Application Analysis of Immobilized Bioremediation Preparation in Oil Spill Contaminated Shore

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Abstract. The thesis uses self-developed immobilized bioremediation preparations to carry out on-site trials of bioremediation of oil spilled contaminated beaches, with a view to solving the practical problem that traditional dry powder / liquid bioremediation preparations are difficult to be applied on site in oil contaminated shores. The degradation characteristics of the marine petroleum degrading bacteria group DC10 were studied, and a degrading bacteria agent based on the bacteria group was developed and used in the bioremediation experiment of Dalian Oil Spilled Beach, and the intertidal and upper tidal belts were investigated by the degrading bacteria agent. The degradation of oil pollution was evaluated by analysing the degradation rates of C17 / hopene, C18 / hopene, and total alkane and total aromatics. The study found that the degradation rate of the degrading bacteria group DC10 to petroleum under laboratory conditions was higher than that of the constituent strains. The degradation rate of petroleum in one week was about 60% higher than that of the control, and it could degrade most alkanes and aromatics.

1. Introduction
Coastal oil spill pollution is a form of oil spill pollution that drifts from the sea to land to the shore or leaks directly from the land to the shore. It is the most direct form of pollution that affects society and the environment. My country has 18,000 km of mainland coastline, and there are a large number of beaches, animal habitats, bird migration routes, mangroves and other national key nature reserves, which are extremely valuable environmental resources and economy. Resources, combined with the shallow shore beach topography and complex landforms and the effects of tidal fluctuations, in the event of oil spill pollution from the shore beach, it will directly affect the living environment of rare animals and plants, and have a disastrous impact on the national economic development and people’s lives, and caused great damage to the ecological environment of the beach.
The existing marine oil spill disposal technology is mainly based on physical and chemical methods. However, the high cost of physical methods and the secondary pollution caused by chemical methods have made more and more scholars turn their attention to bioremediation methods, especially for the treatment of oil pollution on the shore, bioremediation can better degrade the oil pollution on the shore and the cost is low. It is considered to be the most effective method to deal with oil pollution, especially oil pollution on the shore. There have been cases of successful application of biological remediation methods to remove oil pollution from ocean shores abroad, but most of them still focus on biological promotion methods, such as spraying nutrients. The use of petroleum-degrading bacteria to accelerate the degradation of oil pollution is rarely used. Based on the research background, this study takes “fixing formulation” as the main idea, and designs and builds a 300m² real-world simulated oil spill contaminated shoreline bioremediation field test system. The self-developed immobilized bioremediation preparations were used to evaluate the effectiveness of on-site application of shoreline bioremediation products, and provided technical support for the on-site application of bioremediation technologies for oil spill-contaminated shores and beaches [1].

2. Characteristics of oil spill from shore

2.1. There are many types of shores and beaches, and the geographical environment is complex
Shore beaches can be divided into rocky shore beaches, gravel shore beaches, beach shore beaches, and sedimentary shore beaches. In the beaches contaminated by oil spills, oil spills can penetrate into deep depths on medium and coarse sand beaches, gravel beaches and sloughs, which are difficult to remove and cause long-term harmful effects. Cluster. In addition, shore pine, soft soil, and poor bearing capacity make it difficult for personnel and conventional machinery to operate, causing difficulties in oil spill emergency and oil pollution clean-up.

2.2. Poor disposal effect and great difficulty
Regardless of whether the source of oil spills comes from land or sea, once entering the shore, the conventional emergency equipment is not applicable because the oil is attached to the shore, and its cleaning efficiency and difficulty are much greater than those on the sea and land. According to the experience of oil spill emergency disposal site, the oil spill on the shore is lost, and conventional equipment such as oil skimmers and oil collecting nets cannot be used. The oil spill of the “16.16” oil pipeline explosion in Dalian caused a large area of coastline to be contaminated with crude oil and could not be thoroughly cleaned with conventional equipment in a short period of time.[2].

