Biogeosciences Discuss., 11, 16773–16797, 2014
www.biogeosciences-discuss.net/11/16773/2014/
doi:10.5194/bgd-11-16773-2014

Biogeosciences Discussions
Open Access

The shift of microbial population composition accompanying the injected water flowing in the water-flooding petroleum reservoirs

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Received: 23 October 2014 – Accepted: 3 November 2014 – Published: 5 December 2014

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Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

In water-flooding petroleum reservoir, microbial populations in injected water are expected to migrate into oil-bearing strata and reach production wells. To demonstrate this, we firstly investigated microbial compositions in a homogeneous sandstone reservoir. The results indicated that the injected water harbored more microbial cells than produced water, and the shared populations and their abundance accounted for a minor fraction in injected water, while dominated in produced water, suggesting that most populations in injected water did hardly reach production wells in this reservoir. We further investigated microbial communities in water samples collected from wellhead and downhole of injection wells and production wells in a heterogeneous conglomerate reservoir. The results indicated that, except for the community reconstruction mainly resulted from dissolved oxygen, most populations were simultaneously detected in the wellhead and downhole of injection wells and production wells, suggesting that most microbial populations in injected water reached the production wells. This study suggest that microbial populations in injected water can pass through reservoir strata and reach production wells, but the reservoir heterogeneity, interwell spacing, sieve effect of strata and dissolved oxygen exert significant influence on microbial migration and distribution in reservoirs.

1 Introduction

Water flooding is an efficient oil recovery process and is employed worldwide. After long-term water flooding, complex ecosystems comprising diverse microorganisms formed in petroleum reservoirs. These microbial populations and their metabolites, such as polysaccharide, organic acids and bio-surfactants, can improve reservoir properties by blocking preferred water flow paths, lower interfacial tension between brines and oil phase, and decrease oil viscosity. These characteristics have been used to improve oil recovery. Due to the increasing global energy demand and depletion of

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oil reserves, microbial enhanced oil recovery (MEOR) is currently in intensive development. To date, a large number of laboratory researches and field trials have been performed on stimulating reservoir microbial microorganisms to enhance oil recovery (Abdel-Waly, 1999; Zhang et al., 2012; Bao et al., 2009; Gao et al., 2013; Li et al., 2014).

Microbial populations are important components of reservoir ecosystems, and serve as critical roles in MEOR process. Microbial migration and community succession along with injected water flowing into oil-bearing strata have a direct impact on gathering representative samples before and after MEOR process, and further influence nutrients selection and evaluation of oil displacement efficiency of production wells. Microbial migration in reservoir strata also has a direct impact on the application of MEOR approaches based on injecting nutrients or microorganisms into reservoirs. However, these problems have received little attention hitherto, and are thus poorly characterized. After long term water-flooding, microbial populations in injected water are expected to migrate into oil-bearing strata and reach production wells. However, using 16S rRNA gene clone library method, several studies suggested that despite being flooded by same injected water, each production well harbored specific microbial communities (Tang et al., 2012; Ren et al., 2011). Unfortunately, these studied injected and produced water samples were only selected for once at the same time. Due to neglect the interval that the injected water flows into production wells and the fact that the dissolved oxygen and reservoir pressure will produce significant influence on communities, these obtained results couldn't provide sufficient knowledge about microbial migration and distribution in injection and production wells. Moreover, because of low-throughput of clone library method, many infrequent microbial taxa are usually undetected, making it difficult to compare microbial communities in detail.

Whether microbial populations in injected water could pass through oil-bearing strata and reach production wells? How microbial communities fluctuate along with injected water flowing into reservoir strata and production wells? To explore these issues, microbial populations and their abundance in injection and production wells in a homoge-

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neous sandstone petroleum reservoir with permeability of 522×10^{-3} μm^2 and interwell spacing of 300–425 m were investigated by 16S rRNA gene pyrosequencing and real-time fluorescent quantitative PCR (qPCR). At the same time, we investigated microbial communities in water samples collected from wellhead and downhole of injection wells and production wells in a heterogeneous conglomerate water-flooding petroleum reservoir with permeability of 362×10^{-3} μm^2 and interwell spacing of 100–150 m. High-throughput sequencing provides the opportunity to compare microbial populations with unprecedented levels of coverage and detail. These results presented here will expand our knowledge about microbial migration and community succession in process of injected water flowing into petroleum reservoirs, and guide the application of MEOR.

2 Materials and methods

2.1 Water samples collection and DNA extraction

Lu and Liu field block reservoirs are located in Xinjiang Oil Field, northwest China. Lu field block is a homogeneous sandstone reservoir with an average permeability of 522×10^{-3} μm^2, and has been water flooded since 2001. In the selected well group (an injection well and four production wells), the injection well Lu3084, located at the center position of the production wells, have a direct influence on the neighboring producers, with inter-well distances of 300–425 m. Liu field block is a conglomerate reservoir with an average permeability of 362×10^{-3} μm^2 and interwell spacing of 100–150 m, and has been water flooded for about 30 years. In the selected well group, including of two injection wells and three production wells, the production well T90 was located at the center of the injection wells T86 and T93, while production wells T95 and T96 were located at the edge of this field block, and were mainly flooded by the injection well T93 (Fig. 1). Although the injection wells have a direct influence on neighboring production wells, the conglomerate reservoir heterogeneity is very strong (the data

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were provided by Xinjiang Oilfield Company). The detailed reservoir characteristics and physicochemical property the collected water samples were listed in Table 1.

