Effects of surfactants on the remediation of petroleum contaminated soil and surface hydrophobicity of petroleum hydrocarbon degrading flora

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ABSTRACT

It has been proven that surfactants used in the remediation of petroleum hydrocarbon contaminated soil have great application potential. In this study, the effects of five surfactants (SDBS, Tween80, Tween60, rhamnolipid and TRS-1) on leaching of petroleum hydrocarbons from soil were investigated through orthogonal experiments, and petroleum hydrocarbon components were analyzed by GC/MS. The effects of surfactants on the degradation of petroleum hydrocarbon were analyzed by the changes of microbial growth curve and surface hydrophobicity. The results showed that surfactant type, temperature and surfactant concentration had significant effects on the removal rate of petroleum hydrocarbon. Tween80, rhamnolipid and TRS-1 have good bio-friendliness and a high removal rate of petroleum hydrocarbons (up to 65%), suitable for the restoration of the soil used in the experiment. And Surfactants exhibited a higher removal rate for small molecules and petroleum hydrocarbons with odd carbon atoms. Surfactants have a certain modification effect on the surface of relatively hydrophilic bacteria under the initial conditions, making their surface properties develop in the direction of enhanced hydrophobicity, and the hydrophobicity has increased from less than 20% to about 40%.

Keywords: Cell surface hydrophobicity, Hydrocarbon chains, Soil washing, Surfactant bio-friendliness

1. Introduction

Petroleum is currently the most widely used fossil fuel in the world. Land transportation (pipeline, truck, rail, etc.) and maritime transportation (ship, etc.) are usually the main methods of crude oil transportation, and pipeline transportation is the most economical and efficient way. Oil spills caused by traffic accidents and corrosion of pipelines during oil transportation are prone to sudden pollution [1, 2]. Improper management of waste from petroleum extraction and petrochemical enterprises will also cause critical soil contamination [3, 4]. In addition, underground oil storage tanks that have exceeded their service life also have the risk of oil leakage and large oil spills, causing serious soil and groundwater contamination [5].

Researchers are developing effective methods to repair petroleum-contaminated soil, which has promoted the progress and development of soil remediation technologies [6]. Among numerous methods, surfactants have been widely applied and concerned due to their unique advantages [7]. Surfactants have hydrophobic and hydrophilic groups, which can effectively change the properties of the oil-water interface [8]. Mainly acting on the soil-water interface and the water-microbial interface to enhance the desorption of petroleum hydrocarbon from the soil and promote the use of petroleum hydrocarbon by microorganisms [9-11]. Surfactants play a vital role in the in situ remediation of contaminated soil. Compared with other physical and chemical methods, this method has a relatively simple operation process and low cost [12-14]. In recent years, the development of degradable surfactants has almost eliminated the adverse effects of surfactants in the environment [15, 16].
The results showed that the removal rate of petroleum hydrocarbon reached 62% and 71%, respectively. Tween80 surfactant has a significant effect in removing hydrophobic organic matter in the soil, can effectively promote the remediation process, and has also been used in the remediation of petroleum hydrocarbon contaminated soil [19]. Some other non-ionic, anionic, cationic and biological surfactants have been researched and applied in the remediation of soil hydrophobic organic matter pollution [20]. In addition, the use of bio-friendly surfactants to remediate petroleum hydrocarbon contaminated soil has the advantages of good environmental benefits and low cost. It has broad development prospects and important application value in soil petroleum hydrocarbon pollution remediation[21, 22].

Based on existing researches, removal of petroleum hydrocarbon by surfactants depends on many factors: different surfactant types, washing time, washing temperature, surfactant concentration, the age of contamination and soil properties (Soil composition, organic matter content, etc.) [23-25]. However, there are few reports on the optimal use conditions of surfactants and the elution efficiency of surfactants for different petroleum hydrocarbon components during the repair process at present [26, 27]. Surfactants are often used in combination with microbial repair technologies to achieve complete remediation of petroleum-contaminated soil [11, 19, 28, 29]. However, there are still different views on the role of surfactants in microbial repair [29]. Therefore, it is extremely important to determine whether the surfactant could inhibit the growth of petroleum hydrocarbon degrading bacteria in the process of the combination of surfactant and microbial technology.

