Research on the change rule of microbial community in the microbial flooding process of Baolige oilfield

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Abstract: In Baolige oilfield, through the research and field implementation of microbial flooding technology, some application effects had been achieved. In order to further improve the implementation effect, the microbial community in the produced liquid during microflooding was tracked, monitored and analyzed by high-throughput sequencing (NGS). The results showed that the microflora diversity was plentiful. The species diversity after microflooding was higher than that during microflooding. In microbial flooding stage, due to replenish nutrients and exogenous bacteria to reservoir, the advantage bacteria group was mainly Pseudomonas, Acinetobacter, Thauera and Wallingella. After the microbial flooding, due to stop supplying nutrients, the bacteria community changed. The relative abundance of hydrocarbon-degrading bacteria declined and that of anaerobic aerogen increased.

1. Introduction
Microbial enhanced oil recovery (MEOR) is a comprehensive technology that utilizes beneficial activities and metabolites of microorganism to improve oil recovery [1]. According to the microorganism source, MEOR can be divided into exogenous MEOR and endogenous MEOR [2]. The endogenous reservoir microorganisms refer to the microbial community that enters the reservoir with injecting water in the process of water injection development and maintains a relatively stable quantity and species in a certain period [3]. The reservoir microbial metabolism has many types and great variability. It participates in the circulation of matter and energy in the reservoir and plays an important role in the earth ecosystem. According to metabolic types, the reservoir microorganisms mainly include HOB, TGB, FMN, NRB, SRB and MPB [4]. The influence factors of microbial flooding technology efficiency are quantity, type and biological function of the microbial in the reservoir. So it is necessary to understand the structures, properties and changing laws of oil reservoir microbial community roundly and accurately. The recognition of beneficial bacteria and its mechanism action is also vital. They will provide scientific data and methods for the design and optimization of microbial oil recovery technology and then guide the effective application of MEOR technology. The analysis of microbial community structure in reservoir microbial ecology is the key to microbial oil recovery. At present, researchers have conducted a large number of in-depth investigations and studies.
on the composition and distribution of microbial communities in waterflooding reservoir by pure culture and molecular ecology technology based on 16S rRNA [5].

The average buried depth of Baolige oilfield is 1400m. The formation temperature is 58℃. The salinity is 5726mg/L with NaHCO₃ water type. The development pattern is conventional injection. The formation porosity is from 13.4% to 21.7%, the crude oil freezing point is 29℃, the paraffin content is from 10.7% to 10.8% and the colloid asphalt content is from 46.0% to 51.2%. Under the geological conditions, the crude oil viscosity is from 1000 to 2000mPa.s with high oil-water viscosity ratio (34 to 800). The waterflood fingering leads to low efficiency of waterflooding and the watercut rising rate is up to 20%, leading to serious water channeling. Thus some reserves of heavy oil are difficult to be developed effectively with poorer development effect [6]. In Baolige oilfield, overall microbial flooding has been carried out for seven years and reservoir fluid bacteria concentration is up to 10⁶/mL. In order to increase microbial oil recovery technology application, in-depth study on flora evolutionary changes and action mechanism are requisite, which can make effective control strategy and give full play to the synergies between the microbial flora.

2. Experiment

2.1. Source of experimental oil and water samples
The oil and water samples were collected from the produced fluid before and after microbial flooding in Baolige oilfield.

2.2. Experimental reagents and instruments
Fast Prep-24 sample rapid processing system (MP of USA).
  Mastercycler PCR instrument (Eppendorf of Germany).
  High speed refrigerated centrifuge (Beckman Coulter).
  Power Soil DNA Isolation Kit (MOBIO Laboratories, USA).
  Qubit DS-DNA Assay Kit Bradford (Invitrogen, USA).
  Hollow Fiber Membrane (MOTIMO, China).
  DNA polymerase Ta ka-ra Ex Taq TM (Dalian TaKaRa, China).

2.3. Experimental methods

2.3.1. Pretreatment of reservoir samples and total DNA extraction. The experimental procedures are as follows: (1) Water samples of 10L are taken from oil&water samples after standing for stratification. They are filtered and concentrated by hollow fiber of 0.22μm diameter. (2) The concentrate is transferred to a centrifuge tube, centrifuged for 30min to get the bacteria precipitation. (3) The bacterial precipitation is extracted with the MP Kit FastDNA® SPIN Kit for Soil (MP Biomedicals LLC, Solon, OH, USA) to obtain total genomic DNA which is detected by 1% agarose gel electrophoresis and stored at -20℃ environment. The crude oil samples are extracted with PowerSoil® DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) to obtain DNA which is also detected by 1% agarose gel electrophoresis, and then stored at -20℃ environment.

