Journal of Bioscience and Bioengineering
VOL. xx No. xx, 1–6, 2018

www.elsevier.com/locate/jbiosc

Compatibility between weak gel and microorganisms in weak gel-assisted microbial enhanced oil recovery

Yi-Bin Qi,[1,2,*] Cheng-Gang Zheng,[1,2] Cheng-Yuan Lv,[1,2] Zeng-Min Lun,[1,2] and Tao Ma[1,2]

Petroleum Exploration & Production Research Institute, SINOPEC, No. 31 Xueyuan Road, Haidian District, Beijing 100083, PR China[1] *and SINOPEC Key Laboratory of Marine Oil & Gas Reservoir Production, No. 31 Xueyuan Road, Haidian District, Beijing 100083, PR China*[2]

Received 27 March 2017; accepted 15 February 2018
Available online xxx

To investigate weak gel-assisted microbial flooding in Block Wang Long Zhuang in the Jiangsu Oilfield, the compatibility of weak gel and microbe was evaluated using laboratory experiments. *Bacillus* sp. W5 was isolated from the formation water in Block Wang Long Zhuang. The rate of oil degradation reached 178 mg/day, and the rate of viscosity reduction reached 75.3%. Strain W5 could produce lipopeptide with a yield of 1254 mg/L. Emulsified crude oil was dispersed in the microbial degradation system, and the average diameter of the emulsified oil particles was 18.54 μm. *Bacillus* sp. W5 did not affect the rheological properties of the weak gel, and the presence of the weak gel did not significantly affect bacterial reproduction (as indicated by an unchanged microbial biomass), emulsification (surface tension is 35.56 mN/m and average oil particles size is 21.38 μm), oil degradation (162 mg/day) and oil viscosity reduction (72.7%). Core-flooding experiments indicated oil recovery of 23.6% when both weak gel and *Bacillus* sp. W5 were injected into the system, 14.76% when only the weak gel was injected, and 9.78% with strain W5 was injected without the weak gel. The results demonstrate good compatibility between strains W5 and the weak gel and highlight the application potential of weak gel-assisted microbial flooding.

[**Key words:** Crude oil; Microbial enhanced oil recovery; Weak gel; Compatibility; Viscosity reduction]

In recent years, several field tests have been conducted in China's oilfields with the goal of enhancing oil recovery. Depending of the characteristics of the reservoir, microbial flooding, polymer flooding and other developed methods have been implemented in some of these oilfields to extend the development life (1,2). Microbial enhanced oil recovery (MEOR) research was boosted by the petroleum crisis (1970s) and later became a scientific substantiated EOR method (3).

In MEOR, isolated microorganisms and nutrient solution are injected into test wells. Alternatively, a nutrient solution alone is injected into the reservoir to activate the indigenous microorganisms. In either case, the microorganisms grow and reproduce in the reservoir, and produce metabolites to enhance oil recovery (4,5). MEOR is an environmentally benign and low-cost oil recovery technique that has proven effective for high-water-cut (>95%) reservoirs (6). Accordingly, MEOR has gained considerable attention from academic researchers and oil companies. The mechanism of MEOR is based on the metabolites produced by the microorganisms, which include surfactants, organic acids, solvents, and gas and can effectively improve the efficiency of oil displacement (7–9).

Another strategy for the development of high-water-cut reservoir is polymer flooding. When using water flooding, the injected water is unable to sweep substantial amounts of oil located in zone of low permeability. Thus, sweeping residual oil from low permeability areas is crucial to improve oil recovery in heterogeneous reservoirs (10). Treatment with weak gel is an effective methods to control water mobility in heterogeneous reservoirs, and weak gels are widely used to block zones of high permeability (11,12). In this method, a mixture of a high-concentration polymer and a delayed crosslinker is injected into the target formation and reacts to form a gel, which fully or partially seals the formation at reservoir temperature (13,14). Weak gels have been widely applied as a cost-effective method to reduce excess water production because the gels are able to improve the macroscopic sweep efficiency by selectively plugging high-permeability zones during hydrocarbon production (15,16).

Dunsmore et al. (17) reported that it is difficult to improve the sweep efficiency using microbial flooding, while weak gel flooding changes only the injection profile, which has a limited effect on sustainable production. For this reason, Jang et al. (18) studied the use of the microbial-polymer flooding to enhance oil recovery using indoor experiments. Zhao et al. (19) also proposed a combination of secondary and tertiary oil recovery and discussed the application prospects of this technique. Weak gel-assisted microbial flooding is a kind of compound technology combining their advantages. From a technical perspective, the polymer enters the high-permeability channel of the reservoir to form a barrier, forcing the subsequent injection of microbial solution into the intermediate- and low-permeability channels of the reservoir. The microbial metabolites formed by the microbes (e.g., surfactants, organic acids, solvents) then interact with the crude oil in the reservoir to

* Corresponding author. Tel.: +86 10 82316846; fax: +81 10 82316031.
E-mail address: qybn118@163.com (Y.-B. Qi).

