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Original Research Paper

Impact of a Microbial-Enhanced Oil Recovery Field Trial on Microbial Communities in a Low-Temperature Heavy Oil Reservoir

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ABSTRACT

In this experiment, air and nutrients were injected into a low temperature heavy oil reservoir to initiate an indigenous microbial enhanced oil recovery (MEOR) process with a cumulative increment of 1872 t heavy oil. This study reveals the response of microbial communities in the field before and during MEOR based on culture-dependent enrichment and culture-independent 16S rRNA gene clone library methods. The results showed that it was easy to activate the biosurfactant-producing bacteria *Pseudomonas* in laboratory conditions, and the bacteria were also the dominant present group in the mixed oil-water samples after MEOR. Fermentative and hydrocarbon-oxidizing bacteria increased by 300-500%, and the acetate ion concentration also significantly increased. Microbial activity of *Pseudomonas* and the metabolic products including biosurfactants were proposed to be the primary mechanisms for improving heavy oil recovery. The results of this experiment can serve as a useful resource for monitoring MEOR-related microbial population, and for future related experiments.

INTRODUCTION

A large proportion of China's crude oil is heavy oil that is considerably denser and more viscous than conventional oils. Maintaining high production output is important to sustain increasing energy demands. This is often achieved using costly chemical and physical extraction and maintenance processes for production wells. Reducing crude oil's viscosity is a key treatment step to enhance oil recovery (EOR) of heavy oils (Youssef et al. 2009, Macián-Martínez et al. 2013). EOR processes include chemical and physical methods such as using solvents, surfactants (Tayfun 2003), water, CO_2 flooding, and thermal treatment through steam and hot fluids injections (Wang 2012).

Unlike expensive EOR treatments, microbial enhanced oil recovery (MEOR) has gained attention as an important tertiary oil recovery method that is both cost-effective and environmentally friendly to drive oil in reservoirs (Marchant & Banat 2012). MEOR processes do not consume large amounts of energy. Moreover, microorganisms could synthesize useful products *in situ* by fermenting low-cost substrates or raw materials (Bryant & Lockhart 2002). There are two main MEOR mechanisms: the first involves microorganisms improving the crude oil's physical characteristics through degrading heavy oil to reduce the heavy oil's average molecular weight, thereby increasing mobility (Hasanuzzamana et al. 2007), the second depends upon the byproducts of microbial metabolism that could considerably reduce the oil's viscosity, such as surfactants, solvents, acids and gases. Therefore, using microbial consortia with the necessary required properties to allow such mechanisms to take place *in situ* could be desirable to achieve enhanced oil recovery (Jinfeng et al. 2005).

OVERVIEW OF THE STUDY AREA

There are three main *in situ* strategies for implementing microbial-enhanced oil recovery techniques depending on oil well characteristics including whether the well environment has significant indigenous microbial flora and can support microbial growth. MEOR field trials are therefore carried out by (1) injecting nutrients alone, (2) injecting exogenous microorganisms and nutrients, or (3) injecting indigenous or exogenous microorganism products. Prior to the field trial, it is critical to investigate the microbial populations that reside in oil reservoirs to understanding their nature. If suitable indigenous microorganisms exist, culture-dependent microbial enrichment could be carried out, and main metabolic products could be identified following *in vivo* core-flooding testing to optimize an MEOR process for the reservoir. Indigenous microbial-enhanced oil recovery (indigenous MEOR) has been widely applied to oil reservoirs (Youssef et al. 2009). However, MEOR field trials in heavy

oil reservoirs have not been reported.

