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Biostimulation of biogas producing microcosm for enhancing oil recovery in low-permeability oil reservoir

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Abstract

Indigenous microbial enhanced oil recovery (IMEOR) has been successfully applied in conventional oil reservoir, however in low-permeability oil reservoir, is still facing the misunderstanding of mechanism. In order to profile the role of indigenous microcosm in oil recovery, the phylogenetic diversity of microbial community inhibited in reservoir after stimulated with the optimized nutrients in vitro were investigated by MiSeq platforms sequencing of 16S rRNA gene amplicons. Results showed the microbial community structure after stimulation was dramatically changed that the abundance increasing of functional microorganism with ability of producing biogas, biosolvent and biosurfactant was obviously detected under anaerobic condition, such as the genus of *Clostridium*, *Bacillaceae*, *Enterobacteriaceae*, *Oleomonas, Marinobacter, Pseudomonas, Marinobacterium and Dietzia*. Core flooding tests within sandstone were implemented and indicated that these enriched microorganism were closely related to the incremental oil recovery, especially biogas-producing bacteria made main contribution with obvious evidence of pressure increment during core flooding test with no observation of decreasing of surface tension and emulsification. These results suggest that the stimulation of indigenous biogas producer is promising strategy for improving oil recovery in low-permeability oil reservoir.

Keywords: Low permeability; biostimulation; indigenous; biogas; oil recovery.

1 Introduction

Great amount of low-permeability reservoirs have become technical hurdle of enhanced oil recovery (EOR) in China. Characteristics of the low-permeability reservoir are low stratum pressure, low Permeability, good water absorbing capacity and difficult to recover exploit.[1] Water flooding is by far the most commonly used and lowest-cost approach in petroleum industry.[2] Problems like injected water channeling along the high permeability zone, fast water-cut rising and low yield often happen during the water flooding in the low-permeability reservoirs.[3,4] In order to solve them and enhance the oil recovery, various approaches have been trying to make technically breakthrough of improving the oil recovery on these reservoirs, such as chemical flooding, CO_2 foaming flooding, and fracturing acidification. However, these technologies require more effort and have higher energetic, economic, and environmental costs. In addition, the feasibility of injecting chemical agents into low-permeability formation sometimes remains challenging. Therefore, indigenous microbial enhanced oil recovery (IMEOR) technology as economically-efficient and environmental-friendly candidate through the stimulation of indigenous microorganisms by introducing nutrition to improve oil recovery has gained increasing attentions in the academic and industrial field.[5, 6]

MEOR technology is an environmentally friendly tertiary recovery method which involves the application of microbial community and their metabolic products including biogas, biosurfactants, biomass and acids to extend the production life of oil wells. These metabolic products play indispensible roles with multiple mechanisms

for improving oil recovery, especially the biosurfactant and biogas.[7,8,9] Indigenous microbes, having better adaptability to the oil reservoir environment, were widely used in MEOR process.[10] Numerous indigenous species, such as *Pseudomonas* sp., *Acinetobacter* sp., *Bacillus* sp., *Rhodococcusv* sp., *Clostridium* sp. and *Arthrobacter* sp., with great ability of degrading crude oil and producing biosurfactant and/or biogas, play a dominant role for enhancing oil recovery.[6,11] Thus, microbial community diversity was always investigated to evaluate the feasibility or potential of IMEOR, particularly with less energy consumption and cost than exogenous technology.[12, 13,14]

It is well proved that oil degraders and biosurfactant-producing bacteria play important roles in the MEOR processes,[15, 16] however low oxidation reduction potential of petroleum reservoir generally limited this property. Although oxygen was strategically introduced with injection water, it was rapidly consumed by the aerobic microorganism near the wellbore area prior to entering the deep subterranean where the anaerobic biogas-producing bacteria became prevailed and could be good alternates for IMEOR process. Nevertheless, it is not negligible that anaerobic microorganism could produce an amount of biosurfactant.

Lack of nutrients is the main factor that impedes the massive propagation of the microorganisms in the reservoir, such as carbon, nitrogen, and phosphorus sources, although crude oil in reservoir could be used as carbon source.[17] With the injection of nutrients, microbes in the reservoir could be stimulated and produce useful metabolites to improve oil recovery. Extensive researches have been conducted to

investigate the microbial diversity of water sample from reservoir to target the benefit microorganisms which then are stimulated by the well-designed nutrients. However, most of these researches generally focused on the bio-stimulation of biosurfactant-producing microorganism not only in the laboratory studies but also in numerous field tests, with negligible effect of biogas, in medium or high permeability reservoir.[18,19,20] It is worthy to highlight that, few detailed reports have demonstrated how the nutrients influence the microbial community, and how the functional microbial groups, particularly such as biosurfactant-producing bacteria and biogas-producing bacteria, could be directly activated in the low-permeability oil reservoir. Therefore, it is important to figure out the possibility of IMEOR in low-permeability oil reservoir by stimulating the biosurfactant and/or biogas producing anaerobic microorganism.

