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Conversion of petroleum to methane by the indigenous methanogenic consortia for oil recovery in heavy oil reservoir

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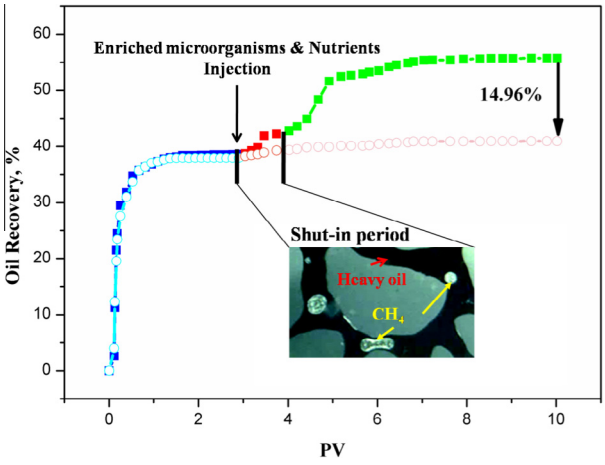
HIGHLIGHTS

- Heavy oil was degraded by the enriched methanogenic consortia.
- Methane production during the biodegradation of heavy oil.
- Viscosity of the degraded heavy oil was reduced with the dissolution of methane.
- In-situ bioconversion of heavy oil to methane has great potential on EOR.

GRAPHICAL ABSTRACT



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ABSTRACT

Microbial enhanced oil recovery has been well acknowledged and becoming an advanced technology for oil recovery. Compared with current techniques, a newly technical strategy of the in-situ heavy oil gasification to methane for oil viscosity reduction was proposed and successfully proved via enriching the methanogenic consortia from the brine of oil reservoir with heavy oil as carbon source. During 200 days anaerobic culturing, 2.34 g of heavy oil was degraded coupling with 1514 μmol of methane production. Phylogenetic diversity analysis showed that the enriched consortia composed with sequences affiliated with the *Firmicutes*, *Proteobacteria*, *Deferribacteres* and *Bacteroidetes*. The recovered archaeal phylotypes were close to the *Methanobacteriales* and *Methanosarcinales*, which could convert the produced small molecules (formic acid and acetic acids) to methane. The viscosity of the degraded heavy oil was reduced by 72.45% at 20 MPa after the dissolution of the produced methane. Core flooding tests finally showed that the oil degradation and methane production by the enriched methanogenic consortia made 14.7% of the tertiary enhanced oil recovery. These results demonstrated a promising and practical strategy of microbial technology on oil recovery by activating the methanogens in heavy oil reservoir.

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1. Introduction

Worldwide energy demand is continuously increasing with the current pace of development, and oil continues to play crucial role in total energy consumption. Exploration of petroleum resources traditionally follows a pattern where the better quality and more accessible resources, like light oils, are extracted firstly before programming to general lower quality, less accessible resources that require more efforts and have higher energetic, economic, and environmental costs [1]. Reserves of heavy oil, ultra-heavy oil and natural asphalt all over the world are about 100×10^9 t [2]. With the depletion of conventional oil reserves, the production of these unconventional fossil resources achieved a boost especially with the significant increases in production from tar sands and heavy oil reservoir around the world. Unparalleled attentions are shifting toward the development of heavy oil. However, to improve oil recovery on this type of unconventional resource is commonly facing great technical challenging due to high viscosity and density, poor fluidity, and easy absorption. Conventionally, thermal recovery techniques including steam stimulation and steam drive methods [3–5], and cold production techniques including alkaline drive, polymer drive and miscible flooding [6–8], have been well developed and widely applied for the heavy oil recovery. In these traditional techniques, reducing viscosity has become the key strategy in heavy oil exploitation, transportation and refining. At present, the main ways to reduce the viscosity of heavy oil are thermal recovery (by means of heating cables, electric heating oil-pumping rod, and heat-conducting oil), dilution method by using light oil, and phase-behavior by emulsification. However, these techniques generally have extreme high cost, and negative effects on the surrounding environment [4,5]. Therefore, extensive interests in microbial enhanced oil recovery (MEOR) for heavy oil recovery have been increasing as cost-effective and environmentally friendly candidate [9].

Microbial enhanced oil recovery (MEOR) technology has been well recognized and successfully applied in the development of the conventional oil, which is a way to utilize microorganisms or their metabolites to improve the recovery of crude oil from reservoirs [10,11]. The main mechanisms of MEOR involve [12]: (1) biosurfactants production could reduce the surface/interfacial tension and alter the wettability of solid surface; (2) degrade the heavy component of crude oil to improve the oil quality; and (3) gas production could dissolve in crude oil and then reduce its viscosity, or increase reservoir pressure. Most of microorganisms that have performed successfully in numerous researches or field pilots for oil recovery, no matter in conventional or unconventional reservoir, are aerobic or anoxic bacteria. Few efforts have been relatively involved into the application of anaerobic microorganism on improving oil recovery, significantly due to that aerobic bacteria have showed greater performance on biosurfactant production and crude oil degradation than anoxics or anaerobics [11,13]. However, these bacteria always cannot fulfill the expecting functions after injected or stimulated in subterranean of oil reservoirs. This is also the key reason that the mechanism of MEOR has not been qualitatively and quantitatively characterized, resulting in MEOR technology has not been widely spread throughout the whole petroleum industry. Recently increasing studies demonstrated that anaerobic microbes dominate subsurface environments, despite slow reaction kinetics and uncertainty as to the actual metabolic activities (such as degradation and emulsification of heavy oil, methane generation) occurring in oil reservoirs [14–18]. However, most of laboratory researches or oilfield trials are limited to the utilization of anaerobic biosurfactant-producing bacteria and its metabolites to form the oil-in-water emulsion, which has limited effects on viscosity reduction at subterranean [19].

