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Relationship between Microbial Community Characteristics and Flooding Efficiency in Microbial Enhanced Oil Recovery

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Abstract

Microbial enhanced oil recovery (MEOR) is the research focus in the field of energy development as an environmentally friendly and low cost technology. MEOR can bes divided into indigenous microbial oil recovery and exogenous microbial oil recovery. The ultimate goal of indigenous microbial flooding is to enhance oil recovery via stimulation of specific indigenous microorganisms by injecting optimal nutrients. For studying the specific rule to activate the indigenous community during the long-term injection period, a series of indigenous displacement flooding experiments were carried out by using the long-core physical simulation test. The experimental results have shown that the movement of nutrients components (*i.e.*, carbon/nitrogen/phosphorus) differed from the consumption of them. Moreover, there was a positive relationship between the nutrients concentration and bacteria concentration once observed in the produced fluid. And the trend of concentration of acetic acid was consistent with that of methanogens. When adding same activators, the impacts of selective activators to stimulate the indigenous microorganisms became worse along with the injection period, which led to less oil recovery efficiency.

Keywords

Microbial Enhanced Oil Recovery (MEOR), Nutrient Concentration, Bacterial Concentration, Methanogens, Microbial Activity

1. Introduction

Nowadays, microbial enhanced oil recovery (MEOR) has become more and more

popular due to many advantages including low-cost, environmental-friendly, convenient source and so on [1]. Compared with conventional EOR technologies, MEOR not only consumed less energy but also rarely depended on crude oil prices. Generally, MEOR, consisting of indigenous and exogenous concepts, is a special type of EOR technology which could enhance oil recovery by utilizing microorganisms and their bio-products [2]. Indigenous MEOR is likely to stimulate the beneficial native microbial communities that existed in the reservoir by injecting optimal activator, resulting in higher oil recovery efficiency. On the other hand, exogenous MEOR refers to adding other microorganisms and those metabolisms to increase displacement efficiency and expand swept zone of than primary bacterial community.

Furthermore, compared with exogenous MEOR, the reservoir adaptability of indigenous MEOR is much more extensive. The oil reservoirs are generally characterized by high temperature (up to 180˚C), high pressure (up to 40 MPa), high salinity (up to 20 $g{\cdot}L^{-1}$) [3] [4] [5] [6]. Recent studies have indicated that there have diverse microbial communities. Most microorganisms isolated from oil reservoirs were able to produce bio-products such as biosurfactants and biopolymers being of interest in MEOR since they could be considered as the material basis of indigenous microbial flooding [7] [8]. Many studies have investigated that structure of indigenous microbial communities could be changed by injecting optimal activators. Gao *et al.* (2013) demonstrated that the activator concentration and composition played key roles in efficiency of activating microbial community in reservoir [9]. The continuous supply of activators, Such as peptone, corn syrup or molasses (please mention examples of activators) within the proper concentration and composition was the critical step for the ultimate oil recovery. In order to understand the large-scale mechanism of indigenous MEOR, physical simulation experiments could make an important contribution to optimization of the parameters used in the field trial [10] [11] [12] [13].

In last two decades, most physical simulation tests were run under such conditions as short-time cultivation (*i.e.*, 7 - 30 days) and single round nutrition injection. Thus, a shut-in period was required for consumption of nutrients by microorganisms to utilization (what metabolize by bacteria please mention) [14]. In practice, MEOR in the filed application by injecting multiple rounds of nutrition has lasted for many years. The injected nutrient would migrate over 30 days without microbial consumption to production well which is placed over 150 meters away from the injection well. To our knowledge, the successful distribution of aerobic-anaerobic colonies observed in the long-scale experiment was reported by Yu *et al.* (2012), utilizing long cores with a PV of 2.7 L [15]. In other studies, these experiments were carried out with a 10-days shut-in period. Although indispensable at present long-scale physical models have not yet been used for investigating oil recovery efficiency and nutrient distribution during the continuous flooding [16]. Such general phenomenon cannot be observed effectively in one-round injection of nutritions under the physical simulation's experiments. This paper would focus on the dynamics of microbial community cha-

racteristics, the concentration of activator components and functional bacteria in a series of flooding experiments designed for ten rounds of injection using a dedicated long-scale (1800 mm) sand-pack column. The objectives of this paper are as follows: 1) To investigate the nutrients migration and consumption; 2) To understand the relationship between the nutrients and the biochemical parameters by means of long-scale and long-term physic model flooding experiments, providing an insight for field trial.

