Biological Degradation and Transformation Characteristics of Total Petroleum Hydrocarbons by Oil Degradation Bacteria Adsorbed on Modified Straw

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ABSTRACT: We report a simple and “green” method for the fabrication of polymer-modified straw-supported oil degradation bacteria (PMS-ODB) for biological degradation of total petroleum hydrocarbons (TPHs) in water. The modification of straw was achieved by in situ copolymerization of styrene and butyl methacrylate using methylene-bis-acrylamide as a cross-linker in an aqueous solution containing straw powders. Compared with the control group (ODB loaded on untreated straw), the results obtained from the experimental group show that the polymer-modified straw is beneficial to the growth of microorganisms. As a result, the degradation rate of TPHs reaches 90.12%, which is 50.54 and 7.08% higher than that of the blank group (ODB only) and the control group, respectively. A study on the transformation characteristics of PMS-ODB shows that the degradation rate of alkanes with low, medium, and high carbon number is higher than 90%. w(∑C21−)/w(∑C22+) (the mass ratio of normal alkanes of high carbon/low carbon), w(pr)/w(ph) (the ratio of pristane/phytane), and OEP (the mass ratio of normal alkanes of odd carbon/even carbon) for TPHs in the experimental group were measured to be 0.6186, 0.7248, and 1.4356, respectively, all of which are the largest value among the blank group, control group, and experimental group. These findings indicate that compared with the control group, the modification of straw could enhance the comprehensive biological degradation performance for TPHs, even those highly stable organics, such as carbon n-alkanes and isoprenoid hydrocarbon, which may open a new possibility for degradation of oils or toxic organics in an enhanced biological manner.

INTRODUCTION

The rapid development of modern industry has led to severe environmental pollution, in particular, organic pollution in water such as oil spills and nitrogen-containing aromatic waste (e.g., nitrobenzene) has aroused serious concerns and worldwide attention owing to its high environmental toxicity and biological accumulation, which has caused numerous ecological problems. Thus, there is an urgent need to develop new technologies or functional materials for efficient removal or elimination of oils or organic contaminants from water.

So far, a number of approaches such as adsorption, flotation, flocculation, electrochemistry, membrane separation, and biological methods have been used for organic wastewater treatment. These methods, in most cases, have their respective limitations such as the need of complicated techniques or poor reusability with high operation costs. For instance, the absorption technology has been adapted as one of the promising remedies to this point. However, the inherent drawbacks for most of the conventional absorbents such as clay, activated carbon, and so on, for example, poor selectivity and limited absorption performance, still remain as a big issue. The recently developed superwetting absorbents with superhydrophobic and superhydrophilic wettability have excellent absorption selectivity for removal of oils or lipophilic organics; however, the need of a multistep process or complicated techniques still remains a big obstacle for their scalable fabrication and in turn hindering their practical applications. On the other hand, the environmental and ecological risk of using these absorbents in application remains unclear and the disposal of them after use may also cause new environmental pollution. Therefore, the exploitation of functional materials that fulfill such multivariable performance requirements for efficient removal of oils and organics from water should be of special interest.

Comparatively, biological methods for degradation of oils or organic contaminants have great technological advantages over those mentioned methods, including environmental friendliness, simple operation, low-energy consumption, and, importantly, capability of direct and complete degradation of contaminants into harmless emissions. In general, the microorganisms, in this case, oil degradation bacteria (ODB), are
immobilized on supporting materials to confine to a certain region of space keeping fixed microorganism growth and biological activity to highly efficient degradation of oil pollutants. Therefore, the number of microorganisms on a given supporting material, the biological degradation activity for oils or organics, and the accessible contact area of microorganisms to organics are essential for their biological degradation performance. To date, cellulose has been employed as a supporting material for immobilization of microorganisms to organics are essential for their biological activity to highly efficient degradation of oil pollutants. Therefore, the number of microorganisms on a given supporting material, the biological degradation activity for oils or organics, and the accessible contact area of microorganisms to organics are essential for their biological degradation performance. To date, cellulose has been employed as a supporting material for immobilization of microorganisms in this field. Though great progress has been made accordingly, the poor stability of immobilization of microorganisms as well as the weak affinity of hydrophilic biomass supporting materials to lipophilic oils or hydrocarbon organics which hinders the effective contact of immobilized microorganisms to oils still remains a big challenge for their large-scale applications. In this regard, here we demonstrate an approach for modification of hydrophilic biomass straw with relative hydrophobic copolymers to enhance both the stability of immobilization of microorganisms and the affinity of microorganism-immobilized straw to oils. As a proof-of-concept study, the number of microorganisms, the activity of dehydrogenase, and the degradation rate for oils are enhanced compared with control, which shows great potential for practical wastewater treatment. Furthermore, the transformation characteristics of total petroleum hydrocarbons (TPHs) were also investigated to provide an insight into fundamental understanding of evolution path for future study. This may open a new possibility for degradation of oils or toxic organics in an enhanced biological manner.