Petroleum reservoirs are characterized as extreme environments by the wide range of temperature and salinity, high pressure, and anoxic/anaerobic conditions, and coupled with multiphase fluids of oil, gas and water. Interests in microbiosphere at deep subterranean petroleum-rich strata for MEOR have been driven by the potential presence and multi functions of living microorganisms within. Recent decades, numerous types of microorganisms from various oil reservoirs and their functions has been recognized, including nitrate reducers, sulfate reducers, fermentative bacteria, iron reducers, acetogens and methanogens under anaerobic condition [18,20–23]. Among them, the conversion of hydrocarbons to methane in oil reservoirs is a typical ultimate anaerobic biodegradation process [23,24], and the generated methane is able to dissolve in oil under subterranean pressure to thereby reduce the oil viscosity dramatically [23,25]. Therefore, the converting the residual oil to methane by anaerobic microorganisms under methanogenic condition could be suggested as a newly way to improve the exploitation and development of heavy oil through heavy oil degradation and in-situ methane dissolution. Under this strategy, heavy oil reservoirs could be regarded as "bioreactors" in which diverse physiological types of microorganisms acting in syntrophic association can be stimulated with specific nutrients and significantly degrade heavy oil to methane [26,27]. Although methane production from crude oil by microbial consortia in oil reservoir has been well documented [28,29], none of researches focus their efforts on the in-situ methane dissolution into the degraded heavy oil and its influence on the viscosity reduction as well as on the final heavy oil recovery. Therefore, this study will investigate the anaerobic microorganisms and their activities on heavy oil degradation under methanogenic condition; quantify the characterization of the in-situ methane production and dissolution in the degraded oil; calculate the comprehensive effects on viscosity reduction; and finally evaluate the potential of heavy oil recovery. Compared with the previous attempts to apply MEOR and other approaches on improving heavy oil recovery, this study will not only extend the theoretical understanding of the mechanism of MEOR, and but also create a practical, high-efficiency and low-cost technique to improve heavy oil recovery. In terms of the current oil price, it is obviously more and more sensible and economical to apply this green strategy to the heavy oil recovery.

2. Materials and methods

2.1. Sample collection

Oil and brine samples were collected from Xing block of Daqing oilfield, and were immediately sealed in sterile 150 ml bottles and transported to the laboratory at 4 °C for further research. The reservoir located at the Chinese northwest (latitude 46.79, longitude 125.04) with depths of 850 m to 900 m subterranean, and characterized with temperature of 50 °C, permeability of 375×10^{-3} μm^2, dehydrated dead oil viscosity of 1823.86 mPa s at 50 °C, and total salinity of 3852.26 mg/L.

2.2. Anaerobic enrichments

Pre-enrichment culture of the methanogenic consortia was carried out by the incubation of 1 g oil and 150 ml enrichment-medium with 50 ml of brines at 50 °C until the detection of methane. The pre-enrichment medium contains (g/L): NH_4Cl 2.0, K_2HPO_4 1.5, KH_2PO_4 1.5, $MgCl_2$ 0.2, CH_3COONa 2.0, HCOONa 2.0, citrate 1.0, yeast extract 0.05, cysteine 0.05, resazurin 0.002, pH 6.0–7.0, supplemented with 1 ml of the trace element solution and 2 ml vitamins solutions previously developed [30]. After

330 days incubation, methane was detected and then 50 ml of the pre-enriched culture was transferred to 200 ml of the prepared anaerobic heavy-oil-medium (namely HOM medium) in 500 ml serum bottles sealed with butyl rubber and aluminum stoppers, and incubated at 50 °C. The uninoculated media were used as control. Six parallel culturing were set as interval samples for analysis during the time course of the experiments. The HOM contains (g/L): heavy oil 40, NH_4Cl 12.0, K_2HPO_4 6.0, KH_2PO_4 6.0, $MgCl_2$ 1.0, yeast extract 0.05, cysteine 0.05, and supplemented with 2 ml trace elements and 0.2 ml vitamins solutions as the above mentioned. All incubations were carried out at 50 °C in triplicate. All nutrient media were prepared following the anaerobic technique described previously [31,32].

2.3. Degradation of heavy oil and methane production

To characterize the degradation of heavy oil under anaerobic condition, the residual oil was extracted from the interval samples by a mixture of n-hexane and dichloromethane (1:1, v/v), and analyzed by gas chromatography according to the described method [33]. In addition, oil was also separated into saturates, aromatics, non-hydrocarbons, resin and asphaltenes using column chromatography using several different developing-solvents and then weighted to evaluate the degrading-ratio [34]. Methane was routinely monitored using gas chromatography and quantified based on methane standards. The concentration of CH_3COO^- and COO^- were measured following the reported method [35].

2.4. Surface tension

The surface tension of cultures during the methanogenic degradation were measured at 25 °C by a digital tensiometer (Kruss, K10ST, Germany) using the ring method for detection of biosurfactant production.

2.5. Phylogenetic diversity of anaerobic microorganism

DNA from the enriched cultures was extracted and purified by using commercialized kit (QIAGEN, China) and the Genomic DNA purification kit (Takara, China) respectively.

The 16S rRNA gene was amplified with primers (304f, 5′-CCCTAYGGGGYGCASCAG-3′) and (1000r, 5′-CCCTAYGGGGYGCASCAG-3′) with PCR Master Mix in 50 ul reactions [26,36]. Thermal cycler program was conducted as follows: 95 °C for 3 min, 30 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 90 s, and a final elongation step of 72 °C for 10 min. Amplicons were purified with using the Qiaquick PCR Purification kit (Qiagen, USA), and then were cloned into *Escherichia coli* with pGEM®-T Easy cloning vector (Promega, USA). The obtained white colonies were randomly picked and cultured overnight at 37 °C in 1 ml LB medium with ampicillin (50 mg/ml).

The sequencing was determined with automated ABI 377 sequencer (Dye-Terminator Cycle Sequencing Ready Reaction FS Kit; PE Applied Biosystems) using universal M13 sequencing primers. The resulting sequences were determined for orientation and then preliminarily compared with those available in the GenBank database of NCBI (http://www.ncbi.nlm.nih.gov) using the BLAST service to determine their approximate phylogenetic affiliations. Partial sequences that differed by more than 3% were considered to be distinct OTUs and one among sequences was chosen to represent each OTU for phylogenetic tree construction. The coverage of each clone libraries was calculated by the equation $C = (1 - (n_1/N)) \times 100$, where n_1 is the number of OTUs represented by only one clone and N is the total number of clones examined. Sequences were aligned with the CLUSTAL-X software. Phylogenetic trees were constructed based on the Kimura two-parameter model [37] and the neighbor-joining algorithm [38] using the MEGA6.0 software. Bootstrap analysis with 1000 replicates was applied to assign confidence levels to the nodes in the trees.

