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Research Article

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On the environmental pollutants disposal producing from volatile petroleum based on micro-biological degradation

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ABSTRACT

All the strains screened out from contaminated soils were capable of degrading diesel oil while consortium and strains Q18 had stronger degradation efficiency. It's found that the existence of alfalfa and mustard could promote the degradation of diesel oil significantly. The existence of plant and the growth of roots may have changed the soil environment to make it more suitable for the growth of strains and degradation. In addition, the number of strains in the mustard rhizosphere was larger than that in the alfalfa rhizosphere, which showed that mustard had stronger ability on the activation of strain. This may be related to the different growth characteristics of mustard and alfalfa. In the experiment, Rhodococcus Q18-Indian mustard complex had the highest rate of degradation of diesel oil.

Key words: Diesel oil; Micro-biological; Oil Pollution; Environmental Treatment

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INTRODUCTION

With the rapid development of the petroleum industry, a large number of oil contaminants leaked into the soil in the petrochemical complex areas, gas stations, automobile factory and other places, which resulted in serious soil and groundwater contamination. Micro-biological degradation of oil pollution is the focus of the present study, while the mechanism of mutual promotion and restriction of restoration system of microorganisms cooperated with plants will also be investigated.

Microbial degradation of pollutants is often affected by a number of environmental factors, such as soil environment, the growth of plant as well as root exudates. The plant growth and development, and other physiological and metabolic activity maybe provide a better breeding micro-environment for the growth of micro-organisms [1-6]. It is generally believed that root exudates can promote the growth and metabolism of rhizosphere microorganism, thus enhancing the biodegradation of organic matter [7]. The plant provided microorganism the growth habitat in root zone and released lots of secretions (sugars, acids and alcohols, etc.) to the soil environment, the amount of which accounted for about 10% to 20% of photosynthesis production [8]. The rapid decomposition of the fine roots also added organic carbon to the soil, which strengthened the microbial degradation of petroleum hydrocarbons. In the presence of plants, the degradation rate of toxic organic pollutants such as polycyclic aromatic hydrocarbons can increase by 2% to 4.7% [9]. Planting corn and soybeans in the contaminated soil, with the addition of different agents of AMF, the degradation rate of oil pollutants could reach to 78% after a growing season [10]. Rhizosphere may play an important role on the biodegradation of toxic organic pollutants (such as PAHs) in the topsoil. Plants and micro-organisms may also coordinate in the process of metabolism, such as the release of a special substrate by plants, which induced the synthesis of two-oxygenase or other enzyme playing an important role in the degradation [11]. As a result, it is an effective method to grow plants in the contaminated soil to improve the environment and promote microbial degradation.

EXPERIMENTAL SECTION

Samples were taken from the contaminated soil in the vicinity of oil storage tanks (2 m deep) in a gas station in Beijing. Separated through enriched cultivation, the strains with strong degradability of diesel oil were screened out from contaminated soils and named Q10, Q14 and Q18.The strains Q10, Q14 and Q18 were identified and classified as Pseudomonas, Flavostrain and Rhodococcus separately. The strains Q10, Q14 and Q18 were mixed in accordance with the volume ratio of 1:1 for the preparation of consortium[12]. The mustard seeds and alfalfa seeds were placed in refrigerator to catalyze at 4℃. After a week, the seeds were soaked for 10min with 10% H2O2, rinsed with de-ionized water. And then they germinated in water. 250W metal halide lamp was used for lighting to provide the necessary solar energy needed for growth of the plants, while the intensity of light was 4900μW cm-2 and the length of lighting 12h d-1.

5 group experiments were designed for degradation of diesel oil. As showed in Table 1, pollutants, strains as well as plants were the factor changed in different groups while other experimental conditions maintained the same.

The experiments were carried out in the loam, which was taken from the site without diesel oil pollutions. The soil samples were air-dried at room temperature, through a 2mm sieve, and then heated at 120℃ for 30min to sterilize[13]. The basic physical and chemical properties of the soil were showed in Table 2. According to the experimental design (Table 1), diesel oil was added to the soil need pollutions by the ratio of 5g kg-1 and mixed completely; so was the 25ml basin bacterial suspension. In accordance with the final design, the plant seedlings were dug out from the seedbed, washed with distilled water and transplanted in the soil.

