Study on the application potential of lipopeptide fermentation broth in oil recovery

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Abstract
Microbial flooding is a new enhanced-oil-recovery technology for the petroleum industry. In this study, a strain of \textit{Bacillus subtilis} named SL-2 was isolated from oil-well-produced fluid. \textit{B. subtilis} produces the lipopeptide surfactin. The fermentation broth of \textit{B. subtilis} contains lipopeptide biosurfactants (1.2 g/L) with good surface and interfacial activities. Adding 7\% of this fermentation broth to water reduces its surface tension from 72 to 33.9 mN/m and the interfacial tension between hexadecane and water from 40 to 1 mN/m. Environmental adaptability analysis revealed that the lipopeptide biosurfactants can tolerate a salinity of 50 g/L NaCl and a temperature of 120°C, and that they have a strong emulsifying effect on the oil phase as well as good emulsification stability owing to their high interfacial shear viscosity. Water contact angle measurements showed that this fermentation broth changes the wettability properties of rock surfaces from hydrophobic to hydrophilic. A new bio-oil-displacement system was developed by combining the lipopeptide fermentation broth with xanthan gum, a biopolymer with viscosifying properties. The new bio-oil-displacement system has the dual functions of improving oil-displacement efficiency and expanding sweep volume. The results of laboratory simulation experiments revealed that the oil recovery for a bio-composite system containing 7\% lipopeptide fermentation broth is 18.7\% higher than that for water flooding. Therefore, the system has good field application potential. Direct preparation of the oil-displacement system using the lipopeptide fermentation liquid can reduce the purification cost of the biosurfactant lipopeptide, which is economically conducive to the application of this bio-oil-displacement technology.

KEYWORDS
biosurfactant, EOR, fermentation broth, lipopeptide surfactin, oil displacement efficiency

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1 | INTRODUCTION

Conventional primary and secondary oil recovery technologies only obtain ~40% of the geological reserves of crude oil, so ~60% of the crude oil remains. Therefore, tertiary oil recovery, also termed enhanced oil recovery (EOR), must be used to exploit the residual oil in a reservoir after primary and secondary recovery. Accordingly, EOR technology has been paid increasing attention in recent years. Common EOR methods include thermal flooding, chemical flooding, miscible flooding, and microbial-EOR (MEOR), which is a method for improving oil recovery that involves injecting microorganisms or microbial products into the well. Here, the biosurfactant product can reduce surface tension and oil–water interfacial tension, change the wettability of the rock surface, and promote emulsification of crude oil and water, so as to improve oil recovery. Compared with chemically synthesized surfactants, biosurfactants have the advantages of low toxicity, good biodegradation, good environmental compatibility, renewable raw materials, and good emulsifying performance. It is thus a typical green environmental protection technology. At present, biosurfactants that can be used in MEOR include rhamnolipid, locust glucolipid, lipopeptides, and other small molecules. There are also high-molecular-weight biosurfactants composed of a mixture of heteropolysaccharides, lipopolysaccharides, lipoproteins, and proteins.

In terms of cost, She et al. have demonstrated that MEOR has a much lower total cost than other EOR techniques in field studies. In a previous study, Li et al. purified the lipopeptide from a Bacillus fermentation broth, and the purified lipopeptide had a lower critical micelle concentration (CMC) value and good stability under extreme temperature, salinity, and other conditions. Liu et al. improved oil displacement efficiency by increasing the content of C15-surfactin in a fermentation broth. Field trials have also been performed, with Rebecca et al. achieving 11%-EOR in a field in the Phoenix area in the 1990s. Liu et al. carried out research on endogenous microbial oil flooding technology and performed experiments simulating the deepburial, high-temperature, and high-salt conditions in the Xin Block of Sheng Li oilfield in China. The results showed that the displacement efficiency can be improved by 8.5%, the oil production was increased from 1.0 to 1.8 t/day, and the water content decreased by 14%. This oil displacement effect is remarkable. Kong et al. has studied ways of flooding oil with endogenous microorganisms by injecting them with nutrients and air. The air injection volume used in microbial oil recovery is 1:8–1:10 liquid/gas ratio.

Although there are many reports on the characteristics of lipopeptides and their application potential in MEOR, the acquisition cost of pure biosurfactants is relatively high and the technology is complex. Considering a large amount of oil produced, it is unrealistic to apply biosurfactants on site. Furthermore, in endogenous microbial flooding, which is a mainstream microbial oil flooding technology, the air injection volume is very high. Due to difficulties in terms of gas injection and low utilization rate, the growth of aerobic bacteria is slow, the oil displacement efficiency is low, and the risk of gas injection is high, so there are certain potential safety hazards. To further reduce costs and increase security, oil displacement efficiency and stability can be greatly improved by using a surface fermentation process to make a single strain grow and ferment in an above-ground fermentation device. This is then injected into the well with xanthan gum.

