Effect of Bioactive Water on the Biodegradation of Naphthalene Contaminated Soil

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Abstract. In this experiment, the strain NW6 with good growth of naphthalene as the sole carbon source and energy source was screened from petroleum contaminated soil after isolation and purification by enrichment culture. The influencing factors of strain were studied. The results showed that the optimum growth conditions for strain NW6 were pH 8, 40 °C. In the contaminated soil for sand, loess and black soil, the degradation rate of naphthalene by NW6 was 28.05%, 24.35% and 21.34% respectively. Adding bioactive water, the degradation rate was increased compared with distilled water. The enhanced rates of BW against strain NW6 in three naphthalene contaminated soils were respectively: sand 83.28%, loess 103.56%, black soil 60.40%. It showed that the bioactive water could promote the degradation of microorganisms.

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are a class of Persistent Organic Pollutants (POPs) that are ubiquitous in the environment and have strong teratogenic, carcinogenic and mutagenic effects on humans and other organisms [1]. Naphthalene is often used as a model compound for the study of two benzene ring degradation studies due to its unique chemical structure. It is widely found in natural environments such as air, water and soil. It has been identified as a strong carcinogen by the World Health Organization and its environmental risks can't be ignored [2]. Therefore, how to effectively repair PAHs contaminated soil has become a major problem in the field of environmental protection at home and abroad and has to be solved urgently [3].

On the basis of microbial remediation technology, there are also some emerging technologies that can be combined with soil pollution remediation. For example, bioactive water technology is also known as BMW technology (Bacteria, Mineral, Water). It is the crystallization of this kind of reflection that isn't a livestock and poultry manure treatment technology developed under the "end management" idea, but a waste resource utilization technology that conforms to the "circular economy" idea [4].

In this experiment, the aim is to isolate a kind of naphthalene degradation strain as the sole carbon source. The growth characteristics and the enhanced degradation effect of contaminated soil with the bioactive water were studied. Applying bioactive water [5] to microbial remediation of soil pollution is the biggest innovation of this experiment, and it has great practical significance for microbial remediation of polycyclic aromatic hydrocarbon contaminated soil.
2. Materials and Methods

2.1. Soil
The petroleum-contaminated soil in the laboratory was used as an isolation source for the degradation bacteria. The loess, black soil and sand were used to degradation experimental. The properties and soil texture of these soils were shown in Table 1.

| Types    | Item          | pH   | Water content (%) | SOM(%) | Clay(%) | International System |
|----------|---------------|------|-------------------|--------|---------|----------------------|
| Loess    |               | 7.02 | 8.57              | 0.89   | 15.9    | Clay loam            |
| Black soil|              | 6.83 | 12.76             | 6.78   | 10.8    | Sandy loam           |
| Sand     |               | 8.35 | 3.28              | 0.29   | 4.1     | Loamy sand           |

2.2. Isolation bacteria
In this experiment, the method of Xu Xiaoyi [6] was consulted to isolation naphthalene degrading bacteria, named NW6. The cultured strain was kept in a -70°C refrigerator.

2.3. Determination of optimum growth conditions
MSM: NaCl 0.01 g, KH₂PO₄ 0.5 g, K₂HPO₄ 0.5 g, MgCl₂•6H₂O 0.2 g, MnSO₄ 0.339 mg, ZnSO₄ 0.428 mg, CaCl₂•6H₂O 0.026 g, H₈MoN₂O₄ 0.347 mg, Yeast extract 0.2 g. The reagents mentioned above were dissolved in 1 L DW.

The strain NW6 was added to the mineral salt medium with naphthalene as the sole carbon source (g/L). The growths under different pH, temperature and salt concentration conditions were determined after 24 hours.

