Effect of EOR Chemical Flooding on Local Microbial Ecological Characteristics in Oilfield Mining Area

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Abstract: The 16SrDNA of total microbial DNA in 26 soil samples from three mining areas of an oil field was analyzed by high-throughput sequencing. The study found that the local microbial species in the mining area were rich, the pedigree was developed, and the dominant species were obvious. The differences in soil bacterial and fungal communities among the three mining areas were far greater than those within the group of four types of samples in the three mining areas. The study shows that the impact of chemical flooding on the local biodiversity of the oilfield is not different, and the impact on the local biodiversity of the oilfield mining area is large. There is no destructive impact on the environment.

1. Introduction
In recent years, with global demand for oil increasing year by year, the original secondary oil recovery technology has not been able to meet the development needs of the times, and tertiary oil recovery technology came into being. Our country attaches great importance to the development of tertiary oil recovery technology. Domestic oil companies have listed EOR technology as the key research point and carried out a lot of research and exploration, which provides technical support for further improving oil and gas recovery and making full use of oil resources. Chemical flooding of tertiary oil recovery is suitable for water flooding oilfield, accounting for 76% of the potential of various EOR methods, and is the main research direction of EOR research in China [1]. At home and abroad, the research on EOR chemical flooding focuses on chemical flooding technology and its mechanism. The impact of polymer, surfactant, alkali and so on used in chemical flooding on the environment and environmental effects are rarely studied or involved.

Once chemical flooding chemicals enter the environment of the oil field and mining area, the first contact is the soil environment. Microorganisms in the soil environment participate in the process of energy flow and material circulation, and their community structure is more considered as a sensitive biological indicator for early warning of ecosystem changes [2-3]. Therefore, soil microorganism is an important factor that can directly represent soil pollution. In this paper, the high-throughput sequencing platform Ions5™XL was used to sequence the 16SrDNA of total microbial DNA in three mining areas of an oil field. The impact of chemicals used in the oil extraction process on soil microorganisms was analyzed, and the overall situation of microorganisms in the mining area was evaluated scientifically and objectively.

2. Experimental materials and methods

2.1. Sample collection and analysis
In this study, three representative sampling points were selected for sampling. Four 10 m × 10 m fixed
experimental plots (blank area, injection station area, injection well area and combined station area) were set up in three sampling sites, and three 1 m × 1 m sample plots were set in each sample plot. Soil samples of 0-20 cm depth were collected in each plot by diagonal sampling method. Remove stones, plant roots and other debris, mix evenly, seal with sterilization self-sealing bag, label and put it in the ice box to bring them back to the laboratory. A total of 26 samples were obtained and numbered to carry out the experiment.

2.2. DNA extraction, purification, PCR amplification and sequencing
Soil bacterial DNA was extracted by Fast DNA SPIN Kit (MP Biomedicals, USA) according to the extraction steps in the instruction manual. The concentration and purity of soil total DNA were determined by nanodrop, Nd-1000, Germany. Bacterial specific primers (338F/ 806R) were used to amplify the v3-v4 region of 16SrDNA gene of soil bacteria. The forward primer was 338F (5'-ACTCCTACGGGAGGCAGCA-3') and the reverse primer was 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Amplification parameters: pre denaturation at 98℃ for 2min, denaturation at 98℃ for 15s, annealing at 55℃ for 30s, and extending for 5min at 72℃ for 30 cycles. The amplification system was 25μL, 5×reaction buffer 5μL, 5×GC buffer 5μL, 2.5mmol·L⁻¹ Dntp 2μL, 10μmol·L⁻¹ Primer 1μL, 10μmol·L⁻¹ post primer 1μL, DNA template 2μL, ddH₂O 8.75μL, Q5 DNA polymerase 2.25μL. IonS5™XL sequencing platform was used for single ended sequencing of PCR amplification products, which was entrusted to Beijing Cell Genome Biotech Co.Ltd.