With this in mind, the oil displacement potential of the SL-2 strain fermentation broth was evaluated. The composition of the fermentation broth as well as its surface tension, interfacial tension, emulsification performance, stability, and oil displacement efficiency of the fermentation broth at different dilutions were evaluated. The fermentation liquid shows good oil recovery performance, and the EOR value of 18.7% is higher than that reported in the present study, demonstrating its suitability for field application. At the same time, since emulsification is one of the core mechanisms of MEOR, we discuss the mechanism of emulsification stability of the biosurfactant.

2 | EXPERIMENTAL METHODS

2.1 | Preparation of fermentation broth

To obtain high-concentration lipopeptide fermentation broth, the following procedures were carried out:

The tested SL-2 strain was stored in the strain bank of the Microbial Percolation Laboratory, Institute of Porous Flow and Fluid Mechanics, Chinese Academy of Sciences. The strain activation medium was Luria-Bertani (LB) medium, its nutrient proportions (mass percentages) were as follows: NaCl 1%, peptone 1%, yeast powder 0.5% (all chemicals were of analytical grade). Culture conditions: temperature, 37°C; rotation speed, 180 r/min; liquid volume, 100/250 ml; incubation time, 12 h.

The fermentation medium was as follows: sucrose 50 g/L, NaNO₃ 3.4 g/L, NH₄Cl 1.1 g/L, KH₂PO₄ 2.5 g/L, Na₂HPO₄·12H₂O 30 g/L, MgSO₄·7H₂O 0.8 g/L (all chemicals were of analytical grade). The pH of the system was 7.5. First, 7.5 ml of the seed liquid prepared
in the above process is put into a 250-ml flask containing 150 ml fermentation medium (i.e., an inoculation dose of 5%, v/v). The flask oscillated at 180 r/min and 37°C for 72 h.

2.2 | Analysis of fermentation broth components

After fermentation, 1.2 ml methanol was added to 300 µm cell-free supernatant, which was then shaken for 1 min and centrifuged at 4°C 10,000 r/min for 10 min. The supernatant was taken as the sample for testing and analysis. The lipopeptide detection instrument was an SR-3000 Solvent Rack fitted with an Amethyst C18-P column (5 µm, 4.6 × 150 mm). Liquid phase injection conditions were as follows: the mobile phase was 90% chromatography-grade methanol and 10% formic acid solution (formic acid content 0.05%). The injection volume was 20 µl, the flow rate was 0.7 ml/min, and the column temperature was 35°C. A UV detector was used, and the wavelength was set at 214 nm.

After analysis, high performance liquid chromatography (HPLC) traces of a lipopeptide standard mix (Sigma) and fermentation samples were compared to determine the quality of the product, and the product composition was quantified using the peak areas.

2.3 | Detection of fermentation broth interface properties

2.3.1 | Surface tension

Distilled water was used to gradient dilute the fermentation broth, and the volume percentages of broth in the systems were 1%, 3%, 5%, 7%, 10%, 20%, 30%, and 50%. With increasing dilution, the surface tension decreases, and there is a sudden change in the surface tension at a certain point. The surface tension was measured with a surface tensiometer (FTA1000 Drop Shape Instrument) at 25°C, and the inflection point was found. This is the critical concentration against oil displacement potential, which is defined as critical micellar dilution (CMD).

2.3.2 | Oil/water interface tension

To study the interfacial activity of the fermentation broth, the oil/water interface tension between the fermentation broth and crude oil, hexadecane, or simulated oil (crude oil/kerosene [1:9, v/v]) was measured using a Texas-500c rotating drop interfacial tension analyzer. The sample tube is filled with the system to be tested. It is then sealed and attached to the rotating drop interfacial tension meter so that the sample tube is parallel to the rotating axis and concentric with the rotating axis. The liquid is spun at an angular velocity of 5050 s⁻¹ and, under the action of centrifugal force, gravity, and interfacial tension, the simulated oil forms long spherical or cylindrical droplets in the system, the shape of which is determined by the rotational speed and interfacial tension. By measuring the length and width of the droplets, the density difference between the two phases, and the rotation speed, the interfacial tension between the water and the simulated oil can be calculated.

2.4 | Temperature and salt resistance

To study the temperature and salt resistance of the lipopeptide, 100 ml of fermentation broth was incubated in the temperature range 25–120°C for 1–2 days. The surface tension after cooling to room temperature was measured on Days 1 and 2.