### RESULTS AND DISCUSSION

**Material Characterization Analysis.** Figure 1a shows the Fourier transform infrared spectroscopy (FT-IR) spectra of corn straw (CS) and polymer-modified straw (PMS). It is clear that there are new absorption peaks in PMS compared with CS; the characteristic absorption peaks at 2376 and 2334 cm$^{-1}$ are assigned to the vibration of benzene ring, and the absorption peak at 1733 and 1066 cm$^{-1}$ belongs to the telescopic vibration of the C=O bond and C−O−C bond in butyl methacrylate. The characteristic absorption peak at 1546 cm$^{-1}$ is assigned to the telescopic vibration of C=C skeleton in the benzene ring. The peak at 617.79 cm$^{-1}$ is attributed to the vibration of the C−H bond outside the benzene ring. The absorption peak at 766 cm$^{-1}$ belongs to the bending vibration of C−H bond benzene ring. The vibration absorption peak of the hydroxyl group associated with PMS at 3401 cm$^{-1}$ weakens, indicating that the hydroxyl position is not completely a graft reaction and the material still maintains the properties of cellulose after the reaction.

The X-ray diffraction (XRD) patterns of CS and PMS are shown in Figure 1b. It can be seen that the XRD diffraction patterns of CS has changed before and after modification. According to the intensity of the peaks corresponding to $I_{22.5°}$ and $I_{16.8°}$, the formula of crystallization index is expressed as follows:

$$X\text{-ray crystallization index } = \frac{I_{22.5°} - I_{16.8°}}{I_{22.5°}} \tag{1}$$

The crystallinity of CS and PMS was calculated to be 0.403 and 0.273, respectively. It can be seen that the crystallinity of PMS is 0.130 lower than that of CS. Such a decrease in the crystallinity of CS may be due to the formation of amorphous copolymers, which in turn indicates the occurrence of successful graft copolymerization. As shown by the scanning electron microscopy (SEM) images of PMS in Figure 1c, the surface of CS consists of a compact fibrous structure, showing that the modification of straw by the polymer is obvious, which makes the straw a more rough surface with larger specific surface and more adsorption sites. Such an increase in surface roughness is beneficial to the adsorption and immobilization of mixed bacteria on the surface. The hollow cellulose tube in the straw not only facilitates the diffusion of the substrate and metabolites but also provides enough space and oxygen for maintaining the normal physiological metabolism of microorganisms.

To investigate the effect of polymer modification of CS on its surface wettability, the water contact angle (WCA) for CS and PMS was determined. As shown in Figure 1d, when a water droplet was placed on the surface of CS, it can be absorbed quickly. However, it can maintain a spherical shape when it was placed on the surface of PMS, indicating a strong water repulsion. The WCA was measured to be 0° for CS and...
128.5° for PMS, respectively. Such an obvious change of wettability of CS before and after polymeric modification, that is, the hydrophilic CS turns to be hydrophobic, is attributed to both the enhanced surface roughness and the lipophilic chemistry nature of copolymer of styrene and butyl methacrylate, which are two key factors for construction of a strong hydrophobic surface. As expected, the strong hydrophobicity of PMS would be beneficial to enhance the affinity of PMS to oils or polycyclic aromatic hydrocarbons which might increase its biodegradation performance.

