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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.