To study the influence of salt concentration on the product, the NaCl concentration of the fermentation broth was set to 0.5, 1, 3, 5, 7, 10, 15, 20, 30, 40, 50, 70, 100, or 120 g/L. After preparation, the broth was incubated at 37°C for 2 days, and then the surface tension was measured at room temperature.

2.5 | Wettability

To evaluate the potential effect of the biosurfactant on the wettability of the rock surfaces in a reservoir, an artificial rock core was cut into thin slices, soaked in crude oil, and then dried. Fermentation broth, 7% fermentation broth, or water (blank control) were dropped on the rock surface, and an FTA1000 Drop Shape Instrument was used to measure the contact angle.

2.6 | Emulsification of crude oil

To determine the emulsification index, 10 ml of the fermentation broth was added to 10 ml of simulated oil (or hexadecane). The mixture was then vortexed at high speed for 2 min and allowed to stand at 25°C for 24 h. EI₂₄ is defined as the emulsion layer height as a percentage of the total liquid column height after 24 h. The emulsification mechanism of the fermentation liquor was also investigated. First, a rheometer (GXZJ-B013035) was used to measure the interfacial
shear viscosity (rotor type-2D), which reflects the interfacial film strength. A zeta potentiometer was used to determine the zeta-potentials of different oil emulsions.

2.7 | Simulation of biosurfactant flooding

Artificial core tests were performed using the conditions shown in Table 1 (an artificial core was used to conduct the flooding experiments). The artificial core flooding experiment is schematized in Figure 1. First, the gas permeability was measured, then the dehydrated crude oil is used to saturate the oil. The pores of the core were filled with oil and aged for 7 days. After aging, primary water flooding was carried out. The injection water used in this displacement experiment was separated water from the oil-well-produced fluid. When the water cut reached 98%, 7% fermentation broth + 0.1% biological xanthan gum (to improve the swept volume of the lipopeptide) was injected. A peristaltic pump was used to inject a 1 pore volume (PV) injection system into the core at a constant flow rate of 0.1 ml/min. After the injection volume reached 1 PV, subsequent water flooding was carried out until the water content of the produced fluid exceeded 98%, at which point the displacement was stopped and the displacement efficiency was calculated. To exclude the influence of xanthan gum on the experiment, its physical effect was evaluated separately, and the core data used are shown in the second column of Table 1. To match the reservoir conditions of the oilfield studied, a production temperature of 55°C was adopted.

Oil displacement efficiency (%) = ([oil displacement after injection system + oil displacement by subsequent water flooding]/total oil displacement) × 100

3 | RESULTS AND DISCUSSION

3.1 | Fermentation broth component analysis

Qualitative analysis can be performed by comparing fermentation broth samples with a standard lipopeptide mix using HPLC. As shown in Figure 2, the peak emergence times for the two samples are similar, and the number of peaks is the same, which indicates that the product contains lipopeptide biosurfactants. A lipopeptide contains a hydrophilic peptide chain and an oleophilic β-hydroxy fatty acid group, so it is an amphiphilic compound. Therefore, lipopeptides can reduce the surface tension of water and its interfacial tension with oil, change the wettability of a rock surface, and promote the formation of water/crude oil emulsions. Accordingly, crude oil that is difficult to recover can be stripped from rock fractures and sand gaps and be recovered with water using lipopeptides. The four highest peaks correspond to four lipopeptide products with different structures. These are C13-surfactin, C14-1-surfactin, C14-2-surfactin, and C15-surfactin. Liu et al.’s study showed that the higher the proportion of C-15-surfactin, the higher the oil displacement efficiency and oil washing efficiency of a surfactin mixture. As can be seen from Figure 2, the fermentation broth in this study has C-15-surfactin as its most abundant component, so it

![Image of artificial core flooding test apparatus](image)

**FIGURE 1** Schematic of artificial core flooding test apparatus

| Permeability (mD) | Length (cm) | Diameter (cm) | Saturated water (ml) | Saturated oil (ml) |
|-------------------|-------------|---------------|----------------------|-------------------|
| 200               | 8           | 2.5           | 7.4                  | 6.2               |
| 230               | 8           | 2.5           | 6.4                  | 5.4               |

**TABLE 1** Core parameters
could be reasonably expected to exhibit excellent performance in oil displacement.

The lipopeptides can be quantified by measuring their peak areas, and the final lipopeptide yield of the process is 1.2 g/L. In addition to lipopeptides, the main components of the fermentation broth are small-molecular-weight compounds such as acetic and lactic acid. They are environmentally friendly biodegradable compounds that do not cause pollution to the environment when used for oil displacement and are safer than other chemical oil displacement agents. Furthermore, acetic and lactic acid can dissolve limestone and calcareous cement, so as to increase the permeability and porosity of rock and thus improve crude oil displacement by water.

