Study of the Characteristics of Two Immobilized Microbial Materials in Degradation and Evolution of Petroleum Hydrocarbon

Xiaofang Luo, Lihua Chen,* Shujing Zhao, Zhongchun Lei, Miaomiao Xia, and Chuantao Zhang

ABSTRACT: To enhance the degradation efficiency of oily wastewater, polyacrylamide (PAM)—sodium alginate (SA) and poly(vinyl alcohol) (PVA)—sodium alginate (SA) were mixed and used as spherical supporting materials for the immobilization of microbials, which were employed as a platform to study the degradation of total petroleum hydrocarbons (TPHs) in the oily wastewater. The degradation and evolution of normal paraffin (n-paraffin) series have been studied by determining the crude oil group composition of the residual oils by the gas chromatography—mass spectrometry (GC—MS) analysis. The results show that the half-lives of the PAM—SA-immobilized microorganisms are 6.21 days, which is 2.11 days less than that of PVA—SA, indicating that more nutrients are provided by PAM—SA for microbial growth, which can accelerate the degradation of TPHs. As can be seen from the GC—MS analysis, the main peak carbons of the n-paraffin series are moved backward after 14 days of degradation, implying the degrading advantage of n-paraffin with low carbon numbers. The $\Sigma C21/\Sigma C22$ value of PAM—SA was measured to be 0.749, which is greater than that of PVA—SA (0.051), indicating that PAM—SA has a superior ability to degrade normal paraffins with high carbon numbers. After 14 days of degradation, an odd—even predominance (OEP) (the mass ratio of normal alkanes of odd carbon/even carbon) value of 1.075 for PAM—SA was obtained, which is slightly larger than that of PVA—SA (0.967), indicating a better degradation performance of PAM—SA, especially for the degradation of the even-carbon normal paraffins with high carbon numbers. The Pr/Ph of PAM—SA is 0.938, which is also greater than that of PVA—SA (0.844), indicating that the ability of PAM—SA for the degradation of isoprenoids is superior to that of PVA—SA under the immobilized conditions. Based on these results, in terms of immobilization of microorganisms, PAM—SA, instead of PVA—SA, is more advantageous for the degradation of TPH in the oily wastewater.

1. INTRODUCTION

The severe pollution caused by organic contaminants or oils has attracted much more attention worldwide. In particular, it is worth noting that soil or water would be possibly polluted by petroleum in the process of oil exploitation, transportation, storage, and use, resulting in environmental contamination and health risk.1–3 Therefore, it is imperative to find out environment-friendly environmental remediation method to address the issues of petroleum contaminants.4–6

Among those existing methods for oily wastewater treatment, degradation of oil contaminates by microorganisms has been highly favored owing to its advantages of low cost and no secondary pollution.7–9 In general, a single bacterial strain can degrade only a few specific hydrocarbons or to a certain stage due to the complexity of petroleum composition and the difficulty in degrading. However, if multiple oil-degrading bacterial strains with different enzyme activities are mixed for the degradation of oil contaminates, its degrading efficiency will be significantly enhanced compared to that of the single microorganism due to the synergism of the creature. For instance, Chen et al.8,9 showed that mixed bacterial strains can efficiently degrade normal paraffin and transform organics’ unstable spatial configuration to stable configurations. Based on the previous studies, the activity-maintaining time after putting the bacterial strain is also key to the treatment of wastewater using the microorganism approach.10 Along this line, the addition of the microbial agent after the immobilized treatment has been proven to have a higher degradation rate in comparison to the direct addition of the free microbial agent according to a previous investigation.11 Compared with directly applying microorganism for the degradation of organic contaminants, the immobilization of microorganism is a reusable technique that immobilizes free microorganisms or enzymes in a fixed space using certain methods while...
maintaining high activity, which features high bioburden, prevention of cell loss, strong tolerance to adverse environments, high biological stability, and convenience of storage and use. To date, adsorption, cross-linking, covalent bonding, and embedding are four traditional methods for immobilizing microorganisms. For example, Zhang et al. discovered that the oil degradation rate after 50 h is greater than 85% under the effect of the microbial agent using the nanoporous SiO2 as an adsorbent to prepare immobilized microorganism agents.

