Preparation of a gel-type microbial-missile for biodegradation of oil pollution

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Abstract. The removal of oil spill pollution is a global problem. In order to solve the problem of low biodegradation efficiency, a series of microbial-missile materials with high floatability, hydrophobicity and biological affinity were prepared to enhance the degradation of oil spill. Hydrophobic macroporous materials with polar groups inside and hydrophobic aliphatic polyester outside were prepared by physical and chemical methods using SA/PVA as substrate for gel-type microbial-missile. The experimental results show that the saturated adsorption rate of macroporous gel-type microbial-missile to oil particles is as high as 18.17 g/g, the contact angle is 149.30°, the average density is 0.5883 g/cm³, the average mechanical strength is 2.52 mN, the penetration rate of 40 s is 82.72%, and the degradation rate of crude oil in 7 days is 88.95%.

1. Introduction
The pollution caused by the entry of petroleum is a worldwide serious marine pollution [1]. At present, the main methods of oil spill cleaning include physical method, chemical method and biological method [2]. Petroleum hydrocarbon-degrading bacteria has the advantage of clearing up oil film more thoroughly than physical and chemical methods, and is considered as the fundamental way to solve the pollution of petroleum hydrocarbons [3,4]. However, due to the specific gravity of petroleum hydrocarbon-degrading bacteria, most of them are in sedimentation state after being added to water, which hinders the effective contact between petroleum hydrocarbon-degrading bacteria and oil slick [5]. Therefore, a floating, hydrophobic and biocompatible immobilized carrier is likely to achieve a qualitative leap in bioremediation of marine oil spill pollution.

Sodium alginate (SA) is a by-product of extracting iodine and mannitol from kelp or Sargassum of brown algae, containing a large amount of -COO ⁻ [6,7]. SA can rapidly form gel under extremely mild conditions. When Ca²⁺, Sr²⁺ and other cations exist, ion exchange reaction between Na⁺ and two valence cations on G unit and G unit stacking form a cross-linked network structure, thus forming hydrogels[8]. The conditions for forming alginate gel are mild, which can avoid the inactivation of sensitive substances such as sensitive drugs, proteins, cells and enzymes [9]. In this study, SA was used as a substrate to explore an oil-affinity carrier that can track and disperse oil pollution, referred to as “microbial-missile”.

2. Experimental materials and methods

2.1. Reagents and materials
Reagents such as dichloromethane, n-hexane and methyl trichlorosilane (MTS) were purchased from China Pharmaceutical Group. Agar, peptone, beef extract, glucose and yeast powder were purchased from Beijing Oberstar Company. All strains come from China Microbial Species Preservation Center. Crude oil, diesel oil and heavy oil are presented by COSCO.

2.2. Preparation of gel-type microbial-missile and immobilized microbial
SA and PVA were fed according to the experimental design in order to prepare the carrier material. Under the condition of 85℃ water bath heating, the mixture was stirred by a rotor agitator, and silyl coupling agent was added to the mixture. When foam was observed in the mixture, the heating was stopped, and when the temperature dropped to 40℃, the mixture was mixed with the bacterial culture medium in equal volume and stirred for 5 min. The mixed liquid droplets were added to 2% calcium chloride saturated boric acid solution by sterile syringe, and the spheres were formed under the cross-linking effect. After 24 h of cross-linking, the carrier was washed 2~3 times with normal saline and stored in 4℃ refrigerator for reserve.

Bacterial consortium enrichment solution (including *Bacillus sp.*, *Alpha sp.* and *Pseudomonas sp.*) with good degrading effect on crude oil were obtained by incubation in Zobell 2216E medium at 24℃ for 3 days at constant temperature. The prepared carrier was weighed at 5.0 g and added to it. After shaking for 3 hours, the culture medium was filtered out and the gel-type microbial-missile was obtained.

