Study on Degradation of Oily Wastewater by Immobilized Microorganisms with Biodegradable Polyacrylamide and Sodium Alginate Mixture

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ABSTRACT: In this work, four immobilized kinds of mixed microorganisms were prepared by uploading oil-degradation bacteria into the cross-linked biodegradable polyacrylamide and sodium alginate mixture supporting material, which were employed for efficient degradation of oily wastewater. The morphology of immobilized microbial pellets was characterized by scanning electron microscopy after 7d and 14d of duration. The components of residual crude oil were determined by gas chromatography–mass spectrometry, and the microbial degradation and evolution of n-alkanes, terpenoids, and steroids were studied. The results show that the oil degradation rate for experimental group I (sample containing 1% crude oil) and experimental group II (sample containing 3% crude oil) reaches as high as 70 and 40%, respectively, after 14d of degradation of saturated hydrocarbon total petroleum hydrocarbons. For different oil components, the degradation degree is in the order of tricyclosditerpanes > homohopanes > norhopanes. The order of the degradation degree of steroids with different carbon atoms is $C_{27} > C_{28} > C_{29}$. In terms of evolution characteristics, it can be seen from the biological evolution parameters of n-alkanes that only a slight degradation for odd-even carbon by biodegradable bacteria was achieved, whereas high degradation for isoprene alkanes was observed. According to the biological evolution parameters of hopane and sterane, hopane $C_{31\alpha} \beta$-22S/22S + R and sterane $C_{29\alpha} a 20S/20(S + R)$ were all greater than 0.4, that is, they are all strongly degraded by microorganisms.

INTRODUCTION

Petroleum is one of the main energy resources of modern industry worldwide. In the process of petroleum exploitation, transportation, storage, and use, oil leakage often occurs because of improper safety measures and sudden accidents, which results in a large amount of oily wastewater and environmental pollution, so there is an urgent need to exploit the efficient strategy for disposal of oily wastewater.

To date, a number of approaches have been developed for the treatment of wastewater, including separation, absorption, and solar steam generation. Ajmia Chouchene studied a combined process of absorption on sawdust, a low-cost renewable absorbent, and an energetic valorization via combustion was studied. Li developed a new oil-bearing wastewater separation device dissolved air flotation tower and studied the application prospect of this column in separating emulsified oil droplets from oil-bearing wastewater. Among these mentioned methods for the treatment of oily wastewater, the microbial method is favored for its low cost and no secondary pollution. Compared with other technologies, immobilized biotechnology has the following advantages: It is beneficial to increase the concentration and purity of microorganisms in the bioreactor, maintain high-efficiency strains, have strong stability, and easy to control reaction. Therefore, in recent decades, immobilized biotechnology has been rapidly developed and widely used. Though great progress has been achieved in this direction, the big challenge still remains in connection to the difficulty for degradation of petroleum with complex composition by a single strain or the degradation can only be performed to a certain stage. More worse in some cases, this issue can only be addressed by a synergy effect of a broad variety of microorganisms. In this regard, the selection, optimization, and combination of various microorganisms for degradation of oils have been studied. For instance, Chen showed that the mixed strains could not only degrade n-alkanes but also better promote the biological transformation of hopane compounds. Ramboarisona E studied how a mixture of bacteria, with nitrogen, phosphorus, and iron added to seawater, can degrade crude oil very effectively, especially in the case of petroleum. In view of above investigations, the synergy effect of different microorganisms could indeed improve the degradation efficiency of petroleum with complex composition; however, the direct application of microorganisms has obvious limitations, for example, unstable, easy to be lost, degradation efficiency dramatically influenced by surrounding environment, and so
forth. Accordingly, immobilized microorganism technology is a modern engineering technique which can restrict or locate free microorganisms in a certain specific space by physical or chemical methods to keep the density and activity of microorganisms. Based on these merits, the immobilized microbial technology (IMT) has been extensively adopted to enhance the oil treatment capacity and adaptability of microbial species to harsh environment. On the other hand, in comparison with that of direct application of microorganisms, IMT have great advantages such as high bioload, low microorganisms loss, strong tolerance to environments, high biostability, easy storage, and so forth. The traditional methods of immobilizing microorganisms are mainly adsorption, cross-linking, covalent bonding, and embedding. In these cases, as a matter of fact, the chemical nature of the substrates for immobilizing microorganisms is of great importance in addition to their porosity, loading capacity, and so on. The substrates with desired chemical composition would not only facilitate the loading and immobilizing of microorganisms but also could provide nutrition for feeding and proliferation of microorganisms. In contrast, the substrate is not rich in chemical composition, which is not conducive to the growth of microorganisms.