A composite immobilization method combining two or more immobilization methods can be used to overcome the defects and disadvantages of the single immobilization method, so as to obtain a microbial immobilization system with a high degree of immobilization and high microbial activity. In addition, compared with the traditional suspension biological treatment process, immobilized microorganism technology has high treatment efficiency and stable operation, can be purified and maintain high-efficiency dominant strains, has large reactor biomass, high specific surface area, and good entrance and solid—liquid separation effect; immobilized microspheres are more adaptable to the outside world than free bacteria. With the increase of degradation time, the surface of microbial pellets collapses and the internal structure is damaged. As a kind of widely distributed organic macromolecular material in nature, sodium alginate (SA) has the advantages of good mass transfer performance, no biological toxicity, a wide range of sources, and low price. Polyacrylamide (PAM) can be produced as a strong immobilization carrier featuring biological corrosion resistance, high mechanical strength, and strong plasticity, etc. along with SA. Moreover, mixing poly(vinyl alcohol) (PVA) and SA appropriately as an immobilized carrier can produce an immobilized biological spherule with good biological compatibility and strong stability and high permeability.

In this work, the degradation of the total petroleum hydrocarbons (TPHs) in the oily wastewater was studied using PAM, PVA, and SA as mixed immobilized carriers in combination with the biological microbial agent for the promotion of degradation efficiency. Meanwhile, the analyses of "fingerprints" and biological evolution parameters were conducted on n-alkanes in petroleum on the basis of analyzing mixed bacteria with gas chromatography—mass spectrometry (GC—MS), so as to clarify the synergism of immobilized microorganisms on biodegradation and understand the characteristics of petroleum degraded by PAM—SA- and PVA—SA-immobilized microorganisms. It can achieve the objectives of improving the performance of the biomicrobial agent and effectively restoring water (Figure 1).

### 2. MATERIALS AND METHODS

#### 2.1. Experimental Materials

1. **Oil Sample.** The oil sample was placed in a vacuum drying chamber upon being dissolved in the normal hexane. A standard oil sample could be obtained and stored in a dry environment as a standby after the normal hexane was completely evaporated. The oil sample was obtained from a petrochemical plant in Lanzhou.

2. **Mixed Microbial Agents.** The petroleum-degrading bacteria adopted in this study were screened and isolated from long-term oil-contaminated wastewater by the laboratory from the previous research project. Four predominance strains were obtained, including pseudomonas aeruginosa, acinetobacter lwofii, bacillus, and bacillus subtilis, with the volume ratio of 1:1:1:1, all of which can effectively degrade petroleum hydrocarbons, conforming to the requirements of the experiment.

#### 2.2. Experimental Design and Methods

1. **Experimental Design.** The experiment was carried out in a flask of 100 mL. The sample was placed in a shaking incubator at a constant temperature of 37 °C for shaking treatment after sufficient mixing. When the in situ remediation of microorganisms was simulated, the crude oil content was 3% of the concentration gradient of the quality of the oil-contained wastewater. Three sets of parallel tests in each experiment were performed to obtain the average. The specific scheme is shown in Table 1.

| Table 1. Experimental Design |
|--------------------------------|
| classification | CK | EG I | EG II |
| standard oil (g) | 1.2 | 1.2 | 1.2 |
| sterile water (mL) | 40 | 40 | 40 |
| PAM—SA ball (g) | 0 | 10 | 0 |
| PVA—SA ball (g) | 0 | 0 | 10 |

#### 2.2.2. Preparation of Immobilized Microbial Spherule

1. **PAM—SA-immobilized Carrier.** PAM (0.3 g) and 0.5 g of SA were weighed and placed into a small beaker of 50 mL for well stirring with the addition of 17.5 mL of sterile water before being wrapped with the preservative film. In this way, the liquid inside the small breaker was gel-like upon full fusion. After heating it to the fluid state in a sterile room, 10 mL of the microbial solution was added to the liquid (it should be noted that the heating temperature should not exceed 40 °C, preventing the loss of part of the activity of microorganisms due to overheating). Then, 20 mL of the liquid was injected into the medical syringe upon well stirring with the installation of the prepared saturated calcium chloride solution. After placing for 3–6 h, it was washed and stored in a refrigerator at 4 °C.