3.2 | Properties of the biosurfactant

3.2.1 | Surface tension

As shown in Figure 3, surface tension changes rapidly when the fermentation broth is diluted to 7%. Thus, this
concentration is a critical point for oil displacement. The surface tension at the inflection point is 33.9 mN/m and the CMD is 7%. These results show that the fermentation broth reduces surface tension efficiently at a low concentration. Low surface tension helps surfactant products migrate well in a reservoir, which leads to a change in the physical properties of the strata, oil, and water in the reservoir, and achieves efficient oil displacement.24

3.2.2 | Interfacial tension

According to the results of surface tension analysis, the minimum effective concentration of the fermentation broth is 7%. Therefore, interfacial water/oil tensions were determined for systems at this concentration. As seen in Figure 4, the interfacial tension for each oil phase is decreased to ~1 mN/m by the prepared system, and that for hexadecane, the standard material, is decreased to <1 mN/m, so the biosurfactant meets the requirements of EOR.11 Furthermore, it can be seen in Figure 4 that the interfacial tension between the two phases decreases in the order crude oil > hexadecane > simulated oil. Therefore, with a decrease in light-component content, the interfacial tension decreases, so the effect of the system on light components is better.

Crude oil contains aromatic compounds, the interactions between which are predominantly π–π interactions, which are stronger than the van der Waals forces between alkane molecules. Thus, surfactant molecules cannot disrupt the interactions between aromatic compounds, so they do not concentrate at oil–water interfaces. The lower the interfacial tension effect is, the worse the effect of the crude oil is. Hexadecane and the simulated oil (mainly C10–16 alkanes) are alkanes. The higher their molecular weight, the higher the van der Waals force between them, and the less likely a surfactant will enrich at the oil–water interface. Therefore, the surfactant decreases the interfacial tension of light components more effectively.25

3.2.3 | Stability

The surface tension (γS) of the original solution was 25.58 mN/m. It was treated at different temperatures for 1 to 2 days. The sample was then cooled to room temperature and analyzed with a surface tensiometer (FTA1000 Drop Shape Instrument) at 25°C to determine the change in γS value. The results in Table 2 show that the surface tension of the fermentation broth changes little when treated at 25–120°C for 2 days. The surfactant withstands heating at 120°C and exhibits wide adaptability to formation temperature.

The salt tolerance of the surfactant was measured, and the results are shown in Figure 5. The products in the fermentation broth exhibit good tolerance to salt. When the concentration of NaCl reaches 50 g/L, the surface tension is still decreased to 43 mN/m. Therefore,
the tolerance of the fermentation broth to NaCl is very good. Because Glu1 and Asp5 in the surfactin structure contain COO\(^-\), it can form salt bridge with the Na\(^+\) counterion to achieve a stable structure and maintain activity, so it has good tolerance to NaCl.\(^{26}\)

Owing to the complex formation conditions, temperature, salinity, and other conditions fluctuate widely. Therefore, good temperature and salt tolerance are important for normal system functionality.\(^{27}\) The lipopeptide in the fermentation broth can withstand the high temperature of 120°C, and it can be used at different temperatures. The salt-tolerance test results show that the lipopeptide has a high tolerance to NaCl. When the concentration reaches 50 g/L, it still exhibits good surface activity.

### 3.2.4 Wettability evaluation

A contact angle of 0° represents total hydrophilicity, while an angle of 180° represents total hydrophobicity.\(^{28}\) As shown in Figure 6, the contact angle between the rock surface and water is reduced from 105.01° to 27.65° by the fermentation broth and to 39.24° with the 7% fermentation broth. Thus, it reverses the properties of the rock surface from hydrophobic to hydrophilic.

Wettability increase is an important mechanism of microbial oil displacement.\(^{28,29}\) The change in wettability is mainly due to the amphiphilic structure of the biosurfactant easily adsorbing onto reservoir rock, which makes the oil-wet surface of the reservoir rock become water-wet and is conducive to spalling of the oil film on the rock surface.\(^{30}\)

### 3.3 Bio-surfactant flooding results

#### 3.3.1 Emulsifying ability

It can be seen from Figure 7 that the biological surfactant is more effective than petroleum sulfonate (provided by Daqing
Huali Energy Biotechnology Co. Ltd. The effective concentration is 40%, the amount used in the experiment was 0.3 wt %, a chemical surfactant commonly used in oil recovery. Furthermore, the fermentation broth has a good emulsifying effect on hexadecane and simulated oil, and the emulsifying effect for simulated oil is stronger than that for hexadecane. The fermentation broth also has a better emulsification effect on hydrocarbons with higher light-component contents, which is consistent with the results of the interfacial tension measurements. Compared with the chemical surfactant, the biosurfactant has a better emulsifying effect.

