Functional microbial stimulation for oil recovery enhancement based on microbial community analysis

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ABSTRACT

This study stimulated functional microbial adaptation to the extreme environment of the Luliang oilfield reservoir and assessed the impact of indigenous microbial enhanced oil recovery treatment on the microbial community and oil recovery. By high-resolution pyro-sequencing, *Acinetobacter*, *Pseudomonas*, *Sphingomonas*, *Thauera*, *Arcobacter* and *Hyphomonas* were regarded as suitable candidates and a customised nutrient mixture was selected to activate the target microbes. The interfacial tension of crude oil and water decreased from 29.76 mN m$^{-1}$ to 0.65 mN m$^{-1}$ and the emulsion stability index increased from 5% to 92% after customised nutrient culture. The oil biodegradation experiments after the cultivation exhibited positive effects in degrading aromatics, resins and asphaltenes fractions and improving the mobility. Six target genera were activated markedly after 96 h of incubation. The oil recovery was enhanced from 51.2% to 60.7% using fermentation broth injection. Thus, injecting a customised nutrient mixture with injection brine into the stratum to stimulate functional microbes has the potential to enhance oil recovery in the Luliang oilfield.

INTRODUCTION

Stimulation of indigenous microbial enhanced oil recovery (IMEOR) has been widely used in oil exploitation to successfully increase oil production [1,2], especially in oil fields where residual oil is no longer recovered efficiently by ordinary water flooding [3]. IMEOR often involves the injection of nutrients with a small amount of inoculum [4] with the objective of stimulating microbes to produce biosurfactants or emulsifiers in the reservoir to emulsify the crude oil, modify the properties of crude oil and enhance oil recovery [5]. Therefore, hydrocarbon degrading bacteria, as functional bacteria in IMEOR, have been the target of in situ nutrient stimulation and have been studied to extract additional oil [6]. A previous study using 16S rRNA gene pyrosequencing showed that the injection of nutrients in the Huabei oilfield in China resulted in a microbial community dominated by hydrocarbon-utilising genera, which was determined by the type of available nutrient [7]. This is thought to be closely related to the incremental oil production [8].

In recent stimulation studies, field candidates of IMEOR were evaluated based on the remaining oil in place, average porosity and average permeability [9]. The optimum nutrients and incubation conditions for the given oil fields were optimised by laboratory experiments [9–12]. A novel study used an exogenous *Bacillus subtilis* to strengthen the IMEOR process [13]. IMEOR relies on the combination of various mechanisms, such as oil emulsification, oil bio-degradation, gas production, acid production and viscosity reduction [11,14,15]. In a large-scale IMEOR pilot field, 210,000 tonnes of crude oil were produced cumulatively by injection of nutrients with water [16].

However, many stimulation experiments in the laboratory, could not even confirm whether the activated bacteria could survive in the oil reservoir, let alone achieve displacement efficiency in the oilfield. Therefore, our target bacteria, which existed in both...
the injection and formation brine, and had a much higher abundance in the formation brine, were regarded as suitable candidates for MEOR. For this purpose, stimulation experiments aimed at the target bacteria were performed and the enhanced oil recovery potential was assessed in this study.

Materials and methods

Reservoir characteristics and sample collection

In this study, three brine samples from the Luliang block in the Xinjiang Oilfield were collected. The depth of the petroleum reservoir was 1200 m, with a temperature of 34 °C and pressure of 14.99 MPa. Brine sample Y1 was injection brine from injected well Y1; Y3 and Y5 were formation brines collected from produced wells Y3 and Y5, respectively. The samples were sieved thoroughly to remove oil and sediment and achieve homogeneity. A portion of each sample was collected in a 200 mL centrifuge tube, placed in an ice-box and transferred to the laboratory. The tubes were stored at −80 °C until DNA extraction.