2. **PVA—SA-immobilized Carrier.** PVA (0.3 g) and 0.5 g of SA were weighed and placed into a small beaker for well stirring with 50 mL of sterile water and soaked for 24 h, which can form a gel after the sterilization using a high-pressure steam sterilizer. After the gel was cooled to the room temperature, 10 mL of the bacterial solution was taken into the gel for rapid stirring, which was sucked with a pipette and instilled into the solution of saturated boric acid and calcium chloride for the cross-linking effect. The spherule was formed after being soaked in the saturated boric acid and calcium chloride solution for 24 h and soaked for a period of time after flushing the agent remaining on the spherule with sterile water.
two to three times for culturing the spherule and maintaining a certain activity of its spherules.

2.2.3. Determination of the Degradation Rate. Samples with different concentrations were prepared with the standard oil. The absorbance of the solution was measured by ultraviolet spectrophotometry at the absorption wavelength of 226 nm with n-hexane as a reference for drawing a working curve based on the data arranged. The standard equation, \( y = 0.0192x - 0.0221 \) can be obtained with the correlation coefficient \( R^2 \) of 0.9993, where \( x \) is the concentration of 10 g of sludge (sand) sample with the unit of mg/L; \( y \) is the effective and nondimensional absorbance of the extracting solution using 50 mL of a normal hexane soxhlet extraction.

2.2.4. Determination of GC–MS. Ten grams of the degraded sample was taken for soxhlet extraction. Five milliliters of the extract was taken to dehydrate with anhydrous sodium sulfate, filtered by 0.22 \( \mu \)m organic solvent-resistant filter membrane, blow-dried with nitrogen, then dissolved in 1 mL of n-hexane as a sample. One milliliter of \( \mu \)m n-hexane was regarded as a sample injection after being dissolved to analyze the masses of various series of compounds in the dissolved petroleum using GC–MS. The peak area of compounds in every series was converted into a relative percentage before calculating the absolute residue (\( \mu \)g) of compounds in each series with the absolute content of the added internal standard (44-deuterated heneicosane). Although a great number of substances with the same mass can be found in the components of petroleum mixtures, they have different structures. When substances are bombarded into fragments by electron flow, their characteristic fragment ions appear with the particle fragments of \( n \)-alkanes of 57, 71, 85, etc. since the structure of each molecular substance in the petroleum component is different. Therefore, substances of the same series can be extracted with the characteristic fragment value in the GC–MS ion source of TPHs.

Analysis conditions: gasification temperature is 260 °C; carrier gas is He; column temperature is 200 °C; and the column is SE-30 (50 m). Mass spectrum conditions: the electron energy is 70 eV and the mass range is between 40 and 450.

3. RESULTS AND DISCUSSION

3.1. Research on the TPH Degradation Kinetics. The amount of residual oil on each day is calculated by substituting the absorbance value measured in each experiment into the relationship between the amount of residual oil and the absorbance, which is in line with the first-order kinetic equation upon analysis. If the negative logarithm is taken in the first-order kinetic equation, it will give

\[
\ln P (\text{oil}) = \ln P (\text{oil}) - kt
\]

where \( k \) is the degradation velocity of the microorganism, \( \ln P (\text{oil}) \) is the amount of residual oil at any time, and \( \ln P (\text{oil}) \) is the amount of residual oil on the 0th day. The equation of the half-life period can be

\[
T_{1/2} = \frac{\ln 2}{k}
\]

The TPH degradation trend presented in Figure 2 is in line with the first-order kinetic equation, which indicates that environmental factors have a slight effect on oil degradation. According to TPH degradation kinetic parameters under different conditions in Table 2, the half-life periods of the blank group, PVA–SA, and PAM–SA are 22.55, 8.32, and 6.21 days, respectively. With microorganisms immobilized PVA–SA and PAM–SA, the half-period can be reduced by 14.23 and 16.34 days, respectively. This is because the immobilization of PVA–SA and PAM–SA can contribute to improving the property of the oil–water interface, accelerating the migration rate of oil contamination from solid phase to aqueous phase, promoting the coalescence and adsorption of oil drops in small particle size, and speeding up microbial degradation of oil contamination. The half-life period of microorganisms immobilized by PAM–SA is reduced by 2.11 days in comparison to that of PVA–SA. The oil–water interface is a major activity area of the microbial degradation of oil contamination. Also, the degradation rate of microorganism is affected by the oil-dispersing degree. Based on the result, microorganisms immobilized by PAM–SA could drive the terminal group to fully desorb oil contamination and provide more nutrients to accelerate degradation for the growth of microorganisms in comparison with PVA–SA. The higher the degree of oil contamination in water, the longer the half-life period is. The shortened half-life period shows that microorganisms immobilized by PAM–SA can enhance the activity of the microbial agent and accelerate the degradation rate.

