13

Research on Remediation of Petroleum Contaminated Soil by Plant-Inoculation Cold-Adapt Bacteria

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1. 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 contamination. Micro-Biological degradation of oil pollution was the focus of the present study. Many hydrocarbon-contaminated environments were characterized by low or elevated temperatures, acidic or alkaline pH, high salt concentrations, or high pressure. Hydrocarbon-degrading microorganisms, adapted to grow and thrive in these environments, played an important role in the biological treatment of polluted extreme habitats.

The biodegradation of many components of petroleum hydrocarbons has been reported in a variety of terrestrial and marine cold ecosystems. Cold-adapted microorganisms are potentially interesting for use in environmental biotechnology applications since a large part of the biosphere has low temperatures during at least parts of the year. Many studies have shown that both oil-contaminated and uncontaminated soils in the Arctic, the Antarctic and the Alps contain microbes that can degrade different hydrocarbons deriving from oils[1].For application at cold climate sites, bioremediation approaches are appealing because they have potential to be more efficient and cost-effective than alternative, more energy intensive approaches. Several bioremediation approaches have been reported to be successful for petroleum hydrocarbon-contaminated soils at cold climate sites [2]. Two psychrotrophic bacterial strains isolated from Antarctic seawaters were investigated for their capability to degrade commercial diesel oil at 4 and 20°C over a period of two-months. The result suggested the possible exploitation of two bacterial strains in future biotechnological processes, directly as field-released micro-organisms both in cold and temperate contaminated marine environments [3].

The cold-adapted bacterial communities were also studied. Some research suggested that geographical origin of the samples, rather than petroleum contamination level, was more

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important in determining species diversity within these cold-adapted bacterial communities [4]. Predominant populations from different soils often included phylotypes with nearly identical partial 16S rRNA gene sequences (i.e., same genus) but never included phylotypes with identical ribosomal intergenic spacers (i.e., different species orsubspecies)[5]. Twenty-two polycyclic aromatic hydrocarbon (PAH)-degrading bacterial strains were isolated from Antarctic soils with naphthalene or phenanthrene as a sole carbon source and all these strains showed a high efficiency to degrade naphthalene at 4°C, and some additionally degraded phenanthrene. Phylogenetic analysis showed that all belonged to the genus Pseudomonas except one that was identified as the genus of Rahnella[6]. 32 cold-adapted, psychrophilic and cold-tolerant, yeast strains isolated from alpine habitats with regard to their taxonomy, growth temperature profile, and ability to degrade phenol and 18 phenol-related mono-aromatic compounds at 10°C [7].

Cold-adapted microorganisms inhabit in most parts of the surface of earth and the annual average temperatures of these regions are below 15°C, but these temperatures are far lower than the optimum growth temperatures of mesophilic microorganisms which have been widely used in bioremediation technology at present [8]. In most parts of China, it was cold in winter and cool in spring and autumn, when the microbial survival rate was low and the natural degradation capacity was poor. Cold-adapted microorganisms which had special genetic background and metabolic channels were widely distributed in the nature at low temperatures. The cold-adapted microorganisms could produce a lot of active substances, and show unique characters in many areas at low temperatures [9].

On the other hand, bioremediation was considered to be the most cost-effective and environmental friendly technology to treat oil-contaminated soil currently. And the combination of microbe and plant would be practical and effective.

Plants could be used to remediate organic or inorganic polluted soils. This technique had been applied widely in practice, named as phytoremediation. The plant roots improved the water content in the polluted soil. The increased microbial activities induced by the improved rhizosphere microecosystem could be used to enhance biodegradation of petrochemicals and bioremediation of polluted soil[10].

In this study, oil-contaminated soil in Tianjin and was used for domestication at different temperatures. Two different communities of bacteria depend on crude oil as sole carbon source were found and the main foundation of temperature on the microbial community screening was researched and revealed. The biodegradation characteristic was studied then. The effect of combined remediation by winter wheat and cold-adapted degrading bacterial was examined. In order to explore the effect of combined remediation by bacterial and plant at low temperature on petroleum hydrocarbons contaminated soil, the TPH removal and the catalase activity were examined at different incubation time in different treatments.