Fermentation broth preparation

The nutrient combination (molasses 1%, yeast 0.06% and ethanol 0.2%) was selected based on stimulation of common functional genera between injection brine and formation brines, according to other reports and preliminary experiments. Cultivation was carried out in 250 mL Erlenmeyer flasks containing 100 mL of injection brine supplemented with selected nutrients and 2% crude oil. Flasks were incubated at 35 °C for 96 h with agitation at 120 rpm.

DNA extraction

Brine samples and fermentation broth were centrifuged at 12,000 rpm for 15 min using a Hettich universal 320R centrifuge (Hettich, Tuttingen, Germany) to collect bacterial cells. DNA was extracted from the bacteria using an AxyPrep Bacterial Genomic DNA Miniprep kit (Axygen Biosciences, Union City, CA) following the manufacturer’s instructions. The extracted DNA was diluted in Tris-EDTA (TE) buffer (10 mmol L−1 Tris–HCl, 1 mmol L−1 ethylenediaminetetraacetic acid, pH 8.0) and stored at −20 °C until use.

Pyro-sequencing

The V1-V3 hypervariable regions of the bacterial 16S rRNA gene were amplified using the extracted DNA as a template and primers 27F (5′-AGA GTT TGA TCT GGC TCA G-3′) and 533R (5′-ATT ACC GCG GCT GGT GGT-3′). Polymerase chain reactions (PCR) were performed in a 25-μL mixtures containing 0.5 μL of each primer, 1 μL of template DNA and PCR SuperMix (Invitrogen, Shanghai, China). The following thermal program was used for amplification by a Bio-Rad C1000 Touch thermal cycler (Bio-Rad, Hercules, CA): 94 °C for 5 min; followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; followed by extension at 72 °C for 10 min [17]. An equal amount of the PCR product from each sample was combined in a single tube to be run on a Roche FLX 454 pyro-sequencing machine at Majorbio BioPharm Technology Co., Ltd, Shanghai, China.

Construction of 16S rDNA gene libraries

Amplification of 16S rDNA of bacteria in the fermentation broth was performed using the extracted DNA as a template and primers 8F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1541R (5′-AAG GAG GTG GTG TAT CAA CAG CGG C C-3′) [18]. The following thermal program was used for amplification: 95 °C for 5 min; followed by 30 cycles of 94 °C for 45 s, 52 °C for 45 s and 72 °C for 90 s; followed by extension at 72 °C for 10 min. The obtained 16S rDNA fragments were inserted into Peasy®-T3 cloning vector and transformed into competent cells. The transformed cells were grown to form colonies and 210 clones were randomly selected for verification using primers T7 (5′-TAC GAC TCA CTA TAG GG-3′) and SP6 (5′′-ATT TAG GTG ACA CTA TAG AA-3′) to remove false positive clones. The clones were sequenced at Majorbio BioPharm Technology Co., Ltd, Shanghai, China. The determined sequences were compared with similar sequences from reference organisms in the GenBank database of the National Center of Biotechnology Information (NCBI) using a BLAST search.

Analysis of crude oil

After the cultivation, the culture was extracted with equal volumes of hexane, dichloromethane and chloroform for the subsequent measurements. The extracts were dried at room temperature by evaporation in a safety cabinet and were used as the residual crude oil [19]. Then, the residual crude oil was separated into saturates, aromatics, resins and asphaltene (SARA) fractions using column chromatography with several different developing solvents, and then weighted according to the standard (SY/T 5119-2008) in China. The viscosity measurements of the crude oil were carried out using a Brookfield DV-II programmable viscometer (Brookfield, Middleboro, MA).
Interfacial tension (IFT), biomass and emulsifying properties

To investigate the crude oil-emulsifying performance of the activation system, the biomass, the oil-water IFT, the emulsion stability index and the diameters of the emulsified crude oil droplets were studied in the stimulation test. The fermentation broth was centrifuged at 8000 rpm for 10 min using a Hettich universal 320R centrifuge; the collected cells were dried in an oven at 80°C, and the dry weight of the biomass was determined. The oil–water IFT was measured using a TX-500C IFT meter. The emulsion state of the crude oil in the fermentation broth was observed using an optical microscope. The images of crude oil captured from the microscope were processed using Microimage Analysis & Process software to obtain the drop sizes. To determine the average diameters of crude oil droplets, 500 were taken droplets at each experimental time. The emulsion stability index was defined as the inverse of the change in transmission and back-scattering in unit time and is positively correlated with the stability of the emulsion.