Crude oil emulsification is one of the core mechanisms of microbial oil displacement. The larger the absolute zeta-potential value, the larger the electrostatic repulsion between the droplets in the emulsion, and the less likely it is to sink, so the emulsion is more stable. The higher the interfacial shear viscosity and the stronger the interfacial film, the more difficult the oil/water emulsion is to disrupt, making it more stable.

Considering the data in Figure 7 and Table 3, it can be seen that the absolute zeta-potential values for the fermentation stock broth, 7% fermentation broth, and petroleum sulfonate increase successively, and the emulsifying effect becomes worse, which is contrary to previous research results. Therefore, zeta potential is not the main factor effecting the emulsification of crude oil with a surfactant.

Combining the results shown in Figures 7 and 8, it can be seen that the interfacial shear viscosity for the biosurfactant is greater than that for the chemical surfactant, that is, the interfacial film strength is greater and the emulsifying effect is better, which is consistent with previous studies. Thus, it can be concluded that the interface film strength is one of the important factors affecting emulsifying stability.

3.3.2 | Artificial core testing of fermentation broth

To simulate the microbial oil displacement process, artificial core tests were carried out in the laboratory. As can be seen from Figure 9, the addition of xanthan gum increases the pressure inside the core, leading to a larger ripple volume, which expands the action range of the fermentation broth. The saturated oil volume is 6.2 ml. After primary water flooding, 53.2% of the crude oil can be extracted from the rock core. When the water cut reaches 98%, the prepared system was used for secondary oil flooding, and subsequent water flooding was carried out after 1 PV flooding. Injection system flooding and subsequent water flooding displaced a total of 1.16 ml of oil. The fermentation broth preparation system increases oil recovery by more than 18.7% and thus has good oil displacement potential for in-the-field use. Xanthan gum alone only improves the oil displacement efficiency by 8%, so 7% fermentation broth improves oil displacement efficiency by 10.7%.

Artificial core tests have certain disadvantages. An artificial core is different from natural rock in terms of structure and uniformity, and because the core is small,
even a small error will have a significant impact on the oil-displacement effect. However, the natural core of a target reservoir is not easy to obtain. Although an artificial core cannot completely replace the natural core, it can imitate its porosity, permeability, and other parameters. The experimental results obtained using an artificial core can reflect the oil displacement ability of a surfactant. Accordingly, this is the standard experimental method for simulating oil displacement in the laboratory.

**TABLE 3** Zeta-potential results

| Emulsifier               | Fermentation broth | 7% Fermentation broth | Petroleum sulfonate |
|--------------------------|--------------------|------------------------|---------------------|
| Potential (mV)           | −14.6              | −22.5                  | −29.6               |

**FIGURE 7** Emulsifying effect of different surfactant systems on hexadecane and simulated oil

**FIGURE 8** Relationship between shear viscosity and shear speed

Liu and Feng\(^{36}\) and many others have carried out oil-displacement experiments with artificial cores. Liu et al. carried out binary composite flooding and ASP flooding with artificial cores, which shows that this method is widely used.\(^{37}\)

**FIGURE 9** Relationship between injection pressure and injection volume

4 | **CONCLUSIONS**

The following conclusions can be drawn from the results of this study:
1. *Bacillus subtilis* fermentation broth contains lipopeptide biosurfactants, and its surface tension can be as low as 25.84 mN/m, and the interfacial tension between 7% fermentation broth solution and crude oil can be as low as 1.24 mN/m.

(2) Even at a temperature of 120°C and a salinity of 50 g/L, the biological fermentation broth still exhibits good activity and the capacity to act upon crude oil, demonstrating its excellent temperature and salt tolerance.

(3) Compared with that for a chemical surfactant, the interface film strength for an oil–water emulsion formed by the biological surfactant is higher, resulting in a crude oil emulsion with higher stability.

(4) The results of simulated core oil displacement experiments showed that oil displacement efficiency is increased by 18.7% using the 7% fermentation broth, demonstrating its field application potential.

(5) Compared with purified biosurfactant, the biological fermentation broth is lower in cost and thus has better prospects for field application.

However, the results of our laboratory-based research need to be verified in the field to confirm the practical value of this strategy.

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