2. Material and Methods

2.1. Media and Fluids

In order to activate indigenous microbes, the optimized nutrient formula was selected in the laboratory under condition simulating the Zhan3 block reservoir. The nutrient solution was given as followings: dried corn steep liquor powder, nitrate and phosphate [17] [18]. The injected nutrients solution was prepared by the injected water of Zhan3 block, and the volume of nutrient slug was 30 mL for each cycle (0.05 PV), and the total rounds would be up to 10. The injection concentration of nutrients components is ten times higher than the optimal concentration obtained from the laboratory experiments (C: 982.7 mg/L; N: 438.9 mg/L; P: 143.0 mg/L).

2.2. The Apparatus and Procedure of the Long-Scale Flooding Experiments

The apparatus and detailed procedure of the long-scale flooding experiments has been described in Sun's report [19]. The permeability of the core was 1150×10^{-3} μm^2, the porosity was 0.28 and the pore volume was 573 mL. The nutrient solution was injected within the rate of 1 mL/min, and then the subsequent waterflooding with the brine was carried out immediately within the rate of 0.02 mL/min (*i.e.*, without shut-in period). After that, the concentration of main nutrients components (carbon/nitrogen/phosphorus) could be detected at the output end of model by Analytik Jena multiN/C UV 2000. The detection method was based on the national standard of the People's Republic of China (GB 11893-89). Each round contained both the activator slug and the subsequent continuous water flooding, advancing 10 rounds of microbial flooding in total.

2.3. Isolation of DNA and Microbial Characteristics Analysis

25 mL of production water samples were collected in every day for DNA isolation and DNA extraction. All samples were centrifuged at 12,000×g at 4˚C for 15 min. After removing the supernatant, those pellets were placed at −70˚C and extracted within 1 week. Then DNA would be extracted from pellets using the AxyPrep DNA Extraction Kit (Axygen, USA). Concentration of bacteria and methanogens were performed by iQ SYBR green PCR master mix (Bio-Rad) and iCycler (iQ5) real-time PCR detection system (Bio-Rad), respectively. Quantification of total *mcrA* Gene copies was determined by primers mlas-F

(GGTGGTGTMGGDTTCACMCARTA) and mcrA-R (CGTTCATBGCGTAGTTVGGRTAGT), while quantitation of total bacterial 16S rRNA gene copies was recognized by primers 907R (CCGTCAATTCMTTTRAGTTT) and 517F (CAGCMGCCGCGGTAANWC). The 20-μL PCR reaction mix consisted of 10 μL of iQ SYBR green PCR master mix, 1 μL of template DNA, 0.2 μL of 2 μM primer and 9.6 μL of double distilled water (ddH_2O). Amplification reactions are using the following conditions: an initial denaturation step consisting of 94˚C for 3 min, 30 cycles consisting of 94˚C for 30 s, 50˚C for 30 s, 72˚C for 30 s, and a final elongation for 72˚C 5 min. Each sample is measured three times to ensure the accuracy of the data.

Illumina MiSeq sequencing was conducted at BGI Co., Ltd, following their standard protocols [20]. The raw data were filtered to eliminate the adapter pollution and low quality to obtain clean reads, then paired-end reads with overlap were merged to tags. And tags were clustered to OTU at 97% sequence similarity. Taxonomic ranks were assigned to OTU representative sequence using Ribosomal Database Project (RDP) Na, e Bayesian Classifier v.2.2. At last, alpha diversity was analyzed based on OTU and taxonomic ranks.

