

Measurement of atmospheric DH in H2 is challenging, especially when the abundance of DH is so small. The authors here present some new measurements indicating the extreme isotopic depletion from H2 derived from certain biological sources. While reasonable, and probably correct, I am concerned about the accuracy of the measurements. In particular, I find it difficult to imagine one can use a gas tight syringe for transporting and injecting molecular hydrogen. In the old days, there was concern of H2 diffusing through glass in mass spectrometric systems (e.g., Craig). In fact, it was a result of these technical problems that people largely stopped studying H2 after the 1960's. There was a flurry of papers in the old JGR volumes, and then nothing. Of course, there have been huge advances in isotopic analysis of trace gases, but I still find H2 to be a tricky one to handle. Without proven, reliable, reproducible results from calibration gas mixtures, I am not sure how much of a difference there is between dD=-600 and dD=-700 per mil. My first thought regarding the measurements is that it is largely consistent with theoretical studies; the differences are, relative to uncertainties in the calibration of H2 (at least as presented here), small. One of the goals of using a calibration gas is to be able to intercompare with other laboratories. Unfortunately, this is not possible, for such depleted DH values, with the 'calibration' used here. It would be nice if the authors provided more detail to show that the calibration of H2 is robust. It is a difficult thing to do. Another option is to perhaps revisit the level of uncertainty associated with the reference gas and includes that in the overall reported error.

Reply: We completely agree with the reviewer that H2 is still a challenging gas to measure, but there has been a large advance in H2 measurement methods in recent years, and a huge increase in H2 measurements in the last decade.

Intercomparison experiments within the European project "EUROHYDROS", in which the author also participated, has shown that generally H2 mixing ratio measurements can be made with a reproducibility of less than 1% and the experiments gave an agreement between the 13 participating labs of better than 1.4% (Yver et al. 2011). Four prepared gas mixtures with a defined H2 mixing ratio were prepared from the Swiss Federal Institute for Materials Science and Technology (Empa) and sent between 13 laboratories to test the agreement of their H2 measurements on a RGA. Although our measurement technique is completely different, the agreement with the other labs was <1.5%, which makes us quite confident about the concentration measurements. For transport and storage of samples we normally use 1L borosilicate glass flasks with PCTFE stopcocks from NORMAG. These flasks are known to be stable for a number of trace gases and storage tests made by the Max-Planck Institute for Biogeochemistry, Jena indicate that they are also suitable for H2. This was also additionally tested by intercomparison with the MPI-BGC, Jena. The biogas samples were transported in 12ml glass flasks and measured within 4 and 14 days after sampling. Gas tight syringes were only used for injection to our measurement system. First replicates were than done between 2 – 6 weeks after sampling, and we could not observe a trend in decreasing concentration e.g. expectable by leaking. Thus, we are confident in our shipping procedure and our measurement technique to determine H2 mixing ratios, but certainly we agree that errors from this could not completely be excluded.

The reviewer is correct that for isotope measurements at very strong deuterium depletions, the isotope scale is the most important issue. Our referencing strategy is presented in Batenburg et al. (2011), where we state that our isotope scale is based on mixtures of commercial, certified, pure-H2 isotope standards (ISOTOP, Messer Griesheim, Germany) with H2-free air. There, we also describe possible systematic

errors that could affect the isotope scale. We acknowledge that inter-laboratory comparability is still limited, because no international standards available. Based on our referencing strategy our scale is traceable to the pure H2 isotope standards. We state clearly in our paper that the depleted values of biogenic H2 are outside this calibrated range, but the Keeling plot analysis itself shows that this cannot induce large errors. The data in Table 1 of our paper show that the extrapolated source signature is $\sim$-700 ‰ regardless of whether the actually measured mixture was within the calibrated range (+16 ‰ see line 9 of Table) or -535‰ (line 17 of Table 1). Our results are in good agreement with the theoretical predictions, and this lends further support to the validity of our measurements.

Furthermore, as mentioned in the manuscript, the "Keeling plot" approach that we apply for measurement of the biogas samples implies that the determination of the δD source signature is insensitive to potential losses of material. If such a loss (e.g. from a gas syringe) occurred, the resulting mixture would still fall on the same mixing line in the Keeling plot, just a little bit closer to the value of the reference gas sample. Therefore, we are confident that effects from potential small leaks did not affected our isotope results. Our Keeling plot analysis gives a straight line, based on 12 values with an R2 of 0.999. The range of reproducibility is given in the manuscript.

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