

Research on the Influence of Inoculating Bacterium Suspension on Microbial Community Structure in Petroleum Hydrocarbon Contaminated Groundwater

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How to cite this paper: Xueyang Huang, Guo Wei, Ziting Ding, Huazhi Wang, Liping Bai. (2023) Research on the Influence of Inoculating Bacterium Suspension on Microbial Community Structure in Petroleum Hydrocarbon Contaminated Groundwater. *OAJRC Environmental Science*, 4(1), 22-28. DOI: 10.26855/oajrces.2023.06.004

Received: June 3, 2023

Accepted: June 29, 2023

Published: July 31, 2023

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Abstract

Petroleum hydrocarbon is a common pollutant which has high toxicity and transport property in groundwater. Considering the current supervision demands of groundwater pollution in China, this paper studies the bioremediation method of petroleum hydrocarbon contaminated groundwater. The degradation bacteria were screened and separated through the soil samples collected at a petroleum hydrocarbon contaminated site in Beijing, China. The cultivated bacterial suspension was added by different proportions into the groundwater samples. The influence of different proportions of bacteria suspension on the bacteria community structure in groundwater samples was analyzed, and the bacteria which could use petroleum pollutants as carbon sources and energy sources were studied. The research results showed that addition of bacterial suspension could have significantly effects on the bacterial structure in groundwater sample. Compared with the raw water samples contaminated by petroleum hydrocarbons, the relative abundance of *Proteobacteria* and *Firmicutes phylum* level increased respectively from 88% to 97% and 0.4% to 34.2% after 24 hours. The relative abundance of *Pseudomonas* and *Bacillus genus* level increased respectively from 67% to 99%, and 0.2% to 32.3% after 24 hours. The advantage bacteria in groundwater samples were considered as petroleum hydrocarbon-related degradation bacteria. The research results could provide theoretical basis for the bioremediation of petroleum contaminated soil and groundwater.

Keywords

Contaminated site, groundwater, petroleum hydrocarbon, degradation bacteria

1. Introduction

Groundwater is the important water resource in China, which is mainly used for agricultural irrigation, industrial and mining enterprises and urban domestic water. More than 400 cities in China use groundwater as drinking water. According to the statistics data, 65 % of domestic water in the northern cities of China, 50% of industrial water and 33% of agricultural irrigation water are originated from groundwater.

The petroleum components are the most concerned pollutants in groundwater, and the polluted groundwater is difficult to be detected and controlled. It is important to find cost-effective and efficient remediation technology for degrading hydrocarbon pollutants in groundwater. Bioremediation is an environmentally friendly remediation method, which principle is mainly to stimulate the degradation of pollutants by microorganisms. Microbial remediation is widely used to treat petroleum hydrocar-

bon contaminated soil and groundwater [1, 2]. More than 100 genera and 200 kinds of species of petroleum-degrading microorganisms had been found in nature, which mainly including bacteria (*Pseudomonas*, *Bacillus*, etc.), fungi (*Aspergillus*, *Penicillium*, etc.), actinomycetes (*Streptomycetes*, *Nocardia*, etc.), yeast, etc. [3].

Microbial remediation techniques include microbial enhancement and microbial stimulation [4, 5]. Microbial enhancement refers to the method of accelerating the pollutants degradation through adding degradable bacteria to the contaminated medium [6, 7]. Microbial stimulation refers to the method of adding nitrogen, phosphorus nutrients and electron receptors such as H_2O_2 and O_2 to the contaminated media, which accelerating the degradation rate by stimulating the growth of organisms [8-10]. The conditions required for microbes to decompose organic pollutants are different for the variety of microbes in soil. Therefore, the effect of biological enhancement is more efficient than biological stimulation effects. While, microbial reinforcement has effective remediation results in contaminated soil with poor functional degrading bacteria [6, 7, 11]. The exogenous microorganisms added into the contaminated medium will be antagonized by the competition of indigenous microorganisms, which influencing the effects of bioaugmentation treatment [12, 13]. Ueno and Wu isolated native microorganisms with the ability to degrade petroleum from the oil contaminated soil, and satisfactory results were achieved by adding the enriched microorganisms into the oil contaminated soil [6, 13].

