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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.

P. Gao et al.

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Dear professor,

We thank you for the time and thought you put into reading our manuscript and for your helpful suggestions for improvement. Based on the comments and suggestions, we have been revising our manuscript in an effort to improve it and address the concerns.

General comments: The manuscript entitled "The shift of microbial population composition accompanying the injected water flowing in the water-flooding petroleum reservoirs", by P.K. Gao and colleagues, describes the fluctuation of microbial communities along with injected water flowing into reservoir strata and production wells. Although

there are some interesting findings in this manuscript, it is not acceptable in its present form. I think that experimental design needs to be further improved, some of the interpretations should be better qualified and perhaps even modified. Occasionally, the text may be misleading, since the usage of English is sometimes not adequate. Hence, the manuscript should be carefully revised.

Our response: We agree with your comment that the experimental design and interpretations needs to be further improved. We have made a readjustment to improve the preciseness of the conclusion based on the data obtained in this study. The data obtained here actually provided detail information on the relationship shared by microbial communities in the injection and production water samples. The results revealed the differences of microbial community in injection and production wells in the water-flooding petroleum reservoirs. As a result, we think the title of the manuscript might be more reasonable to be revised as "Differences of microbial community composition between injection and production water samples of water-flooding petroleum reservoirs". The scientific problem is 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, the 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 major novel result of this study is that we analyzed the relationship shared by communities in injection and production water samples.

Major comments:

Question 1: * English should be significantly improved. This manuscript suffers from grammar errors and poor writing, particularly, in the Results and Discussion section.

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Our response: Thanks for your suggestion. We carefully revised the manuscript according to the comments. To improve the paper quality, we will submit the revised manuscript to be edited by English Language Editing Service before submitting the revised manuscript.

Question 2: * The Title should be reconsidered so that it can directly present the novel findings to the readers.

Our response: Thanks for your suggestion. The results showed the relationship shared by microbial communities in the injection and production water samples. The results revealed the microbial community diversification in injection and production wells in two long-term water-flooding petroleum reservoirs. Therefore, we have revised the manuscript title as “Differences of microbial community composition between injection and production water samples of water-flooding petroleum reservoirs”.

Question 3: * Experimental design: the authors chose a sandstone reservoir and a conglomerate reservoir in a Chinese typical oilfield, and analyzed the microbial population composition in injected water and produced water samples by using high-throughput sequencing technology, in order to test whether microbial populations in injected water could pass through oil-bearing strata. Overall the approach is straightforward. However, there are three major shortcomings in this study:

(i)The lack of control: These two kind of old well groups have water flooded for 13- and 30-years. The negative controls are missing. The indigenous microbial community in the same oil-bearing strata cannot be overlooked. A better way is to determine oilfield water samples from newly drilled well for comparison in the same oil-bearing block.

Our response: We agree with your comment that negative controls, which may be water samples from newly drilled well without water-flooding in the same oil-bearing block, are necessary to provide background information about indigenous populations. Unfortunately, there are no such newly drilled well at this stage in the two petroleum reservoir. On the other hand, because the two reservoirs have been water flooded 13-

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and 30-years, the indigenous microbial community in the subsurface might have been disturbed. Thus, it is difficult to obtain the reliable information on indigenous microbial community. We realize that it is less rigorous to delineate the transport of microbial populations in reservoir strata by only detecting the shared microbial populations in both injection wells and production wells using 16S rRNA sequencing method. Because it is not able to demonstrate whether the species detected in produced water are the same ones in the injected water. To improve the preciseness of the conclusion based on the data obtained in this study, we have made a readjustment, which emphasized the differences of microbial community composition between injection and production water samples. We hope the revision will meet your approval.

(ii) Some important geological parameters are missing: In this manuscript, by comparison of Lu and Liu field block reservoirs, the authors concluded that injected water can pass through reservoir strata, but the reservoir heterogeneity, sieve effect of strata and dissolved oxygen affect the microbial migration. However, geological parameters such as source rocks and oil sources of Lu and Liu field, the characteristics of crude oil (heavy oil or light oil), reservoir pressures, the depths of Lu and Liu oil-bearing strata are missing. If there are great differences between Lu and Liu field block in these parameters, the comparison does not make sense.

