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Core-flooding experimental study of oil displacement by using sulfate-reducing bacteria

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Sulfate reducing bacteria (SRB) are anaerobic microorganisms which are widely distributed in global oil reservoirs. They have been reported to play an important role in enhancing oil recovery (EOR). In this study, a *Desulfobacteriaceae* spp. isolated from Daqing oilfield (China), was used as a candidate for microbial oil displacement in a core flooding experiment that two important experimental parameters were optimized, including numbers of injection slugs and bio-retention time. To find out the EOR mechanism, the produced liquid and recovered oil were analyzed. By the results, SRB showed a best distribution in porous medium when they were injected as two slugs. The oil recovery efficiency was proportional to bio-retention time. By optimizing these two parameters (injection slug and bio-retention time), the oil recovery efficiency could be increased to 11.48%. The viscosity of recovered oil was significantly reduced based on bio-degradation of NSO compounds. Therefore, SRB could be a good candidate in use of microbial enhanced oil recovery.

Keywords: SRB, EOR, TPH, injection slug, core-flooding experiment

INTRODUCTION

Sulfate-reducing bacteria (SRB) are genetically anaerobic organisms that were firstly discovered by Hamilton [1]. SRB could use a very wide spectrum of different low molecular organic compounds for growth, including lactate, acetate, proprionate, succinate, pyruvate, ethanol, sugars, etc. Moreover, SRB use Sulfate (SO_4^{2-}) as electron receptor instead of oxygen for their respiration with SO_4^{2-} being reduced to hydrogen sulfide (H_2S) [2-4]. However, SRB is well known as harmful bacteria in the productive process of oilfields. They might cause serious problems (e.g. corrosion of iron in anaerobic conditions and reduction of the property of injection of water injection wells by precipitation of amorphous ferrous sulfide, etc.) in oilfield water systems [5].

Some recent study indicated that SRB might play an important role in microbial enhanced oil recovery (MEOR) [6-7]. For example, SRB could diminish oil viscosity, replenish the declining pressure of reservoir, and change heavy oil to light oil through yields of bio-generated acids, gas (H_2S) and degradation of hydrocarbons (Aliphatic and Aromatic). Also, different types of SRB are widely distributed in global oil reservoirs. Therefore, SRB could be a great target that used for enhancing oil recovery (EOR).

This study were mainly focused on evaluating the oil displacement efficiency by using SRB. To do this, the entire research was divided into two parts. In the first part, the experimental parameters were optimized, including injection slug (fresh SRB culture) and retention periods (SRB cells interact with crude oil inside of the experimental core after injection). Injection slug is one of important factors for diffusion of microbial cells in porous medium (experimental cores), thus affecting EOR efficiency. Single-slug injection will exhibit a highest local concentration in porous medium, but may limit further diffusion efficiency. In contrast, multi-slug injection could obviously improve microbial diffusion efficiency, whereas the local cell concentration might lose remarkably (decrease in bio-reaction intensity). In this research, the total injection volume is chosen as 0.5 PV of pore volume (experimental core) [8]. To optimize the injection slug, three different injection slugs were tested based on EOR efficiency, including single-slug (1×0.5 PV), two-slugs (2×0.25 PV) and three-slugs (3×0.17 PV).

Once the microbes contact with residual oil, they might use their natural carbon sources for metabolism, including growth, reproduction and respiration, etc. During the metabolic processes, byproducts (bio-mass, bio-gases, organic acids, alcohols and even functional enzymes) are released to the environment [9], thereby resulting in the physical and chemical changes in crude oil. All those bio-physical and bio-chemical reactions require sufficient time to take place. Therefore, three different retention periods (3, 5 and 7 days) was optimized in this study depending on their EOR efficiency. These three experimental retention times

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were commonly used in other microbe-related laboratory researches [10].

In the second part, the produced liquid and recovered oil during subsequent water-flooding (after SRB flooding) were analyzed. The produced liquid were analyzed by cell count to compare the diffusion efficiency among different injection slugs. For recovered oil, its viscosity and composition changes of total petroleum hydrocarbons were evaluated in order to find out the EOR mechanism in core experiment. This is the first time to use SRB for evaluating oil displacement efficiency in experimental core study.