2. Materials and methods

2.1 Domestication and purification of petroleum – Degradation strains

Crude oil and petroleum contaminated soil samples were taken from Dagang oil field (1m deep) in Tianjin. Well drainage was also taken from the well in Dagang oil field. Magnesium sulfate (AR.), Di-hydrogen phosphate ammonia (AR.), Sodium nitrate (AR.), Potassium

nitrate (AR.) and di-Potassium hydrogen phosphate (AR.) were obtained from the Beijing Chemical Factory. All the chemicals were used as received. The restriction endonuclease was purchased from Promega. The nucleic acid electrophoresis apparatus (Bio-Rad) and GDS gel imaging system (Gene company) was prepared.

Inorganic drainage liquid medium: NaCl 5 g/L, K_2HPO_4 1 g/L, $NH_4H_2PO_4$ 1 g/L, $(NH_4)_2SO_4$ 1 g/L, $MgSO_4$ $7H_2O$ 0.2 g/L, $KNO_3$3 g/L. Well drainage was used as solvent and pH adjusted to 7.0. 1mL sterilized crude oil was joined to 100mL inorganic liquid medium to make crude oil inorganic liquid medium. 1g soil sample polluted by petroleum hydrocarbons was dissolved with saline water and cultured in a liquid medium supplemented with petroleum oil as the sole source of carbon and energy for 10 days on 10°C and 25°C respectively. Then a certain amount of the above culture solution was inoculated and cultured in a mineral liquid medium supplemented with petroleum oil as the sole source of carbon and energy for 10 days again. The step was repeated again. The culture solution was diluted (10^{-1} ~ 10^{-8}) separately. 50ul culture solution was coated to the solid culture medium and cultured for 5 days. The flats of proper concentration were selected in different temperatures. All the colonies were inoculated to flat plate to sieve again. Purified strains were cultured in test-tube and stored in refrigerators at 4°C.

2.2 Polymerase Chain Reaction (PCR) assays

The purified cultivated strains were used as template and the primers used for the PCR were 8F and 1492R [11]. (Table 1).

Primer	Position	Primer sequences
8F	8-27	5′-AGAGTTTGATCCTGGCTCAG-3′
1492R	1492-1511	5′-CGGTTACCTTGTTACGACTT-3′

Table 1. The sequence and position of primer.

PCR reaction system contained 2 x PCR mixture 25ul (0.1 U Taq Polymerase/ul, 500uM dNTP, 20mM Tris-HCl [pH8.3]), 100mM KCl, 3 mM $MgCl_2$, 2ul primer 8F (5uM), 2ul primer 1492R (5uM), 2ul template and 19ul double steamed water in a final volume of 50ul.

The thermal cycling was as follows: initial denaturation at 94 ˚C for 6min, followed by 30 cycles of 30s at 94˚C, 40s at 52 ˚C, 1.5 min at 72 ˚C, and a final extension of 10 min at 72˚C. The PCR products were analyzed by running 5ul aliquots of the reaction mixtures in 1.2% agarose gels.

2.3 Genotyping of individual isolates by ARDRA (Amplified Ribosomal DNA Restriction Analysis)

Strains were analyzed by ARDRA using two restriction endonuclease enzymes (RsaI and MspI). Restriction analysis of the PCR products was performed in a 10ul reaction containing: 1ul restriction enzyme buffer, 0.3ul restriction endonuclease and 5ul PCR product. Reactions were then incubated at 37°C for 3h and stopped at 60°C. Restriction fragments were separated on 3% agarose gels. Gels were photographed after staining with ethidium bromide with the imaging system Bioprint.

2.4 Biodegradation of petroleum by cold-adapted petroleum-degrading bacteria

Each triangle flask was filled with 200mg sterilized crude oil and 50mL inorganic salt liquid medium (4g/L), pH7.0. Strains were cultured in the beef extract peptone liquid medium for 48h at 25°C and then centrifuged at 4000 r / min for 10 min to prepare suspending solutions. 2mL suspension was inoculated in each triangle bottle. The sample with no suspension was blank sample. All the samples were cultured at 150 r / min at 10°C. Bacteria density was measured at different time. The strains which grew well were chosen and for the next experiments.