There are three main criteria for evaluating the success of indigenous MEOR field trials: (1) the number of microorganisms in produced fluids, (2) the volumetric improvement of oil production, and (3) the reduction of water-cut in the production stream (Youssef et al. 2009). For MEOR processes, especially indigenous MEOR, analysing stimulated microorganisms leads to successful field trials in oil reservoirs and is essential for understanding the microbial mechanisms underlying MEOR. Therefore, monitoring the microbial community structure during an MEOR process is essential for determining structural variations of the stimulated microorganisms and their effects on developing effective MEOR techniques. However, very few studies have monitored microbial communities before and after MEOR trials to understand and evaluate the process's effectiveness. Therefore, it is unknown how injecting nutrients stimulates specific bacterial cluster growth, and how it affects microbial communities in a reservoir in a field trial. In addition, it is unknown how and how much these targeted microbes affect oil recovery. Studies have also not determined the divergent characteristics of microbial communities obtained in laboratories versus those in oil reservoirs. Discovering the answers can lead to better understanding of the process and drive improvements. This paper reports on a comprehensive assessment of microbial communities' structures before and after microbial field trials in a Chinese low-temperature heavy oil reservoir.

MATERIALS AND METHODS

Oil reservoir conditions and sample collection: An indigenous MEOR process was carried out in the Xinjiang No. 6 oilfield (China). As shown in Fig. 1, the field block has four injection wells (T6185, T6186, T6193, and T6194) and nine production wells. Table 1 shows information detailing the oil production characteristics, the gross composition of the crude oil, and the formation water of the oil reservoir. Oil production at the block was initiated by water-flooding in 1974; however, the production fluid's water content rapidly reached 80% soon after, hindering oil recovery due to poor water flooding.

100 mL oil-water samples were collected from the wellheads of the four production wells on July 23, 2010. The samples were pooled together to create the PWB sample. From August 24, 2010 to September 21, 2011, 100 mL activation nutrient solutions consisting of 1.4 g molasses, 0.3 g ammonium chloride, 0.7g nitrate, and 0.12 g sodium polyphosphate were injected into the oil reservoir along with production water though the four injection wells. 15600 m^3 of nutrient-containing water and 62400 m^3 of air were introduced into the oil reservoir base, as depicted in Table 2. On January 31, 2011, four oil-water samples were collected from production wells T6073, T6181, T6190 and T6196. These samples were collected and stored in sterile 500 mL serum bottles and then transported to the laboratory as soon as possible for further analysis.

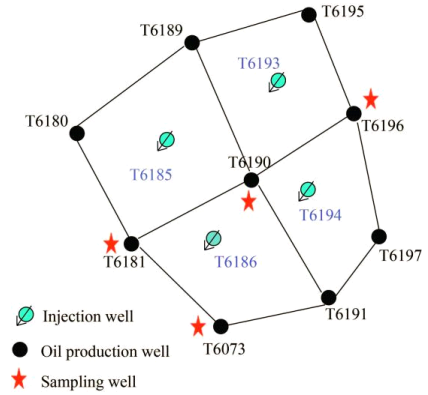

Fig. 1: The well group is composed of four injection wells (T6185, T6186, T6193, and T6194) and nine production wells in Xinjiang No. 6 oil field (China).

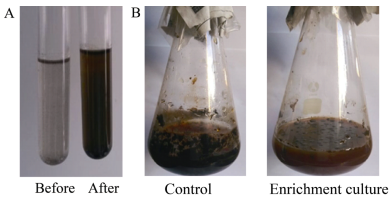

Fig. 2: A indicates changes of produced fluids before and after microbial field trial, B presents emulsification effects after incubated aerobically at 20 °C with shaking (160 rpm) for 7 days.

Enrichment of indigenous bacteria: A medium containing 2g molasses, 0.3g ammonium chloride, 0.7g nitrate, 0.1g sodium polyphosphate and 2g sterilized crude oil per 100 mL of sterilized formation water was prepared in a 250 mL Erlenmeyer flask to enrich microbial strains that were present in the reservoir and likely involved in the enhanced oil recovery process. The enrichment culture (EB) was inoculated with 10% (v/v) of the mixed oil-water (PWB) sample and incubated aerobically at 20°C with shaking (160 rpm) for 7 days.

Additionally, hydrocarbon-oxidizing bacteria (HOB) and fermentative bacteria (FB) in the four monitored production wells were quantified using the most probable number (MPN) technique (Sutton 2010) obtained through a previously described medium that was incubated aerobically at 20°C and shaken (160 rpm) for 7 days (Nazina et al. 2013).