The objective of the present study is to profile the phylogenetic diversity of indigenous microorganisms in water samples from low-permeability after bio-stimulations, and find out the possible mechanism and potentials of EOR by these stimulated microorganisms in low permeability oil reservoir.

2 Experimental

2.1 Sample

Three samples of oil and formation water were collected at the heads of injection well (IW) and production wells (P1 and P2) from a low-permeability sandstone reservoir in Jing' an oilfield located in the Erdos Basin of Shanxi Province, Northwest China. 10 L of each sample was stored in hermetically sterilized plastic bottles at 4°C,

and immediately transferred to the laboratory for further analysis.

The number of microorganisms within various physiological groups such as hydrocarbon oxidation bacteria (HOB), fermentation bacteria (FMB), nitrate reducing bacteria (NRB), sulfate reducing bacteria (SRB) and methane producing bacteria (MPB) in the three samples was determined by the most-probable-number method (MPN).[21, 22] The medium and culture method used for each physiological bacteria group were the same as that used by Nazina et al. and Acosta-González et al.[4,23]

The physical and chemical parameters of the obtained water samples and the MPN analysis of indigenous microorganisms were showed in Table 1.

2.2 Nutrient optimization and culturing techniques

Based on the MPN analysis of indigenous microorganisms showed in Table 1, the biogas-producing microorganism presented the prevalent amount in the collected samples, thus was targeted to stimulation for EOR.[7, 9,24] The effects of carbon, nitrogen, and yeast extract on stimulation of biogas producing microorganism were investigated via single-factor experiments. 100 mL of the brine supplemented with selected nutrients and 2% crude oil was sealed in a 250 mL anaerobic bottle, and the anaerobic cultivation was conducted following the previous method at 40 °C for 7 days.[7] All materials were sterilized at 121 °C for 30 min (except for production brine). The culturing with sterilized production brine was as control. All experiments were conducted in triplicate, and the data were presented as means. The population of microorganisms was determined via plate-counting method.[21] The biogas was qualified by gas chromatography and quantified by using the drainage gas-collecting

126 method.[7, 26]

127 **2.3 Phylogenetic analysis**

128 The above enriched culture of microorganisms stimulated with optimized
129 nutrients were transferred into the medium bottles of HOB, FMB, NRB, SRB and
130 MPB as previous, respectively.[6, 23] After 14-day incubation, cells in these medium
131 bottles were obtained by centrifuging for DNA extraction and high-throughput
132 microbial community analysis.

133 DNA was extracted by using the FastDNA Spin Kit for Soil (MP Biomedicals,
134 Cleveland, USA). 16S rRNA gene was amplified with universal primer set 104F
135 (5'-GGCGVACGGGTGAGTAA-3'), and 530R (5'- CCGCNGCNGCTGGCAC-3')
136 in a 50 μL PCR mixture containing 25 μL of Taq PCR Mastermix (TIANGEN,
137 Beijing, China), 6μL of DNA template, 1μL of Primer 104F, 1μL of Primer530R, and
138 17μL of ddH_2O.The PCR program was conducted as following steps: initial
139 denaturation at 95 ℃ for 2min, 18 cycles begin with of denaturation at 95 ℃ for 30 s,
140 annealing for 30 s at a temperature gradient ranging from 61 ℃ to 53 ℃ (1 ℃
141 touchdown every cycle) and extended at 72 ℃ for 30s; with a final extension period
142 at 72℃ for 5 min. The PCR product was purified using the E.Z.N.A Cycle-Pure Kit
143 (Omega Bio-Tek, Inc., Norcross, USA). And then sequenced on the Illumina Miseq
144 platform.[27]

145 **2.4 Bioinformatics and statistical analyses**

146 For all data sets, reads containing one or more uncalled bases and bases with
147 low-quality scores were removed. FLASH method, described by Magoč and

148 Salzberg,[28] was used to merge the forward and reverse reads when a correct overlap
149 was found. The operational taxonomic unit (OTU) analysis, taxonomic richness, and
150 diversity analysis were conducted according to Caporaso.[29] All sequences were
151 assigned taxonomic affiliations with an assignment cutoff of 0.03. The Ribosomal
152 Database Project (RDP) classifier was used to assign taxonomic data to each
153 representative sequence. The phylogenetic analysis was performed using PyNAST.[30,
154 31]