MATERIALS AND METHODS

All reagents (media and buffers) used were prepared gravimetrically using a Sartorius A200S analytical balance, and made up to volume with room temperature sterile distilled water (dH_2O). All chemicals used in this paper were reached the analytical standard, and have been autoclaved at 121 °C for 20 min for sterilizing before use.

The SRB was isolated from pipe line of water injection well in Daqing Oilfield, China. The isolation was carried out in Postgate medium C (sPGC) [11]. The medium consists of the following: NaCl (0.12 M), $MgCl_2 \cdot 6H_2O$ (5.9×10^{-3} M), KH_2PO_4 (3.6×10^{-3} M), NH_4Cl (0.019 M), Na_2SO_4 (0.032 M), $CaCl_2 \cdot 2H_2O$ (2.8×10^{-4} M), $MgSO_4 \cdot 7H_2O$ (1.2×10^{-4} M), $FeSO_4 \cdot 7H_2O$ (1.4×10^{-5} M), trisodium citrate (1.1×10^{-3} M), sodium lactate (70% w/v, 0.077 M), yeast extract (1 g L^{-1}) and agar (20 g L^{-1}). The pH was finally adjusted to 7.2.

The plates were incubated at 45 ºC for 20 days under anaerobic conditions in a 3.5 L anaerobic jar (Traditional system; Oxoid Company) filled with carbon dioxide and hydrogen which was produced by using anaerogen sachets according to the instructions of the manufacturer. Preparation and inoculation of plates were carried out inside an environmental chamber which contained a mixture of gases (Nitrogen 87%, carbon dioxide 10%, and hydrogen 3%) in oxygen free environment. After incubating for a week, several colonies of SRB were observed. The different bacteria were isolated and allowed to grow on separate plates and were found to be of the same type belonging to *Desulfobacteriaceae* family.

The cell culture was carried out in ATCC medium 1249 type III [12]. Cell culture was prepared by inoculating a single colony from the agar plate into 80 mL of broth in a 120 mL headspace vial. The headspace vial was covered by septa, and subsequently sealed with aluminum cap by capping clamp. Preparation and inoculation were carried out inside an environmental chamber which contained a mixture of gases (Nitrogen 87%, carbon dioxide 10%, and hydrogen 3%) in oxygen free environment. Headspace vials were then grown anaerobically on an orbital shaker (150 rpm) at 45 °C for 20 days (The cell concentration was grown to A600 nm to 1.5 (stationary phase) and stored at 4 °C until required, but no longer than 8 hours.

The crude oil samples were collected from Daqing oilfield with density of 0.851 g cm^{-3}. The permeability of target reservoir is 180.7×10^{-3} μm^2. Reservoir temperature is 45 °C and salinity of formation water is 14,139 mg L^{-1}. Synthetic cores were chosen based on reservoir conditions (Table 1).

The oil displacement experiments were conducted by using the standard core flooding system [13] (Figure 1). Core flooding experiment is composed of a series of steps including, vacuumization of core followed by saturation with formation water, water-phase permeability measurements, determination of crude oil saturation level, aging interaction between crude oil and the core (7 days), water flooding until 98% water cut, chemical flooding slug injection, and subsequent water flooding until 99% water cut. The experiment was conducted at reservoir pressure (9.95 MPa) and temperature (45 °C) with fluid injection rate of 0.2 mL min^{-1}. During the experiments, the pressure differential, oil production, water production and total fluid production were recorded timely to make sure the oil recovery were calculated precisely.