To the sandstone reservoir and the conglomerate reservoir, tracer technique indicated that the time intervals for injected water flowing into production wells are approximately 30–45 days and 7–10 days, respectively (the data were provided by Xinjiang oilfield company). In consideration of the larger microbial size and time needed for migration, the injected water of the sandstone reservoir was collected every 15 days for three times since October 2012, and the produced water samples were collected from the neighboring production wells along with the second injected water sample for three times with 30 days as interval. All the injected and produced water samples were all collected from the wellhead of injection and production wells by the field personnel of PetroChina. In the conglomerate reservoir, the injected water samples were collected in November 2011 from the wellhead and zone near downhole (obtained by backflow) of injection wells. After 7 days later, the produced water samples were collected from the wellhead of neighboring production wells every 7 days for three times. The water samples were completely filled into 15 L sterilized plastic bottles, which were immediately capped and sealed to avoid contamination and oxygen intrusion. 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 well were mixed and resuspended with TE buffer (Tris 80 mM, EDTA 40 mM, pH 8.0), and then lysed using a mini bead-beater (BioSpec, USA) at 200 rpm for 1 min at room temperature with 0.1 mm glass beads. Total genomic DNA was extracted from the suspension solution using an AxyPrepTM Bacterial Genomic DNA miniprep kit (Axygen, USA) according to manufacturer's instructions and then stored at −80 °C for the subsequent study.

2.2 Pyrosequencing of partial 16S rRNA genes and sequences analysis

Broadly conserved primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 533R (5′-TTA CCG CGG CTG CTG GCA C-3′) containing A and B sequencing adaptors

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were used to amplify bacterial 16S rRNA gene, while primers 344F (5′-ACG GGG YGC AGC AGG CGC GA-3′) and 915R (5′-GTG CTC CCC CGC CAA TTC CT-3′) were used to amplify archaeal 16S rRNA gene. PCR reactions were performed following the protocol described in the Supplement (SI). Replicate PCR products of the same sample were mixed within a PCR tube. Then, the amplicons from each reaction mixture were pooled in equimolar ratios based on concentration and subjected to emulsion PCR to generate amplicon libraries. Amplicon pyrosequencing was performed on a Roche Genome Sequencer GS FLX+ platform at Majorbio Bio-Pharm Technology, Shanghai, China.

Sequences generated from pyrosequencing were analyzed using default settings in the open source software package mothur (Schloss et al., 2009). The detailed process is described in SI. Alpha diversity analyses including rarefaction and computation of the Shannon, Simpson, Chao1 metric and phylogenetic diversity (PD) was used to assess biodiversity based on OTUs. The similarity among microbial communities was determined using UniFrac analysis in which weighted and unweighted principal coordinate analysis (PCoA) were performed based on OTUs abundance and phylogenetic relationships. Specific differences in community composition of sample communities were visualized by heatmaps and ggplot using R package. Venn diagrams were implemented by R package.

2.3 Miseq-sequencing of partial 16S rRNA genes and sequences analysis

Bacterial and archaeal 16S rRNA gene V4 region (300–350 bp) were amplified using primer set 515f (GTG CCA GCM GCC GCG GTAA) and 806r (GGA CTA CHV GGG TWT CTA AT) with the protocol described by Caporaso et al. (2011, 2012). A composite sample for sequencing was created by combining equimolar ratios of amplicons from the individual samples, followed by gel purification and ethanol precipitation to remove any remaining contaminants and PCR artifacts. Amplicon sequencing was conducted on an Illumina MiSeq platform at Novogene co., Beijing, China.

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Pairs of reads from the original DNA fragments are merged by using FLASH (Magoc and Salzberg, 2011). Sequences were then analyzed using the QIIME (Caporaso et al., 2010) and UPARSE pipeline (Edgar, 2013). The detailed process is described in SI. The similarity among microbial communities was determined using UniFrac analysis in which weighted principal coordinate analysis (PCoA) was performed based on OTUs composition and phylogenetic relationships. Specific differences in community composition of sample communities were visualized by heatmaps and ggplot using R package. Venn diagrams were implemented by R package.

2.4 Quantification of community abundance

Evaluation of community abundance by real-time fluorescent quantitative PCR (qPCR) was performed using the 16S rRNA gene as molecular markers. Reactions were performed using the FastStart Universal SYBR Green Master PCR mix in a Bio-Rad iQ5 Sequence detection system. The primer set is 8F (5′-AGA GTT TGA T(CT) (AC) TGG CTC-3′)/338R (5′-GCT GCC TCC CGT AGG AGT-3′) (Gittel et al., 2009). The plasmid containing the target gene was used as a standard (Hollister et al., 2010). Amplification efficiencies were calculated from the slope of standard curves. Gene copy numbers in unknown samples were determined based on standard curves constructed from 10 fold serial dilutions of the standard. The specificity of the PCR amplification was determined by melting curves.