3. Results

3.1. Nutrients Migration and Consumption

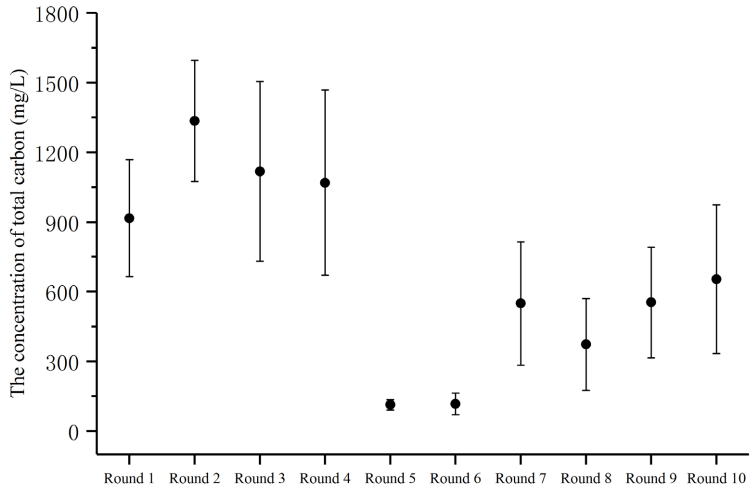
As shown in **Figure 1**, migration velocity of the three components of nutrients in porous media was quite different. The trend of migration and consumption of carbon and nitrogen were similar, with the fluctuating state. The lowest concentration of carbon (122.5 mg/L) and nitrogen (38.3 mg/L) was observed in Round 5. However, the concentration of phosphorus kept constant before Round 8, and afterwards started to rise along with the flooding time.

3.2. Changes of Microbial Concentration in Flooding Experiment

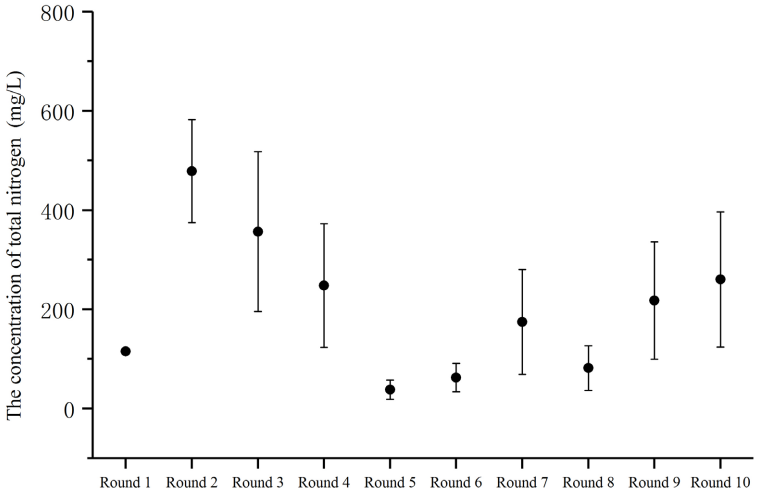
Figure 2 represented the bacterial concentration changed during 10 rounds of nutrient injection in flooding experiment. The changing trend of bacterial concentration was similar to the rule of migration and consumption of taking place for carbon and nitrogen. The minimum bacterial concentration was observed in Round 5. During the process of rounds 1 to 9, nutrient concentration was relevant to the bacteria concentration in produced fluid.

3.3. Microbial Community Structure and Diversity in Produced Liquid

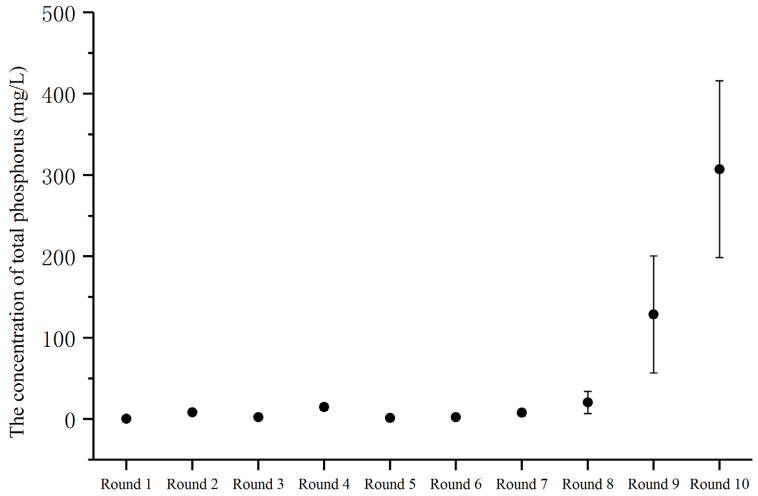
The long-core flooding experiment was subjected to the MEOR treatment for 10 rounds. The microbial community structure and dynamics were analyzed by Illumina MiSeq sequencing. From the sequencing rarefaction curve, sequencing reached saturation, covering 90% of the community (**Figure S1**). OUT-based Shannon index is summarized in **Figure 3**. With multiple rounds of nutrient injection, the diversity of bacterial community decreased first and then increased.



