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A study on the microbial community structure in oil reservoirs developed by water flooding

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ABSTRACT

The success of biotechnological processes for oil recovery depends on adequate understanding of the relationship between the microbial community structure and oil reservoirs conditions. This study was performed to identify the microbial community structures in 10 different types of water-flooded oil reservoirs on Sinopec Shengli Oil Field. These 10 oil reservoirs have a temperature at 55–91 °C, salinity at 3000–20,000 mg/L, and permeability ranging at 207×10^{-3}–6900×10^{-3} μm^2. Some important rules found that very rich diversified bacteria and archaebacteria were identified in the oil reservoirs; these microbial organisms have functions in hydrocarbon-degradation, production of active surfactants and methanogenesis which are very valuable properties required for displacement of oil, and the microbial community structures were affected by temperature, mineralization, permeability and water displacement factors in the oil reservoirs. More abundant archaebacteria and thermophilic bacteria (Thermus, Thermincola, Thermanaeromonas) were found in high-temperature oil reservoirs. In the oil reservoir with temperature above about 90 °C, the content of thermophilic bacteria was as high as 23%, and additionally a hyperthermophilic archaea, such as Geoglobus, was also identified in the microbial community. In oil reservoirs with salinity up to 10,000 mg/L, halophilic bacteria content was 30%, which was twice as much as the reservoirs at lower salinity levels. In high salinity reservoirs, the strictly obligate anaerobic and denitrifying bacteria were not the predominant species. High permeability viscous oil reservoirs after long period of water injection resulted in significant increase of microbial diversity by doubling the species and genera number of microorganisms.

1. Introduction

Microbial enhanced oil recovery (MEOR) processes apply microbial technology to improve recovery of petroleum oil from reservoirs (Sen, 2008). Generally, MEOR operations involve injection of nutrients, typically along with cultivated-exogenous microbes, into the reservoir. The injected nutrients promote microbial propagation as well as production of metabolites (such as gases, biopolymers and biosurfactants) within the reservoir. Produced biomass and metabolites modify the properties of the in-reservoir environment and of the crude oil itself, stimulating more oil to move toward production wells. Compared with other chemical EOR, for example, in which chemicals (such as polymer, surfactant, alkaline etc.) are injected into the reservoir, MEOR is considered to be more cost effective and environmentally friendly. Although pilot operations of MEOR have been trialed in a number of oilfields, the technology remains underdeveloped due to unreliability of the performance (Brown, 2010). It has been suggested that many technical difficulties are associated with the process of injecting exogenous microorganisms into a reservoir. Injected microorganisms often face difficulties in penetrating into the formation (Bernard and Michel, 2005). As the physicochemical (such as pH, pressure, salinity and temperature) properties vary from reservoir to reservoir, it is also challenging to keep exogenous microbes physiologically active within the reservoir.

A major trend for developing the microbial enhanced oil recovery (MEOR) technology is to directly harness the indigenous microorganism resources residing in the reservoirs. Those microorganisms are subjects of many studies in this field, such as identification of the microbial community composition and analysis of the critical factors challenging deployment of the MEOR technology (Bernard and Michel, 2005; Wang and Wang, 2007; Wang, 2010). So far, several case studies on the microbial organisms in oil reservoirs have been performed (Song et al., 2010; Grabowski et al., 2005; Li et al., 2007; Chen et al., 2005; Basso et al., 2005; Nazina et al., 2005; Xiu et al., 2010), but for the difference of oil field reservoir geological conditions, the microbial communities in oil reservoirs have the features of their own. To

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expand the scope of applying MEOR technology, increasing the accuracy when selecting oil reservoirs, and optimizing the MEOR technology, it is necessary for a systematic investigation of the indigenous microbial communities including the species and their distribution in the oil reservoirs. For this purpose, in this study 10 typical oil reservoirs were selected representing different ranges in temperature, salinity and permeability parameters on Shengli Oil Field. The 16S rDNA sequences were used to identify microbial species and distribution patterns, thus to reconstruct the microbial community structures and their metabolic functional properties, which were used to project strategies for deployment of MEOR technology in those reservoirs.