16SrRNA gene clone library construction and sequencing analysis: The oil-water samples and enrichment cultures were centrifuged to pellet the cells. Following the manufacturer's

Table 1: Oil reservoir characteristics and oil production properties of Xinjiang No. 6 oil field (China).

Characteristics	
Environmental parameters of oil reservoir	
Production depth (m)	480
Stratal temperature (°C)	20.6
Porosity (%)	18.96
Properties of crude oil	
Viscosity of degassed crude oil (mPa·s at 20°C)	145.10
crude oil freezing point (°C)	-26
Wax content (%)	3.0
Saturates (%)	41.38
Aromatic (%)	26.29
Resin (%)	22.41
Asphalt (%)	2.16
Formation water	
pH value	7.5
Salinity (mg/L)	8742
F-(mg/L)	8.9
Cl-(mg/L)	26.0
NO3-(mg/L)	180.4
SO42-(mg/L)	164.0
Microbial treatment time	
First injection	8/24/2010
Last injection	9/21/2011

instruction for the TIAN Micro DNA Kit, the genomic DNA was extracted in triplicate to avoid bias and then mixed together. 16S rRNA gene amplifications were carried out with the universal primer pair 27F-1492R following the previously reported PCR-reacting system and thermal cycle program (Zhang et al. 2010). Amplicons were cloned with a TA cloning vector kit (Promega, Madison, USA) according to the manufacturer's instruction. 100 clones in each clone library were obtained to screen for positive clones through PCR with the vector-specific primers T7/SP6. Positive clones were classified into different operational taxonomic units (OTU) based on the amplified ribosomal DNA restriction analysis (ARDRA) profiles (Lagace et al. 2004). Representative clones belonging to different OTUs were selected for 16S rDNA sequencing. The coverage of each clone library was calculated using the following equation: $C = [1 - (n1/N)] \times 100$, where n1 is the number of OTUs represented by one clone, and N is the total number of clones examined. Additionally, a diversity analysis was also carried out as described by Caporaso et al. (2010).

Sequencing was carried out using an ABI PRISM 3730 DNA sequencer (SinoGenoMax Co., Ltd., China). The obtained sequences were manually checked and edited using DNAMAN version 5.2.2.0. Chimeras were detected using the CHIMERA-CHECK program from the Ribosomal Database Project; secondary structure anomalies were also similarly checked (Cole et al. 2003). The final sequences were submitted to the GenBank. Their phylogenetic affiliations were determined using the Basic Local Alignment Search Tool algorithm. A neighbour-joining tree was constructed using Clustalx version 1.83 and MEGA version 4.1 (Tamura et al. 2007). A bootstrap analysis with 100 re-samplings was conducted to assign confidence levels to the nodes in the distance and parsimony trees.

Monitoring chemical properties of production fluids before and after field trial: Ion chromatography was used to characterize the acetate ion as described by Gao et al. (2014). Information regarding the microbial field trial in the oil reservoir was offered by technical personnel from the Research Institute of Experiment and Detection, Xinjiang Oilfield Company.

Nucleotide sequence accession numbers: 16S rRNA gene sequences were submitted to the GenBank database with the following accessions numbers: KJ439801-KJ439813 (clone library of EB and PWB), KJ507680-KJ507704 (clone library of T6190 and T6073), KJ507712-KJ507731 (clone library of T6196 and T6181).

RESULTS AND ANALYSIS

Impact of the MEOR process on produced fluids characteristics: Injecting air and nutrients as an MEOR method significantly enhanced oil production in four production wells in the Xinjiang No. 6 oilfield. During August 2010 and September 2011, a cumulative increment of heavy oil reached 1872 t, and the crude oil in produced fluids emulsified and dispersed homogeneously before and after the microbial trial (Fig. 2A). As depicted in Table 3, the improvement in oil recovery ranged from 0.5-4.3 t/d. Meanwhile, the production fluids' water content decreased from 83.9-91.4% to 56.7-82.5%. These results indicate that the activated indigenous microbe MEOR technique improves the water flooding effect to improve the oil recovery.

