

Interactive comment on “The stable isotopic signature of biologically produced molecular hydrogen (H₂)” by S. Walter et al.

S. Walter et al.

s.walter@uu.nl

Received and published: 16 April 2012

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

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



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 $\epsilon_{H_2-H_2O}$, 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.

BGD

8, C6365–C6372, 2012

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

The slope of 2.2‰ / °C for the relationship between $\epsilon_{\text{H}_2\text{--H}_2\text{O}}$ 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 $\epsilon_{\text{H}_2\text{--H}_2\text{O}}$ (−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 $\epsilon_{\text{H}_2\text{--H}_2\text{O}}$ and temperature. All the obtained $\epsilon_{\text{H}_2\text{--H}_2\text{O}}$, 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 $\epsilon_{\text{H}_2\text{--H}_2\text{O}}$ by Bottinga (1969) might be more preferable to obtain more accurate global average δD value for the biologically produced H_2 .

Reply: We think that this is a misunderstanding and apologize if our approach was not clear. For the calculation of $\epsilon_{\text{H}_2\text{--H}_2\text{O}}$, 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‰. Both values are very close to the predicted value of Bottinga, which is -722‰. In 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 $\epsilon_{\text{H}_2-\text{H}_2\text{O}}$ and the theoretically predicted $\epsilon_{\text{H}_2-\text{H}_2\text{O}}$ 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 H_2 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 H_2 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 H_2 . 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 H_2 ” should be “highly depleted in deuterium of H_2 ”

Reply: will be changed to “highly deuterium-depleted H_2 ”

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

p.12531 L.20 Is this a typo of 60°C ? or is the temperature really 65°C ? The temperature of biogas in second line in Table 2 is also typo? If the temperature is really 65°C, please give the δDH_2 in 65°C together with its theoretically predicted $\epsilon\text{H}_2\text{--H}_2\text{O}$ 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 δDH_2 .

Reply: Will be changed.

Table 2. Please add the theoretically predicted $\epsilon\text{H}_2\text{--H}_2\text{O}$ by Bottinga (1969) in biogas (38°C) 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 $\epsilon\text{H}_2\text{--H}_2\text{O}$, but mentioned for completeness.

Figure 1. The each corrected δD value for a temperature range of 45°C to 60°C 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 38°C.

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

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

Tables 1 and 2. There is contradiction between the corrected δD at 45 ‰ in Table 1 (-734‰) and that in Table 2 (-743‰). Please check.

Reply: Typo, must be -734 ‰. Will 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.

BGD

8, C6365–C6372, 2012

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

C6370



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 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

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

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 ~ -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.

Interactive comment on Biogeosciences Discuss., 8, 12521, 2011.

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)