Table1. Physical properties of experimental cores

Core ID	Length [mm]	Diameter [mm]	Pore Volume [ml]	Porosity φ [%]	Air permeability K_g [10^{-3} μm^2]	Water permeability K_w [10^{-3} μm^2]
ZF-04	200	24.25	13.14	27.07	300	185.90
ZF-05	200	24.80	11.74	24.03	300	196.49
ZF-06	200	24.28	14.14	28.77	300	172.68
ZF-07	200	24.36	12.56	25.69	300	169.27
ZF-09	200	24.37	13.07	26.51	300	190.32

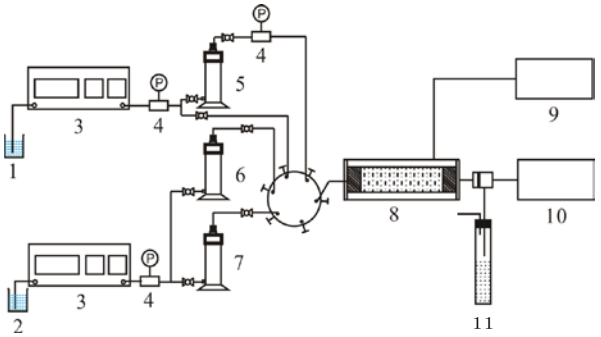

Fig. 1. Schematic of the core flooding setup. 1. Brine; 2. Water; 3. Injection pump; 4. Pressure gauge; 5. Microbial culture tank; 7. Oil tank; 8. Core holder; 9. Confining pump; 10. Back-pressure pump; 11. Produced liquid collector.

During the subsequent water flooding, the produced liquid was collected every 0.1 PV. 100 µL of produced water with appropriate dilutions were pipet out and spread onto a Postgate medium C agar plate. The plates were incubated at 30 ºC for 14 days under anaerobic conditions in a 3.5 L anaerobic jar (Traditional system; Oxoid Company) filled with carbon dioxide and hydrogen which was produced by using anaerogen sachets according to the instructions of the manufacturer. Preparation and inoculation of plates were carried out inside an environmental chamber which contained a mixture of gases (Nitrogen 87%, carbon dioxide 10%, and hydrogen 3%) in oxygen free environment. The number of colony forming units (CFU) mL^{-1} were then calculated.

Viscosity of recovered oil sample was measured by using a NDJ-8S digital viscometer (Nirun Intelligent Technology, China) at 45°C.

30 mg of recovered oil were consecutively extracted with hexane, dichloromethane, and chloroform (100 mL each). All three extracts were pooled and dried at room temperature by evaporation of solvents under a gentle nitrogen stream in a fume hood. After solvent evaporation, the amount of residual TPH was then determined gravimetrically. After gravimetric quantification, the residual TPH was fractionated into alkane, aromatic, asphaltene, and NSO (nitrogen, sulfur, and oxygen-containing compounds) on a silica gel column. To do this, samples were dissolved in hexane and separated into soluble and insoluble fractions (asphaltene). The soluble fraction was located on a silica gel column and eluted with different solvents. The alkane fraction was eluted with 100 mL of toluene. Finally, the NSO fraction was eluted with methanol and chloroform (100 mL each) [14]. The alkane and aromatic fractions were then analyzed by GC-MS.

RESULTS AND DISCUSSION

To optimize the number of injection slugs, three injection plans were designed, including sing-slug, two-slug and three-slug injection. Three days bio-reaction period was used as retention time in this experiment.

Two-slug assay showed the best oil displacement efficiency among the three assays, in which the EOR was improved by 6.69% after subsequent water flooding (Figure 3). In contrast, single-slug (Figure 2) and three-slug assays (Figure 4) only could improve EOR by 4.71% and 4.05%, respectively. Of the three, three-slug assay showed the lowest EOR efficiency. The results indicated that the optimal injection plan is two-slug injection.

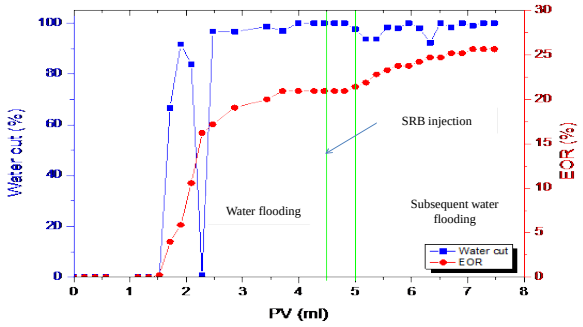

Fig. 2. SRB EOR efficiencies of 3-day retention assay with single-slug injection.