Core flooding experiments for oil recovery

The effect of the nutrient activator was evaluated by artificial core flooding experiments. A schematic diagram of the experimental setup for the core flooding experiments is shown in Figure 1. The oil from the Luliang production well was used as the oleic phase, whereas the injection brine came from well Y1. Artificial cores made from oil sand of Luliang were 29.7 cm in length and 3.8 cm in diameter. Porosity and permeability were also achieved based on the same block. First, the artificial oil-bearing core was injected with injection brine until no more oil was recovered from it. Subsequently, the columns were injected with 0.4 pore volumes (PV) of formation water, 0.4 PV of a nutrient package prepared by the formation water, and 0.4 PV fermentation broth, respectively. After incubation at 35°C for 7 days, ultimate oil recovery and enhanced oil recovery were calculated with successive water flooding.

Results and discussion

Composition of the microbial communities

The microbial community structure of the injection brine and formation brines from oil wells Y3 and Y5 in the Luliang oilfield were analysed using 454 pyro-sequencing. In total, we obtained 10,738 high quality sequences from the three samples, and 3056–3861 sequences were obtained per sample (an average of 3579). Of these sequences, 84% could be classified. The dominant classes (relative abundance >3%) across the three samples were Alphaproteobacteria, Betaproteobacteria, Epsilonproteobacteria, Deltaproteobacteria and Deferribacteres, and these classes accounted for 72% of the bacterial sequences.

Analysis of common functional bacteria

The reservoir environment was unsuitable for the growth of bacteria that existed in the injection brine, but not in the formation brine. However, the microbes that were present in the formation brine but not in the injection brine were non-dominant in the formation brine and represented non-functional bacteria in this experiment. The common bacteria that existed in both the injected brine and formation brine, and had a higher abundance in the formation brine compared with injection water, were more suitable for growth in the reservoir.
As shown in Table 1, a total of 15 genera were common in the three samples. Some functional genera showed increased abundance. For instance, only 0.04% of Sphingomonas was detected in injection brine Y1, but 28.58% was detected in oil well Y5 and 0.92% in Y3, representing an increase of more than 728 times in Y3 compared with that in Y1 and also increased in Y5. All of these bacteria are capable of degrading hydrocarbons in either anaerobic or aerobic conditions, and have been commonly found in other oilfields [7]. Acinetobacter and Pseudomonas, which are known to produce metabolites (i.e. biosurfactants and extracellular polysaccharides) to emulsify crude oil, are hydrogen-oxidizing bacteria and are a focus for MEOR [20–22]. Sphingomonas is reported to be able to degrade aromatic compounds. Thauera, a denitrifying facultative anaerobe, can anaerobically degrade aromatic compounds. The relative abundance of Arcobacter was 0.2% in Y5, which was 55 times higher than its abundance in Y1. The relative abundance of Hyphomonas increased by 9-fold in Y3 compared with that in Y1 and also increased in Y5. Arcobacter and Hyphomonas, which can reduce nitrate and participate in hydrocarbon degradation, inhibit the growth of sulphate-reducing bacteria (SRB). Their presence helps slow down the damage from facility corrosion. The results showed that the six functional genera, which were provided by the injection brine, were able to adapt to the extreme environment of the oil reservoir and showed rapid growth. To improve oil recovery, the nutrient composition for MEOR stimulation was adjusted according to the needs of these six functional genera, especially for Acinetobacter, Pseudomonas, Sphingomonas and Thauera, which could degrade crude oil and were adapted to the oil environment in Luliang oilfield.