3. Experimental materials and research methods

3.1. Materials

3.1.1. Degradation bacteria and culture medium. The marine oil-degrading bacteria group DC10 used in this research includes two species (3 strains) of marine obligate hydrocarbon-relieving bacteria, one of which (2 strains) is alkane-eating bacteria and the other (1 strain) is sea bacteria; the above three strains have been deposited on August 20, 2009, and April 16, 2010, respectively, to the China General Microbial Culture Collection Centre, the deposit numbers are CGMCCC No. 3735, CGMCCC No. 3736, CGMCCC No. 3244; 2216E medium, refer to Han Ping's method.

3.1.2. Main experimental instruments. Electronic balance; full temperature shaking incubator; large-capacity high-speed refrigerated centrifuge; gas chromatography-mass spectrometer; nitrogen blowing concentrator; fermentation tank; ultraviolet-visible spectrophotometer; automatic nutrient analyser.

3.1.3. Crude oil contaminated seawater samples. According to the "Ocean Monitoring Specification" (GB17378.3-2007), two 1 000 mL crude oil contaminated surface seawater was taken as parallel
samples at each station using a brown wide-mouth bottle, manually sampled, refrigerated at 4 ℃ during transportation, and the samples arrived immediately after the laboratory, pre-treatment and analysis tests were carried out. 20 mL of seawater was taken to test the biological toxicity; 500 mL of water samples were taken to test the petroleum hydrocarbon content in the seawater.

3.2. Experimental method

3.2.1. Preparation of petroleum degrading bacteria. The 97CO-5, 97CO-6, PY97S cryopreserved strains were inserted into 100mL 21616E liquid medium, 150r / min, 25 ℃ shaker culture to the logarithmic growth period (97CO-5, 97CO-6 culture 30h, PY97S culture 48h), the first- and second-stage seed liquids are gradually amplified to the platform stage, and then connected to a 200L air-lift fermenter at a ratio of 1:10. The culture conditions are: aeration of 0.25m³ / h, temperature of 25 ℃. The rotation speed is 150r / min; when the bacteria reach the maximum growth density, the cultivation time of the 97CO-5 in the flora is about 36 h, the cultivation time of 97CO-6, and MY97S is about 50h.

3.2.2. Experiment of the optimal ratio of nitrogen and phosphorus nutrients and initial concentration of bacteria DC10 to degrade petroleum. The activated 3 strains of petroleum hydrocarbon-degrading bacteria were evenly mixed to construct the bacterial group DC10. Then, the bacterial group DC10 was connected to the above-mentioned different nitrogen and phosphorus nutrient ratio and initial concentration of seawater petroleum medium at a rate of 2%. At 150 r / min, 20 ℃, protected from light for 10 days, observe the apparent changes in the process of oil degradation, and then determine the oil degradation rate by gravimetry. Use the oil degradation rate as an indicator to determine the optimal nitrogen for bacterial degradation by DC10, the ratio and initial concentration of phosphorus nutrients are shown in Table 1.

| Processing number | Proportion of nitrogen and phosphorus | Initial nitrogen concentration | Initial phosphorus concentration |
|-------------------|--------------------------------------|-------------------------------|----------------------------------|
| A                 | 20:01                                 | 300                           | 15                               |
| B                 | 20:01                                 | 100                           | 5                                |
| C                 | 20:01                                 | 50                            | 2.5                              |
| D                 | 20:01                                 | 20                            | 1                                |
| E                 | 20:01                                 | 5                             | 0.25                             |
| F                 | 10:01                                 | 300                           | 30                               |
| G                 | 10:01                                 | 100                           | 10                               |
| H                 | 10:01                                 | 50                            | 5                                |
| J                 | 10:01                                 | 20                            | 2                                |