Acetate, a fermentation product of indigenous acidogenic bacteria, is an important intermediate product (Nazina et al. 2004). Compared to the concentration before the field trial, the acetate ion concentration increased significantly, ranging from 4.9-525.7 mg/L (Table 3). Nazina et al. (2007) reported similar trends of increasing acetate ions when the microbial oil transformation accompanied acetate ion accumulation. Higher acetate concentrations in the production fluids could subsequently decrease heavy oil viscosity, and then improve oil well production (Youssef et al. 2009).

Indigenous bacteria in the four monitored production wells reached 10^8 cell/mL, compared to 10^6 cell/mL before the field trial. HOB and FB increased from 10^2 and 10^4 cell/mL to 10^7 cell/mL, respectively, except for the FB in production well T6073. HOB and FB activation occurred by injecting nutrients and air; the significantly increasing FB

numbers may explain the increase in acetate concentration (Youssef et al. 2009). These results show that indigenous bacteria have been effectively activated in treated production wells.

Response of the stimulated microbial populations in different production wells during the field trial: The microbial communities of the four different sampling production wells were characterized to determine the stimulated microorganisms during MEOR. The positive clones of four clone libraries were clustered into 6-19 OTUs; coverage values ranged from 90.70-97.72%. As given in Table 4, sample T6073 had the highest bacteria richness, approximately two times higher than samples T6181, T6190 and T6190. Sample T6073 also had the highest bacterial diversity, as demonstrated by a 2.21 Shannon index. Sample T6190 had the lowest richness and diversity.

Dominant OTUs were analysed at the genus level to understand the differences and similarities between the microbes that inhabited the four monitored production wells. Fig. 4 illustrates the relationships between the microbes in different samples. Microbes belonging to the *Pseudomonas* genus predominated in all except for the T6196 well, and represented 43%, 79%, 80% and 21% of detected groups in production wells T6073, T6181, T6190 and T6196, respectively. *Thalassolituus oleivorans* represented 63% of the detected genera and was the dominant genus in the T6196 well. To our knowledge, *Thalassolituus* (Yakimov et al. 2004), *Acinetobacter* (Sipos et al. 2010), *Pseudomonas* (Celik et al. 2008), and *Shewanella putrefaciens* all utilize hydrocarbons either solely or in association with other microorganisms and may improve oil recovery (Bahobil 2011).

Both nitrate and sulphate ions were detected in the oil reservoir's formation water (Table 1). Denitrifying bacteria *Arcobacter* sp. *Desulfuromonas michiganensis*, *Comamonas denitrificans,* and sulphur-oxidizing bacterium (SOB) *Thioalkalivibrio sulfidophilus* were also detected. However, *Desulfotignum toluenicum* was only identified in the T6073 well produced fluids.

Changes of microbial community structure in the heavy oil reservoir before and during the microbial trial: In general, oil reservoirs have low redox potentials, and hence mainly harbour anaerobic and facultative microorganisms (Youssef et al. 2009). After being subjected to water-flooding since 1974, oil reservoirs have experienced changes in oxygen content. As a result, microbial communities, especially those inhabiting the areas near the injecting wells, change in accordance with the change in oxygen in the environment (Lysnes et al. 2009). The studied oil reservoir has been producing oil for approximately 42 years; hence, oxygen contents in different parts of the reservoir are unknown and complex. Monitoring microbial communities before and during the microbial field trial is also important for identifying potential microorganisms and understanding the microbial mechanisms of MEOR.

The mixed samples collected from the four production wells were inoculated into the medium in a laboratory. Fig. 2B shows the emulsification effects and the droplet size distribution of the heavy oil after a 7 day-long aerobic cultivation. The emulsification effect increased after the stimulated microbes and their metabolites had acted upon the heavy oil sample. In the control treatments, the crude oil was clumpy and floating in the liquid. The results indicated that potential MEOR-active microorganisms can survive within the mixed oil-water sample obtained from the heavy oil reservoir. It is interesting to note that micro-emulsion formations can lead to increased oil dispersal into the culture solution to mobilize entrapped oil.