### Table 1. Common genera and their abundance proportions in the three samples.

| Phylum          | Class             | Order             | Family            | Genus          | Y1     | Y3     | Y5     |
|-----------------|-------------------|-------------------|-------------------|----------------|--------|--------|--------|
| Proteobacteria  | Alphaproteobacteria | Caulobacteriales | Hyphomonadaceae   | Hyphomonas     | 0.71%  | 6.79%  | 0.81%  |
|                 |                   | Rhodospirillales  | Rhodospirillaceae  | Magnetospiillum | 0.08%  | 0.03%  | 0.60%  |
|                 |                   | Rhizobiales       | Rhizobiaceae      | Novispiillum   | 0.51%  | 2.71%  | 0.40%  |
|                 |                   | Sphingomonadales  | Sphingomonadaceae  | Sphingobium    | 0.16%  | 0.68%  | 0.12%  |
|                 |                   | Rhodobacterales   | Rhodobacterales   | Sphingomonas   | 0.20%  | 0.92%  | 28.58% |
| Betaproteobacteria |                    | Spirochaetes      | Spirochaetaceae   | Thioclava      | 0.08%  | 10.82% | 0.29%  |
| Delta-proteobacteria |                  | Desulfovibrionales | Desulfovibrinaceae | Desulfovibrio | 0.04%  | 0.06%  | 0.09%  |
| Gammaproteobacteria |                  | Pseudomonadales   | Moraxellaceae     | Acinetobacter  | 0.04%  | 0.18%  | 0.14%  |
|                 |                   | Oceanospirillales | Oceanospirillaceae | Pseudomonas    | 0.04%  | 0.22%  | 0.23%  |
|                 |                   | Campylobacterales | Campylobacteraceae | Marinobacterium | 0.16%  | 0.03%  | 0.03%  |
|                 |                   |                  |                  | Arcobacter     | 0.20%  | 0.25%  | 10.96% |
| Bacteroidetes    | Bacteroides       | Bacteroidales     | Parphyromonadaceae | Proteiniphilum | 0.82%  | 5.10%  | 0.20%  |
|                 | Spirochaetes      | Spirochaetaceae   |                  | Spirochaeta    | 0.12%  | 0.09%  | 0.43%  |
|                 |                   |                   |                   |                | 3.10%  | 47.59% | 50.76% |

**Bacterial community after stimulation**

Using a nutrient activator containing 1.5% molasses and 0.06% yeast, the functional genera Acinetobacter and Pseudomonas of Daqing oilfield in China were stimulated [23,24]; however, Thauera and Sphingomonas were activated at relatively low abundance. An Acinetobacter strain was found to be enriched and produced an emulsifying agent when grown on ethanol medium in a fed batch reactor [25]. Based on these studies and pre-experiments, the nutrient activator with molasses, yeast and ethanol was selected to stimulate the common functional genera in the injection brine.

We constructed a gene library to detect bacterial species in the enrichment culture incubated for 96 h, and then sequenced 191 clones (approximately 1500 bp) from the library. The Shannon–Wiener curve (Figure 2) for the library was close to saturation, which indicated that the sequencing results had good coverage of the species in the culture.

We determined 27 genera from 191 clones in the enrichment culture. As shown in Table 2, the dominant genera (relative abundance >3.53%) in the enrichment culture were Acinetobacter, Pseudomonas, Arcobacter, Sphingomonas and Hyphomonas and these genera accounted for more than 71.4% of the bacterial sequences. The abundance of genera Acinetobacter and Pseudomonas increased rapidly, and they became the most dominant genera, at 740-fold and 515-fold, respectively, higher abundance than that in the produced water Y1.
respectively. The relative abundance of *Arcobacter* and *Sphingomonas* increased by 47 and 200 times compared with that in Y1, respectively. The genus *Thauera* was also detected, but was not abundant. However, the abundance of *Thauera* was also higher than that in injection brine Y1 and formation brines Y3 and Y5. This indicated that the target genera were greatly activated after 96 h of incubation in the selected nutrients, and the two most dominant genera were hydrogen-oxidizing bacteria, *Acinetobacter* and *Pseudomonas*.