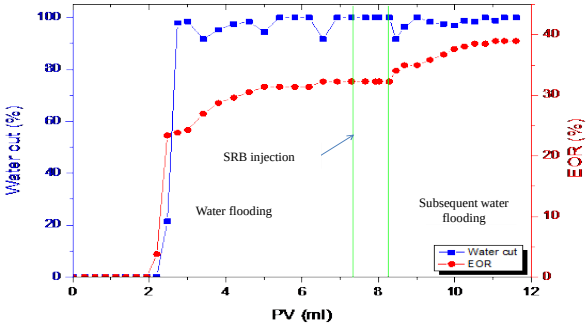

Fig. 3. SRB EOR efficiencies of 3-day retention assay with two-slug injection.

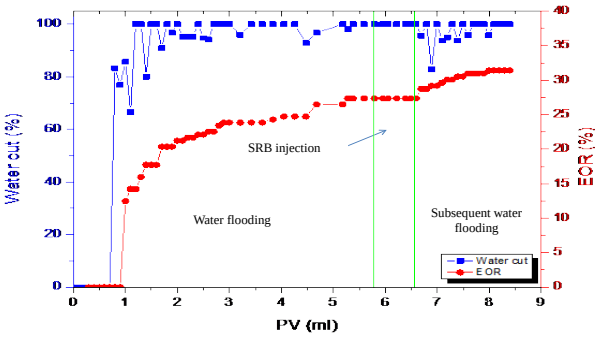

Fig. 4. SRB EOR efficiencies of 3-day retention assay with three-slug injection.

The reason that two-slug assay showed the best EOR result is unclear. However, the highest EOR efficiency might be caused by the best diffusion of SRB cells inside of the core. There were four contact areas between SRB culture and the residual oil in the cross-section of the experimental core in two-slug assay, whereas there was only two in single-slug assay. In contrast, small multi-slugs might lose more cell concentrations during they went through the porous medium, even if they created more contact areas (six contact areas in cross-section in three-slug assay). Moreover, small slugs (low in total cell numbers) might limit the diffusion of microbial cells.

To optimize the retention period, the other two bio-reaction times (5 and 7 days) were tested by their following EOR efficiency. Two-slug injection was used in this experiment. As shown in results, SRB could improve EOR by 9.34% and 11.48% after bio-reacting with residual oil in 5 (Figure 5) and 7 (Figure 6) days, respectively. This might indicated that the EOR efficiency was proportional to the bio-reaction period.

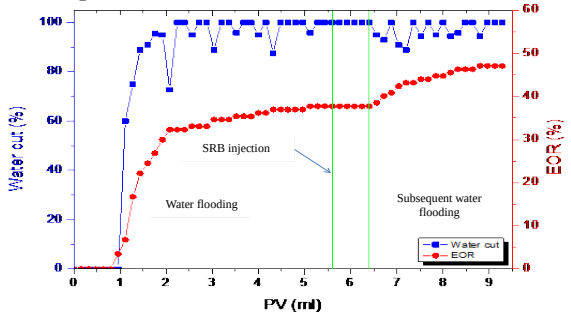

Fig. 5. SRB EOR efficiency of 5-day bio-retention assay with two-slug injection.

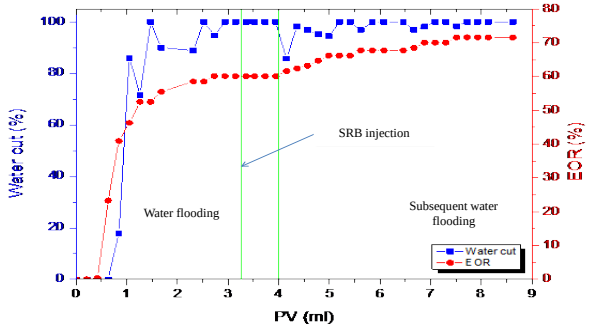

Fig. 6. SRB EOR efficiency of 7-day bio-retention assay with two-slug injection.