So far, unfortunately, the investigation involving the optimization of various microorganisms and immobilizing them into an ideal substrate for construction of efficient IMT for oils degradation is rare because it is difficult to incorporate these factors into one material. Therefore, the development of efficient IMT containing optimization of various microorganisms as well as suitable loading substrates should be of special interest for the oil treatment.

In this work, we report the immobilization of four kinds of mixed microorganisms, which was prepared by uploading oil-degradation bacteria into the cross-linked biodegradable polyacrylamide (PAM) and sodium alginate (SA) mixture supporting material, for efficient degradation of oily wastewater. The degradation rate of saturated hydrocarbon total petroleum hydrocarbons (TPH) was studied by such immobilization of microorganisms. In addition, the evolution characteristics for n-alkanes sterane and hopane were systematically investigated. The results show that the immobilization of mixed microorganism could have a better degradation degree of TPH than that of free bacteria.

Also, the findings of the investigation on evolution characteristics would be expected for providing of useful guidance for future optimization of mixed microorganisms for TPH degradation.

**Experimental Section**

**Oil Sample.** The oil sample is dissolved in n-hexan, and it is heated in a water bath at 68 °C. The standard oil sample is obtained after evaporation of n-hexane and stored in a dry environment. Oil samples from a petrochemical plant in Lanzhou oil.

**Mixed Bacteria Agents.** The petroleum degrading bacteria used in this study were collected from long-term petroleum-contaminated wastewater by the laboratory in the previous research topics, namely, X1, D1, A1 and A2, wherein X1 belongs to the genus *Pseudomonas*, D1 belongs to the genus *Achromobacter*, and A1 and A2 belong to the genus *Bacillus*. Four strains of bacteria were cultured in three stages to prepare a bacterial solution. A mixed suspension of the bacteria was prepared in a ratio of 1:1:1:1 and placed in a 50 mL Erlenmeyer flask for 2 days. When the number of colonies reached $1 \times 10^8$ when it is in/mL, it can be taken out and stored in a refrigerator at 4 °C.

**Preparation of Immobilized Microorganism Pellets.** PAM (0.3 g) and 0.5 g of SA were added into a 50 mL beaker, they were thoroughly stirred with 17.5 mL of sterile water, and placed on a plastic wrap for 12 h to completely fuse into a gelatinous shape. It was heated to the flow dynamics in a sterile room, 0.4 g of corn stalk was weighed together with 10 mL of bacterial liquid (note that the heating temperature should not exceed 40 °C, so as to not overheat and make some bacteria inactivated), stirred well, and then 20 mL was sucked into a medical syringe and dropped into the prepared saturated calcium chloride solution. It was placed for 3–6 h, washed, and stored in a refrigerator at 4 °C, and the pellets were prepared as shown in Figure 1.

**Characterization.** The morphologies of samples were examined by using a scanning electron microscope (JSM6701F, JEOL, Ltd.) after coating the samples with an Au film.