Microbial communities in the production fluids from the four production wells before and during the field trial were significantly similar (Fig. 3). *Pseudomonas* were the predominant group of PWB (53%) and EB (65%) before the field trial, while γ-Proteobacteria was also the dominant group in T6073 (44%), T6181 (90%), T6190 (81%), and T6196 (92%) during the microbial field trial. With the exception of the T6073 well, coverage values for the production wells were higher than that of PWB (Table 3).

However, enrichment cultures in the laboratory were carried out in stable aerobic conditions, leading to preferential growth of aerobic biosurfactant-producing communities. *Pseudomonas* was in the EB samples and the four monitored production wells during the microbial field trial, as shown in Fig. 4; the *Pseudomonas* ratio ranged from 18-21%, indicating that the molasses could be contributing to rapid growth and the predominance of *Pseudomonas* at the expense of other bacterial strains. In fact, members of *Pseudomonas* sp. were not considered indigenous to oil reservoirs (Youssef et al. 2009), but numerous microbial community studies from oil reservoir samples have reported the presence of *Pseudomonas* (Zhang et al. 2010). However, no sequences were detected for the *Pseudomonas* in the PWB clone library; this could be explained by the selective enrichment of some microbes or as a result of a PCR bias and TA clones due to the low strain proportions in the total microbial community (Sipos et al. 2010, Taylor et al. 2007). Therefore, it is inevitable that some rare microbial groups would be missing from the results. Pham et al. (2009) suggested that it is useful to apply more than one technique in microbial diversity studies.

Additionally, the sulphate-reducing bacterium *Desulfotignum toluenicum,* detected only in T6073, can utilize ac-