3.3. Experimental design

After the slope of the beach and sand slope was laid at 10 °, weigh 20kg of crude oil in the test area and add 40L of diesel oil, stir quickly with an electric mixer until the crude oil is evenly dispersed, and transfer the crude oil mixture to the sprayer at twilight. Spray evenly on the sea sand surface of each test pool and wash it for 15 days to ensure that the physical and chemical properties of seawater, sediments and crude oil pollutants are stable. After 15 days of simulated oil spill pollution, immobilized bioremediation agent 1 was evenly added to the surface of the bank of the test pool 2 at twilight, and immobilized biological agent 2 was evenly added to the surface of the bank of the test pool 3. The dosage is 0.5kg / m²; the nutrient salt is evenly added to the surface of the intertidal zone of the test pool 4, the dosage is 0.7g / m²; the test pool 1 is the blank control group without any treatment [3].
3.4. Analysis method
The preparation of crude oil water containing components (WAF) is carried out according to the method of Barron et al. This method is the current technical document referenced by the international marine oil spill ecological risk assessment and toxicity test, and is also the method adopted by the US EPA in the Gulf of Mexico oil spill accident. As follows: add 50 g of petroleum to 2 L of simulated seawater in the faucet bottle, magnetically stir for 16 h, and stand for 8 h. Take the dissolved petroleum hydrocarbon solution from the bottom of the faucet bottle as the experimental sample. Take 3 for each experimental sample Parallel, use DTX880 instrument for detection, the detection system is 190 μL sample + 10 μL of bacteria solution to be tested, the test process is maintained at 30 °C, test every 15 min, the test time is 6 h. Each station takes 3 parallel, using the instrument LB 9507 was tested. The test system consisted of 990 μL sample + 10 μL of bacteria solution to be tested. The test process was maintained at 30 °C and tested every 30 min. The total test time was 6 h.

4. Results

4.1. Removal effect of each component hydrocarbon in oil sands
Table 2 shows the change of TPHs removal rate of oil sands in the upper-tidal zone, inter-tidal zone and sub-tidal zone of the four test pools after the system has been running for 3 months. As shown in Table 2, the degradation effects of oil sand TPHs in the three sampling blocks have the same rule, that is, test pool 2 > test pool 3 > test pool 4 > test pool 1. Compared with the blank control group and the nutrient-only group, the test pool with immobilized biological agents had the best degradation performance of petroleum hydrocarbons. A high concentration of microbial flora can be formed for a long time, thereby effectively improving the crude oil degradation effect and environmental tolerance [4].

| Sampling block | Test pool name | TPHs removal rate   |
|---------------|---------------|---------------------|
| Tide belt (a) | Test Pool 1 # | -15.05 ± 1.37       |
|               | Test Pool 2 # | 95.00 ± 2.77        |
|               | Test Pool 3 # | 66.69 ± 6.24        |
|               | Test Pool 4 # | 59.92 ± 5.55        |
| Intertidal zone (b) | Test Pool 1 # | 32.14 ± 2.33       |
|                 | Test Pool 2 # | 98.20 ± 1.02        |
|                 | Test Pool 3 # | 86.74 ± 5.76        |
|                 | Test Pool 4 # | 77.12 ± 7.24        |
| Subtidal zone (c) | Test Pool 1 # | -106.34 ± 8.11      |
|                   | Test Pool 2 # | 69.76 ± 5.37        |
|                   | Test Pool 3 # | 54.12 ± 7.72        |
|                   | Test Pool 4 # | 46.29 ± 3.24        |

4.2. Optimum nutrient conditions for bacteria group DC10 to degrade petroleum
The oil degradation rate of the bacterial group DC10 under different nitrogen and phosphorus ratios and initial concentration conditions after 10 days was determined by gravimetry. As can be seen from Figure 1, the petroleum degradation rate of the bacterial group DC10 with different nitrogen and phosphorus ratios and initial concentrations the impact is greater. Without the addition of nutrients (treatment R), the degradation rate of bacteria DC10 to petroleum is lower (29.61%), which is only 2.87% higher than that of the negative control (treatment S). Compared with the treatment of nutrients (treatment R), the treatment of the initial concentration of 5 different nitrogen and phosphorus in each of the three nitrogen and phosphorus ratios (20: 1, 10: 1, and 5: 1) has a certain effect on the
degradation of bacteria. The promoting effect of the three different nitrogen and phosphorus ratios on the initial concentration of nitrogen and phosphorus shows a consistent law for the bacterial degradation of the bacterial flora DC10, that is, the promotion effect is more obvious under the conditions of high, medium nitrogen, and phosphorus concentrations, and the low The promotion effect is not obvious under the conditions of nitrogen and phosphorus concentration [5].