**Experimental Design.** In the oscillatory incubator with constant temperature and humidity, oily wastewater from 100 mL conical bottle was used to repair the simulated microorganism in situ in the environment of the coexistence of immobilized microorganism, and the high efficiency microbial mixture was applied to the remediation of petroleum polluted wastewater. The experiment was divided into two groups: experimental group I and experimental group II. The crude oil content was 1 and 3% of the quality of oily wastewater, respectively. The test period was 14 days, and the gas chromatography–mass spectrometry (GC–MS) was measured once on the 7th and 14th day. The specific programmes are shown in Table 1:

**Table 1. Experimental Design**

| classification | BG | EG I | EG II |
|----------------|----|------|-------|
| standard oil/g | 0.4| 0.4  | 1.2   |
| sterile water/mL| 40 | 40   | 40    |
| PSM ball/g     | 0  | 10   | 10    |

**Determination Method of GC–MS.** The degraded sample (10 g) was taken for soxhlet extraction. The extracted solution of 5 mL was dehydrated by using anhydrous sodium sulfate, filtered by using a 0.22 μm organic solvent resistant filter membrane, blew dry with nitrogen, then dissolved in 1 mL of n-hexane as a sample. The quality of each series of
compounds in degraded petroleum was detected and analyzed by GC–MS. The peak area of each series of compounds was converted into the relative percentage content, and the absolute residue (μg) of each series of compounds was calculated by the absolute content of the internal standard (44-deuterated hexadecane). There are many substances of the same mass in the components of petroleum mixtures, but the structure of the substances is different. In the GC–MS ion source of TPH, because of the different structure of each molecular matter in the petroleum component, when bombarded into fragments by electron current, there will be their own characteristic fragment ions. The particle fragments of n-alkanes are 57, 71, 85 ..., therefore, the same series of substances can be extracted by using the characteristic fragment value.

Analytical conditions: gasification temperature 260 °C, carrier gas He, column temperature 200 °C, column SE-30 (50 m). Mass spectrum conditions: electron energy 70 eV, mass range 40–450.

**Analysis of Biological Evolution Parameters of n-Alkanes.** The alkane main peak carbon and \( \sum C_{21-} / \sum C_{22+} \) parameters reflect the tendency of high-carbon alkanes to low-carbon alkanes to be converted by alkanes, and the larger the parameter, the stronger the ability of bacteria to degrade high-carbon alkanes. The more the main peak carbon shifts, the stronger the ability of bacteria to degrade high-carbon alkanes. The OEP value reflects the ability of the bacteria to degrade odd-even carbon alkanes. The smaller the value, the stronger the ability to degrade odd-numbered carbon-alkanes, whereas the greater the value, the stronger the ability to degrade even carbon alkanes. The ratio of planting \( \{w(pr)/w(ph)\} \) is the ratio of decane to phytane, which is commonly used in isoprenoids hydrocarbons. The larger the value, the more difficult it is to degrade the isoprenoids and the more obvious the oxidation.

**Analysis of Biological Evolution Parameters of Hopane and Sterane.** The maturity parameters commonly used for hopane and sterane are C31-αβ-22S/22(S + R) and C29-αααα-20S/20(S + R), both of which are mature at >0.4, <0.2 immature, between 0.2 and 0.4 is a low maturity marker. However, the value of low-mature samples that are more severely degraded by bacteria will be greater than 0.5, indicating mature organic matter characteristics. The norlupane/lupane value (or demethylated effect) was often used as a parameter for degradation of organic matter in decane samples, and the greater the value, the greater the degree of degradation. In addition, the rate of mature conversion of sterane C29-ααααSS20 (S + R) values of low-mature samples suffered from more serious bacterial degradation, which appears to be delayed, that is, the C31-αβ-22S/22 (S + R) value of hopane was significantly higher than the sterane C29-αααα/20(S + R) value. The ratio of \( T_{s} \) to \( T_{m} \) was often used as the degradation degree of the sample organic matter, and the smaller the value of the parameter \( T_{s}/T_{m} \), the greater the degree of degradation. This difference in the hopane and sterane maturity parameters of the same low-evolution sample was evidence that the sample has undergone bacterial degradation.