2. Materials and methods

2.1. Selection of oil reservoirs

For selection of the experimental oil reservoirs, several key factors including oil reservoir temperature, salinity and permeability that affect microbial growth were considered. On Shengli Oil Field, oil reservoirs are buried deep and the stratum temperature is high (mostly above 60 °C). Temperature is a key element affecting microbial distribution; hence the oil reservoir temperature criterion was set at 55–95 °C. In this oil field, formation of water salinity is basically under 20,000 mg/L, which meets the requirements for microbial growth. The salinity level of the oil reservoirs was at 2500–22,000 mg/L. The current MEOR technology uses materials with permeability higher than above 50×10^{-3} μm^2 (Liu, 2009), and microorganisms are very useful due to their smaller sizes. In this study the oil reservoir permeability range was 200×10^{-3}–7000×10^{-3} μm^2. The three factors, temperature, salinity and permeability, were combined to divide the oil field into 10 plots (Table 1) and the indigenous microbial community structures in the respective oil reservoirs were subjected to analysis.

2.2. Sample collection and total DNA extraction

For each oil reservoir, two oil wells were chosen. 10 L of liquid secretion on the wellheads was collected into sterile containers, stored on ice and transported back to the laboratory. The cell density in samples was about from 10^4 to 10^6 cell/mL. Samples were centrifuged repeatedly under 12,000*g* at 4 °C for 15 min to precipitate microbial cells. Genomic DNA was extracted from pellets using an Axygene bacterial genomic DNA mini-extraction kit (TakaRa). Quality of DNA was examined on agarose gel, and then used in the analysis described below or stored at −20 °C. Genomic DNA was extracted within 48 h.

Table 1
Basic information of the experimental oil reservoirs.

Code no.	Formation temperature (°C)	Total formation water salinity (mg/L)	Average permeability (10^{-3} μm^2)	Timing of water injection (Date)
R-A	55	14,000	2750	1991.12
R-B	60	11,000	352	1989.6
R-C	61	9432	1879	1987.6
R-D	62	5143	2526	1977.5
R-E	66	2797	1673	1993.1
R-F	69	4006	1810	1974.10
R-G	71	8647	3000	1987.6
R-H	80	9000	207	1990.11
R-I	80	21,000	6900	1997.2
R-J	91	11,000	675	1998.6

2.3. Amplification and analysis of 16S rDNA genes

PCR amplification of indigenous bacterial 16S ribosomal DNA genes was performed in 25 μl reaction volume, which contained 2 μl of dNTP (2.5 mM), 2.5 μl of 10× PCR buffer, 0.2 μl of Ex Taq (5 U/μl), 4 μl of template DNA (approximately 100 ng), primers 27f/1492r (20 μM) each at 0.4 μl, and 15.5 μl of sterile water. The PCR started with initial denaturation at 94 °C for 5 min, then 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, and followed by a final extension at 72 °C for 10 min. For the amplification of archaebacteria, 16S rDNA, the PCR reaction mixture was the same as for bacteria except primers were replaced by Ar3F/Ar9R (20 μM) each at 0.4 μl. The reaction cycle was after an initial denaturation at 95 °C for 5 min, there were 5 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and then 30 cycles of 92 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The 16S rDNA PCR clones were used to construct a library, which were sequenced. The 16S rDNA sequences were searched in GenBank database to identify the indigenous microbial species in the oil reservoirs.

2.4. Statistical analysis of microorganism species

Rarefaction analysis and coverage were applied to estimate the representation of the phylotypes in the archaeal library. The rarefaction curve was produced by plotting the number of phylotypes observed against the number of clones sequenced using the Analytic Rarefaction 1.3 software (http://www.uga.edu/*strata/software/index.html). The coverage of clone libraries was calculated using the equation $C[1-(n1/N)]100$, where C is the homologous coverage, $n1$ is the number of phylotypes appearing only once in the library, and N is the total number of clones examined.