In fact, MEOR is dependent on the influence of a large number of bacteria and the stimulated bacteria are the most important elements. If the bacteria, which were stimulated and exhibited enhanced oil recovery in laboratory experiments, did not survive in the oil reservoir, it would be impossible to achieve the goal of enhanced oil recovery. In this study, our functional target bacteria could be highly abundant as a result of natural selection in the stratum, without nutritional stimulation. A natural rule-based process

### Table 2. Genera in the enrichment culture and their abundance proportions.

| Class                  | Order               | Family            | Genus                | Enrichment culture | Y1    | Y3    | Y5    |
|------------------------|---------------------|-------------------|----------------------|--------------------|-------|-------|-------|
| Alpha proteobacteria   | Caulobacterales     | Caulobacteraceae  | Phenyllobacterium    | 0.5%               | 1.61% | 0.12% | 0.00% |
|                        | Hyphomonadaceae     |                   | Hyphomonas           | 3.7%               | 0.71% | 6.79% | 0.81% |
|                        | Rhizobiaceae        |                   | Rhizobium            | 1.0%               | 0.04% | 18.69%| 7.80% |
| Rhizobiales            | Rhizobacteraceae    |                   | Roseovarius          | 0.5%               | 15.84%| 0.00% | 0.32% |
|                        |                     |                   | Paracoccus           | 0.5%               | 0.04% | 0.00% | 0.00% |
| Rhodospirillales       | Rhodospirillaceae   |                   | Rhodospirillum       | 0.5%               | 0.43% | 0.00% | 0.06% |
|                        |                     |                   | Roseospirillum       | 0.5%               | 0.24% | 0.00% | 0.00% |
|                        |                     |                   | Tistrella            | 1.0%               | 0.16% | 0.00% | 0.00% |
|                        |                     |                   | Novispirillum        | 1.0%               | 0.51% | 2.71% | 0.40% |
|                        |                     |                   | Oceanibaculum        | 1.0%               | 0.82% | 0.00% | 0.03% |
| Betaproteobacteria     | Rhodocyclaceae      | Rhodocyclaceae    | Sphingomonas         | 8.4%               | 0.04% | 0.92% | 28.58%|
|                        | Desulfuromonadaceae | Desulfuromonadace | Malonobacterium      | 0.5%               | 0.27% | 0.03% | 0.00% |
|                        |                     |                   | Pelobacter           | 0.5%               | 0.08% | 0.00% | 0.00% |
| Epsilon proteobacteria | Campylobacteraceae  | Campylobacteraceae| Arcobacter           | 9.9%               | 0.20% | 0.25% | 10.96%|
| Gammaproteobacteria    | Oceanospirillaceae  | Oceanospirillaceae| Marinobacterium      | 2.6%               | 0.16% | 0.03% | 0.03% |
|                        |                     |                   | Thalassolitus        | 1.0%               | 3.33% | 0.00% | 0.00% |
|                        |                     |                   | Achromobacter        | 30.9%              | 0.04% | 0.18% | 0.14% |
|                        |                     |                   | Pseudomonas          | 21.5%              | 0.04% | 0.22% | 0.23% |
| Anaerolineae           | Anaerolineae        | Anaerolineae      | Longilinea           | 0.5%               | 0.39% | 0.00% | 0.00% |
| Clostridia             | Clostridales        | Incertae_Sedis    | Dethiosulfatabacter  | 0.5%               | 0.04% | 0.00% | 0.00% |
| Planctomycetacia       | Planctomycetaceae   | Planctomycetaceae | Planctomyces         | 0.5%               | 4.23% | 0.00% | 0.00% |
| Mollicute              | Acholeplasmatales   | Acholeplasmatales | Acholeplasma         | 0.5%               | 0.27% | 0.00% | 0.06% |
| Thermodesulfobacteria  | Thermodesulfobacterales | Thermodesulfobacteriae | Caldimicrobium | 1.0% | 0.00% | 0.00% | 0.03% |
|                        |                     |                   | Unclassified         | 2.6%               | 29.09%| 30.74%| 17.09%|
|                        |                     |                   | Uncultured           | 5.8%               | 4.16% | 10.14%| 1.04% |
| Total                  |                     |                   |                      | 100%               | 66.27%| 71.83%| 67.73%|

Figure 2. Shannon–Wiener curve of the enrichment culture.
was performed that added nutrients to enhance the abundance of the functional target bacteria. Therefore, the stimulation experiments based on this principle were more likely to be repeated in the oilfield, thus showing the advantages of this experiment.

