



Deposit reduction in a high pour point oil reservoir due to the activity of indigenous bacterial communities



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ABSTRACT

Physical simulation experiments were performed to simulate the reservoir environment and evaluate deposit reduction and oil recovery by indigenous bacteria inhabiting different types of water. The bacterial communities in the samples were identified based on 16S rRNA gene amplicons. Activation resulted in the predominance of *Bacillus* sp. and *Clostridium ultunense* in the samples. The oil was investigated before and after stimulation using the GC, GC–MS and ESI FT-ICR MS techniques to evaluate and analyse the deposit reduction on different scales. The n-alkanes ($n > 25$) in the sample obtained from the physical simulation experiment using production fluid containing nutrients showed a 68.66% reduction, whereas the amount of wax decreased from 46.27% to 38.75%. Additionally, naphthalene and its homologues decreased by approximately 34.0%. The typical polar compounds containing N1, O1 and O2 class compounds also significantly changed, indicating a marked biodegradation of the alkyl side chains of polar compounds and the pathway of wax metabolism by indigenous bacterium indirectly. The indigenous bacteria in the production fluid more effectively degraded the wax and heavy components. Bacteria such as *Acinetobacter* sp., *Pseudomonas* sp. and *Bacillus* sp. played important roles in deposit reduction and were beneficial to the EOR.

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1. Introduction

Oil reservoirs containing a high proportion of wax and heavy components are difficult to develop (Manshad et al., 2012). The predominant waxy material components in crude oil are complex mixtures of n-alkanes with carbon chain lengths ranging from C17 to C36 in addition to other heavier components such as resins and asphaltenes (Zhang et al., 2014; Zheng et al., 2014). Wax and heavy components usually remain stable and homogenous within crude oil (Kryachko and Voordouw, 2014). Temperatures usually drop at the wellbore during extraction and water flooding, often leading to reservoir damage due to the deposition of wax and heavy

components. Therefore, the enhanced oil recovery (EOR) of the high pour point reservoir is influenced by the deposition of these materials. Deposit removal technology based on chemical and physical treatments have been applied in field trials (Youssef et al., 2009). The chemical methods use chemicals to inhibit the deposition processes or to dissolve and disperse the deposit; however, the chemicals used are hazardous and costly (Sood and Lal, 2008). Physical methods include thermal fluid washing, electromagnetic plug removal and others, but the scope of most physical processes is limited and the thermal methods do not work well when the fluids cool down (Zhang et al., 2014). Compared with the chemical and physical processes, microbial methods are nonhazardous and more economical.

The indigenous microbial communities include both bacterial and archaeal communities that are variable under different reservoir conditions (Kaster et al., 2009; Li et al., 2007). Bacteria and

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archaea play different roles in the reservoir. Bacteria drive the initial and subsequent transformation of hydrocarbons into methanogenic precursors, whereas archaea are critical for the terminal process of methane production (Li et al., 2012; Wang et al., 2011a). Previous investigations of water-flooded petroleum reservoirs showed that the injection water and production fluid were complex ecosystems comprising a large number of bacteria (Le et al., 2015; Li et al., 2014). Aerobic bacteria that grow near the wellbore after the injection of the treated water consume most of the oxygen. The middle transition area of the production and injection wells (known as the “low-oxygen zones”) typically contains facultative anaerobic bacteria. Although oxygen is consumed in the farthest parts of the reservoir and the production wells during water flooding, the predominant bacteria are primarily anaerobic bacteria. Following water injection in an oil well, the reservoir's temperature and the inhabiting environment gradually change (Orphan et al., 2000). Therefore, the functional bacteria vary in both the injection water and the production fluid and the interaction between the bacteria and deposit often depends on the prevailing environmental conditions (Cai et al., 2015).

The efficiency of the microbial treatments is directly linked to the presence of a wide range of functional bacteria in different environments. Furthermore, different functional bacteria play important roles in the deposit reduction process (Affandi et al., 2014; Bachmann et al., 2014; Hasanuzzaman et al., 2007; Kryachko and Voordouw, 2014). However, to date no study has systematically analysed the deposit reduction of the high pour point reservoir by microbial flooding. The objective of the present study was to investigate the possible mechanism and the potential for deposit reduction by various bacteria in different fluids from the high pour point reservoir. Therefore, we performed the physical simulation experiment to simulate the reservoir conditions. The diversity of the bacterial community structure based on 16S rRNA gene amplicons in both the injection water and the production fluid from the HeNan oil field (China) before and after the physical simulation experiment were discussed, with a focus on the bacteria after the physical simulation experiment previously reported as the functional bacteria in wax, resin and asphaltene biodegradation and EOR (Atlas, 1995; Ayed et al., 2015; Capelli et al., 2001; Hasanuzzaman et al., 2007; Kryachko and Voordouw, 2014; Sherry et al., 2013). The components of the deposits were systematically analysed by chemical analysis (thin layer chromatography, gas chromatography (GC), gas chromatography-mass spectrometry (GC–MS) and electrospray coupled with Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS)) on different scales from the components of the crude oil (saturates, aromatics, resins, and asphaltenes (SARA)) to the compounds in the deposit (n-alkanes, aromatic hydrocarbon and the polar compounds). Furthermore, we investigated the possible relationship between the degradation of the deposit components, the functional bacteria and the changes in the wax and the heavy components during the process was discussed, which result in enhanced oil recovery (EOR).