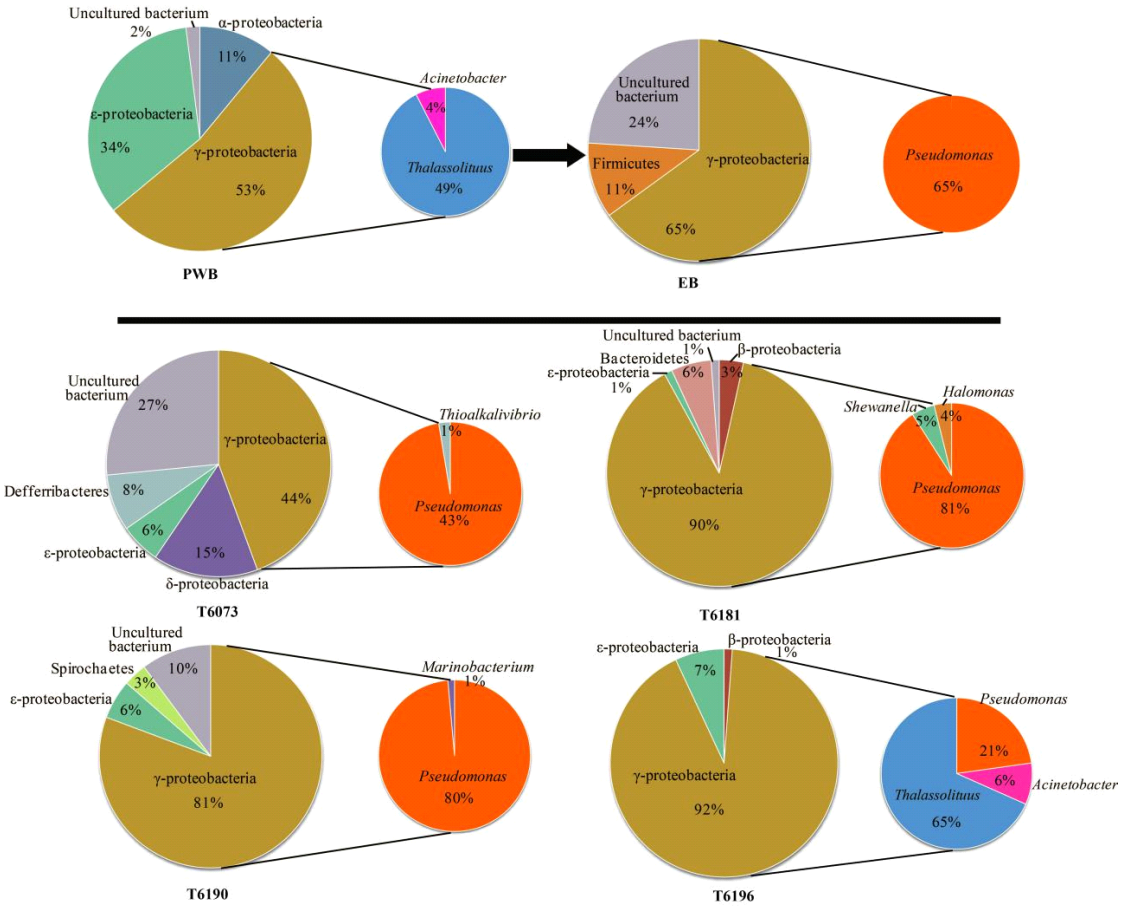

Fig. 3: Relative abundances of microbial community in clone libraries PWB and EB before field trial, and T6073, T6181 T6190, T6196 after field trial.

etate as a carbon source; acetate concentration in production well T6073 decreased slightly from 9.2 mg/L to 5.7 mg/L, which supports our results. Strains of the nitrate-reducing bacteria *Arcobacter* sp. were detected in PWB, T6073, T6190 and T6196; other denitrifying bacteria, such as *Shewanella putrefaciens*, *Halomonas nitritophilus*, *Desulfuromonas michiganensis*, *Comamonas denitrificans,* and the sulphur-oxidizing bacterium *Thioalkalivibrio sulfidophilus* were also detected (Fig. 4). Stimulating NRBs or SOBs and inhibiting SRBs through nitrate addition has successfully inhibited SRBs activity based upon the competition mechanism (Das & Mukherjee 2007).

Potential microorganisms and mechanisms of the field trial of oil recovery: It is generally believed that effective MEOR application involves a combination of multiple microbial mechanisms utilizing bacterial groups with different physiological and metabolic abilities (Youssef et al. 2009). In the present study, *Pseudomonas* sp. formed the dominant microbial group from the monitored production well samples. It is well-known that *Pseudomonas* strains could produce biosurfactants of rhamnolipids that lower the interfacial tension between hydrocarbon and aqueous phases and, consequently, improve crude oil mobility (Yan et al. 2012). They were also frequently reported to be potential microbes for MEOR (Bordoloi & Konwar 2008). Additionally, members of the genus *Acinetobacter* were also identified in the monitored production wells; they can produce bioemulsifier emulsan and improve oil recovery (Chen et al. 2012), which is in agreement with the crude oil in produced fluids emulsified and dispersed homogeneously in our study. To our knowledge, biosurfactants could also play an important role in altering the wettability of a reservoir rock and, thus, improve oil mobility (Perfumo et al. 2010).

Additionally, it has been also reported that *Pseudomonas* and *Thalassolituus* strains often degraded alkanes, aliphatic, and aromatic fractions, resulting in a decrease in crude oil viscosity (Hasanuzzamana et al. 2007, Yakimov et al. 2004). In this approach, stimulating aerobic hydrocarbon metabolism in the vicinity of the injection well results in the production of acetate, other organic acids, and alcohols, which