Among all the treatments with added nutrients, treatments G and M had the highest degradation rate of petroleum, respectively 53.46% and 53.04%, which were increased by 26.72% and 26.30% respectively compared with the negative control (treatment S). Therefore, the bacterial flora DC10 The optimal degradation conditions are as follows: the ratio of nitrogen and phosphorus is 10: 1, the initial concentration of nitrogen and phosphorus is 100 mg / L, 10mg / L; or the ratio of nitrogen and phosphorus is 5: 1, the initial concentration of nitrogen and phosphorus is 100 mg / L, 20 mg / L.

![Figure 1. Degradation rate of bacteria DC10 to petroleum under different nutrient conditions](image)

### 4.3. Degradation rate of degrading bacteria group DC10 on petroleum

The weight of residual oil in the culture medium after the biodegradation of the degrading bacterial group DC10 was determined by gravimetry, and the degradation rate was calculated (Figure 2). The results showed that after the negative control (NC) was cultured on a 7d shaker, the loss rate reached 20.51%, which was mainly caused by the volatilization of light components in petroleum. The degrading bacteria group DC10 (composed of JP97S, 97CO-5, and 97CO-6) degraded oil to 82.47%, which was about 60% higher than the control. The degradation effect of the bacterial flora DC10 was significantly better than that of the three single bacteria that constituted it under pure culture conditions, and the synergistic effect between the single bacteria was obvious [6].
5. Conclusion
In this study, immobilized bioremediation technology was used to carry out on-site verification tests on oil spill-contaminated shores, and good remediation results were obtained, providing technical reserves for the industrial promotion and enterprise compatibility of two independently developed immobilized bioremediation preparations. The outdoor simulated oil spill beach restoration experiment has laid a good foundation for the future restoration of the shore ocean environment during actual ocean oil spills, and provides a reference program. However, in terms of the improvement and application of oil spill repair bacteria, as well as the optimization of the bioremediation effect evaluation system, there is still a lot of research work to be carried out and improved.

References
[1] Hong, S., Lee, S., Choi, K., Kim, G. B., & Khim, J. S. Effect-directed analysis and mixture effects of ahr-active pahs in crude oil and coastal sediments contaminated by the hebei spirit oil spill. Environmental Pollution, 199(1) (2015) 110-118.
[2] Sanni, G. O., Coulon, Frédéric, & Mcgenity, T. J. Dynamics and distribution of bacterial and archaeal communities in oil-contaminated temperate coastal mudflat mesocosms. Environmental ence and Pollution Research, 22(20) (2015) 15230-15247.
[3] Berinstein, A., Sellers, H. S., King, D. J., & Seal, B. S. The diversity of phytoplankton populations in oceanic, coastal and estuarine regions. Journal of Breast Cancer, 18(3) (2015) 3171-3178.
[4] Marzooq, H., Naser, H. A., & Elkanzi, E. M. Quantifying exposure levels of coastal facilities to oil spills in bahrain, arabian gulf. Environmental Monitoring & Assessment, 191(3) (2019) 16001-16016.
[5] Hainsworth, J. Enbridge seeks discussions with canada on proposed b.c. coastal oil tanker moratorium. International environment reporter, 38(24) (2015) 1667-1668.
[6] Kirsten, B., David, R., Bijayalakshmi, N., Lei, L., Fiona, Y., & Vicki, E., et al. Are the traditional medical uses of muricidae molluscs substantiated by their pharmacological properties and bioactive compounds? Marine Drugs, 13(8) (2015) 5237-5275.