2.5 Sequence accession numbers

The raw reads were deposited in the National Center for Biotechnology Information (BioProject ID: PRJNA246768, http://www.ncbi.nlm.nih.gov/bioproject/246768).

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3 Results

3.1 Microbial communities in the sandstone reservoir

In the sandstone reservoir, 249–538 bacterial OTUs and 45–130 archaeal OTUs were detected. The individual rarefaction curves, Shannon curves, and Phylogenetic diversity curves tended to approach saturation plateau (Fig. S1). The results of qPCR indicated that the copy number of 16S rRNA in the injected water was 8.25×10^6 copies mL^{-1}, while 1.5×10^6 to 2.75×10^6 copies mL^{-1} in the producted water samples. Phylogenetic analysis indicated that the injected water (Lu3084) was dominated by *Proteobacteria* (50.43 %), *Cyanobacteria* (15.51 %) and *Chloroflexi* (9.12 %). Among *Proteobacteria*, *Betaproteobacteria* (20.42 %) and *Alphaproteobacteria* (19.63 %) were numeically dominated, while a small quantity of *Deltaproteobacteria* (5.49 %), *Gamaproteobacteria* (4.44 %) and *Epsilonproteobacteria* (0.32 %) were detected (Fig. 2aI). Produced water Lu3073 was dominated by *Proteobacteria* (65.35 %), *Spirochaetes* (13.38 %) and *Bacteroidetes* (12.38 %). *Gammaproteobacteria* (23.96 %), *Deltaproteobacteria* (22.16 %), *Alphaproteobacteria* (13.47 %) and *Spirochaetes* (13.38 %) dominated at class level (Fig. 2aI). In produced water Lu3095, Lu1039 and Lu2180, *Proteobacteria* composed 78.58–95.75 % of the bacterial comunities. *Alphaproteobacteria* (15.43, 26.77, 53.54 %), *Betaproteobacteria* (23.48, 50.57, 12.94 %) and *Epsilonproteobacteria* (2.79, 4.38, 25.54 %) were dominant (Fig. 2aI).

More than 95 % of the archaeal sequences were assigned to *Methanobacteria*, *Methanococci* and *Methanomicrobia* (Fig. 2aII). 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.

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3.2 Microbial communities in the conglomerate reservoir

A total of 52 719 to 129 106 16S rRNA gene sequences were analyzed and assigned to 2623 to 3414 genus-level OTUs. In combination with the relative abundance, the number of bacterial and archaeal sequences was figured out, with the number of sequences per sample ranged in size from 51 273 to 128 980 and 85 to 1445, respectively (Fig. S2). In contrast to the sandstone reservoir, *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Spirochaetes* and *Synergistetes* were simultaneously detected in both the injected and produced water, composing 85.7–94.1 % of the whole bacterial comunities. Similar with the sandstone reservoir, *Proteobacteria* were more detected in produced water. At the class level, *Gammaproteobacteria*, *Epsilonproteobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Bacteroidia*, *Bacilli* and *Clostridia* composed 74.5–83.7 % of bacterial comunities in both the injected and produced water samples (Fig. 2bI).

The archaea were mainly assigned to *Methanomicrobia*, *Methanococci*, *Methanobacteria*, *Thaumarchaeota*, *Parvarchaea* and *Thermoplasmata* (Fig. 2bII). Among them, *Methanobacteria*, *Methanococci* and *Methanomicrobia* composed 64.3–94.6 % of archaeal comunities. Compared with the injected water botained from the wellhead of 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 genera level, *Methanocorpusculum*, *Methanococcus*, *Methanocalculus* and *Methanosarcinales* were dominant, accounting for 61.6–89.3 % of the archaeal communities in the injection wells and production well T90.

3.3 Shared microbial populations between injection and production wells

The shared microbial OTUs and genera between communities in injection and production wells were investigated using Venn diagram, histogram and heatmap. Venn diagrams indicated that 16.3–32.81 % bacterial OTUs and 13.73–51.61 % archaeal OTUs were shared between each produced water sample and the injected water in

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the sandstone reservoir (Fig. 3a). These shared bacterial OTUs accounted for a minor proportion in total bacterial community in injected water (4.6–24.71 %), but dominated in each produced water sample (43.23–76.18 %) (Fig. 4aI). Furthermore, only 13 bacterial OTUs and 3 archaeal OTUs were shared by both the injected water and produced water samples (Fig. 3a). Accordingly, the shared genera only accounted for 2.26 % of bacterial community in injected water, but dominated in each production well (12.02–36.5 %) (Fig. 4aII). Similar with the bacteria, three archaeal genera belonging to *Methanobacterium*, *Methanococcus* and *Methanolobus* were detected in both the injected and produced water samples, comprising 13.58 % of total archaea in injected water, while 90.4–96.89 % in each produced waters (Fig. 4aIII).