2.3. Characterization test of samples
Contact angle measuring instrument (Easy-Drop, RUSS company, Germany) measures surface contact angle. Scanning electron microscopy (JCM-7000, JEOL, Japan) was used to observe the internal appearance of the carrier. Ultraviolet spectrophotometer (UV-1800, Shimadzu, Japan) Measure residual oil concentration. Adsorption performance test: including material oil absorption ability, oil retention ability test [10]. Oil absorption ratio is used to characterize the oil absorption capacity of materials. The oil absorption capacity is measured by mass difference ratio method. As seen formulas (1) and (2), the mass of the sample before adsorbing oil was recorded as \( m_1 \). The mass of the sample after absorbing oil was recorded as \( m_2 \). After 15 min, the sample was weighed again, and the mass of the sample was recorded as \( m_3 \).

\[
G_s = \frac{(m_1 - m_3)}{m_1} \quad (1)
\]

\[
q = \frac{m_3 - m_1}{m_2 - m_1} \times 100\% \quad (2)
\]

3. Results and analysis

3.1. Reaction principle of preparing immobilized microbial community carrier with PVA/SA as substrate
The reaction principle of preparing hydrogel microbial-missile carrier material with SA/PVA as substrate is shown in figure. 1. The SA molecular chain contains a large number of hydroxyl and alkyl groups. CaCl₂ is used as crosslinking agent. The calcium alginate polymer is obtained by crosslinking reaction between the two. The prepared gel carrier with SA/PVA as the substrate not only has the advantages of biocompatibility, modification, and complete degradation, but also has the characteristics of three-dimensional network structure and interstitial water combination. Therefore, the adsorption and degradation of spilled oil by floating macroporous gel-type microbial-missile is a promising alternative to solve the problem of marine oil pollution.
Figure 1 Principle of hydroxyl cross-linking reaction for preparation of microbial-missile using PVA/SA as substrate

3.2. Adsorption performance test

According to formula (1) and (2), the final saturated oil absorption rate $G_s$ (g/g) and the final sustained release oil retention rate $q(\%)$ are calculated(Table 1). The adsorptive capacity of the prepared carrier for crude oil and diesel oil is quite different, which indicates that the variety of oil is related to the adsorptive capacity.

Table 1 adsorption test results of macroporous gel-type microbial-missile for different oil products

| No. | Type     | $m_1$ (g) | $m_2$ (g) | $m_3$ (g) | $q_a$ (g/g) | $q_k$ (%) |
|-----|----------|-----------|-----------|-----------|-------------|-----------|
| 1   | Heavy oil| 0.150     | 1.864     | 1.791     | 12.43       | 96.10     |
| 2   | Crude oil| 0.150     | 1.154     | 1.055     | 7.70        | 91.38     |
| 3   | Diesel oil| 0.150    | 0.654     | 0.527     | 4.36        | 80.71     |

3.3. Static contact angle measurement

The hydrophobicity of macroporous gel-type microbial-missile is characterized by static contact angle. The test results are shown in figure 2. The average contact angle of macroporous gel-type microbial-missile is $149.300^\circ$, which is between $90^\circ$ and $150^\circ$, and is nearly $150^\circ$, showing hydrophobicity. Hydrophobic carriers contact and adsorb with weak polar oil slick under the impetus of ocean wind and current, which provides carbon source for the survival of bacteria in carriers, and is conducive to improving biodegradation efficiency.

3.4. Density, mechanical strength and mass transfer performance of macroporous gel-type microbial-missile

The density of carrier material is measured by drainage method. The mechanical strength of the carrier is measured by front pressing method. The mechanical strength of each carrier is indicated by pressure gauge. The mass transfer performance of the particles was tested by color infiltration method. The results are shown in Table 2, Table 3 and Table 4 respectively.