2. Materials and methods

2.1. Sample collection

The V block oil reservoir with an area of 3.1 km² located in the northwest of the HeNan oilfield (China) was selected for this investigation. Oil production at this block commenced in 1977; the original oil in place (OOIP) was estimated to be 280×10^4 t with a current OOIP recovery of 47.25% and a high water-cut of 95%. The crude oil produced in the V block has a pour point of 45 °C and a high wax content of 46.72%. The original formation pressure is

1977.8 psi, and the temperature is approximately 65 °C.

Crude oil, injection water and production fluid samples were collected from production well V149 and injection well V149-3. The water–oil samples were retrieved from wellheads and stored in sterile plastic bottles to avoid contamination and oxygen intrusion. The samples were transported to the laboratory for analysis within 24 h. Artificial cores from the Northeast Petroleum University of China were used in the physical simulation experiments (Xu et al., 2012).

2.2. Physical simulation experiment

For the culture-dependent experiments performed in our laboratory, we used our previously reported medium composition containing (grams per liter): crude oil, 20.0; (NH₄)₂HPO₄, 1.6; KNO₃, 0.2; and molasses, 20.0. The pH was adjusted to 7.0 to stimulate the bacteria and ensure nutrient availability (Zhang et al., 2012). The physical simulation experiments were performed to simulate the reservoir environment and to obtain the EOR under different conditions. Standard core flooding equipment was used as previously described (Bao et al., 2009) and included a Teledyne ISCO pump (Teledyne ISCO 100DX, USA), core holder, confining pressure pump, high pressure vessels and oven. The schematic of the dynamic experimental setup for the physical simulation experiment is shown in Fig. 1. The cores, high pressure vessels and medium for simulation were sterilized. The core samples were first evacuated for approximately 2 h and then saturated with the injection water or the production fluid. The cores were placed inside the core holder at a confining pressure of 1000 psi, and the initial liquid permeability was determined by flowing the injection water or the production fluid across the cores at a constant flow rate of 0.5 ml min^{−1}. The dewatered and degassed oil of well V149 was injected into the core to establish initial oil saturation at 65 °C. After the saturation of water and oil, the core holders were kept at 65 °C for 12 h. Subsequently, sterile water was used for the water flooding until the water-cut of the outlet reached 98% and the recovery of the water flooding was calculated.

The recovery of the second round of water flooding was obtained after injecting medium diluted with the injection water or production fluid and incubating for 15 days. After the incubation, the outlet of the water-cut of the sterile water flooding reached 98% water to oil. Fermented liquid (the liquid collected from the outlet) was extracted with dichloromethane, and the organic phase was collected for analysis following the incubation.

2.3. DNA extraction and PCR amplification

DNA extracts were obtained from the original injection water and production fluid samples and the samples after stimulation. 100 ml water samples and 2 ml of fermented liquid were centrifuged at 11 363 G for 10 min to pellet the cells. Genomic DNA was extracted from the collected cells following the protocol of the TianGenamp Bacteria DNA Kit (TianGen Biotechnology, Beijing, China). The extracted DNA was detected by agarose gel electrophoresis. The bacterial 16S rDNA was amplified from the bulk DNA using the universal bacteria-specific primers 27F (5'-AGAGTTT-GATCCTGG CTCAG-3') and 1492R (5'-CTACGGCTACCTTGTACGA-3'). The 25 µl PCR reaction mixture contained 2.5 µl of 10 × PCR buffer (Mg²⁺ plus), 10 nmol of deoxynucleotide triphosphates, 1 U Taq DNA polymerase (TaKaRa), 10 pmol of each primer and 1 µl of template DNA. The thermal cycling conditions were an initial denaturation at 94 °C for 5 min, 40 cycles of 94 °C for 30 s, 56 °C for 60 s, 72 °C for 90 s and a final extension step of 72 °C for 10 min. The amplified fragments were approximately 1450 bp in length.

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