Totally 310 clones were sequenced and categorized according to their sequence similarity. The partial 16S rRNA sequences obtained in this study were submitted to the NCBI Genebank databases under accession numbers JN128118-JN128124 for bacteria and JN128125- JN128126 for archaea.

2.6. Heavy oil viscosity reduction

The viscosity of heavy oil after methanogenic degradation was measured by viscometer (Brookfield, USA) at 50 °C. In order to determine how the produced methane influences the viscosity of the degraded heavy oil, a steel piston container was developed (shown in Fig. 1) and the oil viscosity was measured according to the method as described by Shuler et al. [39], with viscosity standard fluids (Brookfield, USA) to calibrate the curve of the torque against viscosity. Firstly, 25 g of the degraded heavy oil was transferred into the mixing container (Fig. 1) after dehydration and degasing. Secondly, a amount of pure methane was introduced into the mixing container to purge the space and keep the absolute pressure of container 1 atm. Thirdly, push piston rod with constant rate by syringe pump, and monitor the viscosity and pressure simultaneously.

2.7. Oil recovery evaluation

The potential application of the indigenous bacteria and their metabolites for MEOR was evaluated by using the sandpack (Fig. 1) core-flooding technique [11]. All tests were implemented at 50 °C with dehydrated dead heavy oil (viscosity of 1823.86 mPa s at 50 °C). The parameters of the core flooding tests were presented in Table 1. The core flooding tests were carried out as follows: firstly, formation water saturation; secondly, after oil saturation, the first water flooding was conducted until no further oil was observed in the outlet of the core; thirdly, 1.0 pore volume (PV) of cell solution prepared by mixing the microbial cells of 200th-day cultured sample with the autoclaved mineral medium were injected into the water-flooded core, followed by a 100-days shut-in period at 50 °C; 1.0 PV of the autoclaved mineral medium without microbial cells was set as control. Finally, the second water flooding was performed until no further oil was observed from the outlet of the core.

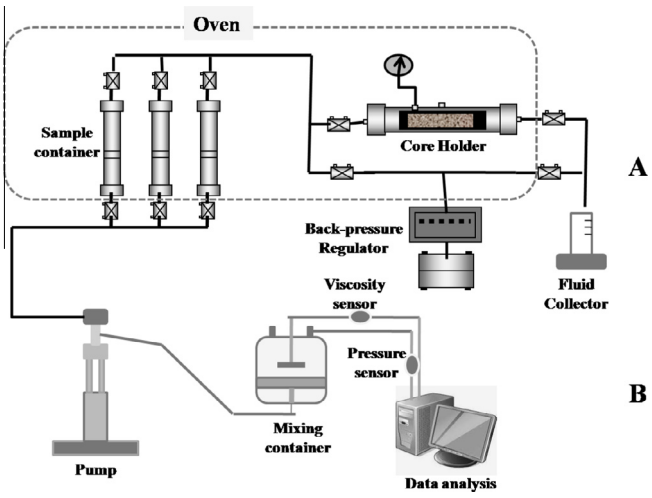

Fig. 1. Schematic of the developed physical simulation system: A, core flooding test; B, high pressure rheometer for methane and heavy oil.

Table 1
Parameters of core flooding tests.

Sample core	Dimension cm × cm	PV (ml)	Porosity (%)	Permeability (μm^2)	Initial water saturation (ml)
MEOR	3.8 × 30	81.78	26.78	0.270	18.30
Control	3.8 × 30	81.07	28.80	0.294	18.71

Note: data was average value of two parallel tests.

3. Results and discussion

3.1. Methane production

Methane production as an indicator of the methanogenic metabolism was monitored in incubations amended with Xing heavy oil. The enriched methanogenic consortia showed the great ability of producing methane when using heavy oil as sole carbon source (Fig. 2). In the process of the heavy oil degradation, a lag period of the methane production (~80 days) was observed; and then the activity of methanogens achieved an substantially increasing, finally resulting in 1514 μmol of methane production and 25% degradation ratio of heavy oil over the 200 days incubation. As the first stage (0–20 days) presented in Fig. 2, the intensity of heavy oil degradation was weak resulting in <30 μmol of methane production. Bioconversion of petroleum hydrocarbons to methane exclusively requires the syntrophic communities of the acetogenic bacteria and methanogenic archaea based on the thermodynamics point of view [16,17,20,22,23]. A large number of literatures have well documented that the degradation of petroleum at methanogenic condition generally follows the trend: the most degradable straight chain n-alkanes, the more resistant branched-acyclic and monocyclic hydrocarbons, the most resistant polycyclic steroidal and triterpenoidal hydrocarbons, and finally aromatic hydrocarbons [40]. The saturated hydrocarbon fraction in heavy oil, generally accounts for small percentage, was enwarped or dispersed in resin and asphatene, which will lead to the poor availability of the readily-edible saturated fractions to microorganisms. This is possible reason that a small amount of methane was produced in the first stage, while the small molecules like formic and acetic acids began to accumulate. In second stage (20–80 days), the degradation rate of the heavy oil was extensively increased, which has higher degradation rate than the first and third stage, but the methane production was still low (<40 μmol). The syntrophic activities of crude oil degradation has been intensified, and the concentration of small molecule substrates (formic acid and acetic acid) that are essential for methane production reached their maximum points of 257.12 mg/L and 165.34 mg/L respectively (Fig. 2).

The surface tension in this stage was drastically decreased from 56.24 mN/m to 32.45 mN/m, indicating the production of the surface/interface active materials (namely biosurfactants). Although the structural and quantitative analysis of such biosurfactants was not conducted in this study, the presence of these surface/interface active materials could explain the extensive degradation of heavy oil in second stage. Numerous literatures have reported the positive correlation between biosurfactant production and hydrocarbon degrading capability [11–13,40]. In third stage (80–200 days), the methane concentration increased rapidly to 1514 μmol at 200th day with the sharply decreasing of the concentration of the produced formic and acetic acids.

