

***Interactive comment on “Identify the core bacterial microbiome of hydrocarbon degradation and a shift of dominant methanogenesis pathways in oil and aqueous phases of petroleum reservoirs with different temperatures from China” by Zhichao Zhou et al.***

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The manuscript contains interesting data and is sufficiently well written such that it should be suitable for publication after minor modifications. The main contributions of the manuscript are to help define a common core microbial community in petroleum reservoirs and to hypothesize that the dominant methanogenic biochemical pathway and associated microorganisms shift in water versus oil phase microorganisms, and

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that the relative importance of hydrogenotrophic and acetoclastic pathways is different in low versus high temperature reservoirs.

Major comments: Nowhere in the text are the temperatures that correspond to low, moderate, and high petroleum reservoirs described. The revised manuscript should specify temperatures in the abstract and the introduction as a minimum.

Reply: Thanks to this suggestion. We added the detail temperature information in the Abstract (Line 23) and Introduction (Line 101) according to the reviewer's suggestion.

Since aqueous phase and oil phase microbes were recovered from only 7 locations consisting of 2 low, 4 moderate, and one high temperature reservoirs it is premature to state conclusions that the relative importance of hydrogenotrophic and acetoclastic pathways is different in low versus high temperature reservoirs. Rather, the revised manuscript should discuss the observations made by inspecting these data and offer a hypothesis that requires further testing.

Reply: Thanks for the comments here. Since the limited locations and samples in this study, it is truly premature to make a conclusion as the reviewer pointed out. As a result, we summarized the observations and confirmed the distribution pattern by three taxonomical investigating methods, e.g., *mcrA* gene-based (clone library), methanogen 16S rRNA gene-based (clone library), and MiSeq-based archaea 16S rRNA gene methods (Fig. 3). All of these three methods support a similar community structure, suggesting that the results in our manuscript are reliable and could serve as a hypothesis that requires further testing with more samples for the ubiquity of the observation.

Table 1 shows that the concentration of acetic acid in the water phase of these 7 samples does not correlate with the reservoir temperature and does not support the claims made regarding the importance of acetoclastic methanogenesis derived from the genetic data. This should be discussed.

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Reply: Thanks for the insightful comments. We double-checked the original acetate data, and are sure about the reliability of them. The chemical datasets were measured from the aqueous phase of each well production water sample by ion chromatography as stated in the Materials and Methods. We observed the community shifting from hydrogenotrophic methanogenesis to acetoclastic methanogenesis in high temperature reservoirs from oil phase to water phase, but this is not necessarily equivalent to that acetoclastic methanogenesis is more important in high temperature reservoirs and the corresponding acetate-utilizing rate will be faster than the other eco-niches. Meanwhile, as in a complex microbial community, the other members and processes could also potentially contribute to the consumption of acetate, such as syntrophic acetate oxidization pathway coupling with hydrogenotrophic methanogenesis. Therefore, it is not suitable for us to make a reasonable conclusive deduction at this moment.

Without the in situ microbial activity data and other quantitative measuring methods on the chemical reactions (stable isotope labeling), it is still unreliable to address the direct connection between the microbial function/activity and acetate concentration in any petroleum reservoirs. In the manuscript (Line 330-331), we introduced more discussion about the acetoclastic methanogenesis and syntrophic acetate oxidization processes.

Another issue is that the production of glycine betaine as an osmoprotectant in high salinity petroleum reservoirs has been shown to serve as the main source of methane, via methylotrophic methanogenesis, in several reservoirs. The reservoirs studied here are low salinity and no evidence of methylotrophic methanogenesis was found, but the discussion of a core microbial community should be improved by discussing genetic data from higher salinity reservoirs as it compares with the results of this study.

Reply: Thanks for the comments. Actually, we found the molecular evidence of methylotrophic methanogenesis in P1 aqueous and oil phase samples; they included different groups within Methanosarcinales (Fig. 3). We could not conclude that they are obligate methylotrophs, while, to some extent, the existence and abundance in certain petroleum sites suggest the availability of methyl-containing compounds, e.g.,

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glycine betaine in saline environments (Ollivier et al., 2010, oil reservoir ecosystem). It is suggested that this osmoprotectant could be accumulated or synthesized by the halophiles, and the degraded methyl-compounds from fermentative processes will subsequently fuel the growth of the methylotrophic methanogens. We added the corresponding discussion in the manuscript (Lines 307-316).

Are there any prior publications describing the recovery of microbial cells from oil-phase samples, or is this a new technique reported for the first time? If this technique is new, that should be highlighted in the revised manuscript.

Reply: Thanks for the comments. To the best of our knowledge, there is not any report available on obtaining microbial cells from oil-phase samples directly. The reason might be: 1) difficulties to preserve production fluids (oil/water mixture) and maintain the viability of the cells when retrieving them from high pressure/temperature oil-wells; 2) good fractionation method to separate the cells from oil phases through extraction. The current studies on oil-phase microbes are all based on metagenomics approach.

Line 86 change pervious to previous. Reply: Revised as suggested (Line 87).

Line 275 replace ture with true. Reply: Corrected as suggested (Line 286).

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