155 **2.5 Core flooding test**

156 The enhancement of the recovery associated with the optimized nutrients was
157 tested using a core flooding approach which stimulated the oil reservoir environment
158 of Jing' an oilfield.[32, 33] Schematic of the dynamic experimental setup for physical
159 simulation experiment is shown in Figure 1.The cylindrical cores were 20cm in length,
160 2.5cm in diameter and packed with 100 mesh sized acid-washed silica sand had
161 permeability in the range of 128 to 164 mD, as show in Table 2. The core models
162 were saturated with injection water of Jing' an. Each core model was then flooded
163 with crude oil of Jing' an until residual brine saturation was achieved. After aging at
164 40 °C for 24 h, the core models were flooded again with injection water until the
165 water cut in the effluent of core models was higher than 98% which means that the
166 core reached its residual oil saturation. The residual oil was then calculated by
167 measuring the amount of oil produced during the water flooding process.

168 The next steps in the experimental work depend on the type of the experiment.
169 One set of experiment were designed as control groups, the core were shut in for 10

days at 40 °C after the first water flooding without injecting nutrient. The other set of experiment were designed to access the potential of nutrients system as an in situ MEOR. The experiment were performed with 0.4 PV prepared formation brine containing optimized nutrients, the core were shut in for 10 days at 40 °C after nutrient injection. The amount of oil recovered in this stage was measured.

3 Results and discussion

3.1Screening and evaluation of nutritional system for direct stimulation

Based on the MPN results of three water samples, the biogas-producing microorganism was strategically selected to be stimulated. Sample P2 was chose to screen the nutritional system for direct stimulation. Considering the cost and H_2S hazards,[6, 34] molasses, nitrate and yeast were chose as nutrient and theirs effects of concentrations on biostimulation were shown in Fig.2. The quantity of cells and biogas production were not enhanced with no addition of molasses (in Fig.2a). But the opposite phenomenon was observed in samples with molasses during 7 days incubation, especially when 1.2% of molasses were added. Continuous increasing of molasses led to the decreasing in both gas production and microorganism population. HOB generally was activated using petroleum or carbohydrate, but the growth of microorganisms was slow when petroleum as sole carbon source, especially at the subterranean. Compared with petroleum, molasses can be easily utilized by indigenous microorganisms and the nutritional system contained molasses results in the rapid growth of microorganisms, especially FMB. Therefore, the molasses are beneficial to microorganism growth and biogas production.

Up to 80% of all corrosion damage in oil field-operating machinery were attributed to the metabolic activity of sulfate-reducing bacteria (SRB), which resulted in severe economic losses.[35] Nitrate was used to inhibit SRB growth by stimulating NRB in the petroleum reservoirs. A fine balance between carbon and nitrogen is also required for cell growth and biogas production. The effect of nitrate addition on the microorganism growth and gas production was shown in Fig.2 (b). The optimal SRB inhibition was observed when the $NaNO_3$ concentration was in the range of 0.2 to 0.3% and the maximum biogas and biomass production were also obtained. Nazina reported field trials in which the injection of water with 100 to 150 mg/L of nitrate caused SRB inhibition in a reservoir containing low levels of sulfate and sulfide.[2] However, a higher nitrate concentration is needed in the Jing' an oil reservoir.

The effect of yeast concentration on biostimulation is shown in Fig.2 (c). Although the microorganism growth became relatively stable when the yeast concentration is above 0.06%, the maximum biogas production appeared at yeast concentration 0.08%.

Therefore, the optimized nutrition system included 1.2% molasses, 0.25% $NaNO_3$, and 0.08% yeast. The population of microorganisms in various physiological groups was determined after stimulation via the MPN method. The results showed that after stimulation, the population of microorganisms increased rapidly, and that of SRB maintained at a low level (Table S1). After biostimulation, the content of volatile fatty acids increased rapidly (Table S2), which is similar to previous studies.[22] Fatty acids with small molecules can stimulate the growth of biogas-producing

microorganisms, whereas one of the MEOR mechanisms, which has an important function in the improvement of oil recovery especially in carbonate reservoirs, is acid production.

3.2. Diversity analysis of enriched functional microbes

A total of 403602 high quality sequences were obtained from 16 libraries. The sequence data quality was analyzed using FastQC, as shown in Fig. S1.The rarefaction analysis based on OTUs at a 0.03 cut-off level shows that the curves become flat at high values of sequence numbers, which indicates a good coverage of the species in the samples.