To evaluate the suitability of surfactants for microbial repair techniques, this work studied the remediation of 5 surfactants (sodium dodecylbenzenesulfonate (SDBS), rhamnolipid, Tween80, Tween60 and TRS-1) on the contaminated soil taken from Shengli Oilfield. During the research, we paid attention to the elution effect of different surfactants for different petroleum hydrocarbon components during the repair process at present [26, 27]. Surfactants are often used in combination with microbial repair technologies to achieve complete remediation of petroleum-contaminated soil [11, 19, 28, 29]. However, there are still different views on the role of surfactants in microbial repair [29]. Therefore, it is extremely important to determine whether the surfactant could inhibit the growth of petroleum hydrocarbon degrading bacteria in the process of the combination of surfactant and microbial technology.

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2. Materials and methods

2.1. Contaminated Soil, Culture Media and Chemicals

The petroleum hydrocarbon-contaminated soil used in the experiment was taken from Shengli Oilfield, with a sampling depth of 5-20cm. The oil content is about 7%. This soil was sieved using a 2mm sieve to remove large coarse materials such as leaves and stones. And petroleum hydrocarbon-degrading bacteria were isolated from it for experimental use.

MIE medium: 2.44 g/L NaH2PO4, 1.52 g/L KH2PO4, 0.5 g/L (NH4)2SO4, 0.2 g/L MgSO4·7H2O, 0.05 g/L CaCl2·2H2O, 4.5×10⁻³ g/L MnSO4·7H2O, 0.1×10⁻³ g/L CuSO4·5H2O, 0.1×10⁻³ g/L FeSO4·7H2O, 2 g/L glucose. Its pH was adjusted to 7.0 ± 0.2 with NaOH/HCl.

The culture medium is sterilized by a vertical high-pressure steam sterilizer (LDZX-30KBS, Shanghai Shen’an Medical Equipment Factory) for use.

Unless otherwise specified, the chemical reagents used in this experiment were purchased from Sinopharm Reagent Co., Ltd., with a purity of ≥ 99.0%.

2.2. Determine the Best Conditions for Surfactant-Assisted Hydrocarbon Leaching

The petroleum hydrocarbon leaching process is tested according to the soil-water ratio of 1:5. An orthogonal test (Table 1) with four factors and five levels of L25 (45) was designed to explore the cleaning effect and optimal cleaning conditions of different surfactants. According to the experimental conditions of each group in the orthogonal experiment table (Table S2), the surfactant solution was mixed with the contaminated soil, and the experiment was carried out at 150 r/min on a constant temperature water bath shaker.

2.3. Detection of Petroleum Hydrocarbon Content in Contaminated Soil

Gravimetric methods were used to determine the TPH content in soil. 10 g soil samples were weighed into Soxhlet extractor. 200 mL petroleum ether with boiling range of 60-90ºC was added, heat and extract for 6 h. Then, the samples were dried at 50ºC for 1 h. The TPH content was then determined gravimetrically after evaporation of the solvent [30].

2.4. Analysis of Petroleum Hydrocarbon Components

The samples were extracted with dichloromethane, and the components of petroleum hydrocarbon were analyzed by GC/MS (Shimadzu GC/MS QP2010 Ultra). The analysis conditions were as follows [31]:

Column: Rxi-5sil MS 30.0 m × 0.25 mm × 0.25 μm.
Gas phase conditions: Oven temperature 60ºC; inlet temperature
260°C; heating program 60°C for 1 min, 5°C/min to 130°C for 2 min, 12°C/min to 180°C for 2 min, 7°C/min to 280°C for 10 min; carrier gas flow rate is 1.27 mL/min; split ratio is 5.

MS conditions: the ion source is EI; the ion source temperature is 220°C; the interface temperature is 250°C; the solvent delay time is 4 min; the scanning mode is SCAN; the scanning range is m/z 35-500.