**Analysis of Changes in Microbial Quantity and Degradation Gradient.** The effect of duration time on the microbial quantity and the degradation gradient of three groups are shown in Figure 2. It is well-known that microbes secrete specific extracellular enzymes, use some organic matter as nutrients to be absorbed by microbes, and the rest of the nutrients are oxidized by microbes into simple organics or inorganic substances, purifying oily wastewater in the early stages. As an indicator of microbial adaptability to the substrate, microbial quantity has a good correlation with the degradation effect of petroleum hydrocarbons. It is clear that the microbial quantity of the experimental group reaches the maximum value of $1.69 \times 10^9$ g $^{-1}$ after a duration of 3 d and then gradually decreases with the increase of treatment time. Comparatively, for the control and blank groups, the maximum microbial quantity was observed at a duration of 7 d, though they show a similar tendency to that of experimental group. For the entire procedure, the microbial quantity of experimental group is higher than that of control and blank groups. Accordingly, the degradation gradient is proportional to the microbial quantity of all of the three groups before 7 d of treatment. Afterward, the degradation gradient for control and blank groups is still relatively stable; however, for experimental group, the degradation gradient nearly reaches zero. That is, the experimental group has the best degradation performance only within 7 d, which is 49.11 and 6.22% higher than the blank groups is still relatively stable; however, for experimental group, the degradation gradient nearly reaches zero. That is, the experimental group has the best degradation performance only within 7 d, which is 49.11 and 6.22% higher than the blank and control groups, respectively. Notably, the degradation performance of blank group is the lowest one, where the degradation gradient remains nearly unchanged even after 25 d of duration. To better understand the biological utilization ability of microbial for organic substrates, we also investigated the effect of duration time on the dehydrogenase activity and degradation gradient of three groups. As shown in Figure 3, the curves for the three groups are closely similar to that shown in Figure 2. The dehydrogenase activity reaches maximum in 7 d, which is hysteretic to that of the microbial quantity (reaches maximum in 3 d, Figure 2). Such hysteresis may be due to the fact that the proliferation of microbial is overwhelming in the initial stage while the increase in the dehydrogenase activity only occurred after the propagation of the microbial. Hydrogenase oxidizes petroleum hydrocarbons by activating the hydrogen atom of petroleum hydrocarbons and transferring it to a specific hydrogen acceptor, while dehydrogenase activity can be used to reflect the change of petroleum degradation microorganisms. After 7 d, the dehydrogenase activity decreases with prolonging the duration time, which is in good agreement with the tendency of that of the microbial quantity.

To provide a direct observation for the changes on the number of microorganisms adsorbed on PMS, SEM analysis was performed and the SEM images of PMS after different duration times are shown in Figure 4. At the initial stage (0 d), only a few of microorganisms (the micrometer-scale papilla-like structure) were observed on the smooth surface of polymers, which is grafted on the CS. When the duration time reaches 6 d, the papilla-like structure increased and densely aggregated together, that is, the number of microorganisms adsorbed on PMS increased very quickly. In contrast, after a duration time of 15 d, the number of microorganisms decreased and the shape of papilla-like structure is not as regular as that of 6 d, implying that the microorganisms are withering away. The SEM observation is also well consistent with that of the analysis on the microbial quantity and the degradation gradient of three groups (Figures 2 and 3).

**Analysis of Dynamic Adsorption Characteristics of TPHs.** Figure 5 shows the dynamic sorption characteristics of TPHs of all three groups, and the half-life of degradation is calculated by the equation

$$T_{1/2} = \ln 2/k$$

where $k$ is the slope of the dynamic curve. Accordingly, the half-life of blank group, control group, and experimental group was calculated to be 7.31, 6.22, and 5.31 days, respectively. It can be seen that the half-life of experimental group is 2.00 and 0.91 days lower than that of blank group and control group. Obviously, the experimental group shows the shortest half-life.
among the three groups, implying the best degradation performance. The oil–water interface is the main active area of microbial degradation of oil pollution, and the degree of oil dispersion affects the degradation rate of microorganisms. The shortening of half-life indicates that PMS can effectively increase the contact area between microorganisms and oils, enabling the microorganisms to obtain more nutrients, and accelerate the degradation of TPHs by microorganisms.

