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Interactive comment on "The shift of microbial population composition accompanying the injected water flowing in the water-flooding petroleum reservoirs" *by* P. Gao et al.

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Dear Prof. Gu,

Thank you for reviewing our manuscript, and your constructive comments and suggestions. Based on the comments and suggestions, we have been revising our manuscript in an effort to improve it.

General comments:

This published paper on 'The shift of microbial population composition accompanying the injection water flowing in the water-flooding petroleum reservoirs' by Gao et al. cer-

tainly shows some descriptive information on the possible transport of microorganisms through oil reservoir subsurface sandstone materials.

Question 1: * I have to say that the title does not fit with the data obtained because the samples did not include a nonintervention control to allow assessment of the indigenous population for a meaningful comparison. Without this critical sample and information, the transport of bacteria is a claim not supported by convincing data.

Our response: We thank Prof. Gu for giving us the constructive advices. These advices are important and valuable for improving the manuscript quality.

We agree with your comment that it is less rigorous to delineate the transport of microbial populations in reservoir strata by detecting the shared microbial populations in both injection wells and production wells using a 16S rRNA sequencing method.

We have made a readjustment to improve the preciseness of conclusion based on data obtained in this study. The data obtained actually provided detail information on the relationship shared by microbial communities in the injection and production water samples. So, we revised the title of the manuscript as "Differences of microbial community composition between injection and production water samples of water-flooding petroleum reservoirs". The revised manuscript focused on comparing the differences of microbial community composition between injection and production water samples. We hope the revision will meet your approval.

We think that if microbial populations in injected water could flow into reservoir strata and reach production wells, microbial community in injected water are supposed to have a similar community composition with those in production wells? If there is a big difference in community composition, how many microbial populations were shared? To explore these issues, microbial communities and their abundance in water samples collected from wellhead or downhole of injection wells, and production wells in a homogeneous sandstone reservoir and a heterogeneous conglomerate reservoir were investigated using high-throughput sequencing. The results suggest that microbial

community have significant differences between injection and production wells, and the number of shared taxa has a closely relation to reservoir parameters, particularly, strata heterogeneity and interwell spacing.

Question 2: * In the text, the differences of detected pyrosequences between injection water and production water were used as the key variables to delineate the transport (migration) of microorganisms, a major shortcoming with this approach is that some microorganisms will not survive the subsurface environmental conditions due to lack of oxygen, nutrients etc. the approach used in this research plan should be reconsidered.

Our response: Thanks for your suggestion. Since Bastin et al. firstly isolated sulfate-reducing bacteria (SRB) from reservoir in 1926 (Bastin, 1926), culture-independent methodologies, such as fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), quantitative PCR, 16S rRNA clone libraries, and high-throughput sequencing have revealed diverse microbial populations inhabiting petroleum reservoirs. Although these methods could not delineate survive and activity of reservoir microbial populations, culture-dependent methodologies combination with culture-independent methodologies have demonstrated the existence and activity of hydrocarbon-degrading bacteria (HDB), nitrate-reducing bacteria (NRB), sulfate-reducing bacteria (SRB), and methanogens in injection and production water samples. On the other hand, even there is no metabolic activity in the subsurface environmental conditions due to lack of oxygen and nutrients, these populations might lie dormant in reservoir.

The study was performed to illustrate the relationship shared by microbial communities in the injection and production water samples. We think that the high-throughput sequencing can distinguish the differences between microbial communities in water samples collected from wellhead or downhole of injection wells, and production wells.

Question 3: * First of all, I am sorry to say that the quality of this manuscript writing is low and it is hard to read the text for accurate meaning and the precise information.

The writing needs extensive efforts and time to revise to reach to a reasonable level of acceptance. Authors must work hard on this and serious because the results of the information can be compromised seriously when the statements cannot be comprehended well enough by reading.

Our response: Thanks for your suggestion. We will work hard on writing in this and our future paper. To improve the paper quality, we will submit the revised manuscript to be edited by English Language Editing Service.

Question 4: *There is little or any disagreement now that oil reservoirs have indigenous population of microorganisms, but non-indigenous microorganisms are introduced into the reservoir systems when water flooding is introduced. It is always a big challenge to obtain the truly indigenous population of microorganisms in the reservoirs because of the difficulties involved in non-contamination sampling of the subsurface environment without any potential contamination. In a similar but different aspect, the physical characteristics of the subsurface materials, either heterogenous or homogenous as stated in this paper is also a term of personal choice here than substance because of their natural origin and heterogeneity no matter called heterogenous or homogenous. Heterogeneity is the true nature of such materials. Therefore, I have concern on the choice of 'homogeneity' and 'hererogeneity' simply based on the average permeability values because this value is an average numerical number, which cannot be used reliably for transportability of bacteria. Considering the differences in permeability between the two blocks, there should be no disagreement on bacteria can be transported in both subsurface systems, but the rate of transport may be different. If this is the case, what is the key scientific information that can be extracted from the selection of the 2 blocks in this investigation? If the injection of water had only started with this study, the collected water/oil samples can be of some meaning interpretation, but I do not think such is the case with this set of production wells.