1389-1723/$ – see front matter
https://doi.org/10.1016/j.jbiosc.2018.02.011

Please cite this article in press as: Qi, Y.-B., et al., Compatibility between weak gel and microorganisms in weak gel-assisted microbial enhanced oil recovery, J. Biosci. Bioeng., (2018), https://doi.org/10.1016/j.jbiosc.2018.02.011

increase its mobility, allowing the oil to be further displaced by subsequent injection water (20,21). Consequently, this technique can enhance the sweeping volume along with improve the efficiency of oil displacement, greatly enhancing oil recovery.

This paper presents a study of the Wang Long Zhuang block in Jiangsu Oilfield. We evaluated the compatibility of a microbial strain with a weak gel and assessed the technical feasibility of polymer-assisted MEOR at the study site. First, based on investigations of the growth and metabolism of microorganisms in a weak gel, we analyzed the effects of the microorganisms on the rheological properties of the weak gel. Second, the effects of the weak gel on the growth and metabolism of the microorganisms were analyzed by adding the weak gel with different gelation times. Finally, a core-flooding test was conducted to evaluate the compatibility of the microorganisms with the weak gel and assess the feasibility of applying weak gel-assisted microbial flooding at the study site.

MATERIALS AND METHODS

Oil reservoir conditions and sample collection Isolated samples, including formation brine and crude oil (the viscosity of the crude oil is 45.4 mPa s) were collected from the Wang35 well in Jiangsu Oilfield located in East China. The injection water was recycled after separating the oil from the production water. The salinity of injection water is 4484 mg/L and the water type is $NaHCO_3$. The depth of the petroleum reservoir is 924–1038 m, and the temperature is 60°C. The average air permeability of the reservoir is 0.233 μm^2.

Strain screening and identification Crude oil (2 g) was added to a 250-mL Erlenmeyer flask containing 100 mL of mineral salt medium (MSM) with the following composition: 1.0 g/L K_2HPO_4, 1.0 g/L KH_2PO_4, 2.0 g/L $NaNO_3$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L $(NH_4)_2SO_4$, 0.1 g/L $CaCl_2$ and the solution pH was adjusted to 7.0. Formation brine (5% v/v) was inoculated into the flask, and the flask was shaken at 180 rpm in a shaking incubator at 60°C until the oil was emulsified. Five percent of the enrichment liquid was then collected and inoculated with fresh MSM followed by culturing under the same conditions. This process was repeated at least three times before the bacterial strains were isolated. Strain isolation and purification were carried out on MSM agar plates using conventional spread plate techniques. Oil (0.2% w/v) was sprayed on the surface of the pure culture as the sole carbon source. Each potentially different colony was selected from the MSM agar plates, and 15 strains, named W1–W15, were isolated from the oilfield production water. Strain W5 was selected for further study because of its ability to emulsify oil in MSM (22,23).

16S rDNA of strain W5 was amplified by using universal eubacterial primers 8f (5′-AGAGTTTGATCCATGGCTCAG-3′) and 1541r (5′AAGGAGGTGATCCAGCCGCA-3′) for bacterial fragments (~1500 bp) from the total DNA. The gene of 16S rDNA was purified by gel electrophoresis. The purified gene was sent to Shanghai Meiji Biological Technology Company (Shanghai, China) and sequenced (24).

Weak gel The weak gel used in this study consisted of a polymer [partially hydrolyzed polyacrylamide (HPAM), average molecular weight is 25 million; Beijing Hengju Company] and a delayed-crosslinker ($AlCl_3$). The polymer was dissolved in NaCl solution with a salinity of 4484 mg/L.

Preparation and rheological properties of the weak gel The polymer solution with concentration of 1000 mg/L was prepared. The delayed-crosslinker ($AlCl_3$) was then added into the polymer solution at a fixed quality ratio ($m_{polymer}$:$m_{crosslinker}$ = 30:1). After adjusting the pH of the solution was adjusted to 8 using Na_2CO_3, the mixed solution was placed in an incubator at 60°C (Binder, Tuttlingen, Germany) for gel formation. The resulting weak gel was observed by transmission electron microscope (TEM, Philips CM30 TEM, Amsterdam, Netherlands), and its viscosity was determined using a rheometer (Brookfield LVDV-III Ultra, Middleboro, MA, USA) at different shear rates (25).

Determination of the crude oil viscosity Crude oil (30 g) from the Wang35 oil was mixed with 30 mL of microbial fermentation broth (W5, V_{W5}:$V_{weak\ gel}$ = 1:1) in a 250-mL Erlenmeyer flask, and the system was shaken at 180 rpm in a shaking incubator at 60°C for 7 days. After microbial treatment, the petroleum was collected and electrically dehydrated in a pressurized tank. The viscosity was measured as indicated above and the variation of oil viscosity was detected (26,27).

Effect of the weak gel on microbial growth During the initial stage of microbial fermentation, the weak gel differ in colloidal time (pre-gelation, colloid and colloid degradation) and different concentrations of polymer (1000, 2000 and 4000 mg/L) were added into the microbial culture system. Microbial growth was then measured at different times (0, 1, 2, 3, 5, 8, 15, 24, 36 and 48 h).