3.2. Degradation Law of \( n \)-Alkanes. The main component of the crude oil is saturated hydrocarbon. Also, a major component in the saturated hydrocarbon is paraffin C12–C35, including normal paraffin, isoparaffin, and iso-prenoid, which account for 94.6% of the saturated paraffin distillates.

The degraded petroleum hydrocarbons were analyzed by GC–MS with the total ion currents shown in Figure 3. The data of GC–MS of PAM–SA can be referred to the literature. Based on these graphics, the backward movement of the main peak carbon can be observed in the GC–MS diagrams of PAM–SA and PVA–SA after 7 and 14 days of degradation with the main peak carbons of C15, C20 and C14, C29, respectively. The backward movement of the main peak

![Figure 2. Degradation kinetics of TPH.](image)

| Table 2. TPH Degradation Kinetic Parameters under Different Conditions |
|-----------------------------|-----------------------------|-----------------------------|
| serial number | the degradation velocity of the microorganism | the half-life period (days) |
| CK | 0.03074 | 22.55 |
| PVA–SA | 0.08327 | 8.32 |
| PAM–SA | 0.11158 | 6.21 |

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and 32-n-tane, 26-dotriacontane, 27-tetracosane, 19-n-standard), 15-n-heptadecane, 9-pristane, 10-n-4-number in Figure 3 include 1-of PVA MS, carbon numbers of immobilized PAM low carbon numbers can be presented by microorganisms Di

Table 3. Relative Degradation Rates of n-Alkanes with carbon indicates that the advantage of degrading n-alkanes with low carbon numbers can be presented by microorganisms immobilized PAM–SA and PVA–SA.32

As can be seen from the total ion chromatography of GC–MS, carbon numbers of n-alkanes are distributed between C10 and C38 after 7 and 14 days of adding mixed microbial agents to petroleum hydrocarbons. Moreover, it can be observed from Table 3 that the degradation rates of PAM–SA and PVA–SA.

Table 3. Relative Degradation Rates of n-Alkanes with Different Carbon Numbers after 14 Days of Degradation (%)

| material     | C10–C20 | C21–C30 | C31–C38 |
|--------------|---------|---------|---------|
| PAM–SA (%)   | 88.41   | 86.07   | 35.65   |
| PVA–SA (%)   | 90.56   | 73.67   | -21.46  |

in low-carbon-number n-alkanes are 88.41 and 90.56%, respectively, and PAM–SA is slightly smaller than PVA–SA, indicating that the ability of the microbial agent degrading the low-carbon-number normal paraffin is slightly superior to that of PAM–SA under the load of PVA–SA. The degradation rates of PAM–SA and PVA–SA in a medium-carbon-number and high-carbon-number normal paraffins are 86.07 and 35.65% as well as 73.67 and -21.46%, respectively, which indicate that the ability of the microbial agent degrading the low-carbon-number normal paraffin is slightly superior to that of PVA–SA under the load of PAM–SA. The negative degradation rate of n-alkanes with high carbon number shows that PVA–SA has a low selectivity of n-alkanes that are refractory to degrade with high carbon number, which is supported by the backward movement of the main peak carbon.

Therefore, the ability of the bacterial agent degrading medium-high-carbon-number normal paraffin of PAM–SA is superior to that under the load of PVA–SA.