Analysis on Degradation of n-Alkanes. Figure 6 shows the total ion GC/MS chromatogram of standard oils containing n-alkanes and isoprene alkanes after 21 d of degradation by three groups. Because n-alkanes and isoprene alkanes are the main components of TPHs, the degradation of n-alkanes presents the degradation of TPHs. It is clear that the degradation of oils with different carbon numbers is different for three groups. For the blank group, the abundance of residual oils with a carbon number of 15, 17, and 19 is relatively higher than those oils with low or high carbon numbers, while for the experimental group and control group, the oils with a carbon number ranging from 1 to 25 have high abundance. To precisely clarify the relative percent content of each substance in residual oils, the peak area normalization method was employed using 44-deuterated 21 alkanes as the internal standard. The residual amount of each n-alkane was calculated by the absolute content of internal standard by the following equation

\[
\text{percentage content of n-alkanes (\%)} = \frac{\text{residual of n-alkane (\mu g)}}{\text{residual of internal standard (\mu g)}} \times 100\%
\]

The calculated results for residual oil content of n-alkanes are shown in Figure 7. For all n-alkanes with carbon number ranging from 13 to 35, the residual oil content of n-alkanes for experimental group is lowest among the three groups, indicating that the experimental group possesses the best degradation performance. The degradation order of n-alkane is as follows: experimental group > control group > blank group. These results also indicate that the addition of straw is beneficial to the degradation of n-alkanes, and the degradation performance of PMS is better than that of CS. The average degradation rate of n-alkanes with high carbon number (>C31) was found to be 96.83%, which is the highest value, and is 3.52 and 11.28% higher than that of control group and blank group, respectively. The average degradation rate of n-alkanes with medium carbon number (C21–C30) reaches 90.49%, which is 2.02 and 18.84% higher than that of the control group and blank group. The average degradation rate of low carbon number (C13–C20) was calculated to be 93.20%, which is 4.34 and 23.64% higher than that of control group and blank group. The results indicate that the loaded microorganisms using PMS as the carrier could enhance the degradation ability of n-alkanes. Such improvement in the degradation of PMS could be attributed to the fact that the grafting copolymers of butyl methacrylate and styrene result in a hydrophobic modification layer on the surface of CS, which efficiently increases the affinity for the oil molecules and facilitates the absorption of oils on the PMS. On the other hand, such copolymers on PMS could also form a space network structure, which is beneficial to the loading and the propagation of the microorganisms, thus in turn enhancing the degradation of n-alkanes.

Biological Evolution Characteristics of n-Alkanes. The biological evolution characteristics of n-alkanes were also investigated according to the total ion GC/MS chromatogram of n-alkanes and the results are shown in Table 1. As shown in Figure 6, the abundance of residual n-alkanes with low carbon numbers is higher than that of n-alkanes with high carbon numbers, that is, the main peak carbon of n-alkanes degraded by microorganisms will move forward. This phenomenon is due to the fact that the n-alkanes with high carbon numbers were degraded into n-alkanes with low carbon numbers, which in turn increase the abundance of the latter. In fact, such a main peak carbon of n-alkanes moving forward is affected by other factors such as the difference of parent source, environment, species, and degradation degree of microorganisms. Table 1 shows the biological evolution parameter of n-alkanes, compared with the blank group, the main peak carbon of the experimental group, and the control group moved forward, which was a reflection of the strong demethylation of high carbon number n-alkanes made by ODB adsorbed on straw. The maximum value of \( w(\sum_{C21}^{C25}) / w(\sum_{C21}^{C25}) \) of the experimental group was 0.6186, which indicates that PMS, as a carrier of adsorption, could effectively...
improve the degradation of \( n \)-alkanes with medium and high carbon numbers. On the other hand, the bigger the ratio of \( w(pr)/w(ph) \) is, the higher the degree of redox of TPHs by microorganisms, the better the effect of degradation of isoprene hydrocarbon. If a change in isoprenoid occurs, it indicates that the organic matter of oils is subject to degradation of microorganisms. The ratio of \( w(pr)/w(ph) \) of the experimental group and the control group was larger than that of the blank group, which indicates that PMS promoted the degradation of isoprene alkanes and removed a methyl group from a partial phytane and converted it into basalane. The highest ratio of \( w(pr)/w(ph) \) was measured to improve the degradation of \( n \)-alkanes with medium and high carbon numbers. On the other hand, the bigger the ratio of

Figure 6. Total ion GC/MS chromatogram of \( n \)-alkanes, isoprene alkanes after degradation. Note: the substance corresponding to the peak number is 1-\( n \)-tetradecane, 2-\( n \)-pentadecane, 3-\( n \)-hexadecath, 4-\( n \)-hexadecath, 5-pristane, 6-octadecane, 7-phynane, 8-nondacane, 9-\( n \)-eicosane, 10-deuterated 21 alkane, 11-heneicosane, 12-docosane, 13-tricosane, 14-tetracosane, 15-pentacosane, 16-hexacosane, 17-heptacosane, 18-octacosane, 19-nonacosane, 20-triacontane, 21-hentriacontane, 22-dotriacontane, 23-triatriacontane, 24-tetratriacontane, 25-35-alkanes.