This results could be understand by the previous studies. For example, microbial metabolites are amphiphilic molecules which contain both hydrophilic and hydrophobic groups [9]. These metabolites are known as bio-surfactants (e.g. bio-mass, organic acids, and organic alcohols, etc.), which could bind both water and oil molecules, thereby forming a stable emulsification system between oil and liquid, and also decreasing oil/liquid interfacial tension. This will result in a decrease in oil viscosity, thus increasing oil fluidity [15]. Furthermore, SRB has been reported to degrade oil compounds such as alkane and methylbenzene [16, 17]. Degradation of heavy hydrocarbons would also increase oil fluidity. Therefore, the longer the retention period is, the more functional metabolites produced and the greater intense of bio-reaction took place.

In a previous study, *Pseudomonas* spp. has been reported to use for oil displacement experiment [18]. It could improve EOR efficiency by about 5% - 13% with more than 3.5 PV of microbial injection. In contrast, SRB could improve EOR by about 10% with 0.5 PV of injection. This represents that SRB is a good candidate for MEOR. Recently, most of MEOR-related studies are focused on basic mechanisms (e.g. microbial communities in target reservoir, oil degradation mechanism, production of bio-surfactants, and changes of wettability, etc.) [19-22]. However, there is still require more core-flooding studies to compare.

To best understand the influence of microbial injection slugs in porous medium, produced liquid was collected every 0.1 PV to indirectly evaluate cell diffusion efficiency by counting cell numbers. The results were shown in Figure 7. In single-slug assay (blue bars), SRB showed a “mountain-shape” graph. The highest cell number occurred in the middle of experimental cores with concentration of 10^7 cell mL^{-1}. There was no SRB found in the first 0.1 PV of produced liquid, which was similar with three slug assay. Of the three, three-slug assay exhibited the lowest diffusion efficiency in average (green bars). In contrast to those two, SRB cells were found in every collection of produced liquid (red bars). This indicated that microbes were well distributed inside of the entire core. The result might give the evidence why tow-slug injection of SRB showed the highest EOR efficiency.

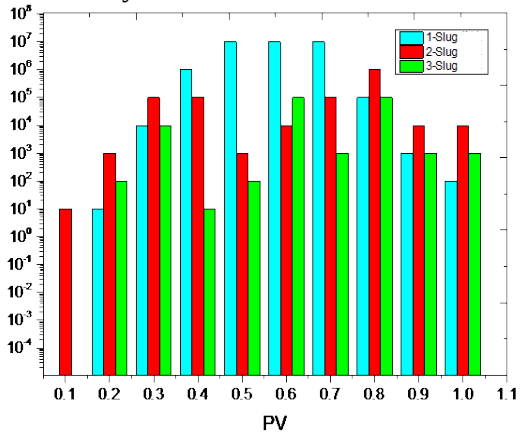

Fig. 7. Cell number count of produced liquid with different SRB slug-injection assays.

To find out the SRB-EOR mechanism during core-flooding experiment, the viscosity of recovered oil was tested (7-day bio-retention). The viscosity of crude oil was also analyzed as control. In comparison with crude oil, the viscosity of recovered oil was significantly reduced by 15% (Figure 8). The reason of decrease in recovered oil viscosity need to be further studied.

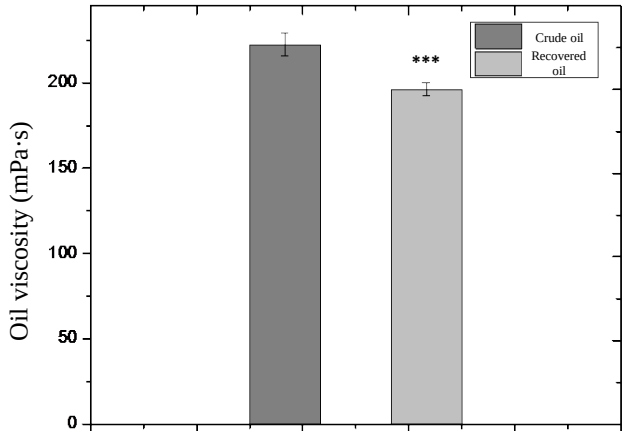

Fig. 8. Viscosity comparison between crude and recovered oil samples. *: significant difference from crude oil assay. The number of stars (*) indicates the significance level. *: P<0.05; **: P<0.01; ***: P<0.005; ****: P<0.001.The significant difference was determined in paired test. n=4, error bars are standard error.