Our response: Thanks for your comment. As you pointed out, the two reservoirs have been water flooded 13- and 30-years, non-indigenous microorganisms might be in-

troduced into the reservoir systems, and the indigenous microbial community in the subsurface might have been disturbed. Thus, it is difficult to obtain the reliable information on indigenous microbial community, even if we obtained water samples from newly drilled well. We planned to delineate the transport of microbial populations in reservoir strata by detecting the shared microbial populations in both injection wells and production wells using a 16S rRNA sequencing method. We now realize that it is less rigorous, because it is not able to demonstrate whether the species detected in produced water are the same ones in the injected water.

Based on the data obtained in this study, we have made a readjustment to improve the preciseness of the manuscript. Because the data illustrated the relationship shared by microbial communities in the injection and production water samples. We think it may be better to compare the differences of microbial community composition between injection and production water samples. We hope the revision will meet your approval.

Question 5: *The 'approximately 30-45 days and 7-10 days, respectively' – I have no way of knowing what do they refer to by the sentence because no designation was offered. I may assume they are associated with the heterogenous and homogenous reservoirs, but such assumption should not be the responsibility of the readers and they must be clearly stated by the authors to avoid any misunderstanding.

Our response: Thanks for your suggestion. We have revised the sentence as "Tracer technique indicated that the time intervals for injected water from injection well flowing into neighboring production wells are approximately 30–45 days in the sandstone reservoir, while 7–10 days in the conglomerate reservoir."

Question 6: *Sampling procedures were inadequately described and I am especially troubled by the statement ': : :by the field personnel of PetroChina.' because the quality of the samples may be compromised for one. In addition, how can the authors interpret the results when they are not involved in the in situ sampling to know the detail steps involved and the effects on the results obtained?

Our response: Thanks for your suggestion. Actually, we involved in the in-situ sampling, and the sampling process was assisted by the field personnel of Oil Field.

To avoid Misunderstanding, we have made correction in the revised manuscript. The revised section is: "All the injected and produced water samples were collected randomly from sampling valves located on the wellhead. The water samples were completely filled into 15 L sterilized plastic bottles, which were immediately capped and sealed to avoid contamination and oxygen intrusion."

Question 7: * Further on the sampling for concentration of bacterial cells, oil/water mixture should separate the oil from the mixture and then concentrate the cells from water phase or both oil and water phases. This detailed information show the understanding of the system you are dealing with and the quality of the cells you would be obtained. Are there any differences in terms of the composition and richness of microbial groups associated with the oil and water phases? Why was the oil phase not treated for extraction of DNA in the similar way as water phase? Actually, recent publication(s) has/have some information on this topic and you should also cited the work here. Were there any quality controls in the extraction of genomic DNA and PCR amplification?

Our response: Thanks for your suggestion. This is a good inspiration to our future research in this direction. According to your suggestion, we have looked through the recent publications, which compared the similarities and differences of microbial communities in oil phase and water phase. We will make a relevant discussion in the manuscript.

In the study, the studied two reservoirs are all serious water flooded, with the water content of up to 80.8% and 86.8%, respectively, implying that most microbes exist in water phase. As you suggested, oil phase also harbors a large amount of microorganisms, with significant different community structure from those of water phase. But, these microorganisms are supposed to originate from water phase.

Before collecting microbial cells, the oil-water mixture was firstly demulsified by heating

at 60 °C for 30 min. To collect as much of the microbial genomes as possible, the collected cells were resuspended with a TE buffer, and then lysed using a mini beadbeater (BioSpec, USA) at 4°C and 200 rpm for 1 min at room temperature with 0.1 mm glass beads. After bead beating, lysozyme was added (final concentration of 1 mg/ml), and samples were incubated at 37 °C for 1 h. Following the lysozyme treatment, 120 μL sodium-dodecyl sulphate (20% SDS, W/V) was added and samples were incubated at 65 °C for 60 min. The obtained genome was determined using gel electrophoresis and 16S rRNA PCR amplification.

Question 8: * 'In the sandstone reservoir' – I do not agree with you to have such a statement and claim simply because there is no strictly control, which did not have any water flooding to show the indigenous population and composition. If the objectives of this study are on migration of microorganisms in subsurface sandstone, I do not think the experimental design can answer the questions effectively. This is a key point in Discussion, I have strong reservation in accepting this. The high-throughput used can be sensitive for detection of microorganisms in samples, but they do not answer the transportability of microorganisms without careful planning, selection of samples (including subsurface) and the analysis involved.