## RESULTS AND DISCUSSION

**Characterization of Materials.** In order to observe the embedding status and whether the bacteria can grow and reproduce well, a scanning electron microscope was used to observe the synthesized microspheres, as shown in Figures 2 and 3. The structure of the microspheres after one and two weeks of degradation was observed by scanning electron microscopy and the electron micrographs at different times of one week. It can be clearly observed that the inner structure of the immobilized pellets is cross-linked with each other, and the pores are dense and uniform, showing a porous network structure, which provides the microenvironment for good growth of microbes inside. Both the surface void structure and the internal skeleton structure indicated that there are sufficient contact areas and opportunities for the growth of microbe substrates in the interior, which is conducive to the transfer of nutrients. Because of the large number of colonies adsorbed on the microspheres, the uneven surface of the microspheres could be observed, which also indicated that the experimental embedding materials had good adsorption performance. At the same time, the electron microscope images of different times after degradation for two weeks were observed. When degradation reached the 14th day, the structure of the pellet broke down, the void became larger and uneven, the pore wall became fragile, and the collapse of the surface of the pellet began to appear. This is
because as the degradation time is lengthened, the prepared PAM−SA hybrid gel gradually hydrolyzes, so that its internal structure was destroyed and the surface collapses.

Determination of Crude Oil Degradation Rate. As shown in Figure 4, the experimental group I degradation rate of about 70%, and the experimental group II degradation rate
is only 40%, and all is in 14 days after the degradation rate of the maximum, so the immobilized microorganisms on the degradation of oil have been completed within 14 days.\(^3\)\(^4\)

Degradation in the early stage of 0–4 days, the experimental group II (3% concentration) crude oil degradation rate is always higher than that of the experimental group I (1% concentration) of crude oil; this may be due to the experimental group II initial chain of the low carbon source for microbial degradation in than experimental group I abundant reasons. On the 4th day, two experiments of the degradation rate is basically the same, all around 24%, but 4
days later, experimental II degradation rate increase slowly, in contrast, the experimental group I oil rate growth speed, the results showed that the microbial degradation rate of 3% crude oil was fast at the early stage and the aftereffect was insufficient.

Determination of Degradation Rate of n-Alkane. The main component of crude oil is saturated hydrocarbon and the total ion current is shown in Figure 5. The main components of saturated hydrocarbons are alkanes C12−C30, including n-alkane series, iso-alkane series, and isoprene hydrocarbon series, which account for 94.6% of saturated hydrocarbon fractions. The total ion currents of the degraded petroleum hydrocarbons were analyzed by GC−MS, and the total ion currents were found in Figures 6−8. According to the previous research results and the GC−MS diagrams of 7 and 14 days of degradation, it can be seen that the two experimental groups have the phenomenon of “main peak carbon forward shift”. The results show that the immobilized microorganism can effectively degrade saturated hydrocarbons with a high carbon number (Figure 9).

As shown above, the carbon number of n-alkanes was distributed in C10−C30 after adding mixed bacteria for 7 and 14 days, and the main peak carbon of n-alkanes moved forward obviously after the action of various kinds of bacteria, which indicated that the bacteria had selectivity to n-alkanes with a high carbon number, and there is a strong effect of demethylation. GC−MS data were used to calculate the degradation rate of n-alkanes after 7 and 14 days. It can be seen from Figure 10 that the degradation rate of experimental group II is higher than that of experimental group I in most carbon numbers at the same time. Therefore, the degradation rate of n-alkanes in experimental group II (3% crude oil concentration) was higher than that in experimental group I (1% crude oil concentration).