Hundreds microbial species had been found to degrade petroleum hydrocarbon compounds, and the most common genera include *Pseudomonas*, *Achromobacter*, *Arthrobacter*, *Micrococcus Genus*, *Nocardia*, *Acinetobacter*, *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Alcaligenes*, *Bacillus*, *Mycobacterium*, and *Rhodococcus* etc. [14]. Sathishkumar et al. isolated 57 strains of oil-degrading bacteria from oil contaminated soil [15], which include *Bacillus sp.*, *Corynebacterium sp.*, and *Pseudomonas sp.* Gao et al. studied the variation of microbial community in soil with different salinity and crude oil concentration [16], and analyzed the active bacterial species in soil through DGGE. The research results showed that *actinomycetes*, *γ -proteobacteria*, *Firmicutes* played a dominant role in the degradation of petroleum hydrocarbons. Microbial community structure exerted a decisive role in the strength and characteristics of ecosystem functions [17]. Shahi A et al. found that variation of microbial community structure was related to the removal of petroleum hydrocarbons.

Soil pollution prevention and control has received more and more attention in China. Due to the time limitation, microbial remediation technology is rarely used in the industry contaminated sites in China before 2016. The Action Plan for soil pollution Prevention and Control of China was issued in 2016, and Law of the People's Republic of China on Soil Pollution Control was issued in 2018. In the above law and issuance, the risk-based idea and method were suggested for control and remediation of soil and groundwater pollution. As for the remediation and regulation for contaminated sites with petroleum hydrocarbon pollutants, it has great significance to study the variation of microbial community in petroleum hydrocarbon polluted medium.

This study focuses on the variation of microbial community structure in oil contaminated groundwater. The dominant bacteria were screened and enriched from the contaminated soil, and then the purified dominant bacteria with different proportion of bacteria suspension were added to the groundwater samples collected from the contaminated site. The variation of microbial community structure was analyzed and evaluated, and the research results could provide theoretical basis for microbial remediation of petroleum contaminated sites.

2. Materials and methods

2.1 Soil and groundwater samples

The soil and groundwater samples used in this experiment were collected from a chemical plant in Beijing, China. Based on the soil and groundwater survey results, this site was contaminated by petroleum hydrocarbon.

2.2 Cultivation medium

The composition of the isolation culture was (g/L) $MgSO_4 \cdot 7H_2O$, 0.7; KCl, 0.7; $Na_2HP0_4 \cdot 12H_2O$, 2.13; KH_2PO_4 , 1.08; NH_4Cl 2.13g/L. The composition of the purification culture was $MgSO_4 \cdot 7H_2O$, 0.7 g/L; KCl, 0.7 g/L; $Na_2HP0_4 \cdot 12H_2O$, 2.13 g/L; KH_2PO_4 , 1.08 g/L; NH_4Cl , 2.13 g/L; agar 1.8%-2.0 %. The Luria-Bertani culture composition contained (g/L): 10 Tryptone, 5 yeast extract, and 10 NaCl. Benzene was added into the culture as carbon source after sterilizing and filtering process.

2.3 Experimental methods

(1) Isolation

The mixture of 10 mL benzene solution, 90 mL isolation media and 1 g soil sample contaminated by petroleum hydrocarbon were prepared and added into a 250 mL conical flask. Then the flask was oscillated at 30°C by 180 r/min for a week to obtain the bacterial suspension. Culture medium with 10 mL benzene solution and 90 mL sterilized purification media was prepared in the culture plate. 200 μ L bacterial suspension was evenly coated on the purified culture medium, and the medium was incu-

bated in cultivation box at 30 °C for a week.

(2) Purification

The inoculating loop was sterilized with alcohol lamp flame, and the cooled loop was picked and streaked on the purified culture medium. Then the medium was inverted and incubated in cultivation box at 30°C for 3 days. The above steps were executed for three times, and the degrading bacteria were got for the experiment.

3. Results and discussion

By sequencing the isolated and purified plate and comparing the bacteria sequence in the database, the selected bacterial genus was *Bacillus*.

3.1 High throughput sequencing of groundwater microbial communities

The growth of microorganisms could be influenced by temperature, oscillation rate, and carbon source concentrations. The optimal logarithmic suspension with 2000 mg/L benzene concentration was enriched at 40°C by 180 r/min. The bacterial suspension is divided into 5 groups and added into the petroleum hydrocarbon contaminated groundwater samples with dosage ratios of 1%, 5%, 10%, 15%, and 20% respectively. The groundwater samples with bacterial suspension were oscillate at 40°C for 24 hours. The absorbance was measured every 2 hours at 600 nm by UV spectrophotometer to obtain the growth curve (Figure 1).