3.3. Analysis of Biological Evolution Parameters of n-Alkanes. Since adding different immobilized carriers might affect the ability of the bacterial agents degrading different hydrocarbons in petroleum, bioevolution parameters can be utilized to reveal the internal mechanism of degradation and evolution of the bacterial agent on normal paraffins in different immobilized carriers.33 As can be seen from Figure 4, the carbon numbers of normal paraffins are distributed between C10 and C38, accounting for more than 60% of the total composition of TPHs, their degradation evolution can well illustrate the overall degradation trend of TPHs. Therefore, represented by normal paraffins, the following evolution parameters are illustrated separately.

The trend of converting paraffins with high carbon numbers to paraffins with low carbon numbers when paraffin undergoes bacterial action can be reflected by the \( \sum \text{C21}/\sum \text{C22} \) and paraffin main peak carbons.\(^{34} \) \( \sum \text{C21}/\sum \text{C22} \) is the ratio of the sum of paraffins with low carbon numbers to the sum of paraffins with high carbon numbers; the greater the parameter, the stronger the ability of bacteria degrading paraffins with high carbon number. To be specific, the more forward the main peak carbon indicates, the stronger the ability of bacteria to degrade paraffins with high carbon numbers. According to Table 4, \( \sum \text{C21}/\sum \text{C22} \) values of PAM–SA after 7 and 14 days are 1.729 and 0.749, respectively, while the \( \sum \text{C21}/\sum \text{C22} \) values of PVA–SA after 7 and 14 days are 2.276 and 0.051, respectively, which are declined with the extension of the degradation period. It shows that with the help of PAM–SA and PVA–SA, the advantage of microorganisms degrading.

Table 4. Biological Evolution Parameter Value of Normal Paraffins

| material     | OEP   | \( w(\sum \text{C21}/\sum \text{C22}) \) | \( w(Pr)/w(Ph) \) |
|--------------|-------|----------------------------------------|-------------------|
|               | 7 days| 14 days                                | 7 days            | 14 days        |
| PAM–SA (%)   | 1.033 | 1.075                                  | 1.729             | 0.749          |
| PVA–SA (%)   | 1.008 | 0.967                                  | 2.276             | 0.051          |

![Figure 3](image1.png)

Figure 3. (a) GC–MS TIC of PVA–SA (7 days). (b) GC–MS TIC of PVA–SA (14 days) Substances corresponding to the peak number in Figure 3 include 1-n-decane, 2-n-undecane, 3-n-dodecane, 4-n-tridecane, 5-n-tetradecane, 6-n-pentadecane, 7-n-hexadecane, 8-n-heptadecane, 9-pristane, 10-n-octadecane, 11-phytane, 12-n-nonadecane, 13-n-eicosane, 14-perdeuterated, n-heneicosane (internal standard), 15-n-heneicosane, 16-n-docosane, 17-n-tricosane, 18-n-tetracosane, 19-n-pentacosan, 20-n-hexacosane, 21-n-heptacosan, 22-n-octacosan, 23-n-nonacosan, 24-n-triacontan, 25-n-hentriacontan, 26-dotriacontan, 27-n-tritriacontan, 28-n-tetratriacontan, 29-n-pentatriacontanone, 30-n-hexatriacontan, 31-n-heptatriacontan, and 32-n-octatriacontan.

![Figure 4](image2.png)

Figure 4. Degradation rates of various substances in normal paraffins.
normal paraffins is normal paraffins with low carbon. The $\sum C_{21}^-/\sum C_{22}^+$ value of PAM–SA after 7 days of degradation was lower than that of PVA–SA, showing that PVA–SA had a stronger ability to degrade normal paraffins with low carbon numbers previously; and the $\sum C_{21}^-/\sum C_{22}^+$ value of PAM–SA after 14 days was greatly greater than that of PVA–SA due to the previous degradation. Since residual normal paraffins that are refractory to degrade with low carbon number are deprived, the advantage of microbial degradation is the gradually transferred normal paraffins with low carbon number to that with high carbon number.\(^{35}\)

After 14 days of degradation, the $\sum C_{21}^-/\sum C_{22}^+$ value of PAM–SA is 0.749, which is greater than 0.051 of PVA–SA, which indicates that residual normal paraffins with high carbon number can be effectively degraded by the microbial agent under a load of PAM–SA.