The classification analysis of bacterial sequences was presented in Fig. 3. The population of HOB group in P1, P2 and IW were 1.3×10^3, 5×10^2 and 1.1×10^2 after biostimulation, respectively. After biostimulation, HOB refer to a kind of bacteria that can use oil as a substrate at aerobic conditions.[36, 37] The HOB group detected in three enriched samples were mainly categorized into two phyla, *Proteobacteria* and *Actinobacteria*. For *Proteobacteria,* it mainly included *Phaeospirillum*, *Oleomonas*, *Pseudomonas*, *Marinobacter*, *Thalassospira*, *Dietzia* and *Parvibaculum*. Compared with other genus, *Marinobacter* which has been reported as halophilic oil degrader, had a relatively high abundance in the production-water samples with an abundance of 48.3% in P2sample and 5.2% in P1 sample, while neglectable amount in IW sample. *Dietzia* has been previously reported as an excellent oil-degrader and biosurfactant-producer,[38] was only detected in the production-water sample. *Phaeospirillum* with abundance of 33.9% in IW sample, has been reported as

neutrophilic facultative-anaerobic, Fe (II)-oxidizing bacteria and denitrificans, but the ability of this genus in hydrocarbon degradation is still unknown.[39]

FMB, an importantly functional microbial group in the reservoir ecology, can produce a short-chain fatty acid and biogas (H_2 and CO_2). The population of FMB group in P1, P2 and IW were 2×10^9, 7×10^8 and 2×10^7 after biostimulation, respectively. FMB in three brine samples after stimulation have the most abundant with *Oleomonas* (65.1%in IW sample), *Desulfovibrionaceae* (38.0%, in P1 sample), and *Bacillaceae* (55.7%, in P2 sample), respectively. *Enterobacteriacea*, *Pseudomonas*, and *Marinobacter* were also detected in three stimulated samples when analyzed with MPN. *Oleomonas* can degrade the crude oil and has been recently described as aerobic biosurfactant-producing bacteria.[40] The genera of *Desulfovibrionaceae* have been reported with an extremely high hydrogenase activity, and can produce hydrogen in natural habitats with limited sulfate.[41] *Enterobacteriaceae* had dominated abundance in the PW sample, with an abundance of 22.1%. It can produce 1.6 moles gas by per mole of utilized sucrose, had great potential in oilfield applications.[42] *Bacillaceae* is one of the most widely distributed bacteria in reservoirs can produce a great amount of gas at actual oil reservoir stimulation conditions.[43]

The population of NRB group in P1, P2 and IW were 7×10^7, 1.1×10^8 and 1.1×10^7 after biostimulation, respectively. For NRB group, the dominant sequence-types in the three stimulated cultures were *Hyphomicrobiaceae* (61.3% in IW sample), *Soehngenia* (33.1% in P1 sample), *Vibrionales* (37.7% in P2 sample).

Fusibacter, *Marinobacterium*, *Paenibacillus*, *Pseudomonas*, and *Marinobacter* were detected with relatively low percentage. *Hyphomicrobiaceae* dominated in the IW sample but were not detected in the PW samples. In fact, *Vibrio* sp. were found to be the most proficient gas-producing strains under conditions that simulated actual oil reservoir conditions. In situ growth of vibrio in sand-packed columns produced amount of gas (CO_2, H_2) and large recoveries of residual oil occurred.[44, 45] Many species of *Hyphomicrobiaceae* were reported to be denitrification bacteria.[46] *Marinobacterium* and *Marinobacter* had a high abundance in the PW samples. *Marinobacterium* and *Marinobacter* are nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB), which contribute to the increase in redox potential through the biological oxidation of sulfide,[34, 47, 48] *Pseudomonas* is one of the most common microorganisms in reservoirs and a kind of NRB, such as *Pseudomonas denitrificans*, *Pseudomonas stutzeri*, and *Pseudomonas fluorescens*, which were isolated from many soil and marine samples.

SRB is generally restricted in MEOR as these bacteria lead to corrosion, reservoir souring, as well as the deterioration of oil and gas. SRB had a relatively high abundance in production water (PW) samples and undetected in the cultures of IW samples. The population of NRB group in P1, P2 and IW were 0.5×10^2, 1.3×10^1 and 0.9×10^1 after biostimulation, respectively. Members of SRB in the PW samples were mainly *Desulfovibrionaceae* (84.4% in P2 and 53.1% in P1) *and Fusibacter* (0.15% in P2 and 24.5% in P1) followed by *Sphaerochaeta. Desulfovibrionaceae* was reported to be a major SRB frequently recovered from oilfields.[14] *Fusibacter*, which was first

isolated from an African saline oil-producing well and has been detected in many oil reservoirs, can reduce thiosulfate to sulfide.[49]