(Note: C10-n-decane; C11-n-undecane; C12-n-dodecane; C13-n-tridecane; C14-n-tetradecape; C15-n-pentadecane; C16-n-hexadecane; C17-n-heptadecane; Pr-decane; C18-n-octadecane; Ph-phytane; C19-n-nonadecane; C20-positivedecane; C21-n-docosane; C22-n-docosane; C23-n-docosane; C24-n-tetracosane; C25-n-pentacosane; C26-n-hexacosane; C27-n-heptacosane; C28-n-octacosane; C29-n-docosane; C30-n-tridecane; C31-n-tridecane; C32-n-docosane; C33-n-triucane; C34-n-tetraadecane; C35-n-trisocadecane; C36-n-trihexadecane; C37-n-tricadecane; C38-n-tridecane.)

It can be seen from Figure 10 and Table 2, in general, the immobilized microbes of PAM materials have obvious degradation effects on different concentrations of crude oil. Especially for normal paraffin with a carbon number between 10 and 22, the degradation effect is remarkable. Among them, the degradation rate of n-alkane in experimental group I (1% crude oil concentration) is higher than that in experimental group II (3% crude oil concentration), especially for the number of carbon atoms in the 10−16 group; the corresponding n-alkanes was developed between the relative degradation rate is as high as 88.41%, while the experimental group II only 50.56%; similarly, the relative degradation rate of 56.07% for the experimental group I of n-alkane with a carbon number between 17 and 22 is also higher than that of the experimental group II of 23.67%. The difficulty of degradation is as follows: low carbon number > medium carbon number > high carbon number. From Figure 10, it can be observed that the lowest peaks of both C17 and C18 appear. It can be concluded that when degraded to seven days, both the experimental group I and the experimental group II, the degradation rates of pristane and phytane are preferentially maximized because pristane and phytane are the most susceptible to degradation. Moreover, both experimental group I and experimental group II had better degradation effects on 14 days than on 7 days, and the degradation effect on the low carbon number was obvious. Moreover, both experimental group I and experimental group II had better degradation effects on 14 days than on 7 days, and the degradation effect on the low carbon number was obvious substance, so it is preferred to suffer from more intense bacterial degradation.

Analysis of Biological Evolution Parameters of n-Alkanes. It can be seen from the evolution parameters of n-alkanes in Table 3, 7d-1%-PAM main peak carbon is nC21, the 7d-3%-PAM main peak carbon advances to nC23, 14d-1%-PAM main peak carbon is nC25, the 14d-3%-PAM main peak carbon advances to nC26. The main peak carbon is obviously moving forward, indicating that the microbial agent is selective for high carbon number n-alkanes and has a strong degradation effect.35 The n-alkane distribution of 7d-1%-PAM is characterized by \( \sum C_{21} = \sum C_{22} + \sum (w(C_{21−s})/w(C_{22})) = 1.000 \), but the distribution of normal paraffins of 7d-3%-PAM is expressed as \( \sum C_{21−s} > \sum C_{22−s} \), characteristics \( w(C_{21−s})/w(C_{22−s}) = 1.729 \), and it indicated that the degradation degree of 7d-3%-PAM was significantly greater than 7d-1%-PAM; similarly, analyzing the values of \( w(C_{21−s})/w(C_{22−s}) \) for 14d-1% and 3%, the degree of degradation of 14d-3%-PAM was found to be significantly greater than 14d-1%-PAM. The OEP values were 1.060, 1.075, 1.033, and 1.075, respectively. In general, the value of experimental group II was smaller than that of experimental group I, indicating that the maturity of the latter was higher than that of the former (or the latter was higher than the former). The values of pristane/phytane were positive,

| component | average degradation (mg) | relative degradation rate (%) |
|-----------|--------------------------|------------------------------|
| C17       | EG I 0.0006603           | 52.23                        |
| C18       | EG I 0.0008587           | 20.81                        |
| C19       | EG I 0.0005943           | 43.30                        |
|           | EG II 0.0006964          | 17.21                        |
|           | EG I 0.0005460           | 40.81                        |
|           | EG II 0.0005858          | 13.04                        |