(a)



(b)



(c)

Figure 1. Changes of nutrients components concentration. (a) Carbon; (b) Nitrogen; (c) Phosphorus.

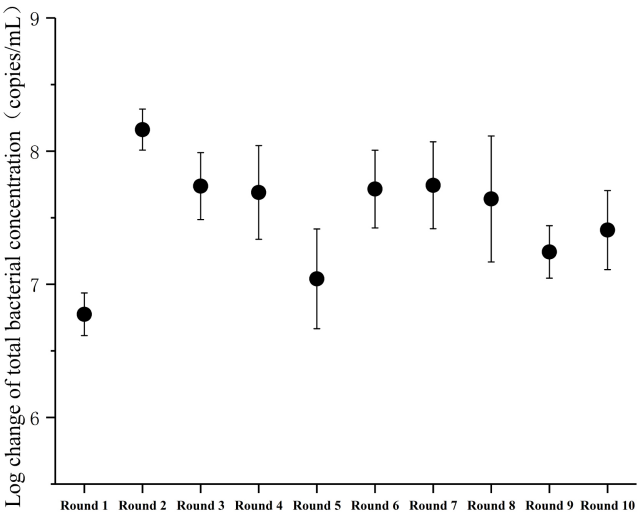
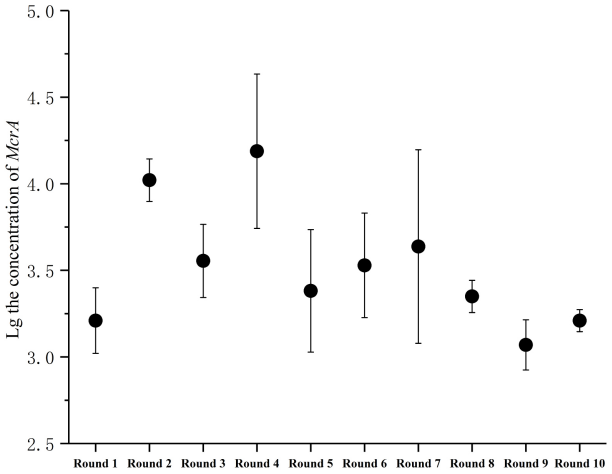
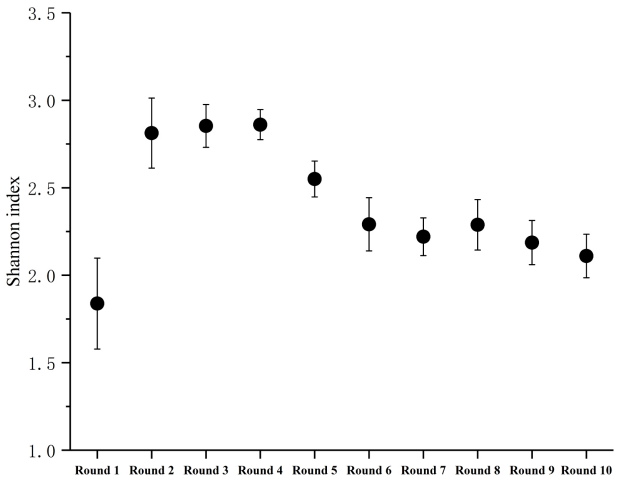


Figure 2. Bacterial concentration of the produced liquid in different rounds.



(a)



(b)

Figure 3. Change of microbial characteristics along with flooding rounds. (a) Concentration of *mcrA* (Methanogens); (b) Microbial community diversity.