The odd–even predominance (OEP) value is changed since changes in the relative abundances of odd and even carbon number of the paraffin series are triggered when organic matters suffer from strong bacterial or thermal actions. The ability of the bacteria degrading paraffins with high carbon numbers can be reflected by the OEP value. To be specific, the smaller the OEP value, the stronger the ability to degrade paraffins with odd carbon numbers.\(^{36}\) As can be seen from Table 4, the mean of the OEP value upon adding the PAM–SA carrier is increased with the extension of the degradation period, while the OEP value upon adding the PVA–SA carrier is decreased with the extension of the degradation period. After comparing the OEP values of different carriers at the same time, the OEP of PAM–SA is increased from 1.033 on the 7th day to 1.075 on the 14th day, indicating that the combination of PAM–SA has a strong ability to degrade normal paraffins with even carbon numbers. The OEP of PVA–SA is decreased from 1.008 on the 7th day to 0.967 on the 14th day, indicating that the combination of PVA–SA has a strong ability to degrade normal paraffins with odd carbon numbers. The overall OEP value of PAM–SA is greater than that of PVA–SA, indicating that adding PAM–SA rather than PVA–SA can better improve the ability of the bacterial agent to degrade paraffins with even carbon numbers in high carbon numbers.

The $w(Pr)/w(Ph)$ is the ratio of pristane to phytane, which is commonly used in isoprenoids. The larger the ratio, the more difficult the significant oxidation of microorganisms on isoprenoids that are refractory to degrade.\(^{37}\) As can be seen from the table, the Pr/Ph value of PAM–SA is increased from 0.913 on the 7th day to 0.938 on the 14th day, while the PVA–SA increased from 0.769 on the 7th day to 0.844 on the 14th day, presenting that demethylation is performed on the isoprenoid alkenes because of the microbial agent during the degradation process from the 7th day to the 14th day; that is, some of the phytane molecules upon removing a methyl group is converted into pristane.\(^{38}\) The Pr/Ph values of PAM–SA on the 7th and the 14th days are greater than that of PVA–SA, which indicates that the ability of the microbial agent degrading isoprenoid alkenes is superior to that of PVA–SA under the immobilization of PAM–SA.\(^{39}\)

From the above discussion, the carbon number of n-alkane is distributed in C10–C38, accounting for more than 60% of the TPH. Its degradation and evolution can fully explain the overall degradation trend of TPHs. In this study, the two immobilized materials both showed the rule of the first degradation of high-carbon-number n-alkanes and the degradation of medium/low-carbon-number n-alkanes. Secondly, OEP can reflect the ability of bacteria to degrade odd/even-carbon-number alkanes. The lower the value is, the higher the ability to degrade odd-carbon-number alkanes is. On the contrary, the higher the value is, the higher the ability to degrade even-carbon-number alkanes is. The degradation results demonstrated that the total OEP of PAM–SA was higher than that of PVA–SA, suggesting that PAM–SA addition can more significantly improve the ability of microbial agents to degrade even-carbon-number alkanes in high-carbon-number alkanes compared with PVA–SA addition. Therefore, the ability of PAM–SA to degrade high-carbon-number n-alkanes is higher than that of PVA–SA.

In addition, the evolution parameters of markers revealed the degradation and evolution characteristics of material structure. The evolution parameters were consistent with the degradation rate. Using various markers to reflect difference in degradation rates difference and evolution characteristics of n-alkanes in crude oil by immobilized microbial agents can be more clearly identified, and meanwhile can help us to have a deeper understanding of the evolution of the structure of various components during the degradation and a more scientific approach the reasonable utilization of bacterial strains or the selection of mixed strains.

4. CONCLUSIONS

(1) The half-life periods of CK, PVA–SA, and PAM–SA are 22.55, 8.32, and 6.21 days, respectively. Microorganisms immobilized by PVA–SA and PAM–SA could lead to the reduction of the half-life period by 14.23 and 16.34 days. Besides, the half-life period of microorganisms immobilized by PAM–SA is reduced by 2.11 days in comparison with that immobilized by PVA–SA. It indicates that immobilizing microorganisms with PVA–SA and PAM–SA can enhance the activity of the microbial agent and enhance its degradation rate.