Qualitative analysis using NIST08, LIB database retrieval. Relative quantitative analysis using peak area integration and area normalization.

2.5. Effect of Surfactants on Petroleum Hydrocarbon Degrading Bacteria

2.5.1 Effect of surfactants on the growth of petroleum hydrocarbon-degrading bacteria

Four surfactants (SDBS, rhamnolipid, Tween80 and TRS-1) with better cleaning effect on petroleum hydrocarbon were selected for experiment. The experiment was divided into 4 groups. Each group took 6 culture bottles with a volume of 500 mL and added 200 mL MIE medium. Added 0 mg, 20 mg, 100 mg, 200 mg, 300 mg and 500 mg of four surfactants to the culture flasks of each experimental group, respectively, so that the surfactant concentrations in the six culture flasks of each group were 0 mg/L, 100 mg/L, 500 mg/L, 1,000 mg/L, 1,500 mg/L and 2,500 mg/L.

The petroleum hydrocarbon degrading bacteria group was resuspended to make an OD600 value of 0.5, and 1 mL of bacterial liquid was inoculated into each experimental group. Take the bacteria in the logarithmic growth period of the experimental group in 2.5.1 were recovered. By detecting the contact angle of bacterial extracellular lipopolysaccharide, distilled water on the surface of the microbial membrane and the surface hydrophobicity (BATH) of the bacterial cell, it reflects the influence of different surfactants on the surface performance of petroleum hydrocarbon degradation cells.

Refer to the experimental methods of Pan M and Redmile-Gordon MA et al [32, 33]. Extraction of bacterial extracellular lipopolysaccharide and proteins were performed using the dilute sulfuric acid method. The content of bacterial extracellular proteins was determined using the Coomassie Brilliant Blue method. The content of bacterial extracellular lipopolysaccharides was determined using the phenol-sulfuric acid method.

Refer to the experimental method of Mohanty S et al [34]. The bacterial solution was filtered using a disc filter membrane with a diameter of 25 mm and a pore diameter of 0.45 μm. Bacteria can form a uniform microbial membrane on the surface of the filter membrane. The medium components were washed with a phosphate buffer solution, and the filter membrane was dried under vacuum at 30°C, and then the contact angle of the distilled water droplets on the surface of the biofilm was detected using a contact angle measuring instrument (Chengde Dingsheng Testing Machine Testing Equipment Co., Ltd., JY-82C).

The recovered bacteria were resuspended with a phosphate buffer solution and adjusted to an OD600 value of 0.6. 5 mL of the bacterial solution and 1 mL of a n-hexadecane solution were added to a test tube. The solution was shaken vigorously for 2 min and allowed to stand for 15 min. The water phase was measured for OD600. Cell surface hydrophobicity BATH = [1- (OD600after mixing / OD600before mixing)] × 100% [35].

2.6. Statistical Analysis

The experiment was repeated three times and averaged. All data calculation and analysis in this experiment were made using origin8.0 software.

3. Results and Discussions

3.1. Contaminated Soil Analysis

The oil content of the petroleum-contaminated soil samples tested was 7.95%.

The GC-MS chromatogram of petroleum hydrocarbon component in contaminated soil is shown in Fig. 1. Details of each petroleum hydrocarbon component are shown in Table S1.

![Fig. 1. GC-MS chromatogram.](image)

The contaminated soil used in this work was characterized by the presence of 23 major hydrocarbon ranging from C11 to C33 (Fig. 1). It was observed that the components were well separated, indicating that the analytical method was feasible. According to the analysis in Table S1, 23 hydrocarbons belonged to alkanes.

3.2. Treatment by Surfactants

As shown in Table S2, when the SDDBS concentration was 2,500 mg/L, the shaking time was 60 min, and the temperature was 30°C, the petroleum hydrocarbon removal effect was the best, with a removal rate of 68.10%. Range analysis showed that the effect of each experimental condition on the petroleum hydrocarbon removal rate was ranked as follows: surfactant concentration > surfactant type > temperature > oscillation time.