3.3 Functional Analysis of enriched microorganisms for EOR

In many studies, the incremental oil production was correlated to the oil degraders and biosurfactant producing bacteria.[50, 51] These microorganisms and their metabolites were always found in the oil field environment, and play important roles in the MEOR process. Although the oil-degrading and biosurfactant-producing bacteria in this study mainly related to the genus of *Oleomonas*, *Marinobacter*, *Marinobacterium* and *Dietzia* was detected in enriched cultures as shown above, HOB merely took a small amount of the whole bacteria community and has weak ability of producing biosurfactant under axoic/anaerobic condition with evidence that no obvious oil emulsification was obsevered. On the contrary, we observed high abundance of *Clostridium*, *Bacillaceae*, *Enterobacteriaceae*, *Pseudomonas* and *Vibrionales* in the samples, which were reported as biogas producing bacteria in many researches.

Bacillaceae appeared frequently in the FMB, MPB culture of production water, *Bacillaceae* accounted for 55.7% and 8.4% in the P2.FM and P2.MP, respectively. *Bacillus* sp. were the most common microorganisms used for gas production for MEOR processes. Spore production by these species is an advantage because spores survive harsh conditions and penetrate deep into the petroleum reservoir. Bacillus sp. also produce oil displacement agent such as acids, gas and alcohols.[52]

Clostridium sp. is one of the most common and effective hydrogen producers.

Clostridium sp. appeared in many samples cultured in the anoxic condition. Accounting for 29.5% in the P2.MB. It is also the dominant species existing in microflora of anaerobic fermentation processes. Many species of *Clostridium* are strong and efficient producers of hydrogen, including *Clostridium butyricum*, *Clostridium beijerinckii* and so on.[53]

Pseudomonas had a relatively high abundance in the culture of NRB. *Pseudomonas* is one of the most common microorganisms in reservoirs and a kind of NRB, some species of *Pseudomonas* such as *Pseudomonas aeruginosa*, *Pseudomonas stutzeri* and *Pseudomonas fluorescens* possess the ability to denitrify nitrate compound and produce nitrogen in anaerobic condition by such series of recation: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$.[54]

Desulfovibrionaceae and *Fusibacter* had a relatively high abundance in the culture which limited S. *Desulfovibrionaceae* and *Fusibacter* were reported as sulfate and thiosulfate reducing bacteria. They were a major group of environmental anaerobic bacteria that play a key role in the global cycle of carbon and sulfur. They also have the ability to use simple organic compound such as lactate, ethanol, formate and butyrate to produce H_2 fermentatively in limiting sulfur conditions.[55]

Biogas producers were closely related to incremental oil production during the MEOR process. Metabolite of biogas producers include gases (CO_2 and H_2), acids and solvents were used to improve oil production from individual wells or to mobilized entrapped oil during water floods. If sufficient CO_2 and CH_4 are made, these gases will result in swelling of crude oil and reduce its viscosity. In situ gas production may

also lead to repressurization of oil reservoirs and hence improve oil recovery especially in mature reservoirs. Organic acid production can lead to the dissolution of carbonates in source rocks, increasing porosity and permeability, and enhancing oil migration. In this study, the biogas producing bacteria appeared frequently in different samples. They were easy to active in the limited oxygen environment supplied with carbohydrates and low molecular weight organic matter. Biogas producing bacteria would be the potential microorganism in the MEOR.

3.3 Core flooding test

Core flooding test was designed to simulate the IMEOR process. Two groups of tests were designed to evaluate the influence of the optimized nutrients system on oil recovery. The results of the core flooding tests are shown in Fig.4.

During the shut-in period after sample injection, the inner pressure of microbial core holder was increasing and reached maximum value with 0.65MPa, while there was no significant increase of pressure in the control core holder, indicating obviously that biogas was produced under this anaerobic condition.

As it was mentioned earlier, the first experiment was set as control groups. It is shown that water flooding resulted in the recovery of 37.62% of original oil in place (OOIP) due to its volumetric sweep efficiency and also the results from the second water flooding revealed that very few oil recovery (0.39% of residual oil) was produced. The other experiment was designed to evaluate the effectiveness of nutrition injection in IMEOR. It is shown that water flooding resulted in the recovery of 37.75% of OOIP due to its volumetric sweep efficiency and also the results from

the second water flooding revealed that 3.7% of residual oil was produced.

Biogas-producing bacteria were used in many MEOR field trials which resulted in large increases in pressure and decreases in oil viscosity,[56] meanwhile, fermented CO_2, acid and solvent production at the sand surface may have led to oil release.