3.2. Degradation of heavy oil under methanogenic condition

It was well documented that microorganisms have showed the great ability of degrading crude oil at various environment [40–44]. Although increasing attentions has been paid on the anaerobic degradation of petroleum compounds [23,29,30,45], the most rapid and complete degradation of the majority of petroleum hydrocarbons is brought about under aerobic conditions [33,46–49]. However the enriched consortia of this study can significantly degrade the heavy oil under anaerobic condition, and exhibited obviously better performance than some aerobic microorganisms on degrading heavy fractions of oil.

Chromatography column separation and chromatography/mass spectroscopy (GC/MS) were performed to characterize the heavy oil degradation of the enriched methanogenic consortia. Four fractions from Xing heavy oil were redistributed after 200 days of anaerobic degradation with the results shown in Table 2 that saturated hydrocarbons, aromatic hydrocarbons, resin and asphaltenes were relatively decreased by 10.57%, 6.03% and 3.63% respectively; while the non-hydrocarbon was relatively increased by 71.26%. The total weight of heavy oil was deceased by 23.4% after anaerobic degradation.

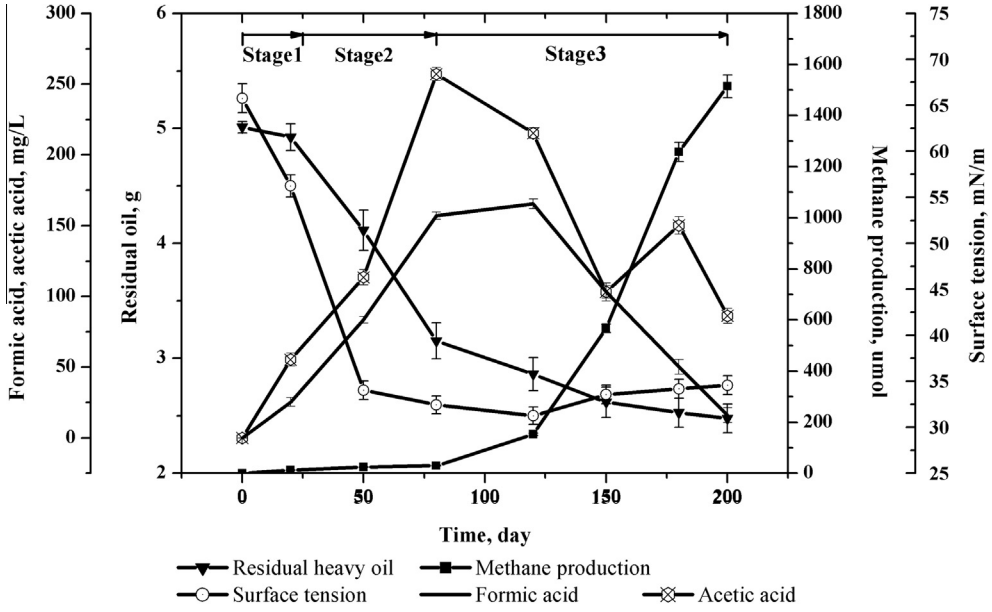

Fig. 2. Microbial activities during the heavy oil degradation by methanogenic microcosm enriched from the brine of heavy oil reservoir, including methane production, surface tension, formic acid and acetic acid generation. The values are mean ± standard deviations ($n = 3$) after deduction of control.

Table 2
The four fractions of heavy oil before and after microbial treatment.

Sample	Heavy oil weight (g)	*w* (%)			
		Saturated hydrocarbons	Aromatic hydrocarbons	Non hydrocarbon	Resins asphaltenes
Original	5.00	33.02	26.53	6.09	34.36
Degraded	3.83	29.53	24.93	10.43	33.11
Change ratio	−23.40	−10.57	−6.03	+71.26	−3.63

The dates are average value of three parallel experiments.
Change ratio (%) = $(w_{Degraded} - w_{Original})/w_{Original}$.