2.4. Determination of degradation rate in contaminated soil
To determination of degradation, 5 g of contaminated loess, black soil, sand (2 mm sieve) was weighed into the sample bottle, adding standard solution of naphthalene (2000 mg/L), and the closed system contaminated was made to 2 mg/L. Soil slurry (soil:MSM = 1:1) was prepared by adding MSM and all samples were incubated for 48 hours. The experimental group was inoculated with strain NW6 2% (v/v) (OD₆₀₀nm = 2.0) and incubated for 2 days. The samples were centrifuge and taken. The naphthalene in soil phase was extracted with methanol (1:1) using the ultrasonicator for HPLC analyzed [7].

2.5. Effect of bioactive water for degradation of contaminated soil
Soil slurry was prepared with mineral salt medium involved in DW and BW respectively for degradation experiment.

3. Results and Discussion

3.1. Analysis of growth characteristics of strain NW6
As can be seen from Figure 1, the NW6 strain can grow in a wide pH range. When the pH is 6, the OD₆₀₀nm value rise and the growth are optimal at the initial pH 8, and the OD₆₀₀nm value reaches 1.024. After that, OD₆₀₀nm began to decrease with the increase of pH and when the pH increase to 10, the OD₆₀₀nm decrease to 0.703. It can be seen from Figure 2, the OD₆₀₀nm value of NW6 cultured at 30 °C for 24 hours is 0.536. As the temperature decreases, the amount of growth gradually decreases and OD₆₀₀nm is only 0.245 at 20 °C for 24 hours. In the range of 35 °C ~ 45 °C, the strain grows vigorously especially at 40 °C. The maximum growth OD₆₀₀nm is 1.276.
The strain NW6 isolated in this experiment could have the largest growth under the conditions of 40°C, pH 8, indicating that the strain had strong adaptability to different environments. This has certain practical significance for the microbial remediation of soil pollution.

3.2. Effect of biodegradation of naphthalene contaminated soil

According to Figure 3, it can be seen that the degradation rates of strain NW6 for three naphthalene contaminated soils are: sand (28.05%) > loess (24.35%) > black soil (21.34%). The concentration of naphthalene of experimental groups in three soils was lower than the control, indicating that the strain NW6 had a certain degradation effect on naphthalene contaminated soil.

Li Zuoyang [8] used the GC-MS to measure the amount of residual PAHs degraded by the strain. The natural degradation rate of naphthalene was the highest, reaching 8.57% by comparing the natural degradation rate.

3.3. Effect of bioactive water on biodegradation of naphthalene contaminated soil

It can be seen from Figure 4 the residual amount of naphthalene of experiment (BW) in three soils was all lower than the control (DW). The degradation rates of control (DW) are 23.75%, 18.51%, 16.06% in the sand, loess, black soil respectively. The degradation rates of Experiment (BW) are 43.53%, 37.68%, 25.76% in the sand, loess, black soil respectively. The enhanced rates are 83.28%, 103.56%, 60.40% in the sand, loess, black soil respectively (Figure 5). The degradation rates were increased in the soil slurry which was made by BW in the experiment with soils. It indicated that the BW promoted the degradation rate of strain NW6 in naphthalene contaminated soil.
For an organic compound, its adsorption is directly proportional to the organic matter content and clay in the soil. According to the experimental results, it can be known that the biodegradation effect of BW on naphthalene contaminated soil was higher than that of DW (Figure 4), indicating that BW could promote naphthalene degradation. The residual amount of naphthalene in the control group of the three soils BW was lower than that of DW. It is possible that BW and soil properties affect the extraction of naphthalene and the difference of the partition ratio, which needs further study.

4. Conclusion
In the experiment of the growth characteristics of the degrading-bacteria, the optimal growth conditions for the strain NW6 were pH 8, 40°C. In the biodegradation contaminated soil, the order of degradation rate from high to low was as follows: sand > loess > black soil. In the soil slurry system for biodegradation, adding bioactive water, the biodegradation rates were enhanced compared with distilled water for three soils. It showed that bioactive water could promote the degradation of naphthalene in soil.

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