As shown in Fig. 2, different surfactants had a significant effect on the removal of petroleum hydrocarbon, which was closely related to the molecular structure of the surfactant [36]. The length of the hydrophilic chain and hydrophobic chain, hydrophilic-lipophilic balance (HLB) of the surfactant molecule have a greater impact on the removal of petroleum hydrocarbon [37-39]. The ex-
Experimental results showed that SDBS, Tween80, rhamnolipid and TRS-1 surfactant illustrated more effective removal of petroleum hydrocarbon than Tween60. The effect of the oscillation time on the experimental results was not obvious. At the beginning of the experiment, petroleum hydrocarbon was continuously dissolved and diffused from the soil into the water phase. With the increase of the shaking time, the dissolution and diffusion process gradually reached an equilibrium state, and the dissolution of petroleum hydrocarbon also reached an equilibrium state [36, 40]. Temperature also has an important effect on the solubilization of petroleum hydrocarbon in the presence of surfactants [40]. As the temperature increases, on the one hand, the number of micelles in the surfactant tends to increase, which is beneficial to the dissolution of hydrophobic organic compounds (such as petroleum hydrocarbon). On the other hand, the solubility of petroleum hydrocarbon will gradually increase. Therefore, proper temperature is very important to improve the efficiency of petroleum hydrocarbon removal [23]. The results showed that when the temperature increased from 25°C to 35°C, the removal rate of petroleum hydrocarbon increased significantly (from 52.7% to 58.4%) (Fig. 2). As the temperature continued to rise, the removal rate of petroleum hydrocarbon rose slowly. Surfactant concentration has the most significant effect on the removal of petroleum hydrocarbon from soil [36, 40, 41]. When the concentration of the surfactant solution is lower than the critical micelle concentration (CMC), it has less effect on dissolving petroleum hydrocarbon. This is mainly due to the presence of surfactant molecules as monomers. During the process of leaching the soil, most surfactant molecules will be adsorbed to the soil or oil-water interface, resulting in poor dissolution of petroleum hydrocarbon. In contrast, when the concentration of the surfactant solution is higher than that of CMC, it has a significant effect on dissolving petroleum hydrocarbon. This is due to the formation of micelles by the surfactant in the solution, and organic pollutants enter the interior of the micelles during the elution process, which will significantly improve the removal effect of petroleum hydrocarbon [42, 43]. As the concentration increases, the removal of petroleum hydrocarbon by the surfactant solution will increase significantly, and eventually stabilize [44]. It showed that surfactant concentration had a significant effect on the removal rate of petroleum hydrocarbon in Fig. 2. When the surfactant concentration was increased from 500 mg/L to 2,000 mg/L, the removal effect of petroleum hydrocarbon was significantly improved. As the surfactant concentration continued to increase, the removal rate of petroleum hydrocarbons gradually stabilized. Based on the above analysis, it was determined that the shaking time was 45 minutes, the shaking temperature was 35°C, and the surfactant concentration was 2,000 mg/L. The better SDBS, Tween80, rhamnolipid and TRS-1 were selected as candidates.

Orthogonal experimental analysis of variance is shown in Table 2. The P values of the factors including surfactant type, temperature and surfactant concentration were less than 0.05, indicating that these three factors had a significant impact on the removal rate of petroleum hydrocarbon. The order of significance from large to small was: surfactant concentration, temperature, surfactant type. The P value of shaking time was greater than 0.05, indicating that it had no significant effect on the removal rate of petroleum hydrocarbon. These conclusions were consistent with the above analysis and the findings of Kingsley Urum et al. [40].

From orthogonal experiments, it determined that the removal rate of petroleum hydrocarbon by Tween60 was lower than the other four surfactants. The appropriate experimental conditions determined were: shaking time of 45 min, temperature of 35°C, and surfactant concentration of 2,000 mg/L.

### 3.3. Verification Experiment

A verification experiment was set up based on orthogonal experiments to verify the results. The experimental results are shown in Table 3. When the experimental conditions were: shaking time of 45 min, temperature of 35°C, and surfactant concentration of 2,000 mg/L, SDBS, Tween60, rhamnolipid and TRS-1 all showed high removal rates (> 60%), while Tween60 showed lowest removal rate.