(2) As can be observed from the GC–MS spectrum analysis, the main peak carbon is moved backward due to the degradation of the microbial agent under the load of PAM–SA and PVA–SA, which shows that the immobilized microorganisms have a significant degradation effect on saturated hydrocarbons with low carbon number. Obvious differences can be observed from the ability of the immobilized microorganisms degrading different carbon number segments in n-alkanes. The degradation rates of PAM–SA and PVA–SA in n-alkanes with medium carbon number and high carbon number are 88.41 and 90.56%, respectively, indicating that the ability of the microbial agent degrading n-alkanes with low carbon number is slightly superior to that of PAM–SA. The degradation rates of PAM–SA and PVA–SA in medium-carbon number and high-carbon number normal paraffin are 86.07 and 35.65% as well as 73.67 and −21.46%, respectively, which indicates that utilizing the PAM–SA-immobilized microbial agent is more likely to enhance the ability of the microbial agent degrading n-alkanes with low carbon number and high carbon number than utilizing the load of PAM–SA. Under the loads of PAM–SA and PVA–SA, the difficulty of the microbial agent degrading n-alkanes is presented as low carbon number > medium carbon number > high carbon number.
Concerning the evolution characteristics of normal paraffins, \( \Sigma C_{21}^-/ \Sigma C_{22}^+ \) is the ratio of the sum of paraffins with low carbon numbers to the sum of paraffins with high carbon numbers; the greater the parameter, the stronger the ability of the bacterial agent to degrade paraffins with high carbon numbers. The \( \Sigma C_{21}^-/ \Sigma C_{22}^+ \) value of PAM-SA is 0.749, which is greater than 0.051 of PVA-SA, indicating that PAM-SA has a superior ability to degrade normal paraffins with high carbon numbers. After 14 days of degradation, the OEP value of PAM-SA is 1.075, which is greater than 0.967 of PVA-SA, indicating that adding PAM-SA can better increase the ability of bacterial agents to degrade the even-carbon normal paraffins with high carbon numbers. The pristane–phytane ratio \( w(Pr)/w(Ph) \) is a common ratio of pinane to phytane used in isoprenoids, and the larger value shows a more obvious oxidation effect of the microorganism on the isoprenoids that are difficult to degrade. The Pr/Ph value of PAM-SA is 0.938, which is greater than 0.844 of PVA-SA. The ability of the bacterial agent to degrade isoprenoids is superior to that of PVA-SA under the immobilized action of PAM-SA, which can help bacterial agents to conduct the demethylation on isoprenoid alkenes, so that part of phytane molecules can be converted into pristane after removing a methyl, enhancing its degrading rate.

As for immobilized microorganisms, PAM-SA material with a better degradation effect on TPH in oil-contained wastewater is more advantageous than PVA-SA.

**AUTHOR INFORMATION**

**Corresponding Author**

Liuhua Chen — College of Chemical Engineering, Key Laboratory for Utility of Environment-Friendly Micro Composite Materials and Biomass in University of Gansu Province, Northwest Minzu University, Lanzhou, Gansu 730030, P. R China; orcid.org/0000-0002-2094-2279; Email: clh@xbmu.edu.cn

**Authors**

Xiaofang Luo — Experimental Teaching Department, Northwest Minzu University, Lanzhou 730030, P. R China

Shujing Zhao — College of Chemical Engineering, Key Laboratory for Utility of Environment-Friendly Micro Composite Materials and Biomass in University of Gansu Province, Northwest Minzu University, Lanzhou, Gansu 730030, P. R China

Zhongchun Lei — College of Chemical Engineering, Key Laboratory for Utility of Environment-Friendly Micro Composite Materials and Biomass in University of Gansu Province, Northwest Minzu University, Lanzhou, Gansu 730030, P. R China

Miaomiao Xia — College of Chemical Engineering, Key Laboratory for Utility of Environment-Friendly Micro Composite Materials and Biomass in University of Gansu Province, Northwest Minzu University, Lanzhou, Gansu 730030, P. R China

Chuantao Zhang — College of Chemical Engineering, Key Laboratory for Utility of Environment-Friendly Micro Composite Materials and Biomass in University of Gansu Province, Northwest Minzu University, Lanzhou, Gansu 730030, P. R China

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