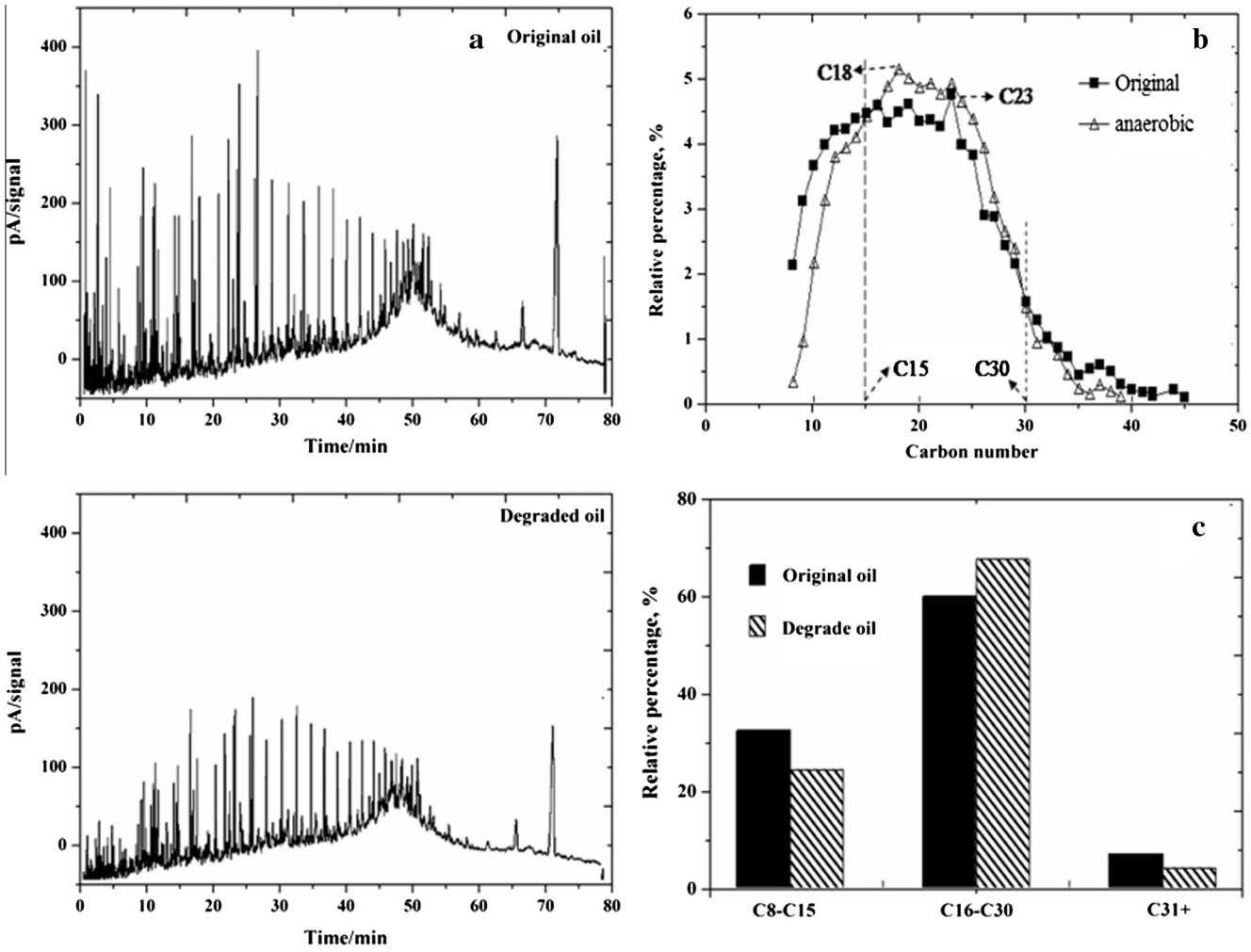

Fig. 3. Gas chromatography analysis of heavy oil degradation by enriched microorganism under anaerobic condition at 50 °C. The values in (b) and (c) were average of three parallel experiments.

Gas chromatography analysis of heavy oil shown in Fig. 3a demonstrated that the redistribution of saturated hydrocarbon fractions in Xing heavy oil before and after methanogenic degradation. Obvious increase in the relative concentration of saturated fractions (C15–C30) was observed from 59.85% to 67.44%, accompanying with a decrease in short-chain hydrocarbon fractions (C8–C15) from 32.56 wt.% to 24.66 wt.% and heavy hydrocarbon fractions (C31+) from 7.59 wt.% to 4.68 wt.% (Fig. 3b and c). Long-chain hydrocarbon fractions (C41–C45) were degraded completely. Numerous literatures have reported the microbial activities on the shifting of the saturated hydrocarbons in crude oil, but few of them detected the complete degradation of long-chain hydrocarbon fractions (C41–C45), and more than 5% increasing of the short-chain hydrocarbon fractions (C8–C15) [17,29,30,33,48,50]. Table 3 showed that the max peak of carbon fraction was drifted from C23 to C18, and obvious increase in the fractions ratio of *w*(C21−)/*w*(C22+) and *w*(C21 + C22)/*w*(C28 + C29) accompanied with a decrease of *w*(pr)/*w*(nC17) and *w*(pr)/*w*(nC18). These results presented the distribution of hydrocarbons have been shifted from heavy fractions to light fractions after anaerobic degradation, which has positive effects on the viscosity reduction.

Although the metabolic pathway of converting heavy oil to methane was not unveiled, the reported mechanism involved in methanogenic degradation of crude oil [26,28,30,34,51], which includes the syntrophic activities of converting the hydrocarbons to the small molecule compounds (like formic acid, acetic acid) and then methane production through methanogens, was well embodied in this study. Based on the degradation results of heavy

Table 3
The parameters of the oil component.

Sample	w(nC21−)/w(nC22+)	w(nC21 + C22)/w(nC28 + C29)	w(Pr)/w(ph)	w(pr)/w(nC17)	w(pr)/w(nC18)	Max peak
Original	1.31	1.91	1.07	0.18	0.16	C23
Degraded	1.42	2.02	1.12	0.13	0.11	C18

The dates are average value of three parallel experiments.

Table 4
Distribution of 16S rRNA gene clones detected in heavy oil-amended microcosms.

OTU	No. of clones	Phylogenetic group	Closet phylogenetic relatives (Accession number)	% sequence identity
Bacteria				
MB-1	85	*Clostridiales*	Uncultured bacterium clone PL-16B6 (AY570599)	98
MB-2	10	*Bacteroidales*	Uncultured bacterium L9B-2 (FN646539)	99
MB-3	13	*Bacteroidales*	*Uncultured Porphyromonadaceas TCB179x (DQ647169)*	98
MB-4	25	*Enterobacteriales*	*Pantoea* sp. (KF017289)	92
MB-5	31	*Syntrophobacterales*	Uncultured *Syntrophus* (HE648201),	86
MB-6	32	*Syntrophobacterales*	*Syntrophaceae* sp. clone B2 (JX473526)	97
MB-7	25	*Deferribacterales*	*Uncultured Deferribacteres* (GD179761)	99
Archaea				
MA-1	64	*Methanosarcinales*	*Methanosarcina barkeri Sar* (AF028692)	97
MA-2	25	*Methanobacteriales*	*Methanothermobacter thermautotrophicus* GC-1 (AY196661)	98

oil presented in Figs. 2 and 3 and Tables 2 and 3, the possible pathway we could infer includes four steps. Firstly, some of the syntrophic microorganisms utilized the original short-chain (C8–C15) of heavy oil to produce the small molecules that could be utilized by methanogens for methane production. This can explain the minimal production of methane in log period of the stage 1 in Fig. 2. Secondly, other specific syntrophic microorganisms converted the aromatic hydrocarbons, resins and asphaltene to the more readily usable composition, such as saturated hydrocarbons and non-hydrocarbons. Some literatures have reported this similar transforming process in microbial community [51–53]. Thirdly, long-chain (C31+) fractions of saturated hydrocarbons were enzymolyzed by specific enzymes from the syntrophic microorganisms to the medium-chain fractions (C15–C31) or directly to the short-chain (C8–C15), and then the short-chain (C8–C15) fractions continued to be converted to the small molecules such as formic acid and acetic acid. This stage accumulated more readily-usable small-molecules for methane production; the data in Fig. 2 showed the concentration of acetic and formic acid reached its maximum point at the end of the second stage. Finally, the small-molecules (formic acid, acetic acid) were converted to methane by methanogens, related to the extensive production of methane in third stage in Fig. 2.