#### Table 2. Variance Analysis

| Source               | DF | SS   | MS   | F-Value | P > F |
|----------------------|----|------|------|---------|-------|
| Error                | 8  | 23.0 | 2.87 |         |       |
| Corrected total      | 24 | 1,096| 49.0 | 17.1    | 0.005 |
| Surfactant type      | 4  | 196  | 49.0 | 17.1    | 0.005 |
| Oscillation time     | 4  | 22.6 | 5.67 | 1.97    | 0.192 |
| Temperature          | 4  | 160  | 40.0 | 13.9    | 0.004 |
| Surfactant concentration | 4  | 694  | 173  | 60.4    | 0.001 |
rate (55.6%) for petroleum hydrocarbon. The results of the verification experiments were consistent with the orthogonal experiments.

### 3.4. Changes of Petroleum Hydrocarbon Composition after Surfactant Treatment

The components of petroleum hydrocarbon in the soil treated with surfactant were analyzed by GC-MS (Fig. 3). It shows chromatograms of petroleum hydrocarbon components in untreated soil in Fig. 3a. The chromatograms of petroleum hydrocarbon components after treatment with different surfactants are shown in Fig. 3 (b)-(f). It was observed that the response value of each petroleum hydrocarbon component decreased significantly in the soil. Peak area integration of each petroleum hydrocarbon component was performed by a chromatographic workstation, and the removal rates of C11 to C33 alkanes were calculated by integral data.

The relative quantitative analysis of the removal rate of each petroleum hydrocarbon component is shown in Fig. 4. When the number of carbon atoms is less than 20, the removal rate of even-numbered carbon-atom alkanes by surfactants is slightly lower than that of odd-numbered carbon-atom alkanes, which is determined by the molecular structure of alkanes. As the number of alkane carbon atoms increased, the removal rate of alkane by the surfactant gradually decreased. When the number of carbon atoms exceeded 20, the removal rate of alkane by the surfactant tended to be stable. The results were consistent with the conclusion of Razika Khalid et al. [45]. It could be caused by two reasons. On the one hand, as the number of carbon atoms increased, the solubility of alkanes in water will gradually decreased, which led to the trend in Fig. 4; On the other hand, alkanes with more carbon atoms had higher viscosity, stronger hydrophobicity and soil adsorption, resulting in lower removal rates [23, 46]. Therefore, in the process of solubilizing petroleum hydrocarbon, the effect of surfactants on light components with fewer carbon atoms was more obvious [47].

We have observed interesting phenomena for alkanes with less than 18 carbon atoms, that is, SDBS exhibited the best removal effect for petroleum hydrocarbon; TRS-1, Tween-80 and rhamnolipids showed close removal rates; Tween-60 had the lowest removal rate among them. The results were caused by factors such as soil properties, CMC of surfactants, hydrophobic chain length, HLB value and adsorption of surfactants by soil [23, 27]. Tween-60 is a non-ionic surfactant. The charge on the surface of soil particles is mainly negative, so it is more likely to be adsorbed [24]. The experimental soil has a higher clay content, which will cause higher adsorption to occur, reducing the effectiveness of Tween-60. Meanwhile, the higher HLB value of Tween-60 also reduces the removal rate of petroleum hydrocarbon. In contrast, SDBS is an anionic surfactant with lower soil adsorption, longer hydrophobic:  

### Table 3. Verification Experiment Results

| Serial number | Surfactant type | Oscillation time / min | Temperature / ºC | Surfactant concentration / mg/L | Petroleum hydrocarbon removal rate / % |
|---------------|----------------|------------------------|------------------|-----------------------------------|----------------------------------------|
| 1             | Tween-80       | 45                     | 35               | 2,000                             | 66.43                                  |
| 2             | Tween-60       | 45                     | 35               | 2,000                             | 55.62                                  |
| 3             | Rhamnolipid    | 45                     | 35               | 2,000                             | 64.51                                  |
| 4             | SDBS           | 45                     | 35               | 2,000                             | 64.21                                  |
| 5             | TRS-1          | 45                     | 35               | 2,000                             | 63.97                                  |
chains, and lower HLB values which lead to its superior removal performance for petroleum hydrocarbon. The other three surfactants performed in-between, and TRS-1 showed better results. We have observed that another problem worth considering was that surfactants exhibited a higher removal rate of paraffins with an odd number of carbon atoms than paraffins with an even number of carbon atoms.