The average density of the carrier is 0.5883 g/cm$^3$, which is less than the density of the water under the standard pressure. The carrier has good floating performance on the water surface.
Table 2 Density determination results of macroporous gel-type microbial-missiles

| No. | 1     | 2     | 3     | 4     | 5     | 6     | Average |
|-----|-------|-------|-------|-------|-------|-------|---------|
| m(g) | 1.0201 | 1.0365 | 1.0157 | 1.0368 | 1.0229 | 1.0268 | 1.0265  |
| v(cm³) | 1.81  | 1.69  | 1.77  | 1.73  | 1.87  | 1.62  | 1.7483  |
| ρ (g/cm³) | 0.563 | 0.613 | 0.574 | 0.599 | 0.547 | 0.634 | 0.5883  |

The average mechanical strength of the carrier is 2.52 mN, which provides an effective protective barrier for bacteria in the marine environment.

Table 3 Mechanical strength determination results of macroporous gel-type microbial-missile

| No. | 1     | 2     | 3     | Average |
|-----|-------|-------|-------|---------|
| $M_i$ (mN) | 38.0  | 36.7  | 38.7  | 37.8    |
| $F_i$ (mN) | 2.53  | 2.45  | 2.58  | 2.52    |

The permeability of carrier increased fastest in 5~20 s, reached osmotic equilibrium in 20 s. The permeability of carrier reached 82.72% in 40 s. It shows that the carrier has good mass transfer performance, low diffusion resistance of substrates and products, and good fluidity of bacteria and secretions in the carrier. It is conducive to immobilization of bacteria to be nutritious and dissolved oxygen, timely discharge of metabolites, thus conducive to the growth and reproduction of bacteria, and improve degradation efficiency.

Table 4 Mass transfer performance determination results of macroporous gel-type microbial-missile

| Time(s) | 5  | 10 | 15 | 20 | 25 | 30  | 35  | 40  |
|---------|----|----|----|----|----|-----|-----|-----|
| Penetration distance (mm) | 0.55 | 1.06 | 1.35 | 1.76 | 1.78 | 1.79 | 1.81 | 1.82 |
| Carrier radius (mm) | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 |
| mass transfer rate (%)   | 25.0 | 48.18 | 61.36 | 80.0 | 80.91 | 81.36 | 82.27 | 82.72 |

3.5. *Biocompatibility of gel-type microbial-missile*

The SEM image (figure. 3) show that the hydrocarbon-degrading bacteria are distributed between the pore of the material and in a good growth state, which shows that the material has good biological affinity. Large pore network structure with coral-like protuberance inside the carrier is not only conductive to the floatation of the carrier, but also conducive to capture dissolved oxygen at the oil-water interface. The three-dimensional mesh structure of large pore can reduce the surface tension between oil/water/gas/solid phases, reduce the rolling angle and thus increase the static contact angle of the surface. The carrier shows oil-affinity, which is conducive to the carrier contacting with oil spill under the impetus of wind, wave and flow.
3.6. Comparisons of degradation rate between gel-type microbial-missile and free-form bacterial consortium

The degradation rates of immobilized and free bacteria were compared. The results were shown in figure 4. The degradation rate of crude oil by immobilized and free bacteria was 57.36% and 42.21% respectively in 24 h. The degradation rate of immobilized bacteria was 1.36 times that of free bacteria. The degradation rate of immobilized and free bacteria reached 88.95% and 72.22% respectively in 7 days. The results showed that the immobilized bacterial consortium showed higher degradation rate than the free consortium. Combining with uncertainties such as wind, wave, current and temperature, the preparation of immobilized natural macromolecule carriers is of great practical value.

4. Conclusion

A kind of floating gel-type microbial-missile carrier was prepared. Through the preparation, characterization and application of the material, the material with inner polar group and outer non-polar lipophilic group was obtained. It can automatically track and absorb oil spill and affinity contact degradation within a short distance, with obvious advantages. The contact angle is 149.30°, and the average density is 0.5883 g/cm³. It has good floater performance and the average mechanical strength is 2.52 mN, which can resist the impact of wind and waves. The mass transfer equilibrium was achieved in 20 s and the permeability reached 82.72% in 40 s. Compared with the free bacterial consortium, the degradation rate of gel-type microbial-missile reached 88.95% in 7 days. It shows that the gel-type microbial-missile material improves the degradation effect of degrading bacteria.

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