In the conglomerate reservoir, most of the OTUs and genera were simultaneously detected in both of the injected and produced water samples (Figs. 3b and 4b). These shared populations accounted for a minor proportion of the total microorganisms in water samples obtained from the wellhead of injection wells, but dominated in water samples obtained from the downhole of injection wells and each production well (Fig. 3b).

3.4 Microbial populations distribution in injection and produciton wells

Microbial populations were clustered according to injection and production wells to highlight the populations that showed the most variability (Fig. 5). In the sandstone reservoir, *Sphingomonas* and *Azospirillum* were found more detected in injected water, while *Arcobacter*, *Marinobacterium*, *Pseudomonas*, *Hyphomonas*, *Novispirillum*, *Proteiniphilum*, *Spirochaeta* and *Rhizobium* were highly abundant in produced water. In the conglomerate reservoir, *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*,

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Rhizobium, *Arcobacter*, *Halomonas*, *Spirochaeta*, *Bacillus*, *Thauera*, *Halomonas*, *Bacteroides* are microaerophilic bacteria, facultative anaerobe or anaerobe.

To further investigate microbial distribution in injection and produciton wells, hierarchical clustering and Unifrac PCoA were performed based on microbial OTUs abound-
5 ance and phylogenetic relationships. To the sandstone reservoir, hierarchical clustering showed that the community in injected water was distinct from those of produced water (Fig. S3). Weighted PCoA distinguished bacterial community of the injected water from those of the production wells, while communities of the production wells were placed at a comparatively decentralized position (Fig. 6aI). Similar with the bacterial com-
10 munities, hierarchical clustering and PCoA distinguished archaeal community of the injected water from those of the production wells, wheras production wells were placed at a close proximity position (Fig. 6aII). In the conglomerate reservoir, communities of water samples obtained from the wellhead of injection wells were clustered into a group in PCoA plot, indicating that communities remained unchanged before injected water
15 flowing into the injection wells (Fig. 6b). Communities of water samples obtained from the downhole of injection wells and neighboring production well T90 were clustered into a group, while production well T95 and T96 were clustered into another group (Fig. 6b). The phenomenon indicated that microbial community reconstructed in the process of injected water flowing into reservoir strata and each production wells.

20 4 Discussion

Petroleum reservoir is an extremely complex environment with multiphase fluids of oil, gas and water. To date, microbial communities in kinds of reservoirs have been intensively analyzed by cultured- and uncultured-dependent methods (Kumaraswamy et al., 2011; Kaster et al., 2009; Lewin et al., 2014; Li et al., 2010, 2014; Pham et al.,
25 2009; Wang et al., 2012). However, knowledge about microbial migration in process of injected water flowing into production wells remains poorly understood. Therefore, microbial populations and their distribution in injected water and produced water of two

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long-term water-flooding reservoirs were investigated by high-throughput sequencing and multivariate statistical analysis. Water backflow of injection wells in the conglomerate reservoir provided us the opportunity to investigate microbial migration and community succession in process of injected water flowing into production wells. The average permeability and interwell spacing of the homogeneous sandstone reservoir is 522×10^{-3} μm^2 and 300–425 m, while 362×10^{-3} μm^2 and 100–150 m in the heterogeneous conglomerate reservoir. Thus, it also provided an opportunity to investigate the influence of reservoir heterogeneity, sieve effect of strata and dissolved oxygen on microbial migration in reservoirs.

Although the sandstone reservoir is a homogeneous sandstone reservoir with a higher permeability, we found that most microbial populations detected in injected water were not detected in production wells. In combination with quantification results showing that the injected water harbored more microbial cells than those of production wells, it is reasonable to speculate that the abundant microorganisms in injected water did hardly reach production wells in this sandstone reservoir. Based on previous researches, the sieve effect, which might be enhanced by the long interwell spacing, are supposed to be the main reason (Ren et al., 2011; LR, 2010). Because of the sieve effect on microbial cells when injected fluid passes through subsurface formation, microbial cells are more difficult to migrate in reservoir strata. The results imply that, long interwell spacing might enhance sieve effect on microbial cells, and restricts microbial migration in the homogeneous sandstone reservoir. Although the sieve effect of reservoir strata might seriously hamper microbial migration, most microbial populations in injected water migrated into oil-bearing strata and reached production wells in the conglomerate reservoir. We found that almost all OTUs and genera detected in injected water were also observed in downhole of injection wells and neighboring production wells in this heterogeneous reservoir, which has a similar permeability with the sandstone reservoir. This phenomenon might be explained by the reservoir heterogeneity and shorter interwell spacing.