3.5. Effect of Surfactants on the Growth of Petroleum Hydrocarbon-Degrading Bacteria

A large number of surfactants have been used in the actual petroleum hydrocarbon contaminated soil remediation process. Whether they will have an impact on the environment, especially on the role of petroleum hydrocarbon-degrading bacteria, must be highly appreciated [48, 49]. The effects of SDBS, Tween80, rhamnolipid and TRS-1 surfactant on the proliferation of petroleum hydrocarbon-degrading bacteria are shown in Fig. 5. As shown in Fig. 5 (a), when the concentration of SDBS in the medium was less than 500 mg/L, it had little effect on the proliferation of petroleum hydrocarbon-degrading bacteria, indicating that low concentration of SDBS not inhibit the proliferation of microorganisms. When the SDBS concentration reached 1,500 mg/L, the growth rate and amount of microorganisms were significantly lower than those in the control group. It was not hard to conclude that the high concentration of SDBS had a significant inhibitory effect on the proliferation of petroleum hydrocarbon-degrading bacteria. This is because high concentrations of SDBS will destroy the cell membrane of microorganisms, inhibit the proliferation of microorganisms, and even kill microorganisms. Therefore, high concentrations of SDBS are likely to cause negative effects on the environment. [50]. Tween80 and rhamnolipid showed bio-friendliness and have no obvious inhibitory effect on the proliferation of petroleum hydrocarbon degrading bacteria when the concentration reached 2,500 mg/L (Fig. 5 (b) and Fig. 5 (c)). This was consistent with recent research reports [45]. Tween80 has good performance in the repair of petroleum hydrocarbon, polycyclic aromatic hydrocarbon and other hydrophobic organic contaminated soils, and exhibits favorable bio-degradability [51, 52]. Rhamnolipid is a microbial surfactant produced by microorganisms. It has good surface activity and has been extensively studied in the remediation of organic contaminated soils with no side effects on the environment [53]; TRS-1 is a plant-based surfactant that has been applied in engineering. However, its effect on microorganisms is not yet clear. In this study, its bio-friendliness was explored (Fig. 5 (d)). When the con-

Fig. 5. Effects of different surfactants on the proliferation of petroleum hydrocarbon degrading bacteria (a) SDBS, (b) Tween80, (c) Rhamnolipid, (d) TRS-1.)
centration of TRS-1 reached 2,500 mg/L in the medium, it had no obvious inhibitory effect on the growth of microorganisms. It showed that TRS-1 was a kind of bio-friendly surfactant, which had great application potential in the complete restoration of petroleum hydrocarbon contaminated soil combined with microbial technology.

3.6. Effects of Surfactants on the Hydrophobicity of Microbial Cell Surfaces

The possible effects of surfactants on the surface of microorganisms during the research are observed in Fig. 6 and Fig. 7. The presence of surfactants usually causes changes in the extracellular protein and lipopolysaccharide content of the microorganism [29, 54]. The changes of microbial extracellular proteins and lipopolysaccharides can expose hydrophilic or hydrophobic groups on the cell membrane, changing the hydrophobicity of the cell surface [55]. The interaction between the surfactant and the microbial cell membrane during microbial growth also affects the hydrophobicity of the microbial surface [11]. The surface of bacteria is rich in hydrophilic groups and hydrophobic groups (such as biological lipid alkane chains, carboxyl groups, hydroxyl groups, phosphate groups, etc.) may adsorb or bind surfactant molecules to change the hydrophobicity of bacteria [11, 29, 56].