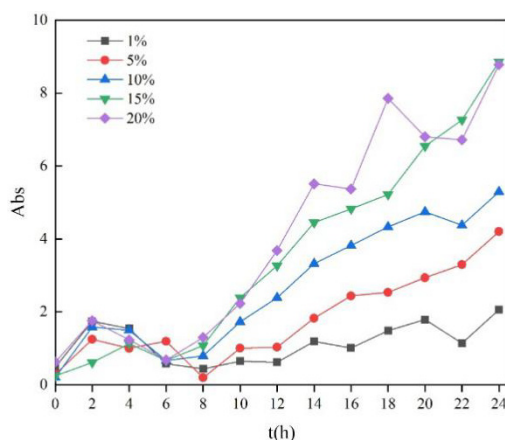


Figure 1. Growth curve of microorganisms.

3.1.1 Sequencing quality evaluation

Dilution curve were drawn through OTU richness statistical and rarefaction analysis. The species diversity difference of the samples could be displayed intuitively in Figure 2.

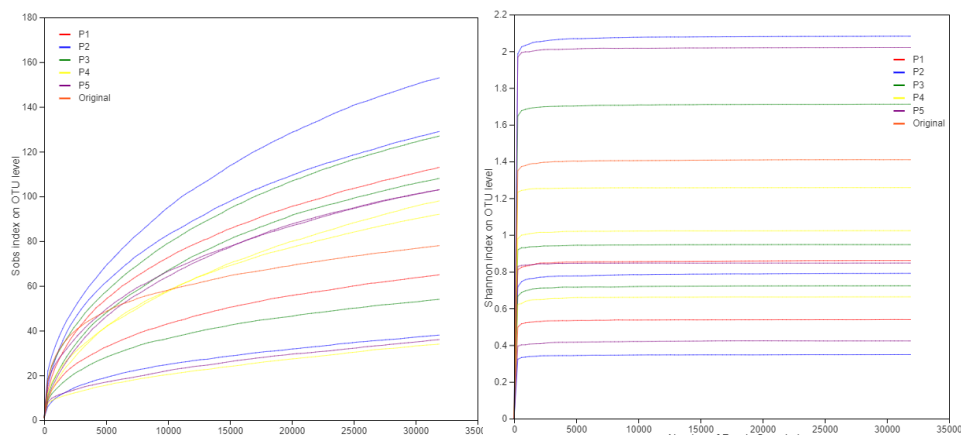


Figure 2. Dilution curve of the samples.

Figure 2 shows that the dilution curves of each treatment tend to be flat, which indicates that the amount of sequencing data is gradual and reasonable. The microbial diversity in all samples was captured, and the amplified sequence could reflect the bacterial community structure of each sample.

3.1.2 Diversity index

Diversity index is a comprehensive index to evaluate the richness and evenness of soil microbial community. The ACE and Chao1 index could be used to measure the species richness. Figure 3 and Table 1 displays the ACE and Chao1 index in this study. Big numbers of ACE and Chao1 index indicate high richness of soil microbial community [18]. Big numbers of the Shannon index and small numbers of the Simpson index indicate the higher uniformity of soil flora [19, 20]. In this experiment, the ACE and Chao1 index value tend to decrease over time.