Arief Nuryadi et al. reported oil recovery enhanced in situ by anaerobic denitrifying medium injection.[57] Additional oil recovery in core flooding experiment was predicted to be the result of re-pressurization by nitrogen biogas production. Macroscopic observation revealed that the injection of *Bacillus subtilis* resulted in more residual oil released than the injection of only nutrient solution.[58] Previous researches had provided evidences that stimulation or injection of bio-gas producing bacteria in the field or core flooding experiments could increase oil production with varied dynamic. Shut-in test experiment with injection of *Clostridium botulinum* (CO_2 producing bacteria) resulted in 43%of oil recovery from OOIP with around 0.35 Mpa pressure increment.[56] Compared to this experiment, result of oil recovery involving nutrition injection was low but reasonable.

In general, the results indicate that biogas-producing bacteria stimulated by the optimize nutrition are the reasons for the additional oil recovery during stimulation. Therefore, nutrients injection can provide a potential stimulation-based MEOR application in the reservoir.

Conclusion

The results show that the potential microbes effective to the IMEOR in the investigated oilfield belong to biogas-producing bacteria. The main functional

368 microbes include *Clostridium*, *Bacillaceae*, *Enterobacteriaceae*, *Pseudomonas* and
369 *Vibrionales*. The optimized nutrition system can efficiently stimulate the growth of
370 gas-producing bacteria, and significantly proven via the core flooding experiment.
371 Although the substantial fundamental studies of oil displacing mechanism need to be
372 done further, obviously low permeability reservoir could be implemented with
373 indigenous MEOR technology to improve oil recovery by stimulating bio-gas
374 producing microorganism.

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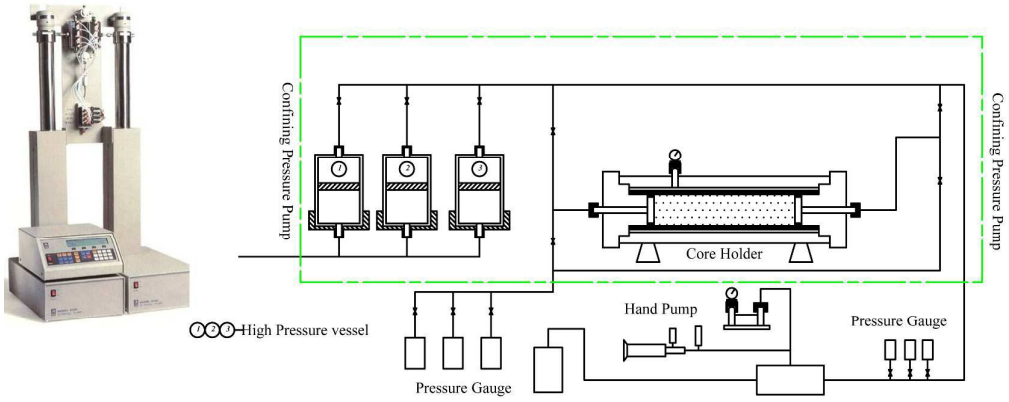

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491 Fig.1. Schematic of the dynamic experimental setup for physical simulation
492 experiment

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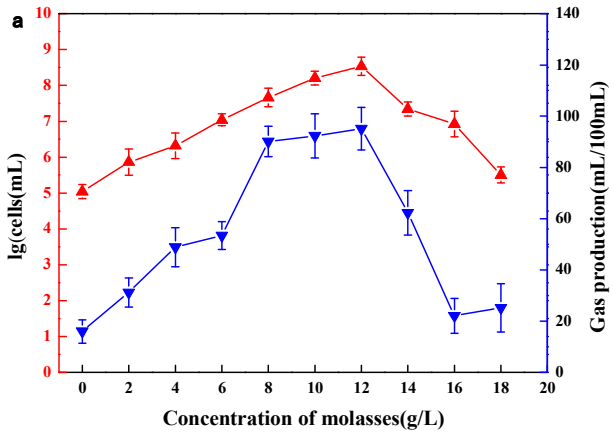

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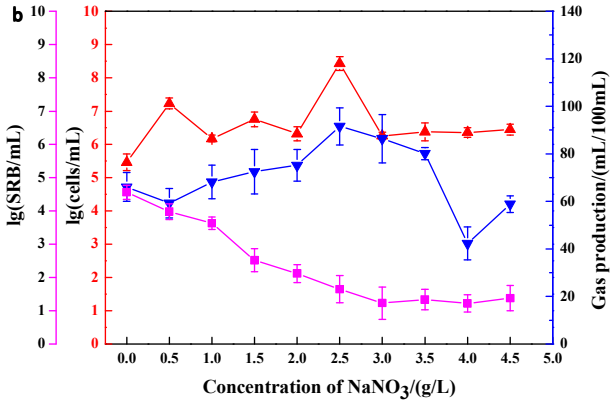