Our response: Thanks for your comment. We have added the geological parameters in Table 1. Although there are some differences in the reservoir characteristics of Lu and Liu field block, the two reservoirs are both located in Junggar Basin of Xinjiang Uygur Autonomous Region, Northwest China. The differences in geochemical parameters between crude oil samples from the two blocks are not obvious, indicating similar oil formation characteristics and maturity (Table 1). The crude oil in both the blocks has a higher content of saturates and aromatics, which are benefit for the growth of hydrocarbon-degrading bacteria. The salinity of Lu block is about 11, 000 mg/L, which is similar with the value at Liu block. The cation and anion among the water samples in each block are similar, with lower content of total nitrogen and total phosphorus, which

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are essential for the survival and growth of microorganisms. The lacking of nitrogen and phosphorus imply the low metabolism level of microorganism.

(iii) Environmental parameters such as dissolved oxygen (DO) or oxidation-reduction potential (ORP), subsurface temperature, and the composition of organic matter of crude oil (as substrate for fermentative bacteria and methanogenic archaea) have not determined in this study. These parameters are crucial for the fluctuation of microbial communities besides injected water. Therefore, it is also necessary to include these parameters in PCoA analysis. Without these information, the conclusion was untenable.

Our response: Thank you for your comment. We agree that these parameters are crucial for the fluctuation of microbial communities besides injected water. According to your suggestion, we have listed the subsurface temperature of the two reservoirs, the component of crude oil, and the concentrations of nutrient factors in Table 1. The subsurface temperature of the two reservoirs is 37 oC and 22.6 oC, respectively. The concentrations of nutrient factors were also measured, such as crude oil properties, total nitrogen (TN), total phosphorus (TP), and ion concentration of formation water (Table 1). The ratio of saturates in the two reservoirs are 71.9Unfortunately, the in situ oxygen concentrations were not measured at that time. But, microbial populations were clustered to highlight the populations that showed the most variability between injection and production wells. We found that Paracoccus, Bacillus, Ochrobactrum, Parabacteroides, Sphaerochaeta, Thauera, Halomonas and Alcanivorax were more detected in injected water, while Arcobacter, Marinobacterium, Pseudomonas, Bacteroides, Oleibacter, Marinobacter and Shewanella were dominant in the downhole of the injection wells and production wells. Among them, Marinobacterium, Paracoccus, Ochrobactrum, Sphingomonas, Alcanivorax and Azospirillum are aerobic bacteria, while Pseudomonas, Rhizobium, Arcobacter, Halomonas, Spirochaeta, Bacillus, Thauera, Halomonas, Bacteroides are microaerophilic bacteria, facultative anaerobe or anaerobe. We think these data reflect the influence of dissolved-oxygen on microbial

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community diversification. On the other hand, Unifrac PCoA analysis was performed based on microbial OTUs aboundance and phylogenetic relationships to extract and visualize the few highly informative components of variation from complex, multidimensional data. The results interpreted the relative similarity and diversification of microbial communities in the injection and production wells. In the biplot, samples with similar community were placed at a close proximity position, while samples with different community were placed at a comparatively decentralized position.

Question 4: * Materials and methods: Amplicon sequencing was performed on two kinds of highthroughput sequencing platform (GS FLX+ and Miseq). Apparently, the outcomes must be different. Did the authors want to make a comparison? I cannot find any clue in this manuscript.

Our response: Thank you for your suggestion. We will make a relevant discussion in the manuscript. For example: Miseq-sequencing produced approximate 52719 to 129106 16S rRNA gene sequences in the conglomerate reservoir. The sequencing depth was approximately 10–20 folds for pyrosequencing used in the sandstone reservoir. However, the current sequencing depth of miseq-sequencing is still limited for detecting archaeal community. This method used in the study simultaneously sequenced the bacterial and archaeal V4 region of 16S rRNA gene, leading 51273–128980 bacterial sequences were obtained per sample, whereas only 85–1445 archaeal sequences were obtained. In contrast, the bacterial and archaeal communities were sequenced independently using pyrosequencing in the sandstone. As a result, 4016–5060 bacterial sequences and 2688–2857 archaeal sequences were obtained. The data suggest that deeper sequencing is necessary for the complex reservoir microbial community, in particular, the infrequent microbial populations.

Question 5: * Discussion: There is no “going home” feeling in this part. Too many hypothesizes were demonstrated. In situ DO in injected and production water and the composition of crude oil should be determined firstly. Then, the content of the true part of the “Discussion” should be carefully revised accompanying with more related new

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references.

Our response: Thank you for your comment. The concentrations of nutrient factors including crude oil properties, total nitrogen (TN), total phosphorus (TP), and ion concentration of formation water, were measured (Table 1). It is a pity that the in situ oxygen concentrations were not measured at that time. But, microbial populations were clustered to highlight the populations that showed the most variability accompanying the injected water flowing into production wells. We found aerobic bacteria were more frequently detected in injected water, while microaerophilic bacteria, facultative anaerobe or anaerobe were dominant in the downhole of the injection wells and production wells. We hope these data could reflect the influence of dissolved-oxygen on microbial community diversification. The "Discussion" has been carefully revising accompanying with more related new references. To improve the paper quality, we will submit the revised manuscript to be edited by English Language Editing Service before resubmission.