2.3.2. Species diversity analysis by Miseq high-throughput sequencing. Experimental procedures and methods refer to GB/T 30989-2014 [8].

3. Results and discussion

3.1. Structure and abundance of reservoir microbial community
Table 1. Identification results of sample microbial community

| Taxon   | The total number | Predominant bacteria number | Predominant bacteria name (Average abundance %) |
|---------|------------------|-----------------------------|-----------------------------------------------|
| Phylum  | 21               | 5                           | Proteobacteria (68.84%), Finnicutes (18.72%), Archaea (7.47%), Bacteroidetes (2.61%) |
| Class   | 53               | 17                          | Alphaproteobacteria (55.53%), α-Proteobacteria (6.26), β-Proteobacteria (4.87), Epsilonproteobacteria (1.64), Deltaproteobacteria (1.49), Class Clostrida (17.6), Thermotogae (0.92), Class Methanobacteria (7.4) |
| Family  | 735              | 63                          | Pseudomonas (37.17), Acinetobacter (13.93), Alishewanella (2.69), Halomonas (1.32) and Nitrincolar (0.13), Sphingomonas (0.18), Brevundimonas (0.11) and Hyphomonas (0.12), Thauera (1.52), Petrobacter (0.26) and Hydrogenophaga (1.41), Arcobacter (1.76) and Wolinella (0.57), Desulfovibrio (0.38), Thermoanaerobacter (0.62), Caldanaerobacter (0.57), Anaerobranca (0.46), Thermacetogenium (1.63), Pelotomaculum (1.07) and Syntrophomonas (0.24), Desulfotomaculum (0.64) and Fusibacter (0.28), Fervidobacterium (0.69) and Thermotoga (0.24), Methanothermobacter (0.51) and Methanomethylovorans (0.24) |

High-throughput sequencing results show that the microbial diversity of reservoir is plentiful. In terms of phylum classification, the reservoir samples belong to 21 groups, but the distribution is very uneven. They mainly distribute in Proteobacteria, Firmicutes, Thermotogae, Bacteroidetes and Euryarchaeota. The dominant bacteria are hydrocarbon oxidation bacteria, Zymophyte, denitrifying bacteria, Syntrophus and Methanogens.

3.2. The variation rules of bacterial species during microflooding

In the early stage of the microbial flooding, species diversity in family is higher. As the continuous replenishment of nutrients and exogenous bacteria liquid to the reservoir, the advantage microflora in reservoir produced fluid is mainly the injected Pseudomonas, Zymophyte, aerobic and anaerobic hydrocarbon-degradation bacteria, while other advantage species decreases rapidly. Pseudomonas and Acinetobacter in Alphaproteobacteria has always been in a major family. Previous studies have shown that these bacteria have a better ability to degrade and emulsify alkanes, aromatic hydrocarbons or their derivatives, indicating that the added nutrients to the reservoir can effectively activate the injected Pseudomonas and beneficial endogenous bacteria in the reservoir, so as to play a role in oil displacement.
Figure 1. Structural changes of dominant bacteria at family level in oil wells at different times

In the whole process of microflooding, the hydrocarbon-degradation bacteria is dominated, while the abundance of Syntrophus and Methanogens is very low, which indicates that the hydrocarbon degradation mainly depends on microbial activity at this stage. After the microflooding, the community structure changes rapidly and significantly. With the consumption of nutrients, the abundance of hydrocarbon-degrading bacteria gradually decreases, and the proportion of anaerobic zymophyte (including Thermoaerobacter, Thermacetogenium, Desulfotomaculum and Syntrophomonas) and Methanogens gradually increases. The methane bacteria number of the well B19-18 increased from 0.9% to 40.6% after 60 days of microbial flooding. The methane bacteria number of well B18-42 increased from 0.05% to 2.4% after 60 days of microbial flooding. The methane bacteria number of well B38-63 increased from 0.3% to 4.1% after 60 days of microbial flooding. All these changes showed that the number of methane produced by bacteria enhanced after microbial flooding. The results are shown in figure 1.