3. Results

3.1. Composition of indigenous microorganisms in the 10 selected oil reservoirs

The analysis of the indigenous microorganisms found a much diversified species of bacteria and archaebacteria in the ten oil reservoirs. Among the bacteria, Proteobacteria is the predominant family, including *Gammaproteobacteria*, *Betaproteobacteria*, *Bacilli*, *Alphaproteobacteria*, *Clostridia*, *Epsilonproteobacteria*, and *Nitrospira* (Fig. 1). The ratio for *Gammaproteobacteria* was 41.4% and 25.0% for *Betaproteobacteria.* The bacterial community consisted of 114 genera, with *Achromobacter*, *Arcobacter*, and *Pseudomonas* as the predominant genera, accountings for 26%, 21% and 18% of total species, respectively.

Archea is the second major microbial species in oil reservoirs. The archaea species under 14 genera, and the predominant species were methanogenic archaea and hyperthermophilic archaea each at 56% and 35% of total species numbers in this family respectively. *Thermococcus* and *Thermofilum* were the *hyperthermophilic*, *hydrogenotrophic Methanothermococcus sp.*, *Methanobacterium sp.* and *Methanoculleus sp.* the *methanolgenic archea*, and *Methanothrix sp.* the acetic acid trophic species.

3.1.1. Effect of temperature on microbial community structures

Temperature is one controlling factor for microbial activity; the microbial community structures are greatly influenced by the environmental temperature condition (Chen et al., 2010). The large gradient of temperatures in the oil field resulted in very different microbial community structures among the ten oil reservoirs.

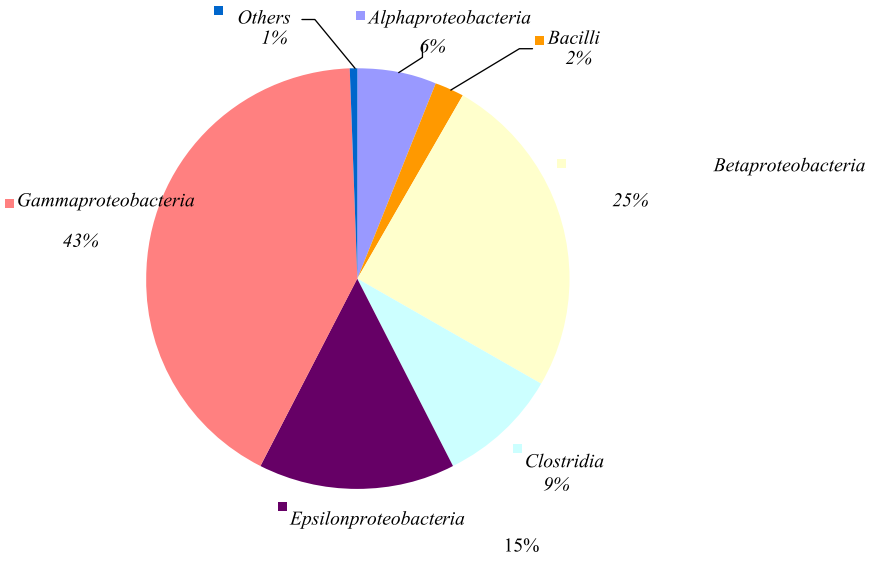

Fig. 1. The diversity of bacteria in oil reservoirs.

3.2. Effect of temperature on bacterial community diversity

In general, the diversity of the predominant bacterial species was reduced at elevated oil reservoir temperature. For instance, the two oil reservoirs, R-A and R-G, had similar salinity and permeability levels, but temperature varied from 55 °C in R-A to 71 °C in R-G. The R-A reservoir at milder temperature contained more diversified bacterial species (15 genera) than R-G (8 genera); moreover, the former reservoir also had a more complex predominant metabolic pathways, such as rom hydro-carbon degradation to active surfactant and acid production, to generate important substrates for methanogenic process.