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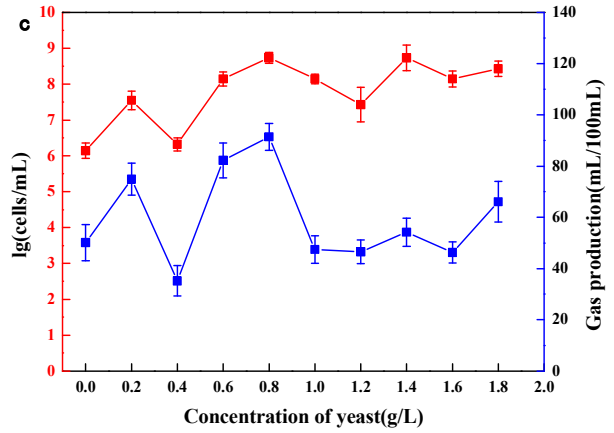


496 Fig.2.The effects of the concentrations of carbon, nitrogen and yeast on biostimulation. (a)The

497 concentration of molasses was variable, the basic medium contained(g/L): $NaNO_3$ 2.0, yeast 0.1;

498 (b) the concentration of $NaNO_3$was variable, the basic medium contained(g/L): molasses 12, yeast

499 0.1; (c) the concentration of yeast was variable, the basic medium contained(g/L): molasses 12,

500 $NaNO_3$ 2.5;▲the number of bacteria in the culture; ▼gas production of each 100ml culture;■the

501 number of SRB in the culture.

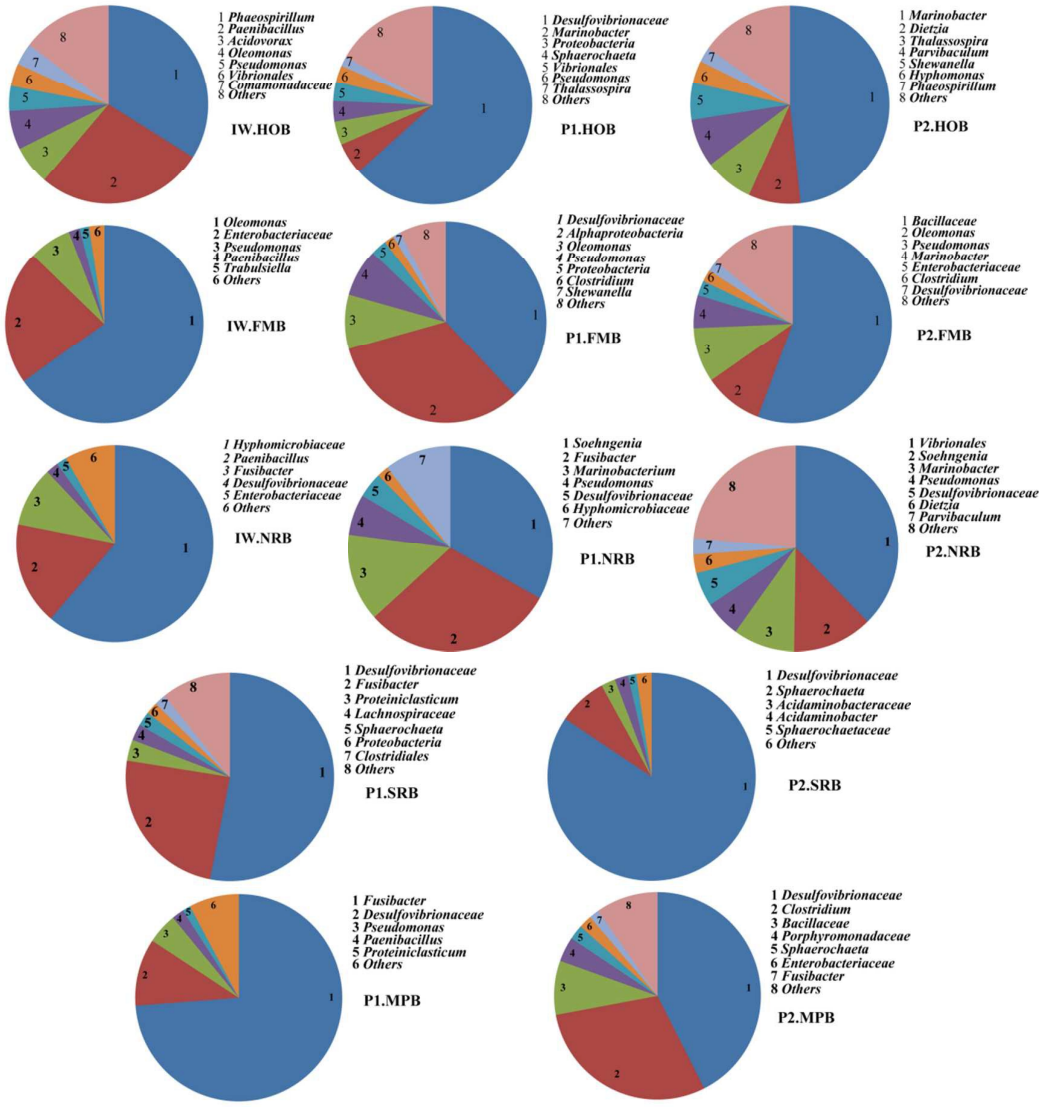
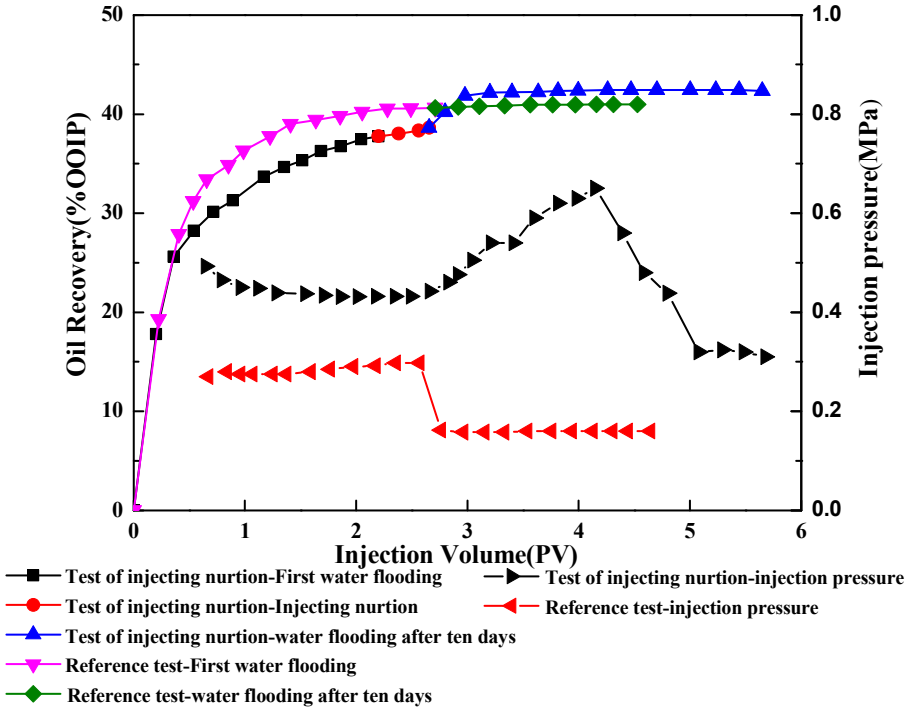