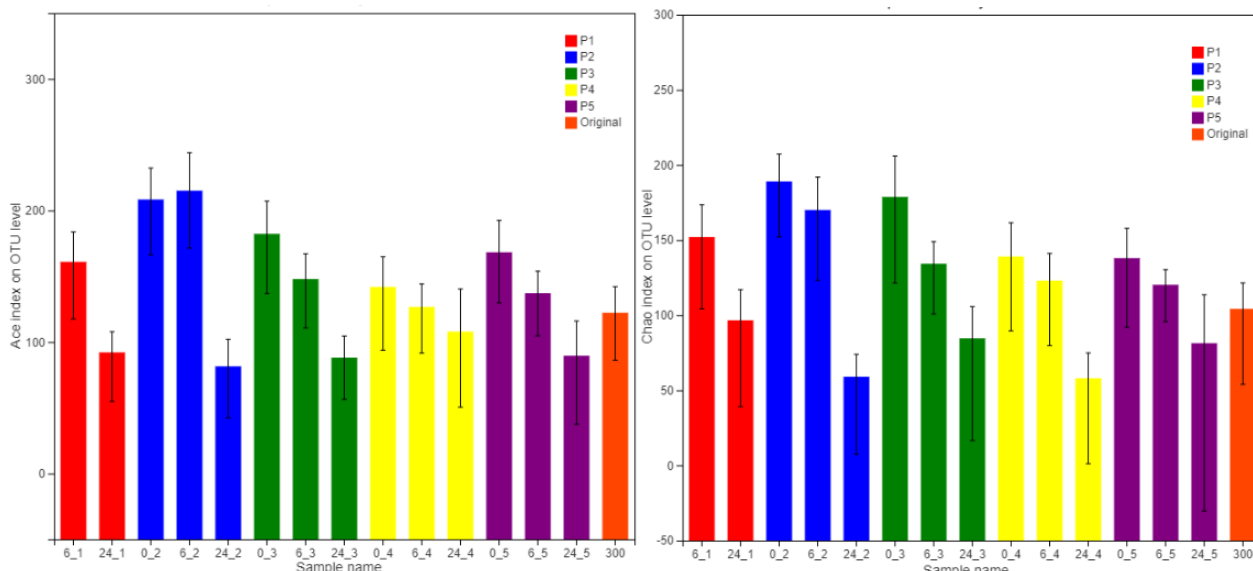


Figure 3. The ACE and Chao1 index results.

Table 1. Microbial community diversity in different samples

No.	Shannon	Coverage (%)	No.	Shannon	Coverage (%)
6-1	0.860238	0.998778	0-4	1.023375	0.998747
24-1	0.539569	0.999279	6-4	0.663037	0.998998
0-2	2.082046	0.998434	24-4	1.25776	0.999499
6-2	0.790283	0.998653	0-5	2.020168	0.998904
24-2	0.349139	0.99953	6-5	0.423286	0.999029
0-3	1.711897	0.998559	24-5	0.846339	0.999467
6-3	0.723491	0.998872	300	1.409682	0.999342
24-3	0.947346	0.999436			

Table 1 shows the results of microbial community diversity in groundwater samples. The Shannon index value of the biological enhanced samples is lower than the raw water sample, which indicating that growth of indigenous microorganisms could be inhibited by the degrading bacteria added into the petroleum hydrocarbons polluted groundwater. The richness and uniformity of soil microorganisms could be also reduced, and these results are consistent with Qi et al. [21]. The Coverage index of the 15 samples is more than 99%, which indicates that the sequencing results can represent the true distribution of bacteria in the samples [3].

3.1.3 Structural changes of soil microbial community

(1) Phylum level

The dominant bacteria groups to degrade petroleum hydrocarbons include *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* [3, 9, 10, 18, 22]. The relative phylum abundance of each sample was analyzed and displayed in Figure 4. *Proteobacteria* and *Firmicutes* are the dominant phylum in all samples, which are common microbial phylum in petroleum hydrocarbons contaminated soil [3, 22, 23]. Compared with the raw water samples, the abundance of *Proteobacteria* in 1%, 5% and 10% bacteria suspension increased from 94%, 91% and 88% to 97%, 99% and 97%, respectively. The relative abundance of *Firmicutes* in 15% and 20% bacterial suspension increased from 0.4% and 2.7% to 34.2% and 16.9%, respectively.