3.3. Effect of temperature on relative abundance of extreme thermophilic bacterial species

Thermophilic bacteria grow at high temperatures; they generally have stronger adaptive response ability to survive extreme environmental conditions (Lei et al., 2001). Those bacteria are major functional groups in oil extraction. In a special case like Shengli Oil Field, oil reservoirs have rather high temperature; hence it is very critical to understand the thermophilic bacterial groups. Fig. 2 contains the diversity and percentage of microbial species tolerant above 50 °C temperatures in oil reservoirs. It can be seen that the thermophilic bacteria are highly enriched in this oil field. The diversity of thermophilic bacterial species as well as the percentage of this group of bacteria in the whole microorganism community was higher in the high temperature oil reservoirs.

But in those warmer oil reservoirs, not only the extreme thermophilic bacteria were identified, but there were also a large number of mesophilic microorganisms which were growing apparently out of the survival temperature range for the respective species.

3.4. Effect of temperature on Archaea community

The '*Archaebacteria*' consist of several distinct subgroups including thermophiles, psychophiles, acidophiles, alkaliphiles, and methanogenus; each subgroup has characteristic metabolic pathways requiring specific growth environments.

The archea in the second main indigenous microorganism in the oil reservoirs; two subgroups, *hyperthermophilic* and *mathanogenic baciteria*, were identified. The methanogens can remove the

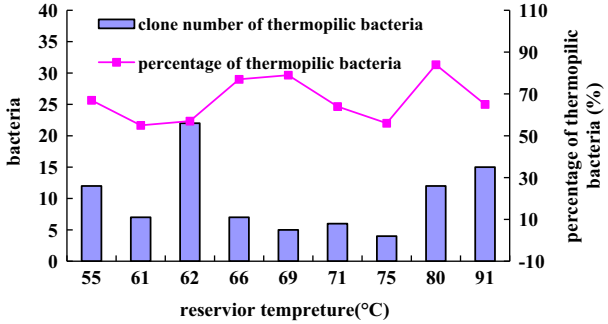

Fig. 2. The number and percentage of thermophilic bacteria clone distribution with different temperature ranges.

inhibitory effect of the end products generated during the anaerobic hydrocarbon degrading process, which will ensure smooth progression of a series of biochemical reactions, thus promoting proliferation, metabolism of the whole microbial communities in the oil reservoirs and enhancing nutrient utilization and oil displacement efficiency. The indigenous microbial community structures in oil reservoirs under four different temperature conditions are described in Fig. 3. The results showed that elevated temperature led to increased diversity of *archaebacteria*, it also changed the community structure of this species and modes of metabolism.

In cooler reservoir (55 °C), the *hydrogenotrophic Methanothermococcus* was the predominant group. In the 60 °C R-B oil reservoir, the *methanogenic archaea* became predominant, the acetic acid type Methanothrix (96%) was the most abundant, and meanwhile a small number of *Thermofilum* also appeared. In the 80 °C oil reservoirs, the *methanogenic archaea* still held the predominant position (68% for the hydrogenotrophic methanogensis and acetotrophic methanogenesis in total microbial community) but other *hyperthermophilic archaea* species and their proportions had a large scale increase. In the 90 °C R-J oil reservoir, besides a small number of *hydrogenotrophic methanogens* (14%), the sulfur-reducing *Thermococcus* and *Thermofilum* and other *hyperthermophilic archaea* were predominant. These results confirmed the significant impact of high temperature on Archaea community structure.