Microbial community structure during the different rounds of displacement period is shown at genus level in **Figure S2** and **Figure S3**. There are a large number of unresolved species in bacterial and archaeal communities. The types of bacteria are significantly more than archaea. The bacterial and archaeal communities in the injected water are significantly different from those in the produced liquid. With the increase of displacement rounds, the structure of microbial community showed continuous dynamic succession, The bacterial community gradually changed from aerobic *Pseudomonas* to facultative and anaerobic bacteria, such as *geobacillus* and *anaerobaculum*. There has obvious rules of cascade activation of aerobic, facultative and anaerobic bacteria in the time scale. The succession of archaeal is not obvious, Finally, *Methanothermobacter* becomes the dominant Archaea in the production fluid. The preponderance of this thermophilic archaea is consistent with the high experimental temperature.

3.4. The Relationship between Microbial Growth, Activators' Migration and Consumption

According to **Figure 3**, the highest value of *mcrA* concentration occurred in round 4. The minimum value of diversity increased gradually from round 3. Based on the results, nutrition concentration was not related to the concentration of *mcrA*/the bacterial biodiversity. The highest concentration of *mcr*A was observed in Round 4. Shannon index was increasing along with the flooding time, which meant that the microbial community structure in the produced water was stable from Round 5 to 10. The observation of *mcrA* concentration shown in the flooding experiment was contrary to Shannon index obtained from Round 5 to 10, tending to enhance the stability of microbial community structure.

4. Discussion

As the critical component of MEOR, the accurate microbial reflection represented the endogenic microbe metabolic activity, increasing the EOR efficiency and providing the scientific guidance for industrial application [17]. Moreover, the nutrient was the main regulator to regulate the microbial community. According to the rules of migration and the consumption, with the presence of an apparent chromatographic effect, the nutrient components would significantly affect the microbial activation efficiency during the process of injection [20].

As shown in **Figure 2**, the lower concentration observed in the produced water was caused by migration of microbe into porous media from Round 1 to 5. According to the trend of migration and consumption of carbon and nitrogen shown in **Figure 1(a)** and **Figure 1(b)**, the lowest concentration of carbon, nitrogen or microbe in the produced water all took place in Round 5 at the same time, which meant the microbial filed could move to occupy the porous media within a specific period of time (*i.e.*, 4 rounds) resulting in the changing concentration of microbe and nutrient in produced fluid. Most of the injected nutrients in round 1 - 4 should be depleted by the adsorption and/or the microbial consumption, therefore the microbe hardly acquires the adequate nutrient for the

rapid microorganism growth in round 1 - 4. Phosphorus is an important element for DNA synthesis, but there was only a few existed in the reservoir produced fluid. The law of phosphorus concentration in produced fluid showed that more time was required for phosphorus to be at equilibrium. During round 6 to 10, the concentration of carbon/nitrogen was increased gradually, and the reasons for this phenomena are as follows: 1) Adsorption and desorption; 2) Lower nutrients efficiency caused by microorganisms depleted in porous media [21].

The concentration of injected nutrition components was optimized using the shake flask laboratory experiments. The nutritional usage ratio by microbes in shake flask was faster than in porous media. Injecting in the early period or increasing the concentration of phosphorus might be the way to accelerate microorganism propagation speed. In the late period of flooding experiment, the concentrations of nutrient elements would rise in produced water, leading to the excessive nutrition. As mentioned above, we could increase the phosphorus concentration to speed up the microorganism propagation in the early period, and also decrease the nutrients concentration to maintain the microbial growth, reducing the cost of injection in MEOR field experiments.

In the previous studies, carrying out 6 rounds of nutritional injection, Shannon index could be used to predict the impact of implementation during the whole process of MEOR, and the high *mcrA* concentration was a symbol leading to reducing the water content definitely, while the Shannon index was the symbol to determine the microbial community stability. The decrease in diversity indicated that activator selectively stimulated some microorganisms in the reservoir microbial community and the efficiency of the oil displacement was higher in earlier stage of flooding experiment. In the longer period of flooding experiments (*i.e.*, 10 rounds nutrition injection), the Shannon index tended to continuously increase in round 5 - 10, where the concentration of water remained at 99% (**Figure 4**) and the final oil recovery was 57.6%. This meant Shannon index was unable to predict the production performance in the late period of both laboratory and field flooding experiments.