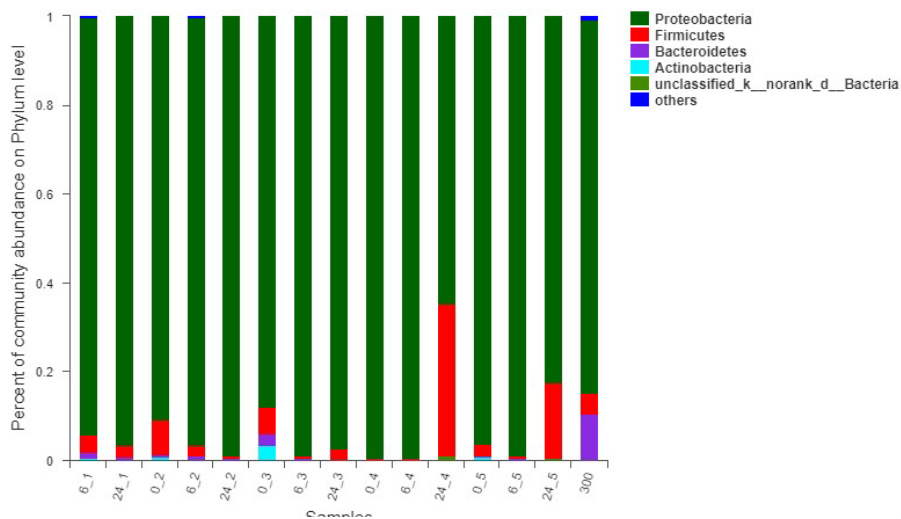


Figure 4. Relative phylum abundance of groundwater microorganism.

(2) Genus level

Figure 5 shows the dominant genus in groundwater samples. Before adding the bacterial suspension, *Pseudomonas*, *Azovibrio* and *Proteiniphilum* were the dominant genus with the relative abundance of 67%, 17% and 9.4% respectively. When the bacterial suspension was added into the groundwater sample after 24 hours, the dominant bacteria was *Pseudomonas* and *Bacillus*. The abundance of *Pseudomonas* in the samples increased from 67% to 90%, 99%, 96% and 76%, respectively.

In the groundwater samples with dosage ratios of 1%, 5%, 10%, the relative abundance of *Bacillus* decreased from 3.1% to 2.1%, 7.1% to 0.6%, and 4.9% to 2.1%. While the relative abundance of *Bacillus* increased from 0.2% to 32%, and 2.2% to 16.9% in samples with dosage ratios 15% and 20%.

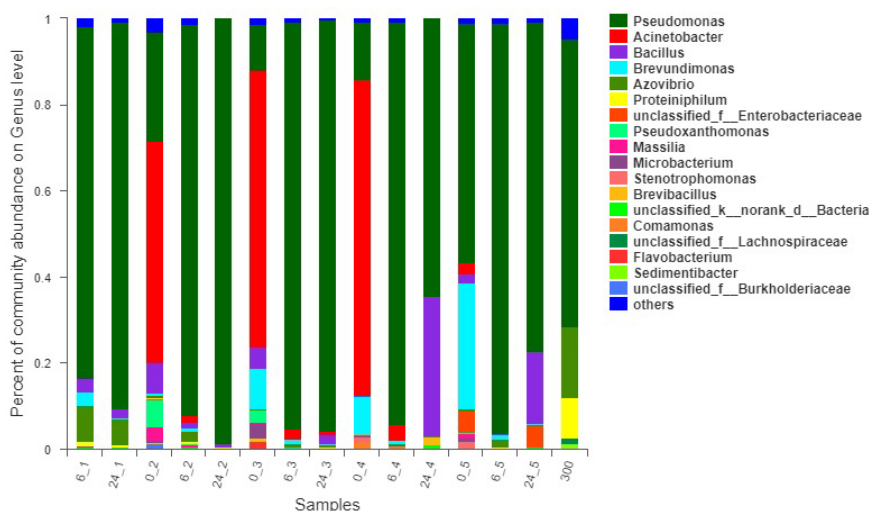


Figure 5. Relative abundance of groundwater microorganism in Genus.

The dominant genera of petroleum contaminated soil include *Pseudomonas*, *Achromobacter*, *Bacillus* and *Acinetobacter* [9, 10, 14-16, 18, 22]. In this experiment, *Proteobacteria* and *Pseudomonas* were dominant species in the groundwater sample. The research results indicate that the strains isolated and purified in this study could use petroleum hydrocarbon as carbon source and energy for growth.

4. Conclusions

(1) *Bacillus* with ability to degrade petroleum hydrocarbons was got by screening, isolating and purifying from contaminated soil.

(2) The community structure in the petroleum hydrocarbons contaminated groundwater samples changed significantly after adding different proportions of bacterial suspension. In the phylum level, *Proteobacteria* was the dominant bacteria. In the genus level, *Pseudomonas* and *Bacillus* were the dominant bacteria.

(3) The advantage bacteria in groundwater samples are petroleum hydrocarbon-related degradation bacteria. The research results could provide theoretical basis for the bioremediation of petroleum contaminated soil and groundwater.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No.41471266).

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