In summary, in most of the oil reservoirs, the indigenous microbial community is mainly comprised of methanogenic archaea, and

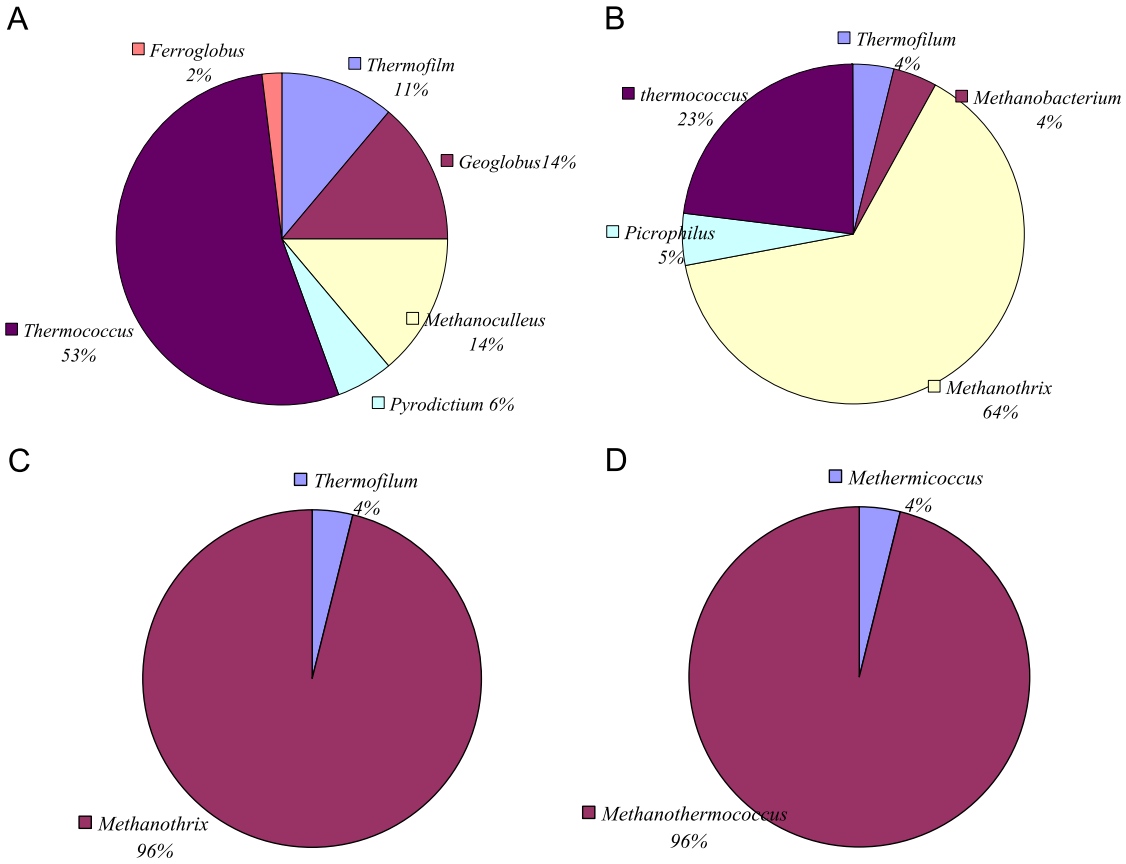

Fig. 3. Archaeal community structure of oil reservoir with different temperatures, (A) R-J (90 °C), (B) R-I (80 °C), (C) R-B (60 °C), and (D) R-A7 (55 °C).

mathanogenic metabolism pathways using hydrogen or acetate as the substrate conducted by those microbes which would provide a sound biological basis for the MEOR technology. Additionally, the Archaea subgroup using the sulfur-reducing metabolic pathway was predominant in the 80 °C oil reservoir. Similar to the situation described above, this sulfate-reducing bacterial community should be taken into serious consideration before deploying the MEOR technology.

3.5. Effect of salinity on bacterial community structures

The formation water salinity affects the osmotic pressure of microbial cells, hence affecting the microbial proliferation of microbes and thus the total community structure. Therefore, salinity is an important factor affecting microbial community formation in oil reservoirs.