Figure 7. Variation of residual oil content of \( n \)-alkanes.

### Table 1. \( n \)-Alkanes Biological Evolution Parameter

| sample          | main peak of alkanes | \( w(pr)/w(ph) \) | OEP  | \( w(\sum C_{21})/w(C_{22}) \) |
|-----------------|----------------------|-------------------|------|--------------------------|
| blank group     | \( nC_{21} \)        | 0.7044            | 0.9537 | 0.3732                   |
| control group   | \( nC_{18} \)        | 0.7150            | 1.2597 | 0.5871                   |
| experimental group | \( nC_{18} \)    | 0.7248            | 1.4356 | 0.6186                   |
be 0.7248 in the experimental group, the higher the ratio, the higher the rate of alkane degradation. Therefore, PMS as the immobilized carrier was the most effective in the degradation of TPHs among the three groups. When microorganisms degrade TPHs, the relative abundance of odd-carbon n-alkanes and even carbon-n-alkanes will decrease or increase. The OEP value of the blank group was 0.9537, close to 1; there is no obvious characteristic of odd—even carbon dominance. The OEP values of the experimental group and the control group were found to be 1.4356 and 1.2597, respectively, showing the degradation advantage of n-alkanes with even carbon number. This result shows that the PMS was beneficial to enhance the degradation ability especially for even carbon number n-alkanes.

**CONCLUSIONS**

In summary, we have demonstrated an efficient approach for the fabrication of PMS-ODB, which was obtained by in situ grafting copolymerization of styrene and butyl methacrylate onto straw followed by loading of ODB. Compared with control group, the results obtained from experimental group show that the polymer-modified straw is beneficial to the growth of microorganisms, and the degradation rate of TPHs reaches 90.12%.

The research of transformation characteristics of PMS-ODB shows that the degradation rate of alkanes with low, medium, and high carbon number is higher than 90% and calculated to be 93.20, 90.49, and 96.83%, respectively. The mass ratio of normal alkanes of high carbon/low carbon, the ratio of pristane/pryhtane, and the mass ratio of normal alkanes of odd carbon/even carbon for TPHs in the experimental group were measured to be 0.6186, 0.7248, and 1.4356, respectively, all of which are the largest value among the three groups.

The findings indicate that compared with the control, the modification of straw could enhance the comprehensive biological degradation performance for TPHs, even those highly stable organics such as n-alkanes with even carbon numbers and isoprenoid hydrocarbon, which may open a new possibility for degradation of oils or toxic organics in an enhanced biological manner.

**EXPERIMENTAL SECTION**

**Immobilized Bacteria Agent.** Six strains of ODB which have been screened and isolated in our group previously were prepared into a suspension according to the volume ratio of 2:2:2:2:1:1 and cultured in a thermostatic incubator. A three-stage expanded culture method was used to check the number of colonies more than $1 \times 10^{10}$ mL$^{-1}$ and make the bacteria agent sealed for storage. Fixing the liquid—solid ratio of 1:1.25, the prepared mixed bacteria solution was sprayed on the straw evenly, cultured for 24 h, the colony number was measured to be larger than $1 \times 10^{10}$ cfu g$^{-1}$, and then the immobilized bacteria agent was obtained.

**Synthesis of PMS.** For the preparation of PMS, 300 mL of deionized water and 30 g of powdered CS were added into 500 mL of four-mouthed bottle at a temperature of 50 °C. After the introduction of nitrogen for 10 min, a certain amount of diammonium cerium(IV) nitrate, styrene, butyl methacrylate, and methylene-bis-acrylamide was added into the mixture at 50 °C. After 2 h, the reaction products were filtered under reaction temperature, washed with deionized water and ethanol for several times, and dried under vacuum. The dried materials were extracted with toluene to remove the homopolymers produced during the reaction and then affording the PMS.