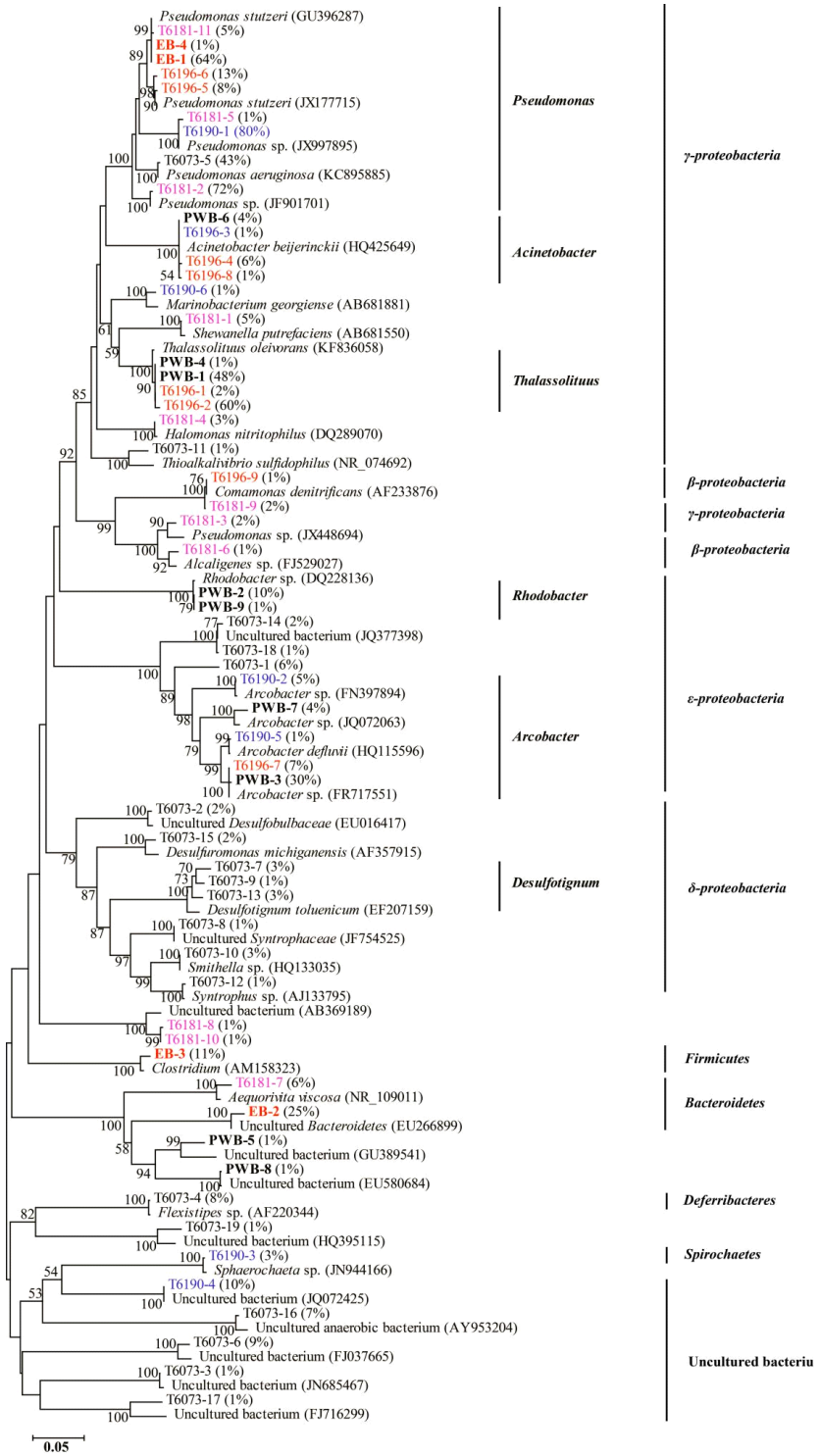

Fig. 4: Phylogenetic tree of 16S rDNA phylotypes of clone library mixed samples (PWB, Bold), enrichment culture (EB, Red and Bold), and samples from the four monitored production wells T6073 (Black), T6181 (Magenta), T6190 (Blue), and T6196 (Red). The tree was based on partial 16S rDNA genes and the closest sequences retrieved from GenBank database. Bootstrap values (n = 100 replicates) of >50% are reported as percentages. The scale bar represents the number of changes per nucleotide site. The percentage represents the ratio of the number of positive clones classified into one OTU in the total of positive clones in each clone library.