Fig.3. Taxonomic classification of bacterial reads retrieved from different samples at genus level from 16S rRNA gene pyrosequencing. IW, P1 and P2 refer to injection water, production water 1, and production water 2, respectively.



507

508 Fig.4.Change of oil recovery versus injected PV in core model

509

510 Table 1. Characteristics of water samples and SARA content of crude oil in the

511 Jing'an oilfield

Parameter	P1	P2	IW
Category	Production well	Production well	Injection well
T(℃)	40	40	-
Water content (%)	88.1	78.4	
Characteristics of the formation water			
Salinity(mg/L)	14,457	24,341	9720
C (%)	1.42	1.23	2.41
N (%)	-	0.618	-
O (%)	4.97	5.27	3.910
Na (%)	17.4	15.8	7.850
Mg (%)	0.869	0.960	0.260
P (%)	0.0421	0.0361	0.0143
S (%)	0.0327	0.0161	0.108
Cl (%)	55.8	55.9	56.10
K (%)	0.789	0.722	0.153
Ca (%)	13.8	13.4	25.70
HOB		5×10^{2}	2.5×10^{1}
FMB		5×10^{3}	2×10^{1}
NRB		2×10^{2}	5×10^{4}
TGB		7×10^{3}	1.1×10^{3}
SRB		7×10^{2}	1.1×10^{3}
SARA content of the oil			
Saturated hydrocarbon (%)		70.11	
Aromatic hydrocarbon (%)		17.39	
Resins (%)		6.25	
Asphaletene (%)		6.25	

512

513 Table 2.The parameters of sand pack columns used in the oil displacement situ

Test project	Diameter (D, cm)	Length (L, cm)	Porous volume (PV, ml)	Porosity (Φ, %)	Permeability to water (Kw, mD)
Control	2.5	20	42.6	43.39	128
Nutrients	2.5	20	43.3	44.12	164

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