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The shared OTUs and genera accounted for a minor fraction of the whole community in injected water, while dominated in downhole of injection wells and production wells, suggesting that microbial community reconstructed in the process of injected water flowing into reservoir strata and production wells. Oxygen, which is known strongly related to bacterial growth and metabolism (Gao et al., 2013), may be one of the main factors to influence community structures. As expected, in injected water, aerobic bacteria, including *Sphingomonas*, *Azospirillum*, *Paracoccus*, *Ochrobactrum*, *Alcanivorax* and *Hydrogenophilaceae*_uncultured bacteria were more detected, while microaerophilic bacteria, facultative anaerobe and anaerobe, including *Pseudomonas*, *Rhizobium*, *Arcobacter*, *Halomonas*, *Spirochaeta* and *Bacteroides* were found have higher relative abundance in produced water (Fig. 5). Apart from the dissolved oxygen, another most striking factor influencing microbial populations' distribution in injected water and production wells might be crude oil. Petroleum reservoirs represent extreme and oligotrophic environments. Although diverse microbial populations, including of hydrocarbon-degrading bacteria, nitrate-reducing bacteria, sulfate-reducing bacteria and methanogens, inhabit reservoirs, only hydrocarbon-degrading bacteria can growth and produce bio-surfactants and small molecular organic substances when growing on crude oil as carbon source. This is consistent with the observed results that hydrocarbon-degrading bacteria, including of *Marinobacterium*, *Pseudomonas*, *Rhizobium*, *Halomonas* and *Oleibacter* were more detected in the downhole of injection wells and production wells. This phenomenon is consistent with our previous observations, which showed that abundant oil degraders or biosurfactant-producing bacteria (including of *Pseudomonas*, *Marinobacterium*, *Dietzia*, *Alcaligenes*, *Shewanella* and *Rhizobium*) were detected in production wells in the process of microbial nutrients stimulation in the conglomerate reservoir (Li et al., 2014). Furthermore, as well as an increase in the density of these microbial populations, a mass of incremental oil were obtained (Li et al., 2014). The conglomerate reservoirs have been proved to be feasible for microbial nutrients stimulation to enhance oil recovery in filed test. However, due to the lack of nutrients, in particular nitrogen and phosphorus, these microorganisms usually

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could not massively propagate and produce sufficient metabolites to improve oil recovery. In consideration of the high water ration stage (> 80 %) and the shift of microbial population in process of injected water flowing into production wells, microbial nutrients stimulation technique rather than injecting exogenous microorganisms is supposed to be more suitable for enhancement of oil recovery in the sandstone reservoir.

As reported in previous researches (Zhao et al., 2012; Tang et al., 2012), the archaea have a lower diversity, and are mainly assigned to methanogens. Similar with the bacteria, only three archaeal genera were detected in both the injected and produced water samples in the sandstone reservoir. These genera comprised a minor fraction of total archaea in injected water, while dominated in each produced waters. In the conglomerate reservoir, most archaea were simultaneously detected in injected water and produced water. However, the relative abundance showed a big difference between the water samples obtained from the wellhead and downhole of the injection wells, suggesting that dissolved oxygen exert a significant effect on archaeal communities. As the terminal process in metabolism chain of reservoir ecosystem, archaea, in particular methanogen, are closely related to bacteria that degrade hydrocarbon and yield volatile fatty acids, which served as substrates for archaea (Voordouw, 2011; Zhao et al., 2012). Therefore, except for the dissolved oxygen, archaeal communities are supposed to be more influenced by bacterial communities and available substrates (Onstott et al., 2010; Zhao et al., 2012; Voordouw, 2011; Magot, 2000).

5 Conclusions

Using high-throughput sequencing, this study comprehensively surveyed microbial migration in process of injected water flowing into reservoir strata of a homogeneous sandstone reservoir and a heterogeneous conglomerate reservoir. The results indicated that microbial populations in injected water did hardly reach production wells in the homogeneous sandstone reservoir, while most populations in injected water passed through reservoir strata and reached production wells in the heterogeneous

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conglomerate reservoir. The results demonstrate that microbial populations in injected water can pass through reservoir strata and reach production wells, but the reservoir heterogeneity, interwell spacing and sieve effect of strata might exert significant influence on microbial migration in reservoirs. Aerobic bacteria, including *Sphingomonas*, *Azospirillum*, *Paracoccus*, *Ochrobactrum*, *Alcanivorax* and *Hydrogenophilaceae*_uncultured bacteria were more detected in injected water, while microaerophilic bacteria, facultative anaerobe and anaerobe, including *Pseudomonas*, *Rhizobium*, *Arcobacter*, *Halomonas*, *Spirochaeta* and *Bacteroides* were found have higher relative abundance in produced water. In addition, hydrocarbon-degrading bacteria, including of *Marinobacterium*, *Rhizobium*, *Halomonas* and *Oleibacter* were more detected in downhole of injection wells and the production wells. These data imply that the dissolved oxygen and crude oil have an observed influence on microbial population composition in the process of injected water flowing into production wells. Our results expand the knowledge about microbial migration and distribution in process of injected water flowing into petroleum reservoirs, and provide guides for gathering representative samples before and after MEOR process and for the application of MEOR approaches based on injecting nutrients or microorganisms into reservoirs.

The Supplement related to this article is available online at doi:10.5194/bgd-11-16773-2014-supplement.

Acknowledgements. This study was supported by the National High Technology Research and Development Program of China (Grant No. 2013AA060902), the National Natural Science Foundation of China (Grant No. 41373074), the Chinese Postdoctoral Science Foundation (Grant No. 2014M561175), and the key technology Research and Development program of Tianjin (Grant No. 14ZCZDSY00016).