**Experimental Design.** The following experiments were designed to evaluate the degradation performance of oil pollution by bacteria adsorbed on the straw. Blank group: TPHs were naturally degraded by 4% standard oil. Control group: 2.5 g of CS was added on the basis of blank group. Experimental group: on the basis of blank group, 2.5 g of PMS was added, and 4% bacterial solution was added according to the mass ratio of liquid phase. Specific treatment measures are shown in Table 2.

Table 2. Experimental Design of Degradation

| sample                  | blank group | control group | experimental group |
|-------------------------|-------------|---------------|--------------------|
| standard oil (g)        | 4           | 4             | 4                  |
| CS (g)                  | 0           | 2.5           | 0                  |
| PMS (g)                 | 0           | 0             | 2.5                |
| microbial inoculum (mL) | 4           | 4             | 4                  |
| nutritive medium (mL)   | 100         | 100           | 100                |

**Microbiological Count.** The active material (0.1 g) was determined by a five-point sampling method; after adding 9.9 mL of aseptic water, shaking, and centrifuging, the suspensions in the wastewater were diluted by gradient, and a suitable solution with a desired dilution degree was coated on the plate with three parallel samples for each concentration, cultured in the incubator at 37.0 °C for 48 h, and the colony count was counted. The degradation gradient was defined as

$$\text{degradation rate of the latter interval} - \text{degradation rate of the former interval} / \text{degradation rate of the former interval}$$

**Determination of TPH Degradation Rate.** The standard oil curves were measured by an ultraviolet spectrophotometer at 226 nm, and the residual oil content and degradation rate were calculated according to the standard equation. The standard equation of TPHs is obtained as follows:

$$y = 0.0189x + 0.0172$$

$$R^2 = 0.9980,$$ where $y$ is the effective absorbance of the extraction solution from oil pollution wastewater extracted by 50 mL n-hexane, dimensionless, and $x$ is the oil concentration of the sample, mg L$^{-1}$.

**Means of Material Characterization. X-ray.** An X-ray fluorescence spectrometer (model S4EXPLORER) from Bruker AXS was used for semiquantitative spectral analysis, Cu K target, graphite monochromator, 50 kV tube voltage, and 80 mA tube current.

**FT-IR.** The American thermoelastic infrared spectrometer (model NICOLET6700 FT-IR) was used for infrared spectral analysis and scanned the area of 500~4000 cm$^{-1}$ to compare the changes of groups under different carbonization temperatures.

**SEM.** The samples were sprayed with gold and fixed on a copper platform with a conductive double-sided adhesive tape, and a Japan Hitachi s-5200 field emission scanning electron microscope was adopted to observe the surface morphology of the samples, the working voltage is 0.5~30 kV, the current was 1~2 mA, and the thickness of gold spraying was 10 nm.
Contact Angle. The static contact angle was tested with a DSA-25S contact angle tester, the contact angle between the droplet and the glass sheet at 25 s is the stable contact angle, and each sample is measured seven times, and the average value is the final contact angle.

Determination of Dehydrogenase Activity. The formula of the standard curve drawn by UV spectrophotometry at 486 nm is

\[ Y = 0.0130X - 0.0125 \]  

\( R^2 \) is 0.9930. Type: \( X \) is the triphenyltetrazolium chloride (TTC) solution concentration and \( Y \) is the absorbance value corresponding to each concentration of the TTC solution.

Biological Evolution Parameter Analysis. The main peak carbon and parameter \( \left[ \frac{w(\sum C_{21})}{w(\sum C_{22})} \right] \) reflect the trend of conversion from high carbon alkanes to low carbon alkanes when alkanes are subjected to bacterial action. The higher the carbon front of the main peak, the stronger the ability of bacteria to degrade high carbon number alkanes. \( \frac{w(\sum C_{21})}{w(\sum C_{22})} \) is the ratio of the sum of low carbon alkanes to the sum of middle and high carbon number alkanes. The higher the value of this parameter, the stronger the degradation ability of bacteria for high carbon number alkanes. The OEP value reflects the ability of bacteria to degrade even and odd carbon alkanes. The higher the OEP value is, the stronger the degradation ability to even alkanes, indicating that the degradation ability for even carbon is strong. The ratio of \( w(\text{pr})/w(\text{ph}) \) is to study the redox degree of organic matter by using the ratio of pristane to alkane in isoprene hydrocarbon. The larger the value is, the more obvious the oxidation of isoprene hydrocarbon.

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