Determination of crude oil degradation rate Crude oil (2 g) from the Wang35 was mixed with 100 mL MSM in a 250-mL Erlenmeyer flask. A total of 5% microbial fermentation broth (W5, V_{W5}:$V_{weak\ gel}$ = 1:1) was added into MSM after sterilization. The flask was shaken at 180 rpm in a shaking incubator at 60°C for 7 days, and the petroleum was then collected by centrifugation. The petroleum was weighed after vacuum drying, and the degradation rate was calculated. The degradation rate is the average weight that strain W5 degradation of crude oil in one day.

Measurement of the particle size of oil emulsion Crude oil (5 g) from the Wang35 was mixed with 5 mL of microbial fermentation broth in a 50-mL Erlenmeyer flask. The flask was shaken at 180 rpm at room temperature for 1 min and then left to stand for 24 h. The emulsified particles in crude oil were observed using a microscope (Olympus BX53, Olympus, Tokyo, Japan). The diameters of 50 random emulsified oil particles were measured, and the average diameter was calculated (28).

Analysis of crude oil composition The composition of the petroleum degraded by strain W5 was analyzed as follows (29): After incubation, the culture was extracted sequentially with equal volumes of hexane, methylene chloride and chloroform. All extracts were pooled, dried at room temperature via evaporation in a safety cabinet, and used as the residual crude oil. The residual crude oil was then separated into saturated hydrocarbons, aromatic hydrocarbons, resins and asphaltene fractions using a silica gel G (60–120 mesh) column (2 cm × 30 cm), and the change in oil composition resulting from microbial degradation was assessed (30).

The identification of biosurfactant As a pre-culture, *Bacillus* sp. W5 was inoculated into a 250-mL shake flask containing 100 mL of Luria–Bertani medium (5 g/L yeast extract, 10 g/L peptone, and 5 g/L NaCl, pH adjusted to 7.0) and cultivated at 60°C under shaking at 180 rpm for 24 h. For lipopeptide production, 5% (v/v) pre-culture was inoculated in a 500-mL shake flask containing 100 mL of lipopeptide fermentation medium (40 g/L glucose, 4.0 g/L NH_4NO_3, 4.1 g/L KH_2PO_4, 4.2 g/L Na_2HPO_4, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.2 g/L $FeSO_4 \cdot 7H_2O$, 0.05 g/L $CaCl_2 \cdot 2H_2O$, and 0.01 g/L ethylene diamine tetraacetic acid, pH adjusted to 7.0) and cultivated at 60°C under shaking at 180 rpm for 72 h.

The lipopeptide was separated from the broth and purified as follows: Cell-free supernatant was obtained by centrifugation (Sigma 3k-15, Sigma, Osterode, Germany) at 10,000 ×*g* for 10 min at 4°C, acidified with dilute hydrochloric acid to pH 2.0, and stored at 4°C overnight. The acid precipitates were harvested by centrifugation (10,000 ×*g*, 10 min, 4°C), extracted with methanol, concentrated by vacuum rotary evaporation (YaRong RE-6000, Shanghai, China) at 50°C for 2 h, and dried overnight at 35°C, leaving the lipopeptide (31,32).

The lipopeptide compounds were separated using a HPLC system (Agilent 1200, Agilent Technologies, Santa Clara, CA, USA), and the molecular masses of the compounds were analyzed by electrospray ionization mass spectroscopy (ESI-MS). For HPLC, the column (Agilent C18) was held at 35°C, and the solvent system consisted of 93% (v/v) acetonitrile with 0.1% trifluoroacetic acid (TFA) and 7% (v/v) water with 0.1% TFA. The eluting flow rate was 0.84 mL/min. The absorbance of the eluate was measured at 210 nm by an Ultraviolet Detector. For ESI-MS, the desolvation gas (N_2) was heated at 150°C, the capillary was held at a potential of 3.5 kV, and the cone voltage was 25 V. The separated lipopeptide compounds were analyzed in positive-ion mode, and the scanning range was 300–2000 m/z (33).

Core flooding test The enhancement in oil recovery associated with the flooding systems was tested using a core flooding approach (34,35). The oil from production well Wang35 was used as the oleic phase, and the formation brine was used as the injection phase. The parameters of the three layers of heterogeneous consolidated cores are shown in Table 1. First, the oil-bearing core was injected with the formation brine until no more oil was recovered from the core. Subsequently, 40% Pore volume (PV) flooding slug (W5, weak gel, 10% PV weak gel was injected, and then 30% PV W5 injected) were injected into the water-flooded core. The core was then incubated at 60°C for 7 days. Finally, formation brine flooding was performed again until no further oil was observed. Discharges from the column were collected to measure the amount of oil

TABLE 1. Parameters of heterogeneous cores.

Permeability layer	Thickness (cm)	Porosity (%)	Length × width × height (cm × cm × cm)	Permeability ($\mu m^2 \times 10^{-3}$)	Average permeability ($\mu m^2 \times 10^{-3}$)
High	1.5	19.8	30.0 × 4.5 × 4.5	320	235
Medium	1.5			240	
Low	1.5			150	

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