Question 6: * The conclusion is too long and should be carefully rewritten.

Our response: Thank you for your comment. According to your suggestion, we have carefully rewritten this section. The revised conclusion section is as below: 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 injection 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.

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Minor comments:

Question 1: * Page 16774 Line 5-8: “The results indicated that the injected water: : : in this reservoir.” This sentence needs to be rephrased.

Our response: Thank you for your comment. We have made a readjustment to improve the preciseness of conclusion based on data obtained in this study. The abstract was revised accordingly. The revised abstract is as follow: In water-flooding petroleum reservoirs, investigation of microbial community is of high interest, since it is strongly related to enhancement of oil recovery. However, little attention has been focused on the relationship between microbial communities in injection and production wells. In the present study, the microbial community composition and structure in the 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. Quantitation PCR indicated that the injected water harbored more microbial cells than the produced water. A small number of microbial populations were shared in the sandstone reservoir, whereas a large number of microbial populations were shared in the wellhead and downhole of injection wells and production wells in the conglomerate reservoir. However, the community structure exhibited a big difference in the injected and produced water samples, with the shared populations accounted for a minor fraction in the injected water, while dominated in the produced water in the both two reservoirs. The results indicate that microbial communities have significant differences between injection and production wells, and the number of shared microbial populations has a closely relation to reservoir parameters, particularly, strata heterogeneity and interwell spacing.

Question 2: * Page 16777 Line 10-12: “All the injected and produced water samples were all collected from the wellhead of injection and production wells by the field personnel of PetroChina.” Once the samples were collected, how long would be the genomic DNA extracted?

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Our response: Thanks for your comment. The transportation from Xinjiang Oil Field to Tianjin is seven days. To avoid misunderstanding, we have made correction in the manuscript. The revised section is as below: “The water samples were completely filled into 15 L sterilized plastic bottles, which were immediately capped and sealed to avoid contamination and oxygen intrusion. After immediately transporting to the laboratory, microbial cells were collected from 5 L of each water sample by centrifugation at 4°C for 15 min at 10,000 × g in a high-speed centrifuge (Beckman, USA). The cell deposits obtained from the same sampling location were mixed and were resuspended with TE buffer (Tris 80 mM, EDTA 40 mM, pH 8.0).”

Question 3: * Page 16779 Line 13-14: why do not quantitate archaeal populations? It would be helpful for the interpretation of methanogenic community later.

Our response: Thank you for your suggestion. According to your suggestion, we have quantitated the number of archaeal populations using genome DNA preserved.

Question 4: Page 16780 Line 3-4; Page 16781 Line 2-3: The diversity of the microorganisms in the sandstone reservoir (249-538) is much lower than it in the conglomerate reservoir (51273-128980). Why? In this respect, the geological and environmental condition of Lu and Liu field might be very different from each other.

Our response: Thank you for your comment. We have made correction in the manuscript. The truth is that 4016–5060 bacterial sequences and 2688–2857 archaeal sequences were obtained by pyrosequencing in the sandstone reservoir, while a total of 52719 to 129106 16S rRNA gene sequences were obtained by miseq-sequencing in the conglomerate reservoir. The sequencing depth of miseq-sequencing was approximately 10–20 folds of the pyrosequencing.

Question 5: * Page 16781 Line 17: The word of "botained" should be revised to "obtained".

Our response: Thanks for reminding us. We have made a correction in the manuscript.

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Question 6: * Page 16784 Line 17: "LR, 2010" should be revised to "Brown, 2010".

Our response: Thanks for reminding us. We have made correction in the manuscript.

Question 7: * Page 16791 Table 1: I guess the temperature was the surface temperature. The temperature of oil-bearing strata should also be given.

Our response: Thank you for your comment. The listed temperature (37 oC and 22.6 oC) is the subsurface temperature of the two reservoirs.

Question 8: * Page 16793 Figure 2: The color representing a designated microbial class should be consistent so that it is easy for comparison. (e.g. The color of Methanococci is blue in Fig. 2aII but red in Fig. 2bII.)

Our response: Thank you for your suggestion. We have made correction in the revised manuscript.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/11/C8191/2015/bgd-11-C8191-2015-supplement.pdf

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Interactive comment on Biogeosciences Discuss., 11, 16773, 2014.