Conflict of interest. The authors declare that obviously there is no conflict of interest regarding the publication of this article.

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Table 1. Characterization of the reservoir characteristics and the collected water samples.

Reservoir characteristics	Lu field block (a sandstone reservoir)					Liu field block (a conglomerate reservoir)						
Temperature	37					22.6						
Average water content, %	80.8 %					86.8 %						
Interwell distances, m	300–425					100–150						
Average permeability, μm^2	522×10^{-3}					362×10^{-3}						
Effective porosity, %	29.9					18.96						
Formation pressure, MPa	10.2					7.2						
Water flooding operation (yr)	13					30						
Well number	Lu3084	Lu1039	Lu2180	Lu3073	Lu3095	T86-0	T86-8	T93-0	T93-7	T90	T95	T96
Source of water sample	Injected water	Produced water				Injected water				Produced water		
Mineralization	10 850	11 690	11 170	10 545	11 102	10 101	11 313	11 399	13 991	13 203	8997	9710
Total nitrogen, $mg L^{-1}$	15.1	11.5	10.6	12.7	11.6	7.6	6.8	10.2	11.5	5.7	6.3	8.5
Total phosphorus, $mg L^{-1}$	20.2	19.1	17.5	18.8	19.5	16.8	15.1	22.6	21.2	18.5	16.2	12.1
$Na^+ K^+$	4524.9	4803.1	4565.3	4308.7	4486.9	3364.3	3630.1	3801.6	4348.5	4014.2	3097	3139.8
Mg^{2+}	21.7	32.07	31.55	26.03	28.83	33.1	63.05	28.37	63.05	68.09	17.46	50.2
Ca^{2+}	191.3	281.9	284.7	181.6	216.4	70.18	77.98	72.78	77.98	96.17	86.36	108.66
Cl^-	5640	6125	5820	5160	5850	3010.24	3629.99	2921.7	3452.92	3098.77	3816.21	3405.92
SO_4^{2-}	116.2	14.04	4.86	23.13	8.86	483.4	89.4	523	362.4	89.9	65.6	165.1
HCO_3^-	356	434.1	464	846.29	511.4	3140.1	3823	4051.8	5686.9	5836.6	1914.9	2840.6

Lu3084, T86-0 and T93-0 represent water samples obtained from the well head of the injection wells; T86-8 and T93-7 represent water samples obtained from the downhole of the injection wells; T90, T95, T96, Lu1039, Lu2180, Lu3073 and Lu3095 represent water samples obtained from the well head of the production wells.

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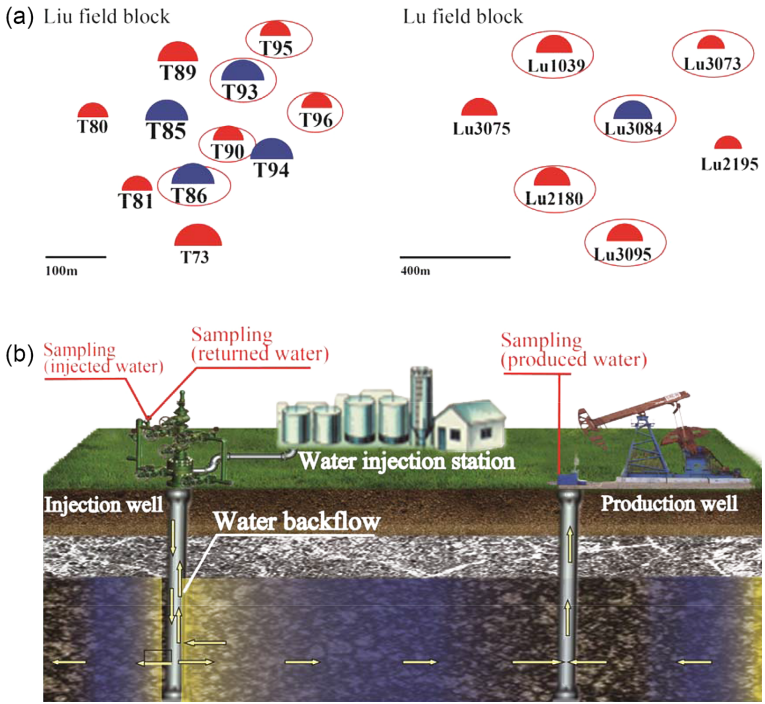

Figure 1. The schematic diagram revealing **(a)** the distribution of the injection and production wells of Liu and Lu field block, and **(b)** the wellhead and downhole of injection wells and production wells, and the place where water samples were collected. The selected T86, T93 and Lu3084 are injection wells, while the selected T90, T95, T96, Lu1039, Lu2180, Lu3073 and Lu3095 are production wells.