Table 2: The injection nutrients for indigenous MEOR field trial in Xinjiang No. 6 oil field (China).

Injection wells	Total of injected fluids (m^3)	Total of injected air (m^3)	Injected fluids per round (m^3/d)	prepared fluids per round (m^3)	Injected air per round (m^3)	Shut for days per round (d)	Round (time)
T6185	3000	12000	20	300	1200	7	10
T6193	4500	18000	30	450	1800	7	10
T6186	3600	14400	24	360	1440	7	10
T6194	4500	18000	30	450	1800	7	10
Total	15600	62400	104	1560	6240	/	/

Table 3: Changes of production fluids, concentration of acetate ion, and indigenous microorganisms obtained from the four monitored production wells before and after field trial.

Characteristics	T6073		T6181		T6190		T6196	
	Before	After	Before	After	Before	After	Before	After
Daily production (m^3)	15.9	13.3	1.9	3.8	7.5	18.2	16.2	10.9
oil content (t)	2.6	5.8	0.2	0.7	0.2	4.5	1.5	2.8
Water content (%)	83.9	56.7	91.4	82.5	97.0	75.5	91.0	74.8
Incremental oil (t/d)[a]	3.2		0.5		4.3		1.3	
acetate content (mg/L)	9.2	5.7	6.3	525.7	4.9	380.0	6.7	202.7
Total number of indigenous microbes (cells/mL)	9.0×10^7	7.7×10^7	2.4×10^6	3.7×10^8	1.0×10^7	2.8×10^8	3.9×10^6	8.2×10^7
Hydrocarbon-oxidizing bacteria (HOB) (cells/mL)	6.0×10^4	1.1×10^7	6.0×10^2	1.1×10^7	6.0×10^4	2.5×10^6	2.5×10^3	1.3×10^4
Fermentative bacteria (FB) (cells/mL)	1.1×10^4	1.3×10^2	6.0×10^4	1.1×10^7	1.3×10^4	7.0×10^6	7.0×10^4	2.0×10^5

[a] the best incremental oil in one day.

Table 4: Analysis of bacterial communities' diversity from the produced waters based on 16S rDNA clone library.

Parameters	Before field trial		After field trial			
	PWB	EB	T6073	T6181	T6190	T6196
Number of positive clones	89	88	86	87	88	86
Number of OTUs	9	4	19	11	6	9
Coverage (%)	94.38	98.86	90.70	95.40	97.72	96.51
Shannon index	1.73	1.25	2.21	1.21	1.06	1.53
Simpson index	0.63	0.51	0.72	0.35	0.35	0.56

is in agreement with the significant increase observed in the acetate concentration with an increasing number of HOB and FB after the field trial in our study.

Notably, a strategy for stimulating NRBs based upon a competition mechanism for electrons was successfully applied to control SRBs (Voordouw 2011). In the study, nitrate-reducing bacteria *Arcobacter* sp., *Halomonas nitritophilus* and *Comamonas denitrificans* were stimulated by injecting nutrients. Therefore, enhancing the activity of such strains could be useful for controlling SRBs and would then further enhance oil recovery.

DISCUSSION AND CONCLUSIONS

In summary, MEOR via nutrient injection increased crude oil output by 1872 t when applied in a heavy oil reservoir. Strains of *Pseudomonas* were present in samples taken from production wells before and during the microbial field trial. It is supposed that increased microbial activities of surfactant-producing bacteria in the production fluids were the main mechanisms for improving heavy oil recovery.

The results suggest that effectively applying MEOR requires a combination of multiple microbial mechanisms utilizing groups of bacteria that reduce oil viscosity and enhance oil mobility. However, we could not definitely determine what microbial activities and products took place during the microbial treatment for heavy oil recovery. Further isolation and metabolic analysis investigations are required. Microbial community analysis in production wells could indicate which microbes may be important in guiding further studies concerning the potential application and mechanisms of microbes in oil fields.

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