3.3. Phylogenetic analysis of 16S rRNA gene libraries

More detailed bacterial community analysis was conducted on consortium samples from day 400 using 16S rRNA gene clone libraries. Seven major different operational taxonomic units (OTUs) were determined from the heavy oil-amended enrichment incubations (Table 4). The coverage of the clone library was 98%. Phylogenetic analysis revealed that approximately 47.51% of the clones (OTU MB-1) in the library belonged to the order *Clostridiales*. The closest cultivated relatives of the MB-1 clones were *uncultured Dethiosulfatibacter* sp. (91% sequence similarity) and *uncultured bacterium* clone PL-16B6 and L9B-1 (99% similarity) which were previously detected in homoacetogenic cultures from the formation-water of low-temperature oil reservoir and in methanogenic cultures from the high-temperature oil reservoir [32,54]. Previous reports showed that *Clostridiales* play predominant roles in the enrichment cultures derived from oil sands with C7 and C8 iso-alkanes as carbon source under methanogenic conditions [16], *Dethiosulfatib*acter has the potential to play important roles as polycyclic aromatic hydrocarbon degraders, and could be detected in cultured sample of mangrove sediments after 14 days exposure to polycyclic aromatic hydrocarbon [15]. The two *Bacteroidales*-affiliated phylotypes (MB-2 and MB-3) were distantly related to the *Uncultured bacterium* L9B-2 with 99% and *Uncultured Porphyromonadaceas* TCB179x with 98% identity respectively. *Uncultured bacterium* L9B-2 was detected in an alkane-degrading methanogenic enrichment culture from production water of an oil reservoir, but its function in pathway of the methane production was not elaborated [30]. *Uncultured Porphyromonadaceas* TCB179x presented the predominance in the microbial community of the produced water from a high-temperature North Sea oilfield [55]. Furthermore, it has been well reported that *Porphyromonadaceas* strain could use various substrates to produce propionate, acetate, formic, a small amount of butyrate and hydrogen [56], which were preferable substrates for the growth of methanogens and methane production. MB-4 accounting for 11.29% of total clones, was closely related to *Pantoea* sp.1 (92% identity), a member of the family *Enterobacteriaceae* within the γ-subdivision of the *Proteobacteri*a. The facultative anaerobic *Pantoea* strain has been reported with great ability of degrading alkanes and aromatic hydrocarbons, and could produce biosurfactant to facilitate the hydrocarbon degradation [57]. This possibly explained the decreasing of surface tension from 56.24 mN/m to 32.34 mN/m at the second stage of heavy oil degradation. In addition, the degradation pathway of polycyclic aromatic hydrocarbon in *Pantoea* sp. has been elaborated thoroughly [58]. Phylotypes of MB-5 and MB-6 shown respectively highest identity with *Uncultured Syntrophus* (86%) and *Syntrophaceae bacterium* enrich clone (97%), both members of the family *Syntrophaceae* within the δ-subdivision of the *Proteobacteria*. The first report of the methanogenic degradation of hydrocarbon showed three species from the *Syntrophaceae* were very highly enriched with 90% of the bacterial population [28]. Numerous literatures proved the dominant role for *Syntrophaceae* in the activation and oxidation of crude oil alkanes via long chain fatty acids (LCFA) to acetate and hydrogen in methanogenic environments impacted systems [14,17,59,60]. The phylotype MB-7, representing 25 clones, was closely related to *Uncultured Deferribacteres* (99%), a putative member of the phylum *Deferribacteres*, which was previously detected in water from petroleum reservoir, although its role in conversion of crude oil to methane was hardly described.

Compared with the bacteria, the diversity of archaeas was simple in the heavy oil-amended enrichment incubations. 16S rRNA gene sequences revealed that the all sequenced clones of archaeas were affiliated into the phylum *Euryarchaeota* and divided into two phylotypes: *Methanosarcinales* MA-1 and *Methanobacteriales* MA-2 (Table 4). The phylotype MA-1 accounted for 71.91% of the archaeal clones and showed high identity (>97%) with *Methanosarcina barkeri* (Fig. 3). *Methanosarcina* is metabolically versatile. It appears to be a generalist with high growth rate, capable of growing on several different substrates, including H_2/CO_2, methanol, methylamines and acetate, for methane production [22,61,62]. The other phylotype MA-2, representing 28.01 % of the archaeal clones, shared >98% sequence identity with 16S rRNA gene from the cultured organism *Methanothermobacter thermautotrophicus* GC-1. Previously the members of this group were well recognized by their exclusive growth on H_2 and CO_2. However, the methane production by *Methanothermobacter* with other precursors has been provenasKosaka elucidated that the isolated *Methanothermobacter* sp. CaT2 is capable of methane production from utilizing formate and propionate oxidation [63].

Based on the thermodynamics, syntrophic bacteria and methanogenic archaea is required in the methanogenic conversion of crude oil. The increasing research on the syntrophic bacterial species from methanogenic hydrocarbon-degrading consortia showed the great functional diversity in metabolic pathways for ultimately converting hydrocarbons to methane [27]. In this study, therefore, the results of phylogenetic analysis and characterization of oil degradation could make a reasonable assumption to explain the material flux from the syntrophic bacteria (five orders include *Clostridiales*, *Bacteroidales*, *Enterobacteriales*, *Syntrophobacterales* and *Deferribacterales*) to methanogenic archaea (two orders include *Methanosarcinales* and *Methanobacteriales*). *Clostridiales* (account for 38.46% of total bacterial clones) mainly degraded the short-chain of the saturated hydrocarbons to small-molecular acids, such as formic acid, acetic acid, propionic acid, and butyric acid. We conducted the GC–MS analysis, and the results showed the formic acid and acetic acid was produced during the degradation (Fig. 2). *Bacteroidales* and *Enterobacteriales* (account for 21.72% of total bacterial clones) preferably degrade long-chain saturated hydrocarbons, aromatics, resin and asphaltene to the long chain fatty acids [64–66]. *Syntrophobacterales* (account for 28.50% of total bacterial clones) mainly convert long chain fatty acids (LCFA) and non-hydrocarbons to acetate and hydrogen [67,68]. Then the all produced small-molecular acids, hydrogen and carbon dioxide were ultimately transferred into the methanogenic pathway for methane production [22]. The phylogenetic tree of the 16S rRNA gene phylotypes was presented in Fig. 4.