**Biodegradation and viscosity alteration of the crude oil**

The proportion of heavy components in the crude oil, such as asphaltenes and resins, strongly affects the crude oil viscosity and the degree of difficulty in oil reservoir development [26]. As shown in Table 3, Luliang Oil contained 61.28% saturated hydrocarbons. After the cultivation, the relative percentage of saturates increased by 6.8%, whereas the percentages of aromatic hydrocarbons and resins decreased by 6.1% and 20.7%, respectively. Meanwhile, the asphaltenes of the degraded crude oil were found to decrease slightly compared with the original oil. The increase in the saturates content mainly resulted from the consumption of the other three fractions. The degradation of crude oil had a clear impact; the viscosity of the crude oil decreased significantly from 45.2 mPa s to 29.7 mPa s. Comparing these results with other reports (in the ‘Bacterial community after stimulation’), it showed that the stimulated bacterial community had the ability to degrade the heavy fractions and change the chemical and physical properties of Luliang crude oil after stimulation by the selected nutrients.

**Emulsifying properties after stimulation**

Biomass, oil–water IFT, the emulsion stability index and the average diameters of emulsified crude oil droplets are shown in Figure 3. A maximum biomass of 1.77 g L\(^{-1}\) was obtained at 84 h. The IFT decreased from 29.76 mN m\(^{-1}\) to 1.21 mN m\(^{-1}\) (72 h) and a stable IFT below 1 was observed from 84 h. The average diameters of the crude oil droplets decreased to 22.15 µm at 72 h. Greater crude oil emulsification began to appear from 48 h, which lagged behind cell growth. The best emulsification was observed at 72 h, while the emulsion stability index increased from 5.1% to 92% during the entire period of nutritional stimulation. In contrast, with the formation brines, IFTs of 21.6 mN m\(^{-1}\) and 20.87 mN m\(^{-1}\) and emulsion stability indices of 13.4% and 12.9% were observed in formation brines Y3 and Y5, respectively. Figure 4 shows the emulsions of the activation system, injection brine Y1 and formation brines Y3 and Y5. The emulsification performance was more pronounced in the activation system than in the injection and formation brines. Emulsification in the activation system was mainly

![Figure 3](image_url)  
**Figure 3.** Kinetics of cell growth (biomass), interfacial tension, emulsion stability and average diameter of crude oil droplets in the enrichment culture.

| Sample      | Viscosity (30°C), mPa s | Saturates | Aromatics | Resins | Asphaltenes |
|-------------|-------------------------|-----------|-----------|--------|-------------|
| Original    | 45.2                    | 61.28     | 13.52     | 15.27  | 9.93        |
| Degraded    | 29.7                    | 65.45     | 12.69     | 12.11  | 9.75        |
| Change ratio | -34.3                  | 6.8       | -6.1      | -20.7  | -1.8        |
attributed to biosurfactants produced by the bacteria rather than cell growth. This result demonstrated that the complex bacteria were stimulated significantly by nutritional stimulation and could use crude oil, metabolise and produce biosurfactants, which led to a reduced IFT and an excellent emulsification performance for crude oil. Reducing interfacial tensions between brines and oil, and forming micro-emulsions, are important mechanisms involved in MEOR [27,28]. In this study, nutrients accelerated the growth of functional bacteria that were adapted to the stratum in the injection brine and promoted crude oil emulsification in 4 days compared with formation brines.