Figure 2. Structural changes of dominant microorganisms at class level in oil wells
Figure 3. Structural changes of dominant microorganisms at phylum level in oil wells

As shown in figure 2 and figure 3, during the micro-flooding stage, when injecting nutrient and exogenous bacteria solution, the microbial community structure of oil wells is single. The hydrocarbon degradation bacterium in Proteobacteria is dominated. Alphaproteobacteria and Epsilonproteobacteria are the main class. At the end of microflooding, with the consumption of nutrients, the proportion of Proteobacteria decreases, while the proportion of Firmicutes and Methanogens increases gradually. Microbial community structure begins to complicate and species diversity increases. The proportion of anaerobic bacteria gradually increases, including Thermotoga, Thermacetogenium and Desulfotomaculum in Methanobacteriaceae. The anaerobic bacteria produce organic acids by using hydrocarbon degradation. Further, interoperate with methanogens, methane is finally produced, which indicates that the methanogenesis metabolism is the main process in this stage.

3.3. analysis of produced fluid

Figure 4. The variation of bacterial concentration and crude oil viscosity of produced fluid in Baolige oilfield

The microbial quantity and crude oil viscosity of the produced fluid from 52 oil wells in Baolige oilfield were continuously monitored. The average bacterial concentration and crude oil viscosity changes are shown in figure 4. The concentration of bacteria in the produced fluid was maintained at $10^6$/mL, indicating that a stable microbial field was formed in the reservoir. The average viscosity reduction rate of crude oil was 48.1%. After injection of bacterial fluid and nutrient fluid, the beneficial bacterial community that was easy to drive oil was effectively activated and the number significantly increased. The relationship curve of microbial and oil well production showed that microbial could achieve the effect of increasing and stabilizing production.
Table 2. Surface tension of produced fluid of oil wells (unit: mN/m)

| Well | Before microflooding | 60 days after microflooding | 120 days after microflooding | average reduction rate, % |
|------|----------------------|-----------------------------|-----------------------------|---------------------------|
| B18-42 | 59.12                | 51.35                       | 50.15                       | 14.16                     |
| B19-18 | 58.35                | 58.35                       | 51.36                       | 10.87                     |
| B38-58 | 60.18                | 60.18                       | 52.22                       | 14.04                     |

Table 3. Gas composition of oil wells (unit: %)

|        | Methane | Ethane | Propane | n-butane | CO₂  | N₂     |
|--------|---------|--------|---------|----------|------|--------|
| Before microflooding | 74.76   | 4.12   | 4.02    | 1.1      | 1.06 | 13.46  |
| 120 days after microflooding | 63.23   | 5.48   | 6.92    | 2.48     | 1.82 | 17.58  |
| 60 days after microflooding | 85.41   | 2.13   | 1.57    | 0.65     | 1.01 | 7.48   |

The monitoring results of production fluid before and after microflooding showed that the content of biological surfactant produced by microorganisms during microflooding in the reservoir was improved and the surface tension decreased by about 12% compared with that before microflooding. After the microflooding, the content of methane increased by 10.65% compared with that before microflooding, indicating that anaerobic zymophyte began to interact with methanogenic archaea to degrade petroleum hydrocarbon and produce methane through oxidation and denitrification. The change of metabolites further indicated that the types and functions of bacteria in the reservoir changed before and after microflooding.

4. Conclusion

1. The biological diversity of reservoir is very rich. In the process of microbial flooding, the microbial community structure in the reservoir is a dynamic changing process. In the process of microbial flooding, the dominant flora is Pseudomonas, Acinetobacter, Thauera and Wallingella.

2. The types and abundance of the dominant bacteria in the reservoir change significantly in the microflooding process. During the period of injecting nutrition agent, the hydrocarbon-degrading bacteria are dominated, while the abundance of Syntrophus and Methanogens is very low. After the microbial flooding, as the consumption of nutrients, the proportion of anaerobic zymophyte (including Thermoanaerobacter, Thermacetogenium, Desulfotomaculum and Syntrophomonas) and Methanogens gradually increases. The anaerobic bacteria produce organic acids by using hydrocarbon degradation. Further, interoperated with methanogens, methane is finally produced. Microbial hydrocarbon degradation and interactional methane production plays an important role in microbial displacement in Baolige oilfield.

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