To better understand the relationship between oil composition changes and viscosity declination during core-flooding experiment, the TPH ratios of recovered oil were analyzed. The TPH composition of crude oil was also studied as the control. After SRB flooding, three fraction ratios of TPH were slightly increased (alkane, aromatic and asphaltene) compared to crude oil samples (Table 2). However, those variations were remained in estimated errors. Of the four fractions, only NSO was significantly reduced when compared with their control counterpart. The reason is unclear. It might be that the compounds in NSO are more readily to be used by SRB. This result indicated that an increase in EOR efficiency was based on the reduction of oil viscosity by bio-consumption of NSO compounds.

Table 2. Fraction changes of TPH between crude and recovered oil samples

Fraction	Contents (Mean ± SD) (%)	
	Crude oil	Recovered oil
Alkane	55.71 ± 0.24	56.38 ± 0.31
Aromatic	11.25 ± 0.28	12.12 ± 0.28
Asphaltene	1.96 ± 0.03	1.99 ± 0.02
NSO	30.97 ± 0.26	27.41 ± 0.18*

*: significant difference from crude oil assay. The number of stars (*) indicates the significance level. *: P<0.05.

Both oil samples were then analyzed by GS-MS to compare with the changes of alkanes and aromatics. However, no statistically significant difference was found in both assays (data not shown). It has been reported that SRB could degrade oil hydrocarbons (e.g. alkane and methylbenzene, etc.) [16, 17]. This might be caused that SRB bio-activity could not be sufficiently exhibited in 7-day retention period. It is believed that SRB flooding efficiency could be further improved by extending retention time.

CONCLUSION

In this study, SRB was the first time to be used as a candidate in core experimental oil displacement. By optimize the experimental factors (microbial injection slugs and bio-retention time), SRB could increase EOR by more than 11% after subsequent water flooding. By analysis of produced liquid (cell number count), two-slug injection showed the best distribution efficiency of SRB cells in porous medium. By analysis of recovered oil (evaluation of oil viscosity and changes of TPH), the oil viscosity was significantly reduced by bio-degradation of NSO compounds. Therefore, SRB can be a good candidate in use of MEOR.

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ЕКСПЕРИМЕНТАЛНО ИЗСЛЕДВАНЕ НА ИЗМЕСТВАНЕТО НА НЕФТ С ПОМОЩТА НА СУЛФАТ-РЕДУЦИРАЩИ БАКТЕРИИ ПРИ ИЗПИТАНИЕ СЪС ЗАЛИВАНЕ НА СКАЛНА ЯДКА

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(Резюме)

Сулфат-редуциращите бактерии (SRB) са анаеробни микроорганизми, които са широко разпространени в глобалните петролни резервоари. Те играят важна роля за подобряването на добива на петрол (EOR). В това проучване, щам *Desulfobacteriaceae* spp. изолиран от петролното находище Дацин (Китай), е използван като кандидат за микробиологично изместване на петрол в експеримент за заливаневане на ядрото, при който са оптимизирани два важни експериментални параметри, включително броя на инжектиране и време за биозадържане. За да се установи механизмът на EOR, бяха анализирани получените течности и петрол. Чрез резултатите SRB показа най-доброто разпределение в порестата среда, когато са двукратно инжектирани. Ефективността на оползотворяването на петрола е пропорционална на времето за биозадържане. Чрез оптимизирането на тези два параметъра (инжектиране на шлака и време на биозадържане), ефективността на добива на петрол може да се увеличи до 11,48%. Вискозитетът на петрола е значително намален чрез биоразграждане на NSO съединенията. Следователно SRB могат да бъдат добър кандидат за използване на микробиално подобрение на добива на петрол.