All the experiments were repeated for three times. After cultured for 3d, 5d, 10d, 15d and 20d respectively, a certain amount of soil were taken from three different parts of the pot and blended completely as the soil sample. 5 g soil samples were weighted respectively to determine the diesel oil content in every sample. Schwab's method was used to extract diesel oil in the soil [11]. The content was determined by ultraviolet spectrophotometer (Varian Cary-50 Probe).

Taking into account the volatility of diesel oil, as well as some non-biodegradable loss, 2 blank experimental groups were designed (Table 1). The first experimental group was as a blank of total experiment. In the second experimental group, the amount of diesel oil reducing as a result of volatile, non-biodegradable and other factors was measured through changes in the content of diesel oil before and after the experiment, and then the experimental results were deducted correspondingly to eliminate the effect of volatile oil, leaching and other non-biodegradable factors. H_2O_2 was used as an extraction agent and the supernatant was measured by UV visible spectrophotometer in 460 nm.

Table 1 Experimentation Template

No.	Pollution	plant	strains
1	-	-	-
2	+	-	-
3	+	-	+
4	+	+	+
5	+	+	-

"+" means "have", "-" means "not have".

Table 2. Physical and chemical properties of the soil

Soil classification (USDA)	pH (in 0.01M $CaCl_2$)	total organic carbon (wt%)	total organic nitrogen (wt%)
loam (Sand 69.5%, clay 18.1%, powder 12.4%)	6.7	1.14	0.32

Change of the strains number in the soil matrix and in the rhizosphere was studied. After cultured for 3d, 5d, 10d, 15d and 20d respectively, 1g soil matrix sample was taken to measure the number of strains in the soil matrix. The number of rhizosphere strains was measured through collecting plant roots, leaching completely with de-ionized water which had been sterilized and oscillation.[14] After cultured for 24h, 1mL solution was taken and diluted. The

concentration of bacteria was by 10^{-2}, 10^{-3} and 10^{-4} times of the original concentration. 0.1ml sample was coated on the flat uniformly that had been prepared and cultured for 24h. Then counted directly and recorded as cfu / mL.

RESULTS AND DISCUSSION

Through the third set of experiments, by adding soil pollutions and strains without plants, the ability of degradation of diesel oil by strains alone could be measured. After degradation for a certain period of time, the content of diesel oil remaining in soil was measured and showed in Table 3. The degradation rates of the diesel oil by strains Q10, Q14, Q18 and consortium were 35.15%, 27.43%, 46.28% and 57.47% respectively in the 5d. All the strains were capable of degradation while consortium and strains Q18 had stronger degradation efficiency.

Table 3 The concentration of diesel oil in soil after biodegraded by bacteria (means±sd)

strains	the concentration of diesel oil/(g kg^{-1})				
	3d	5d	10d	15d	20d
Q10	466.913 ±3.189	428.427 ±5.229	378.233 ±9.266	341.693± 1.611	324.260 ±6.806
Q14	472.307 ±4.010	441.867 ±2.854	401.157 ±4.595	373.757± 3.522	362.83± 1.058
Q18	456.576 ±2.645	405.327 ±2.682	343.773 ±2.812	296.297± 8.706	268.600 ±1.595
Consortium	446.901 ±8.710	385.987 ±9.964	312.930 ±5.609	250.951± 2.926	212.627 ±5.606

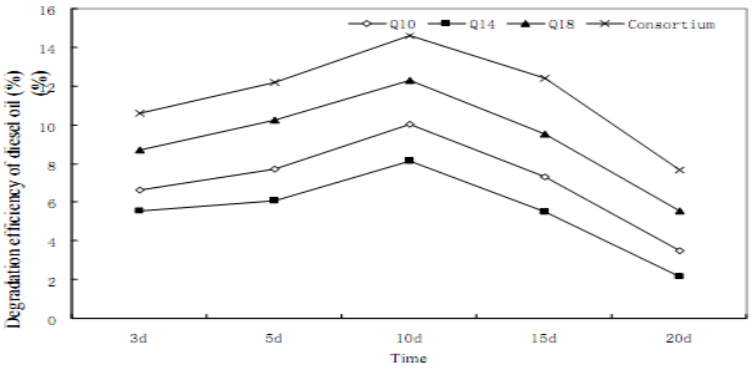

Figure 1. Degradation efficiency of diesel oil by bacteria

The degradation rate of diesel oil was different in different time period. As showed in Fig 1, the trend of degradation rate by the strains Q10, Q14 and Q18 was similar with each other, while the degradation rate kept increasing with the time and reached the maximum in 10d.