The changes in the extracellular protein and lipopolysaccharide content of the microorganisms at different concentrations of the four surfactants are shown in Fig. 6 (a) and Fig. 6 (b), respectively. With the increase of surfactant concentration, the content of protein and lipopolysaccharide decreased first and then stabilized. Due to the destruction of microbial cell membrane by high concentration of SDBS, the content of protein and lipopolysaccharide in SDBS experimental group was significantly lower than other experimental groups at high concentration. The presence of rhamnolipids had little effect on the extracellular protein content of the microorganisms, and the content of lipopolysaccharide showed a slight increase first and then decreased.

The changes in surface hydrophobicity of petroleum hydrocarbon degrading bacteria are shown in Fig. 7. It reflected the changes of the contact angle of distilled water droplets on the microbial membrane in Fig. 7 (a), and the changes of the hydrophobicity of the bacteria are shown in Fig. 7 (b). The contact angle indicates the wettability of distilled water on the surface of the microbial membrane. The hydrophobicity of the bacterial surface increases with the increase of the contact angle. Except for the SDBS experimental group, the surface hydrophobicity of the petroleum hydrocarbon-degrading bacteria in the other experimental groups increased first and then decreased slightly with the increase of the surfactant concentration. Due to the inhibition and destruction of microorganisms by high-concentration SDBS, the surface hydrophobicity of microorganisms first increased and then decreased with the increase of SDBS concentration. BATH is calculated by measuring the adsorption amount of microorganisms in hydrophobic organic matter (n-hexadecane), and has a positive correlation with the hydrophobicity of microorganisms. The hydrophobicity of the microbial surface is positively correlated with the BATH value. With the increase of surfactant concentration, the hydrophobicity of petroleum-degrading bacteria gradually increased and then stabilized. The SDBS experimental group showed that the hydrophobicity of petroleum hydrocarbon degrading bacteria gradually increased and then decreased.

According to the analysis of Fig. 6 and Fig. 7, the change of hydrophobicity of petroleum hydrocarbon degrading bacteria may be caused by the changes of extracellular proteins and lipopolysaccharides and the interaction between surfactants and cell membranes. Reductions in microbial extracellular proteins and lipopolysaccharides often lead to increased hydrophobicity of the cells. In this study, the microbial surface was relatively hydrophilic (the contact angle of the microbial membrane in the absence of a surfactant was 50° to 55°, and the BATH was between 15% to 17%). There will be more hydrophilic groups on the surface of microorganisms, and it is easy to adsorb or bind the hydrophilic groups of surfactant molecules, meanwhile, the hydrophobic groups will extend to the outside, which will increase the hydrophobicity of microorganisms. Studies have shown that the enhanced hydrophobicity of microbial cells was conducive to their use of hydro-

![Fig. 6. Petroleum hydrocarbon degrading bacteria extracellular proteins and polysaccharides.](image-url)
phobic organic matter and had important research significance for improving the efficiency of microbial repair [57].

4. Conclusion

The present experiments have demonstrated that in a surfactant-aided, the petroleum hydrocarbon pollution in the soil has been well repaired. TRS-1 surfactant has good remediation effect on experimental soil, and it has no obvious inhibitory effect on petroleum hydrocarbon degrading bacteria. The type of surfactant, washing temperature and surfactant concentration have a significant impact on the removal rate of petroleum hydrocarbons in the soil, and attention should be paid to them during the application process. Tween80, rhamnolipid and TRS-1 showed good degradability. High-concentration SDBS (< 1,000 mg/L) showed good degradability. High-concentration SDBS (< 1,000 mg/L) have a significant inhibitory effect on the bacteria that degrade petroleum hydrocarbons, and are not suitable for this repair process. For hydrophilic microorganisms, the hydrophobicity of the microorganisms will be enhanced in the presence of surfactants. This is advantageous for the degradation of petroleum hydrocarbon.

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Author contribution

L.J.B. (Associate Professor) participated in the revision of the manuscript and the acquisition of project funds. X.L.M. (Master Student) participated in the design of all experiments and writing of papers. Z.F.F. (Master Student) participated in the inspection and correction of some experiments and papers. J.S.H. (Master Student) participated in some experiments.

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