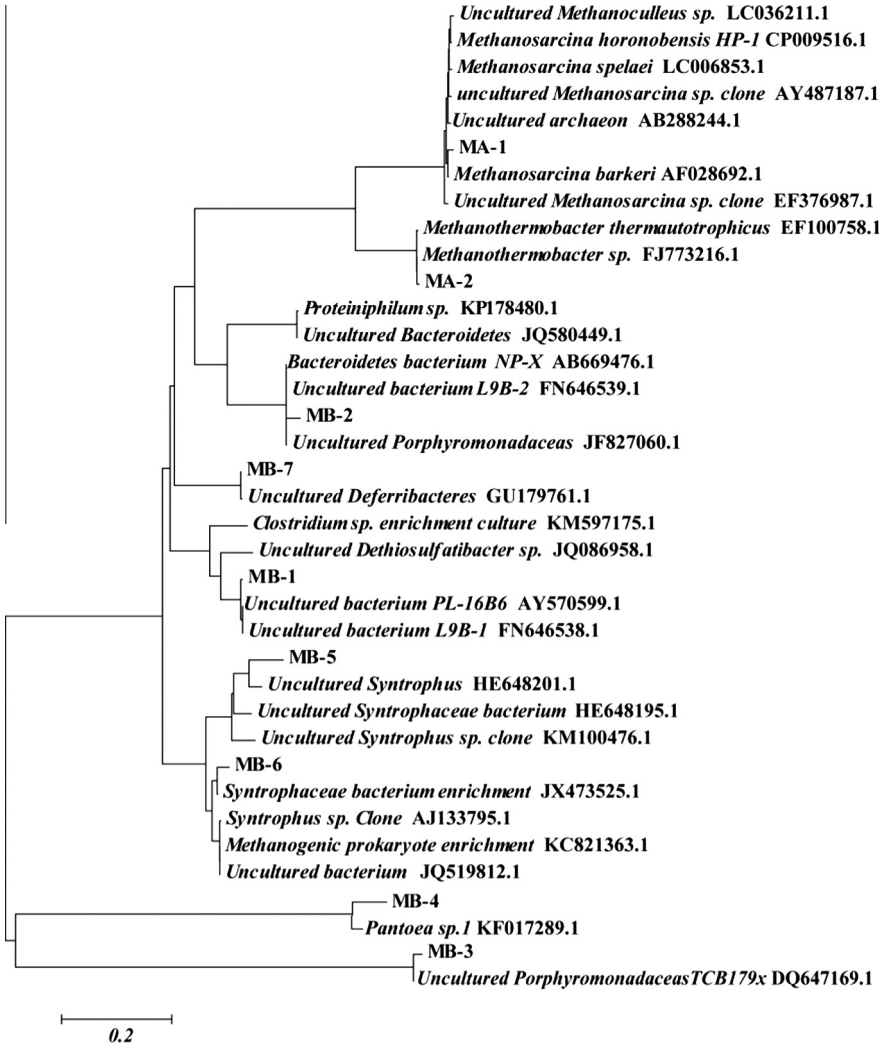

Fig. 4. Phylogenetic tree of the 16S rRNA gene phylotypes retrieved from the heavy oil-degrading methanogenic enrichment cultures in microcosms after incubation of 200 days and closely related sequences selected from GenBank database. Alignments to related sequences (shown with accession number) were performed with Clustal X and MEGA 6.0 software. The topology of the tree was obtained with the neighbor-joining method. Bootstrap values ($n = 1000$ replicates) of >85% are reported. Scale bar = nucleotide changes per site.

3.4. Heavy oil viscosity reduction

Most of conventional methods for reducing the oil viscosity include heating, emulsification, and dilution with light crude oil or its various fractions. However, such methods are expensive and result in pollution of the ambient environment. In this study, the potential utilization of microorganisms to reduce oil viscosity was investigated through the heavy oil degradation and methane dissolution. After 200-days methaogenic degradation, the viscosity of the dehydrated dead oil was reduced from 1823.86 mPa s to 1347.75 mPa s with the production methane dissolution (Fig. 5). It has been well recognized that the high viscosity of heavy oil results from the complex macromolecular structure, which is likely to be similar to the structure associated with asphaltenes and resins, formed via various interactions among these compounds [69]. Chen concluded that the high contents of heavy fractions, heteroatoms (such as O, N, and S), and asphaltenes in heavy crudes lead to their characteristics of high viscosities, and the numerous metal heteroatoms in heavy oil also have a huge contribution to their high viscosities [70]. In this study, the decomposing of heavy fractions (such as resin and asphaltene) shown in Table 2 and Fig. 3) could provide one of the rational explanations to the viscosity reduction (shown in Fig. 5). Importantly, methane dissolution in heavy oil could have significantly contribution to the rendition of the oil viscosity [71]. Within the developed rheometer system (Fig. 1), 5 ml of methane and 25 g of the degraded heavy oil were sealed in piston container at 50 °C. Along with pressure increasing, methane began dissolving in heavy oil and oil viscosity was decreasing. As shown in Fig. 5, the viscosity of the degraded oil was decreased from 1347.75 mPa s to 71.21 mPa s with methane dissolution, while the original oil was decreased from 1823.86 mPa s to 728.73 mPa s with methane dissolution. It indicated that the methane production could reduce the viscosity of heavy oil, and particularly the degraded oil. In this study, the positive effects of the in-situ oil degradation and methane production and dissolution on the viscosity reduction of heavy oil have been well proven; and none of similar research on methane dissolution into the biodegraded oil has been reported.