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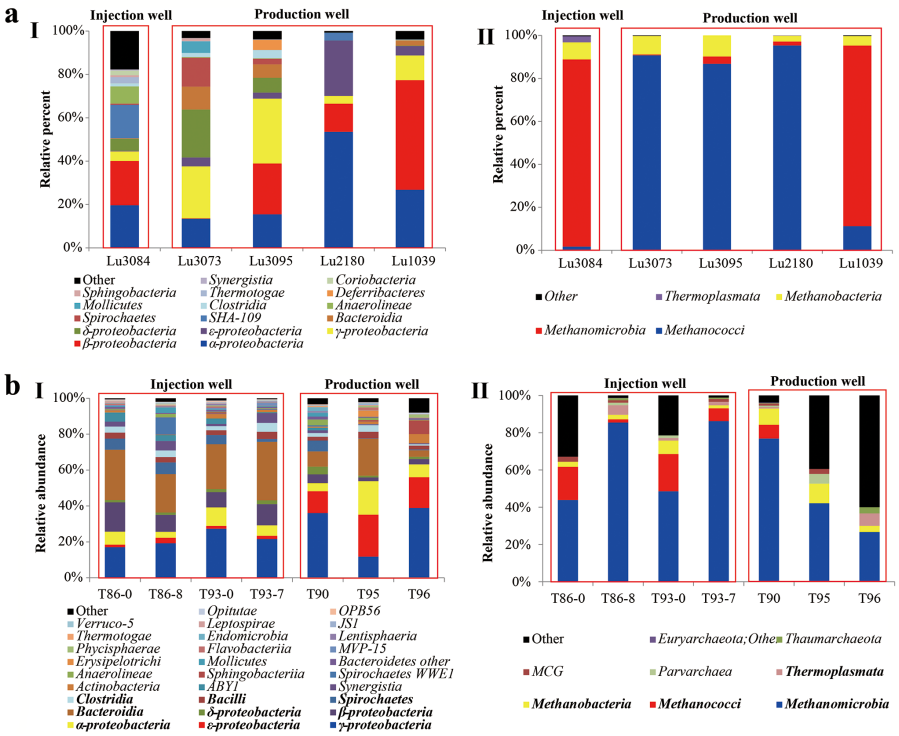

Figure 2. The relative proportion of microbial taxa at class level in injection and production water samples. **(a)** represents the sandstone reservoir; **(b)** represents the conglomerate reservoir; **(I)** represents bacterial taxa at class level; **(II)** represents archaeal taxa at class level. Lu3084, T86-0 and T93-0 represent water samples obtained from the well head of the injection wells; T86-8 and T93-7 represent water samples obtained from the downhole of the injection wells; T90, T95, T96, Lu1039, Lu2180, Lu3073 and Lu3095 represent water samples obtained from the well head of the production wells.

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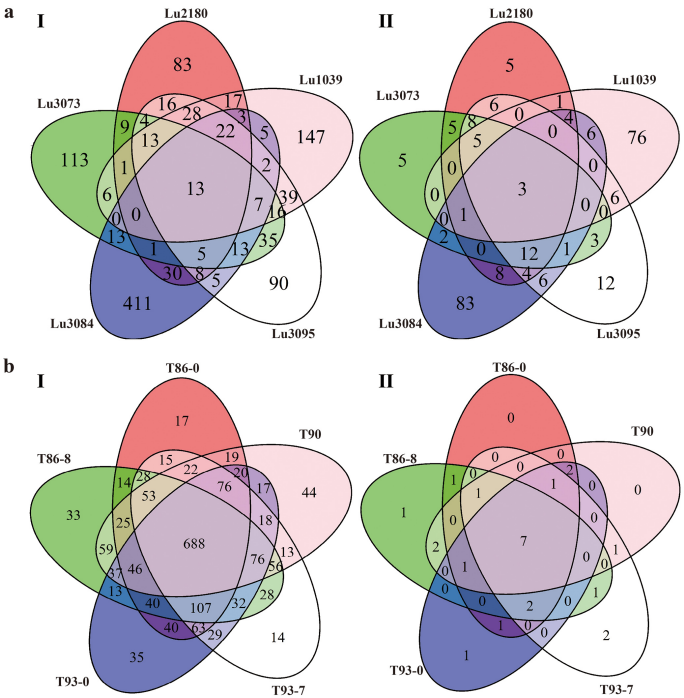

Figure 3. Venn diagram of the bacterial and archaeal OTUs in injection and production wells. **(a)** represents the sandstone reservoir; **(b)** represents the conglomerate reservoir; **(I)** represents bacterial OTUs; **(II)** represents archaeal OTUs.