Enhanced oil recovery in the core flooding experiment

The ultimate goal of MEOR is to enhance the oil recovery or the oil production rate. As shown in Table 4, the oil displacement efficiency increased by 9.5% in the core-flooding tests with the injected fermentation broth and the water drive recovery was 51.2%, which exhibited a great potential to promote the oil influx into the well. The total water drive recovery after injection of the nutrient activator was 58.1%, with 7.6% enhanced oil recovery. In artificial core flooding tests, enhanced oil recovery was demonstrated and such enhancements resulted from the collaborated metabolic activities of the complex enriched bacterial system, especially the six stimulated target genera. The results implied that the nutrients selected for the target genera of Luliang oilfield in this study had great potential to increase the oil recovery.

The results suggested that the method using selected nutrients to stimulate functional bacteria had more benefits, such as raising the quality of crude oil, reducing the oil viscosity and improving the flow characteristics by degrading the heavy fractions and producing biosurfactant to emulsify crude oil. Affected by all these factors, the enhanced oil recovery was similar to that achieved by injecting (bio)surfactants directly [29,30]. However, compared with the injection of (bio)surfactants, this method has low energy consumption and is non-hazardous and cost-effective. Therefore, it could be more advantageous than the direct injection of (bio)surfactants.

Table 4. Results of the core-flooding tests.

| No. | Tested project         | Dimension (cm × cm) | Pore volume (mL) | Oil saturation (mL) | Gas permeability (mD) | Water flooding oil recovery, %OOIP | Total oil recovery, %OOIP | MEOR efficiency, %OOIP |
|-----|------------------------|---------------------|------------------|---------------------|-----------------------|-----------------------------------|--------------------------|------------------------|
| 1   | Fermentation broth     | 3.8 × 29.7          | 75.3             | 50.4                | 580                   | 51.2%                             | 60.7%                    | 9.5%                   |
| 2   | Nutrient activator     | 3.8 × 29.4          | 76.0             | 51.5                | 606                   | 50.5%                             | 58.1%                    | 7.6%                   |
| 3   | Formation brine Y3     | 3.8 × 29.9          | 75.8             | 49.5                | 588                   | 49.9%                             | 52.4%                    | 2.5%                   |
| 4   | Formation brine Y5     | 3.8 × 29.5          | 76.4             | 48.8                | 544                   | 52.5%                             | 54.9%                    | 2.4%                   |
| 5   | Injection brine Y1     | 3.8 × 30.0          | 75.6             | 49.0                | 595                   | 55.1%                             | 57.1%                    | 2.0%                   |

Figure 4. Visible emulsification effect of the enrichment culture, and light microscope images of emulsified oil.

Note: magnification ×400
Conclusions

The results from this study showed that six dominant functional genera: *Acinetobacter*, *Pseudomonas*, *Arcobacter*, *Sphingomonas*, *Hyphomonas* and *Thauera*, were provided by the injection brine, and were able to adapt to the extreme environments of the oil reservoir in Luliang, which provided the basic conditions for IMEOR. Under the given reservoir conditions, biostimulation was carried out and a maximum biomass of 1.77 g L$^{-1}$ was observed in the injection brine supplemented with selected nutrients. With the injection of nutrients, the six functional genera were greatly stimulated and could produce metabolites, such as biosurfactants, to modify the oil emulsifying properties. It resulted in the decrease of interfacial tension to 0.65 mN m$^{-1}$. Meanwhile, a stable emulsion was formed by dispersed crude oil, with the average droplet diameter decreasing to 20.37 μm and the emulsion stability increasing to 92%. After the cultivation, the percentages of aromatics, resins and asphaltenes fractions decreased by 6.1%, 20.7% and 1.8%, respectively. As a result of this, the mobility was improved through the viscosity reduction. Core-flooding experiments showed an enhanced oil recovery of 9.5% with injection of the fermentation broth and 7.6% with the nutrient activator. Therefore, the nutrient combination selected for the six functional genera was confirmed as effective and could be used in stimulation-based IMEOR in the Luliang oilfield.

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