3.5.1. Effect of salinity on bacterial community structure

In general, high formation water salinity level suppressed the diversity of bacteria (Fig. 4). For instance, the R-D and R-C reservoirs have similar levels of temperature and permeability, but differ in salinity. The salinity level was 5143 mg/L in R-D and 9432 mg/L in R-C. Much diversified bacterial communities (23 genera) were identified in the former reservoir compared to the latter one (10 genera). Furthermore, the former reservoir contained predominantly the strictly obligate anaerobic and denitrifying bacteria, but these bacterial groups were not significant in the more saline R-C reservoir. Consequently, salinity is a factor affecting the effectiveness of the MEOR technology. However, the same bacteria, such as *achromobacter*, were found in different salinity level reservoirs.

3.6. Effect of salinity on the content of halotolerant bacteria

The halotolerant bacteria can tolerant above 2% saline solutions. Those bacteria are distributed in salt field and underground brines. In the oil reservoir environment there are also many species of halotolerant bacteria; they produce active surfactants, and are very important for the MEOR technology.

Fig. 5 shows that at below 10,000 mg/L salinity level, the halophilic bacteria start to become part of the microbial community, but the number of species and total bacterial counts are rather low at approximately 15%. When salinity exceeded 10,000 mg/L in the R-I, R-A and R-J reservoirs, halophilic bacteria content raised to a higher level (30%). However, it should be noted that the R-J reservoir at higher temperature (91 °C) did not have the highest salinity level, but still the halophilic bacteria content was 37%. It is because 90 °C is the optimal temperature range for hyperthermophilic microorganisms; the selection pressure against high temperature extremes also has induced tolerance to other stresses. These results indicate that presence of proliferation of halophilic bacteria is affected not only by the salinity, but also the temperature condition. Elevated salinity and temperature both can promote proliferation of halophilic bacterial community growth in oil reservoirs.

3.7. Effect of oil reservoir permeability on microbial community structures

Formation of microbial community structures in oil reservoirs is a result of interaction among multiple elements. The permeability of oil reservoirs affects the mobility of microorganisms and subsequently the microbial community structures. When the pore-throat radius is smaller, the microbes are more affected by

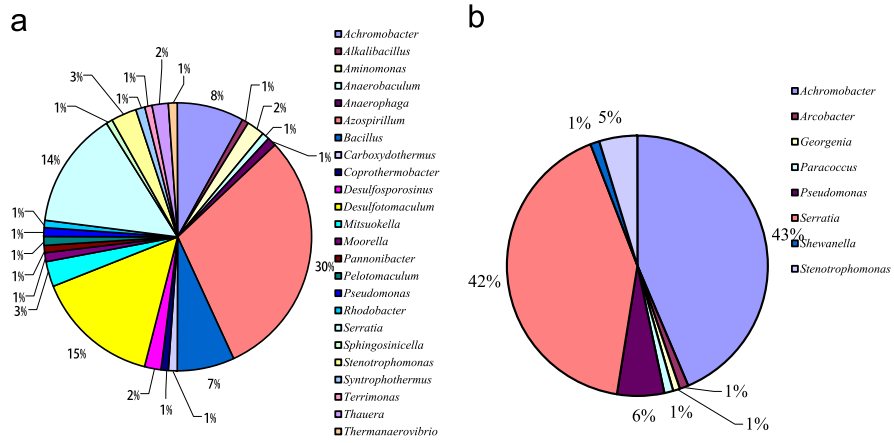

Fig. 4. Effect of salinity on bacterial community structure in R-D and R-C ((a) R-D and (b) R-C).

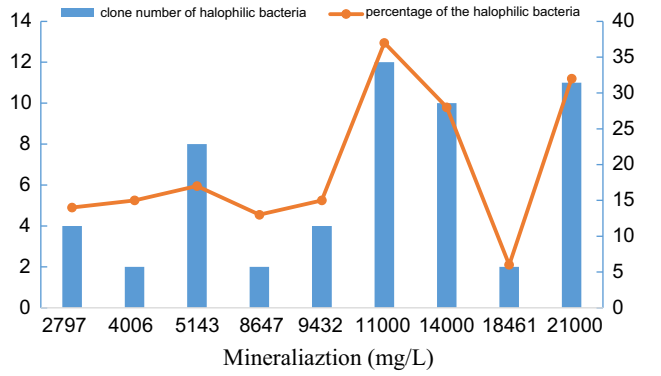

Fig. 5. The influence of mineralization on number and percentage of halotolerant bacteria clone.