Table 6. Biological Evolution Parameters of Hopane and Sterane Compounds

| component  | eigenvalues | 7d (1%) | 14d (1%) | 7d (3%) | 14d (3%) |
|------------|-------------|---------|----------|---------|----------|
| hopane     | Tg/Tm       | 1.110   | 1.212    | 1.121   | 1.314    |
|            | C31αβγ−22S/22S + R | 0.569   | 0.547    | 0.615   | 0.584    |
|            | nor-lupane/lupane | 0.472   | 0.458    | 0.529   | 0.523    |
| sterane    | C29αβγδ/20(S + R) | 0.480   | 0.471    | 0.529   | 0.524    |

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indicating that the sample suffered from strong bacterial degradation, and the values of the experimental group II was greater than the experimental group I. It indicated that the oxidation of the isoprenoids hydrocarbon in experimental group II was more obvious than that in experimental group II.

**Degradation of Hopane and Sterane.** (Note: C21-tricyclodipentane; C22-tricyclodipentane; C23-tricyclodipentane; C24-tricyclodipentane; C25-22s + 22R-tricyclodipentane; C24-four ring post (higher plant markers); C26(1)-β-(H), 21α-ethyl-ethane; C24-tricyclodipentane; C24-tricyclodipentane; C22-tricyclodipentane; C22-tricyclodipentane; C27-18α-(H)-22,29,30-trihopane(Ts); C29-lupane; C29-17α(H), 21β(H)-30-hopane; C29-rearrangement-30-hopane; C30-lupane; C30-17β(H), 21α(H)-30-normotane; C30-17α(H), 21β(H)-hopane; C30(1)-pentacyclic triterpenes; C30(2)-pentacyclic triterpenes; C30-17β(H), 21α(H)-30-normotane; C30-17α(H), 21β(H)-hopane; C31-17α(H), 21β(H)-22s-31-liter hopane; C31-17α(H), 21β(H)-22r-31-liter hopane; C30-γ-paraffin wax; C32-17α(H), 21β(H)-22s-31,32-twoliter hopane; C32-17α(H), 21β(H)-22r-31,32-two-liter hopane; C33-17α(H), 21β(H)-22s-332, 33-3-liter hopane; C33-17α(H), 21β(H)-22r-31,322, 33-3-liter hopane; C34-17α(H), 21β(H)-22s-332, 33, 34-4-liter hopane; C34-17α(H), 21β(H)-22s-332,33,34-4-liter hopane; C35-17α(H), 21β(H)-22s-332,33,34,35-five-liter hopane; C35-17α(H), 21β(H)-22s-332,33,34,35-five-liter hopanes).

As can be seen from Figure 11, PSM-immobilized microbial pellets have obvious degradation effects on hopane compounds, and the degradation trends of hopane in 7 and 14d were basically the same, indicating that the degradation of hopane mainly showed the conversion of chiral carbon to stable configuration, but the degradation rate of hopane was higher than that of 14d at 7d. The degradation of hopane fluctuates significantly because of the alternating appearance of the S and R configurations of the series of substances. The bacterial action promotes the conversion of the chiral carbon R configuration of the pentacyclic triterpenoids to a more stable S configuration. The type of compound is more stable, so the degradation rate is low, the R configuration material is unstable, the degradation rate is high, and the wavy degradation tendency is exhibited. As can be seen from Table 4, the magnitude of the changes in the two groups of data is different, but the overall trend is basically the same, and a comparative analysis can be performed. The specific performance is as follows: the average degradation of tricyclic dipentenoids is 0.792 μg, the relative degradation rate reached 66.65%, which was much higher than the relative degradation rate of 39.20% of the experimental group II. Regarding the relative degradation rate of homohopane and norhopane, degradation of norhopane is slightly inferior to that of homohopane; the overall degradation rate of experimental group II is low, however, the degradation of tricyclopterane, homohopane, and norhopane is consistent with that of experimental group I, that is, the degree of degradation is as follows: tricyclopterane > homohopane > norhopane.

