

Anonymous Referee #1

In this manuscript the authors analyzed the diversity of potentially CO₂-fixing microorganisms using PCR amplification and cloning of marker genes involved in the Calvin-Benson-Bassham cycle, reductive acetyl-CoA pathway, and methane Synthesis in samples of Petroleum reservoirs. They were able to amplify all of these genes from the petroleum reservoir samples they obtained, and discussed the potential role of the different phylotypes for CO₂-fixation in the petroleum reservoirs. This manuscript deals with a rather limited dataset, and I have some concerns regarding the general experimental outline and if the conclusions drawn are really fully justified by the data provided in this study.

(1) The authors state at the end of their introduction that they want to evaluate the potential of in situ microbial CO₂-fixation in the different petroleum reservoirs. However, since they did not use a quantitative but only a qualitative approach to analyze the different marker genes, it is difficult to estimate the genetic potential for the different CO₂-fixation pathways. Here, at least quantitative PCR targeting the different marker genes should be performed to gain insight into the abundance of the different genes and of the corresponding microbial populations. Such quantitative data would provide a more suitable data set to estimate which CO₂-fixation pathways might be quantitatively relevant in the reservoirs.

Reply: Thanks for this valuable comment!

To the best of our knowledge, functional microorganisms involved in CO₂ fixation and conversion in oil reservoir are poorly reported. This manuscript mainly focuses on the investigation of these functional microorganisms in oil reservoir in order to identify, from the microbial viewpoint, if it is possible for in situ fixation and conversion of CO₂ into CH₄ in oil reservoirs with different physiochemical properties. The “potential” used here is not very suitable.

(2) Some conclusions are based on the relative fraction of certain phylotypes within the microbial communities based on each marker gene. What was the coverage of the clone libraries, how well did the authors cover the groups involved in a certain CO₂-fixation pathway?

Reply: The coverage of the clone libraries are more than 76.9%.

Six pathways have been reported involved in CO₂ fixation, i.e. Calvin–Benson–Bassham (CBB) cycle (ribulose-1,5-bisphosphate carboxylase (*cbb*)), reductive tricarboxylic acid (rTCA) cycle (ATP citrate lyase (*acl*)), Acetyl-CoA pathway (CO dehydrogenase/acetyl-CoA synthase (*cdh/acs*)), 3-Hydroxypropionate (3HP) bicycle (malonyl-CoA reductase (*mcr*) and propionyl-CoA synthase (*pcs*)), Dicarboxylate/4-hydroxybutyrate (DC/4HB) cycle (gene/enzyme not known), 3-Hydroxypropionate/4-hydroxybutyrate (3HP/4HB) cycle (acetyl CoA carboxylase (*accC*)). Ribulose 1, 5-bisphosphate carboxylase (Rubisco, specifically, *cbbL*, *cbbM*) are usually used as a biomarker for the Calvin Benson-Bassham (CBB) CO₂ fixation pathway. Specifically, in subsurface environments, CO₂ fixation is usually conducted by chemolithotrophs through CBB pathway.

The gene *fthfs* has been used to investigate the diversity of homoacetogenic bacteria in thermophilic and mesophilic anaerobic sludge (Ryan et al., 2008). The *mcrA*, the α -subunit of MCR (*mcrA* gene), is commonly used for the detection of specific groups of methanogens and the gene encoding [Fe-Fe]-hydrogenase is involved in H₂ generation which is necessary for CH₄ production though CO₂ reducing methanogens.

So, aiming to acquire the basic knowledge on functional microorganisms involved in microbial fixation of CO₂ and conversion it into CH₄ in oil reservoirs, all these 5 genes (*cbbL*, *cbbM*, *FTHFS*, *fefe*-hydrogenase gene, and *mcrA*) were investigated in production water samples from different oil reservoirs in this manuscript.

(3) Regarding the rather limited set of results, the discussion is much too long and should be shortened substantially. Results are repeated in the discussion and some of the background information placed here should rather be moved to the introduction.

Reply: Revised as suggested.

(4) The introduction lacks information about the motivation of this study and especially about the authors' expectations which CO₂-fixation pathways they expect to be represented in the petroleum reservoirs and under which conditions a particular pathway would be favored. As it is now, the introduction primarily lists the different

pathways without a clear link to the system investigated in this study. The authors should also elaborate more on the effect of the different temperatures, which is mentioned in the title but not explained in the introduction. What would be the mechanism behind a relationship between CO₂-fixation pathways or the composition of the CO₂-fixing communities on the one hand and temperature on the other hand? What are the authors' expectations regarding the effect of temperature? Regarding the effect of temperature, the authors should be careful with their conclusions, since no replication or time series of samples was performed. The observed differences between the communities at different temperatures could also be of a rather random nature, or the observed differences could be linked to another factor. It is obvious from table 1 that the water chemistry of the four reservoirs was different with regard to several parameters. Here, multivariate statistics could help to identify other possible relationships.

Reply: Thanks for this valuable comment!

As stated in the introduction part of the revised manuscript, the knowledge about the functional microorganisms involved in CO₂ fixation and conversion in oil reservoir is very limited. This manuscript mainly focuses on the investigation of these functional microorganisms in oil reservoir in order to identify, from the microbial viewpoint, if it is possible for in situ fixation and conversion of CO₂ into CH₄ in oil reservoirs with different physiochemical properties. Unfortunately, the CO₂-fixation pathways represented in the petroleum reservoirs and the conditions under which a particular pathway would be favored were not studied in the present manuscript.

As a matter of fact, the microbial community is influenced not only by temperature but also by the physiochemical properties such as pH, salinity, etc of their inhabiting environments. Thus, the title of this manuscript is changed accordingly to be "The phylogenetic diversity of functional microorganisms for CO₂ fixation and bioconversion revealed by using *mcrA*, *cbbM*, *cbbL*, *fthfs*, *fefe-hydrogenase* genes as molecular biomarkers in petroleum reservoirs with different physiochemical properties".

Multivariate statistics using CCA with "Vegan" package in R has been conducted and added to the revised manuscript.

Specific comments:

p. 1878, l. 4: The CBB cycle was known: : :

Reply: This sentence was revised to be “The CBB cycle is known...”

p. 1882, l. 7: The headers also of the following sections are misleading since only sequencing results of certain marker genes but not results of processes or activities are presented. Please replace by a more appropriate title.

Reply: Revised as suggested. This header has been changed to be “The diversity of *mcrA* gene” and other headers have been checked /changed accordingly in the revised manuscript.

p. 1884, l. 12-14 and l. 27: How much is "less" or "low" similarity? Please be more specific.

Reply: "less" or "low" here means the similarity is less than 80% and this has been added in the revised manuscript.

p. 1885, l. 15-16: This sentence is not clear, please rephrase.

Reply: This sentence is rewritten to be “All these results show that the CO₂-reducing methanogens inhabit predominantly in these four different oil reservoirs with different physiochemical properties.”

p. 1885, l. 24-25: How can the authors be sure that temperature is the only factor underlying the observed differences in community composition?

Reply: This sentence has been deleted. Temperature is only one of the factors which influence the composition of microbial community.

p. 1888, l.6-11: Should this not rather be placed at the beginning of the discussion?

Reply: Revised as suggested.

p. 1890, l. 4-10: In order to fully understand these relationships, quantitative data are needed.

Reply: This paragraph has been rewritten in the revised manuscript.

Great differences exist in relative abundance among all the five functional gene clone libraries established from these four samples (Fig 6). This phenomenon was also observed by Wang, who studied the microbial communities inhabiting in different oil reservoirs.