In the fourth group of experiments, the effect of alfalfa and mustard on the biodegradation of diesel oil by the strains was studied. Throughout the course of the experiment, alfalfa and mustard were growing well, having no obvious symptoms of being poisoned. As showed in Fig 2, the trend of degradation rate by the strains Q10, Q14 and Q18 cooperated with alfalfa and mustard respectively was also similar with which showed in Fig 1. The degradation rate increased gradually at first, and then began to decline. However, the general degradation rate had been enhanced, and the downward trend of the curve was also more moderate. It's found that the existence of alfalfa and mustard could promote the degradation of diesel oil observably.

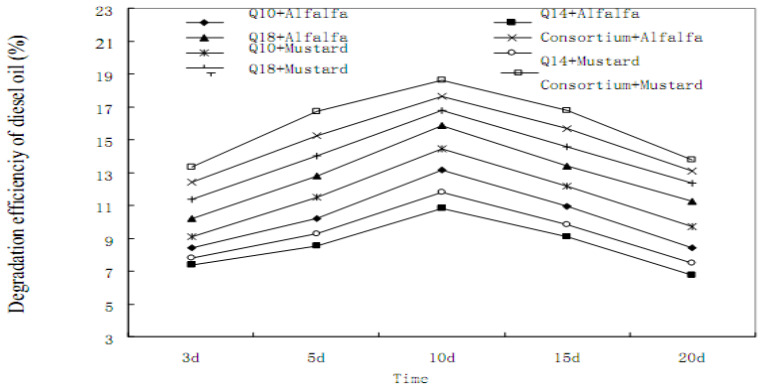

Figure 2. The degradation efficiency of diesel oil by bacteria-plants association

Table 4 listed the total rate of degradation of diesel oil by trains and the complex systems of strains and plants in 20d, and the ability of different strains and consortiums in the biodegradation of diesel oil was compared.

Table 4 The total degradation efficiency of diesel oil at 20d

strains	the total degradation efficiency of diesel oil
Q10	35.15%
Q14	27.43%
Q18	46.28%
Q10- alfalfa	51.18%
Q14- alfalfa	42.59%
Q18- alfalfa	63.55%
Q10- mustard	57.01%
Q14- mustard	46.24%
Q18- mustard	69.17%

The ability of complex system of strain Q10, Q14 and Q18 with alfalfa and mustard in the biodegradation of diesel oil were improved certainly compared with the single strain. In general, mustard - strains complex had stronger ability than alfalfa -strains complex in the degradation of diesel oil. Rhodococcus Q18-Indian mustard complex had the highest rate of degradation of diesel oil. In addition, it's also found that the content of some ions in the soil was also changed after the degradation of diesel oil in the experiment.

Table 5 listed the ion content before and after the degradation by strains Q18 -mustard complex. The total organic carbon content increased while the total organic nitrogen content declined in loam after degradation, which may resulted from the degradation of diesel by strains. The content of Cu, Pb, Cr, Zn and Cd in loam declined to varying degrees, as the mustard might absorb these heavy metal ions. It could be concluded that strains-plants complex was able to change some physical and chemical properties of soil significantly.

Table 5 The chemical properties of soils after biodegradation

	before degradation	after degradation
total organic carbon (wt%)	1.14	7.87
total organic nitrogen (wt%)	0.32	0.11
Cu mg kg^{-1}	32.7	28.3
Pb mg kg^{-1}	38.5	21.7
Cr mg kg^{-1}	9.85	4.21
Zn mg kg^{-1}	182.4	153.5
Cd mg kg^{-1}	0.43	0.27

CONCLUSION

The alfalfa and mustard may enhance the activity of strains and the ability of consortium in the degradation of oil with more developed root systems, as well as faster growth rates. Then in this study, the strains capable of metabolizing diesel oil were isolated from soils polluted by diesel in the gas station. The ability of different strains and consortiums in the biodegradation of diesel oil was compared, and the effect of alfalfa and mustard on the biodegradation of diesel oil by the strains was studied.

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