

Biogeosciences Discuss., referee comment RC2
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Comment on bg-2022-232

Anonymous Referee #2

Referee comment on "Impact of metabolism and temperature on 2H/1H fractionation in lipids of marine bacterium *Shewanella piezotolerans* WP3" by Xin Chen et al.,
Biogeosciences Discuss., <https://doi.org/10.5194/bg-2022-232-RC2>, 2023

In this paper, Chen et al., performed a culture experiment on heterotrophic marine bacterium *Shewanella piezotolerans* WP3 that widely occurs in the deep sea, with different organic substrates and a temperature gradient, to study the impact of metabolism and temperature on the microbial lipid biomarker (*n*-fatty acids) hydrogen isotope fractionation. They showed that central metabolic pathways associated with NADPH production exert an important effect in determining the hydrogen isotope fractionation, and temperature play a secondary role. This study is very valuable for understanding compound-specific hydrogen isotopes in marine sediment records for biogeochemical and paleoclimate studies. I am supportive of publication after consideration of following minor comments.

Minor comments

L20: Add "and" before "relatively small".

L23-24: Specifically add how much hydrogen isotope fractionations are observed under different temperatures.

L25: Please rephrase this sentence "it is most likely controlled.....". For example, "We hypothesized that this may be associated with temperature-induced enzyme activity.....".

L33: Add "are assumed" after "phototrophic algae".

L34-37: Please revise this sentence. For example, "However, increasing studies have found that there are large ranges of hydrogen isotope ratios in lipids (up to 700‰) from variation environmental samples,.....". Note that the fractionation values are up to 700‰, rather than 700%.

L41: Change "thrived" to "thriving".

L74-76: Does *S. piezotolerans* belong to *Shewanella*? If so, please specifically address this here.

L89: Please add some sentences: what and where marine samples does *S. piezotolerans* WP3 to be isolated and enriched?

L104: How did you derivatize fatty acids into FAMES? Please add the chemical experimental process.

L110: How much temperature did you use in the pyrolysis interface?

L115: Add the correction formula and hydrogen isotope value of methyl group.

L130-140: Add some statistical box chart figures as supplementary materials to show the differences in hydrogen isotope fractionation of fatty acids under different substrates.

L225: Change "grown" to "growing".

L265-267: Different individual fatty acids have substantial differences in hydrogen isotope fractionation. Please add some figures as supplementary materials to show the changes in hydrogen isotope fractionation of same individual fatty acid along the temperature gradient.

L273-274: Please rephrase this sentence. For example, "The mechanisms may associated with growth rates and enzyme activities in organisms controlled by temperature."

L324-325: Please revise the grammar.

L326: Change "growing on sugars" to "using sugars as substrates".

L381: Change "growing on" to "using".