On the other hand, the *mcrA* concentration started to decline from round 5 lasting to 10. The methanogenic archaea could propagate utilizing metabolism processes by upstream bacteria, such as acetic acid, and the concentration of acetic acid was considered to represent bacterial metabolic activity. **Figure 5** presented the change of acetic acid concentration in the flooding experiment, and the fluctuations shared the similar trend as *mcrA*. Methanogen's concentration gradually reduced mainly due to lower acetic acid concentration in round 5 to 10. According to the changes of bacteria and nutrient concentration since round 5, it investigated that the efficiency of nutrient was reducing in the late period of MEOR because the vast majority of nutrient had been wasted along with the flooding process. Most of the stranded nutrients in late period of flooding experiment might be utilized by the microbe at the front of core tube [22]. The authors considered that it was critical to increase the *mcrA* concentration for the improvement bacterial activity at the back of core tube to enhance oil recovery.

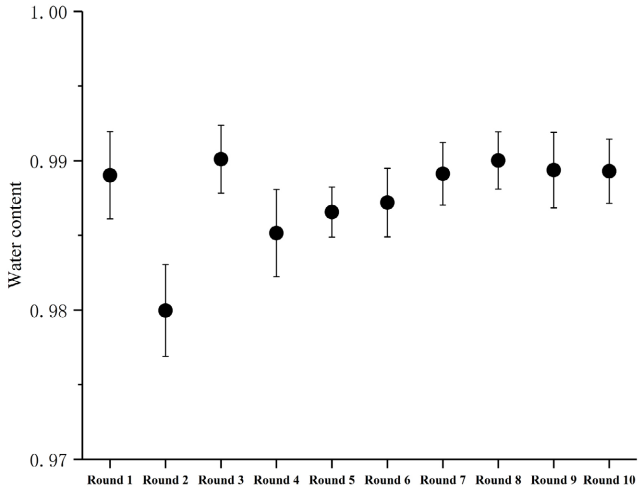


Figure 4. Dynamic change of water cut in the process of flooding.

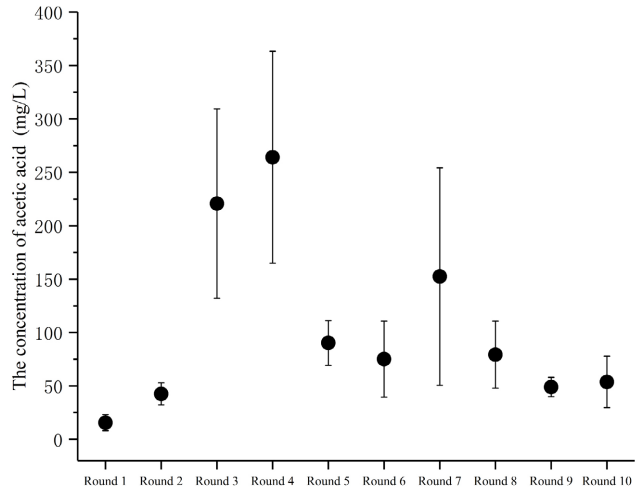


Figure 5. Concentration change of acetic acid in the produced liquid.

More specifically, regulating the microbial communities at the front of core tube and improving the nutrition utilization ratio might be beneficial to raise the *mcrA* concentration and reach the activity level of bacteria in the late period of MEOR, by changing the nutrition composition or injecting patterns.

5. Conclusion

This research could be concluded as followings: Increasing the nutrient concentration could accelerate microorganism propagation in the early period of experiment; reducing the nutrient concentration would maintain the microbial growth and cut down the cost of injection in the late period of experiment; Methanogenic archaea can be used to indicate the metabolic activity while the community diversity can determine the stability of microbial community. After multiple rounds of microbial displacement, the concentration of methanogenic archaea decreased while the diversity index increased, representing the decreased selective activation efficiency of activator. These results indicated that it is critical to ad-

just the activator formula or change the process to improve the biological characteristics and extend the effective period of microbial flooding. Moreover, the experimental results also revealed that Shannon index was not suitable for predicting production performance in the late period of MEOR flooding experiments. Changing the nutrition composition or injecting pattern might be the way to improve microbial activity in the later process of MEOR.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplyment

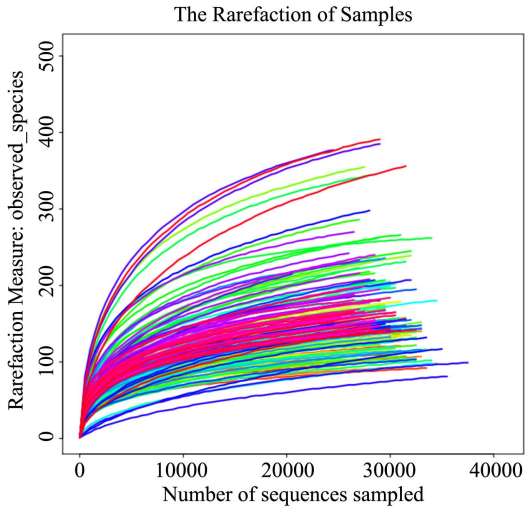

Figure S1. Rarefation curve of high throughput sequencing of physical model production solution samples.

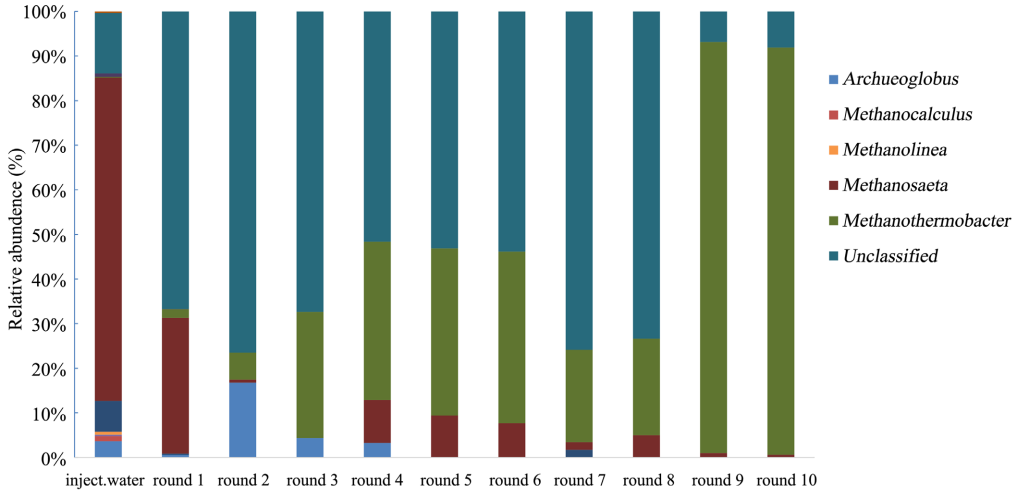

Figure S2. Archaeal community structure of liquid produced by different displacement rounds.

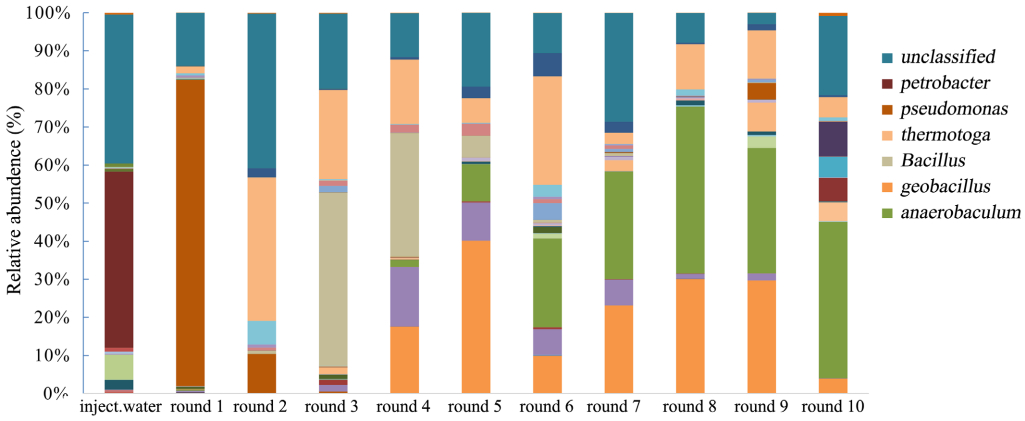

Figure S3. Bacterial community structure of liquid produced by different displacement rounds.