3.5. Enriched methanogenic culture on EOR

In order to evaluate the potential of the enriched methanogenic microorganism on the oil displacing, the cells of the 200th-day culture were collected by centrifuging and re-suspend with 1.0 PV of mineral medium to make microbial solution, and then injected into sandstone core for 100 days shut-in culturing after the first water flooding. Compared with the control core, oil recovery (OR) of the microbial core increased by 4.18% during the sample injection, it was higher than the control by 3.17%. This is possibly due to profile modification of the injected microbial cells as particles. Through numerical and physical simulation, Kim and Fogler verified that the microbial cells could block the pore throat when transporting in porous medium [72]. Nielsen et al. analyzed the positive contribution of microbial cells on EOR efficiency through 1D simulator [73]. During the shut-in period after sample injection, the inner pressure of microbial core holder increased and reached maximum value with 20.45 MPa, while the pressure of control core holder was less than 0.2 MPa, indicating that biogas was produced under such anaerobic condition. Specific analysis on the composition and quantity of gas was performed at 100th day, the biogas included 97.5% of methane and 2.5% of CO_2. After 100 days of shut-in culturing, the results in Fig. 6 showed that the rapid increasing from 42.78% to 51.64% of OR was detected from the microbial core at the first 1.0 PV of the second water flooding. This was possibly due to the synergistic effects of heavy oil degradation, methane production and dissolution, and production of surface/interfacial-active molecules (biosurfactants), although only surface tension of the liquid from the outlet of the tested cores was measured during the whole core flooding process (Fig. 6).

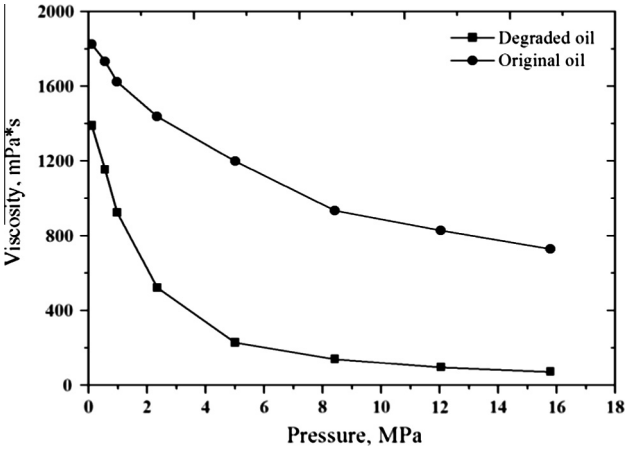

Fig. 5. Effect of methane solubility on the viscosity of heavy oil before and after degradation. The values were average of three parallel experiments.

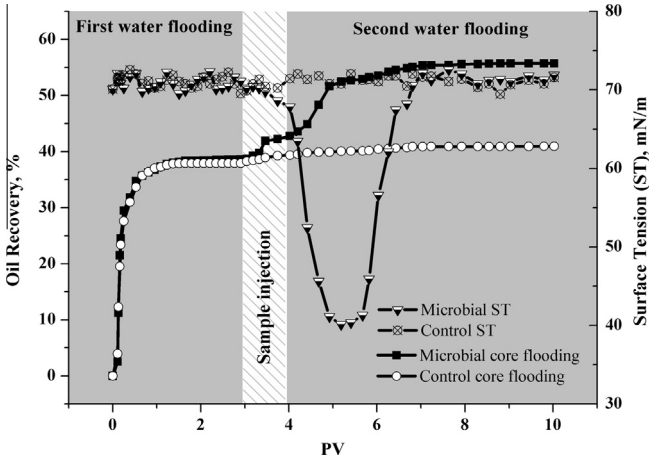

Fig. 6. Core flooding tests of the enriched indigenous methanogenic microorganism from oil reservoir with heavy oil.

The minimum surface tension of the microbial core was 40.12 mN/m (Fig. 6), which was higher than the result in flask (32.45 mN/m shown in Fig. 2). The surface tension of the control core was 65.35 mN/m. Numerous studies have reported that biosurfactants can efficiently improve oil recovery [9,11,74], however herein it was difficult to quantify its contribution on final 55.73% OR of microbial core flooding. According to the data of surface tension, it was easily inferable that the concentration of the surface/interfacial active materials (biosurfactants) is lower than the critical micellar concentration (CMC) which could be calculated in terms of the surface tension shown in Fig. 2 according to the reported methods [11,75]. However, it was obvious that a great amount of methane was produced, resulting in the high pressure in the core. Although the quantification analysis of methane dissolution and its effects on the viscosity of the degraded heavy oil remains a challenging in these core-flooding tests, it is reasonable that the production and dissolution of methane made the significant contribution to the final OR of microbial core flooding, according to the results in Fig. 5. The control core flooding finally obtained 40.94% of OR. Based on OR of the first water flooding, the EOR efficiency of the microbial core was 17.12% (Table 5), which is higher than the EOR efficiency of the current chemical

Table 5
Physical simulation tests of MEOR.

Sample	Culture day (d)	Max pressure (MPa)	First water flooding OR (%)	Sample injection OR (%)	Second water flooding OR (%)	Enhanced oil recovery, EOR (%)
Microbial	100	20.45	38.60	42.78	55.73	17.13
Control	100	0.20	38.27	39.28	40.94	2.17

Note: data was average value of two parallel tests. Max pressure means the maximum valve of pressure in core holder during the sample culturing, this period started from the end of the first water flooding to the beginning of the second water flooding.

and microbial methods for heavy oil recovery within the similar core-flooding procedure [11,76–78]. Therefore, the enriched methanogenic microorganisms obviously have great potentials on EOR, and especially the converting petroleum to methane is a promising technology for heavy oil recovery.

4. Conclusion

Heavy oil reservoir is a widespread petroleum reservoir type all over the world. How to recover the heavy oil efficiently is of importance to guarantee the energy demand. Compared with traditional technologies on EOR of heavy oil, MEOR as low-cost and environmental friendly techniques, has gained increasing interests in recent years. In this study, a newly MEOR strategy by in-situ gasification of heavy oil to methane for EOR was proved successfully in laboratory, and presents great potential application on heavy oil development.

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