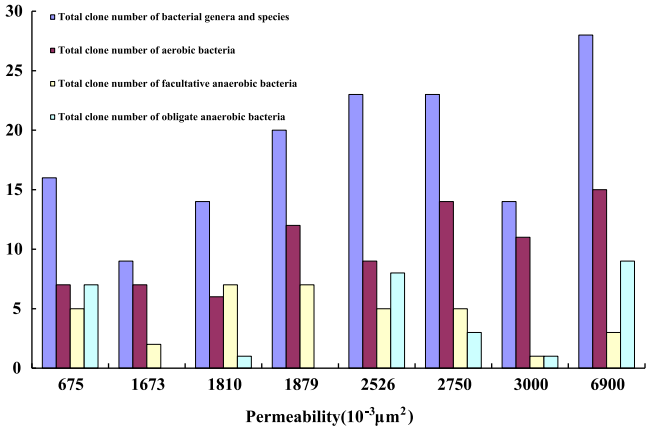

Fig. 6. The influence of permeability on bacteria community structure.

filtration and absorption force; the permeability resistance of microbes is increased concurrently.

The degree of enrichment for the indigenous microbial species under different permeability conditions and the community structures of species with different aerobic properties are described in Fig. 6. It can be seen that as the permeability increased in oil reservoirs, more microbial species were identified which matches results from relevant studies. But data from this study did not confirm there should be a very strong association between the microbial community structures and the aerobic properties of the microbial species.

3.7.1. Effect of the length of water injection treatments on microbial community structure

The water reservoirs selected for this study have all been treated by water injection for over 10 years. Compared to the non-treated oil reservoirs, the indigenous microbial communities are generally all enriched (Nazina et al., 1998). For instance, in the three oil reservoirs, R-E, R-D and R-F, at similar temperature, salinization and permeability, but under water injection for 18, 34 and 37 years, the indigenous microorganisms were grouped into 9, 23 and 14 genera in the three respective reservoirs. Generally speaking, extending water injection period has enhanced the diversity of indigenous microbial organisms in oil reservoirs. These results once again confirmed the long-term impact of water-injection on indigenous microbial community structures in oil reservoirs. Therefore, lengthening water injection time period would lead to more diversified microbial communities, and which in turn will help implementation of the MEOR technology.

4. Discussion

The bacterial community consisted of 114 genera in the 10 selected oil reservoirs, with *Achromobacter, Arcobacter,* and *Pseudomonas* as the predominant genera. In the three genera, *Achromobacter sp.* can utilize hydro-carbon as nutrient resources and it is more adapted to the poor nutrient conditions; consequently those species are distributed in all types of oil reservoirs. The *Arcobacter* and *Acinetobacter* have the capability to degrade aromatic hydrocarbons (Li et al., 2008), predominant species in multiple reservoirs belonged to these two genera. *Pseudomonas* and *Bacillus* have very diversified metabolic pathways and they also have the potential of producing active surfactant compounds and bio-polymer and also for permeability modification (Han et al., 2009), they were identified in 80% of the oil field plots. These dominant species inhabit oil reservoirs universally, together with their metabolic functions in degrading hydro-carbon and producing surfactants, those properties make them the ideal microorganisms to be used in developing the MEOR technology.