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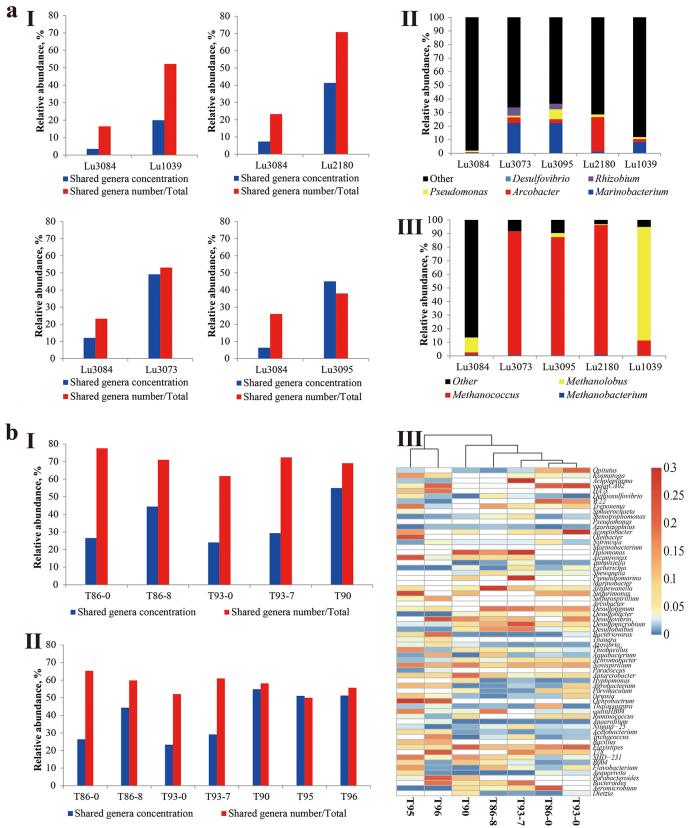

Figure 4. Comparison of shared microbial genera between injection and production wells. **(aI)** represents pairwise comparison between injection and production wells in the sandstone reservoir; **(aII)** shows the shared bacterial genera in both of the injection and production wells; **(aIII)** shows the shared archaeal genera in both of the injection and production wells; **(bI)** and **(bII)** represents comparison between injection and production wells of the conglomerate reservoir; **(bIII)** shows the dominant shared bacterial genera in the conglomerate reservoir.

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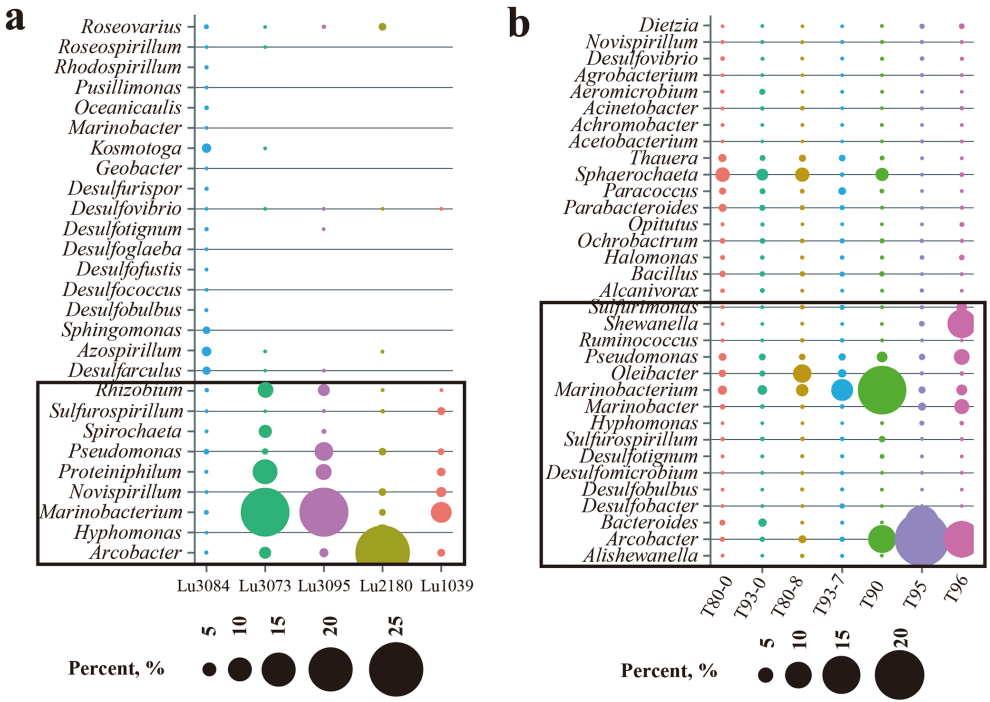

Figure 5. The genera showing the most variability in injected water and production wells. **(a)** represents the sandstone reservoir; **(b)** represents the conglomerate reservoir. The black-bordered box in the layout indicates the genera that were more detected in production wells.

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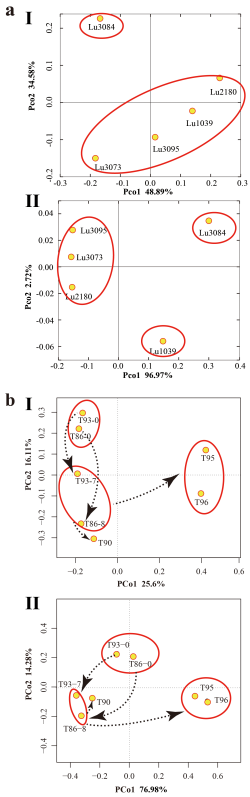

Figure 6. Principal coordinate analysis (PCoA) of microbial communities. **(a)** represents the sandstone reservoir; **(b)** represents the conglomerate reservoir; **(I)** represents bacterial communities distribution; **(II)** represents archaeal communities distribution. Sample points that are close together are more similar in community composition than those that are far apart. In **(b)** arrows show that the community succession in process of injected water flowing into injection wells and neighbouring production wells.

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