Our response: Thanks for your suggestion. We agree with your comment that it is less rigorous to delineate the transport of microbial populations in reservoir strata by detecting the shared microbial populations in both injection wells and production wells using a 16S rRNA sequencing method.

To improve the preciseness of the manuscript, we have revised the manuscript title as "Differences of microbial community composition between injection and production water samples of water-flooding petroleum reservoirs". This study investigated microbial communities and their abundance in water samples collected from wellhead or downhole of injection wells, and production wells in a homogeneous sandstone reservoir and a heterogeneous conglomerate reservoir using high-throughput sequencing. The results imply that microbial community have significant differences between injection

and production wells, and the number of shared taxa has a closely relation to reservoir parameters, particularly, strata heterogeneity and interwell spacing. We hope the revision will meet your approval.

Question 9: * How can you link the microbial groups detected and the possible physiological function in the oil reservoirs? What are the sources of Bcteroides in the production water samples? From the information of archaea detected, which kind of methanogenic metabolism is responsible for CH4 production?

Our response: Thanks for your suggestion. A number of fermentative microorganisms have been isolated from high-temperature and low-temperature oil reservoirs. Many microorganisms in this group possess dual fermentative and respiratory metabolic abilities and could theoretically utilize both strategies for their in situ growth and survival (Youssef et al. 2009). Bacteroidetes include diverse mesophilic fermentative microorganisms. Grabowski et al. first isolated Petrimonas within the phylum Bacteroidetes from oil reservoirs in 2005, making it the first member of this phylum to be isolated from oil reservoirs (Grabowski et al., 2005). Recently, studies based on 16S rRNA gene-based analysis have also revealed the existence of putatively fermentative members of the genus Bacteroides (Grabowski et al., 2005b; Youssef et al. 2009). These fermentative microorganisms might play an important role in reservoir ecosystems, particularly, proving substrates for methanogen to produce methane.

According to your suggestion, we classified the obtained archaeal taxa based on the reported methyltrophic, acetoclastic and CO2-reducing methanogens (Liu, 2008). As reported in previous researches (Wang et al., 2012; Zhao et al., 2012), the archaea identified in both the reservoirs were overwhelmingly methanogens including methyltrophic, acetoclastic and CO2-reducing methanogens. Among them, methyltrophic and CO2-reducing methanogens dominated in both the reservoirs.

In the sandstone reservoir, more than 95% of the archaeal sequences were assigned to Methanobacteria, Methanococci and Methanomicrobia (Fig. 2a II). In the injected

water, 87% sequences were classed into Methanomicrobia, and the dominant genera were Methanosaeta (42.39%), Methanomethylovorans (25.57%) and Methanolobus (10.96%). Methanomicrobia accounted for 84.03% in produced water Lu1039, and Methanolobus (83.46%) and Methanococcus (11.23%) were the dominant genera. The archaeal communities were much more conserved in produced water Lu2180, Lu3073 and Lu3095, with Methanococcus accounting for 95.34%, 90.79% and 86.79%, respectively. The Methanolobus produce CH4 when growing with methylamine as carbon source, while Methanococcus use H2 and formate as carbon sources.

Similarly, Methanobacteria, Methanococci and Methanomicrobia composed 64.3%–94.6% of the archaeal communities in the conglomerate reservoir. Compared with the injected water obtained from the wellhead of the injection wells (T86-0 and T93-0), Methanomicrobia were more detected in the downhole of injection wells (T86-8 and T93-7) and production well T90. At genus level, Methanocorpusculum, Methanococcus, and Methanocalculus were dominant, accounting for 60.3–88.5% of the archaeal communities in the injection wells and production well T90. The tree taxa use H2 and formate as carbon sources to produce CH4.

Question 10: * The Conclusions is too lengthy and shortening is necessary to show the most significant information of this research if any. References should be updated more extensively to include the current published papers to enrich the information reported here.

Our response: Thanks for your suggestion. We have carefully rewritten the conclusion: Using high-throughput sequencing, we comprehensively surveyed the relationship shared by microbial communities in injection and production wells of a homogeneous sandstone reservoir and a heterogeneous conglomerate reservoir. The results imply that microbial communities have significant differences between injection and production wells in both the sandstone and conglomerate reservoir. Even if most microbial populations were shared, the community structure exhibited a big difference in the injected and produced water samples. Aerobic bacteria predominated in the in-

jection well, while microaerophilic bacteria, facultative anaerobe and anaerobe higher relative abundance in production wells. Furthermore, the number of the shared microbial populations have a closely relation to reservoir parameters, particularly, strata heterogeneity and interwell spacing.

The manuscript has been carefully revising accompanying with more current published papers. To improve the paper quality, we will submit the revised manuscript to be edited by English Language Editing Service before resubmission.

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