In those warmer oil reservoirs, not only the extreme thermophilic bacteria were identified, there were also a large number of mesophilic microorganisms present which were growing apparently out of the survival temperature range for the respective species. Similar situation has also been reported in relevant studies (Nazina et al., 1998) and it is generally accepted that after a long period of water injection, the oil reservoir temperature would be reduced to below the original degree level, thus making an environment amendable for mesophilic microorganisms and expanding the diversity of the bacterial community. It is favorable for MEOR that mesophilic microorganisms grow in those high

temperature reservoirs, since mesophilic microorganisms produce active metabolites more easily than thermophilic bacteria.

It is noted that in R-J oil reservoir at 91 °C, a large number of *Thermodesulfovibrio* and *Thermanaeromonas* thermophile sulfate-reducing bacteria and the denitrifying *Pseudomonas*, *Geosporobacter* and *Aquabacterium* were not the predominant species. To utilize this microbial resource for MEOR, appropriate nutrient formulas need to be developed that can promote the proliferation of denitrifying species and meanwhile suppress the thermophile sulfate-reducing bacteria in order to control or prevent corrosive damages.

Water-flooded development of oil reservoirs has a significant impact on microbial community structures. On one hand, water injection reduces the temperature of oil reservoirs resulting in a greater geothermal temperature gradient between the perimeter of water injection wells and deep oil reservoirs. On the other hand, water injection process continuously introduces foreign microbes, nutrients, and soluble oxygen into oil reservoirs. After years of such development, the primitive microbial community structure in oil reservoirs could be altered as well as the metabolic styles and rates, and it is even possible to form new indigenous microbial community structure.

Achromobacter was found in different salinity level reservoirs; it was also found in other oil field reservoirs (Wilfred et al., 2002), and it can restrain sulfide reduce bacteria by denitrification in reservoir.

Generally speaking, extending water injection period has enhanced the diversity of indigenous microbial organisms in oil reservoirs. These results once again confirmed the long-term impact from water-injection on indigenous microbial community structures in oil reservoirs. Therefore, lengthening water injection time period would lead to more diversified microbial communities, and which in turn will help implementation of the MEOR technology.

In general, the oil reservoirs contained rich species of indigenous microbes, and some species have active surfactants production, methanogenesis and hydro-carbon degradation functions. These results have confirmed the potential of using natural microbial resources in the MEOR technology. *Bacillus* and *Clostridia* were also found in some oil reservoirs; they were reported successfully during field trials (Saikrishna et al., 2007); although Clostridia was not a dominant bacteria, proper nutrient could activate it to become a dominant bacteria in MEOR.

The 10 water-flooded oil reservoirs all contained very rich indigenous microbial organisms, including bacteria and archaebacteria. Bacteria belonged to 114 genera, and the predominant groups are functional in hydro-carbon degradation, active surfactant production, acid- and gas-production with potential use for displacement of oil. The archaebacteria were grouped into 14 genera; the predominant subgroups were hyperthemophiles and methanogenesis archaea, and the two types of methanogenic archaea (hydrogenotrophic methanogenesis, and acetotrophic) were both present in oil reservoir environment. The methanogenic microbes can remove the inhibitory effect of the end metabolic products, thus promoting smooth metabolic pathways and enhancing the overall oil displacement function of the whole microbial communities.

The environmental variation determines differences in the diversity level of microbial composition and community structures among oil reservoirs. Lower oil reservoirs temperature, low formation water salinity level, higher permeability and long-term water flooding would all contribute to a higher diversity of indigenous microbial communities. For the microbial community structure, species diversity and content of thermophilic bacteria and archaebacteria increased in high temperature oil reservoir, whereas higher halophilic bacteria content was associated with more saline formation water. Therefore, while some microbial community structure properties differed among oil reservoirs, they should also have some common components of microbial populations. The deployment of the MEOR technology will be determined according to the environmental characteristics of oil reservoirs, and the stimulate formulas and the injection technology should be designed for specific microbial groups in different types of oil reservoirs. Candidate reservoir permeability, porosity, lithology, oil saturation, temperature and salinity should be estimated before any other steps (Saikrishna et al., 2007). Meanwhile, the microbial communities must be considered in the reservoir.

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