Note: C21-pranane; C22-Hi-pranane; C27-βα-20S-diacholestane; C27-βα-20R-diacholestane; C27-ββ-20S-diacholestane; C27-ββ-20R-diacholestane; C27-αα-20S-cholestane; C27-αα-20R-cholestane; C27-αββ-20S-cholestane; C27-αββ-20R-cholestane; C29-ββ-20S-cholestane; C29-ββ-20R-cholestane; C27-ααα-20S-cholestane; C27-ααα-20R-cholestane; C29-βα-20R-24-ethyl-diacholestane; C29-βα-20S-24-ethyl-diacholestane; C27-ααα-20S-cholestane; C27-ααα-20R-cholestane; C29-αββ-20R-24-ethyl-cholestan; C28-αβαβ-20R-24-ethyl-cholestan; C29-αβαβ-20S-24-ethyl-cholestan; 4-Me-24-Me-cholestan; C29-ααα-20R-24-ethyl-cholestan; C30-4-Me-24-ethyl-cholestan; C30-4-Me-24-ethyl-cholestan; C30-4-Me-24-ethyl-cholestan.

As can be seen from Figure 12 and Table 5, the degradation effect of the steroids on the whole of the experimental group I was better than that of the experimental group II. Also, the relative degradation rate of the compound with C27 was 52.23%, the relative degradation rate of experimental group II was only 20.81%; similarly, for compounds with C29 and C30, experimental group I also has obvious advantages. From these two sets of data, it can be concluded that the degradation degree of different carbon numbers of steroids with different carbon atoms is as follows: C27 > C29 > C30.

**Biological Evolution of Hopane and Sterane.** From the biological evolution parameters of Table 6, the four groups of parameters of hopane C31αβ-22S/22S + R and sterane C29αα20S/20(S + R) were all greater than 0.4, that is, they are all strongly degraded by microorganisms, the C31αβ-22S/22S + R parameter value of hopane is greater than sterane C29αα20S/20(S + R), it is fully demonstrated that the microbial degradation of hopane is better than that of sterane. The nor-lupane/lupane value of 7d-1%-PAM is 0.472, the nor-lupane/lupane value of 7d-3%-PAM is 0.529, which indicates that the degradation degree of experimental group II is greater than that of experimental group I. In general, the smaller the T/Tm, the greater the microbial degradation intensity. The T/Tm values of the hopane experimental group I at 7 and 14 days were 1.110 and 1.212, respectively, both of which are less than the values 1.121 and 1.314 of the experimental group II, and this proves that the overall degradation effect of the experimental group I obtained above is better than that of the experimental group II.

### CONCLUSIONS

In summary, we have demonstrated the immobilization of four kinds of mixed microorganism, which was prepared by uploading oil-degradation bacteria into the cross-linked biodegradable PAM and SA mixture supporting material, for efficient degradation of oily wastewater. The degradation rate of saturated hydrocarbon TPH was studied by such immobilization of microorganisms. The results show that the oil degradation rate for experimental group I (sample containing 1% crude oil) and experimental group II (sample containing 3% crude oil) reaches as high as 70 and 40%, respectively, after 14d of degradation of saturated hydrocarbon TPH. For different oil components, the degradation degree is in the order of tricycoloditerpanes > homohopanes > norhopanes. The order of degradation degree of steroids with different carbon atoms is C27 > C28 > C29. In terms of evolution characteristics, it can be seen from the biological evolution parameters of n-alkanes that only a slight degradation for odd—even carbon by biodegradable bacteria was achieved, whereas a high degradation for isoprene alkane was observed. According to the biological evolution parameters of hopane and sterane, the hopane C31αβ-22S/22S + R and sterane C29αα20S/20(S + R) were all greater than 0.4, that is, they are all strongly degraded by microorganisms. The findings obtained from this work would be expected for providing useful guidance for future optimization of mixed micro-
organism for TPH degradation. Furthermore, the transformation characteristics of TPH were also investigated to provide an insight into fundamental understanding of evolution path for future study.

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