2.3. Data analysis method
Based on the IonS5™XL sequencing platform, the raw data was sheared and filtered, chimera was removed, and quality control was carried out to obtain the effective data (clean data) for subsequent analysis[4-5]. Operational taxonomic units (OTUs) clustering analysis was carried out by using uparse software according to 97% similarity level[6]. According to OTUs classification and taxonomic status identification results, QIIME software was used to obtain the composition and abundance distribution table of each sample at different classification levels of phylum, class, order, family, genus and species. In order to compare the diversity of different samples, firstly, all samples in OTUs abundance matrix were randomly resampled at the lowest sequencing depth level of 90%, so as to correct the diversity difference caused by sequencing depth. Then, QIIME software was used to calculate the diversity (Shannon and Simpson) and richness (ACE and Chao1) indexes of each sample. The unweighted UniFrac distance matrix was analyzed by using R software, and the structure distribution of community samples was described by two-dimensional ordination chart. The statistical algorithm of metastats was used to compare and test the sequence quantity difference of each taxon at the level of phylum and genus among samples (groups) [7].

3. Results and discussion

3.1. Sequencing data analysis

| Sample ID | PE Reads | Raw Tags | Clean Tags | Effective Tags | AvgLen(bp) | Effective(%) |
|-----------|----------|----------|------------|---------------|------------|--------------|
| H1C       | 80,059   | 79,753   | 78,707     | 76,858        | 418        | 96.0         |
| H1LS      | 79,971   | 79,572   | 78,197     | 76,414        | 422        | 95.55        |
| H1LW      | 50,826   | 50,633   | 49,968     | 47,898        | 418        | 94.24        |
| H1PZZE    | 55,669   | 55,422   | 54,688     | 53,033        | 417        | 95.26        |
As can be seen from the analysis results in Table 1, a total of 26 samples were high-throughput sequenced, and more than 30000 effective sequences were obtained. A total of 1510381 effective sequences were obtained. From the sample dilution curve on Fig. 1 and Shannon index curve in Fig. 2, it can be seen that the DNA extraction effect of the samples is very good. Meanwhile, it can be proved
that the native microbial species and spectrum in the oilfield mining area are rich. The Department is
developed.

Fig. 1 sample dilution curve

Fig. 2 Shannon index curve of samples
3.2. Analysis of microbial community composition

In order to analyze the variation law of soil microbial community structure in three mining areas of the oilfield, the representative sequences of OTU after clustering were annotated. The results are shown on Fig. 3. Bacterial OTU in soil samples from three mining areas belong to 656 genera, 361 families, 218 orders, 97 classes and 32 phyla. Bacteria or fungi with relative abundance less than 1% and no annotated results at this level were assigned to others. At the phylum level, Proteobacteria, Actinobacteria and Chloroflexi were the dominant bacteria in the soil samples of the three mining areas.

3.3. Diversity analysis

Fig. 4 PCOA cluster analysis chart of 3 blocks (8 samples from block 1, 8 samples from block 2, and 10 samples from block 3)
Fig. 5 Pcoa cluster analysis diagram of blank samples, injection station samples, combined station samples and wellhead samples of 3 blocks (A is blank sample, B is injection station sample, C is combined station sample, D is wellhead sample. Fig. a is peripheral connection diagram; Fig. b is peripheral ellipse; Fig. c is particle diagram)

In order to further clarify the differences in community species composition among samples, pcoa analysis was used to measure soil bacteria in the three mining areas. From the results of Fig. 4, it can be seen that the clustering situation of the three mining areas is obvious, which proves that the microbial species in the three mining areas have certain differences, and the clustering effect of microorganisms in each mining area is different due to regional differences. The pcoa cluster analysis was carried out on the samples from the three mining areas according to the blank samples, injection station samples, combined station samples and wellhead samples. The results are shown on Fig. 5. It can be seen that there is no obvious clustering of the four samples. No obvious separate clustering is found in the peripheral line, peripheral ellipse and particle analysis, and the soil bacterial community groups of the three mining areas are different. It is much larger than the intra group difference of four kinds of samples in three mining areas. The influence of chemical flooding on the local biodiversity of the oil field is less than the impact which is caused by the regional differences of the Oilfield Mining Area.

4. Conclusion
(1) The results of high-throughput sequencing showed that Proteobacteria, Actinobacteria and Chloroflexi were the dominant bacteria in different soils of the three blocks. The native microbial species were rich, the pedigree was developed, and the dominant species were obvious. The relative
average abundance of Proteobacteria was the highest.

(2) Pcoa analysis showed that the differences in soil bacterial and fungal communities among the three mining areas were much greater than those of four types of samples in the three mining areas. The influence of chemical flooding on the local biodiversity of the oil field is less than the impact which is caused by the regional differences of the Oilfield Mining Area. The study showed that chemical flooding had no destructive impact on the environment.

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