60g crude oil was delivered to 1000g clean soil and blended for many times and then the mix was sterilized to prepare petroleum contaminated soil (6%). Different test conditions were set respectively: soil with bacteria D17 added, soil with bacteria D24 added, soil with mixed bacteria (D17 + D24) added and blank contrasts. Petroleum hydrocarbons degradation rate was determined in different conditions at different time. The oil in soil was extracted by accelerated solvent extraction system (APLE, Beijing Titian Instruments Co., Ltd, China) and determined by weight method. The extraction condition by APLE was set as follow: extraction pressure -10 M Pa, heating time- 100 s, static time- 720 s, elution volume- 60%, purge time- 60 s, preheating temperature-170°C, heating temperature-170°C and followed by a cycle. The solvent was hexane/ acetone =1：1 (V/V).

2.5 Combination remediation of petroleum contaminated soil by degrading bacterialD17 and winter wheat

The experimental soils used for this study were collected from an agriculture field in Beijing, China. The soil was dispersed and mixed, then passed through a 5-mm mesh. The soil material was first thoroughly mixed with of oil by different mixing ration and then dried. And the oil content was measured 20 days later while the concentration was 1409.13mg/kg, 2060.26mg/kg and 3273.40mg/kg respectively. The basic soil properties were determined using standard methods recommended by the Chinese Society of Soil science. The basic properties of the soil were shown in table 2. The cold-adapted bacterial used in this study was D17. The experiment condition was showed as Table 3.

The soil of every pollution level was treated as below: first, combined remediation: planted winter wheat and added degrading bacteria. Second, microbial remediation: added the degrading bacteria; Third, the control: no planting and no bacteria. All treatments were shown in Table2.

The study was performed in the incubator which is 1 meter wide, 1.2 meter long and 1meter deep. The soils were put into the incubator and the depth of the soil was 60cm. Transplanted the winter wheat into the incubator with the row spacing of 17cm and added N, P, K slow-release fertilizer to the soil. 360ml bacteria liquid was added to the soil per square meter on February 5, and the OD value was 0.8366. Then 150 ml bacteria liquid was added to the soil per square meter again on April 7th, and the OD value was 0.8433. Simulated conventional irrigation farming conditions during the whole incubation time.

All treatments were incubated in the greenhouse for 126 days, from February 5th to June 10th. The varieties of soil enzymes and oil content with incubation time under different treatments were analyzed. The rhizosphere soil and non-rhizosphere soil of winter wheat

Oil content(mg/kg)	Total N	Total P	Total K	pH
1409.13	0.028%	0.046%	1.74%	8.13
2060.26	0.096%	0.058%	2.02%	8.26
3273.40	0.016%	0.048%	1.65%	8.29

Table 2. Some physical and chemical properties of tested soil.

The catalase was tested by the reduction of potassium permanganate. The results were expressed as an average of three parallel determinations of soil samples.

sample	Pollution level	Oil content(mg/kg)	Experiment treatment
A1	1	1409.13	Combined remediation
A2	2	2060.26	Combined remediation
A3	3	3273.40	Combined remediation
B1	1	1409.13	microbial remediation
B2	2	2060.26	microbial remediation
B3	3	3273.40	microbial remediation
C1	1	1409.13	control
C2	2	2060.26	control
C3	3	3273.40	control

Table 3. Experimental treatment.

were taken on February 5th, March 4th, April 28th, May 8th and June 10th respectively. Record February 5th the first day of the experiment, then the sampling time were the 1stday, the 53rdday, the 83rd day, the93rd day and the 126thday respectively.

Petroleum Hydrocarbon in soil samples was analyzed by using an APLE-2000 system. Data treatments were performed using Excel 2003 and SPSS 17.0 for windows.

3. Results and discussion

3.1 Microbial community of oil-contaminated soil at different temperature conditions

Strains was analyzed by ARDRA and different genotyping was obtained in different temperature conditions. Each genotyping was conducted as an operational taxonomic unit (OTU). All the strains were analyzed with 16srDNA and the sequence subjected to BLAST GeneBank analysis (Table 4 and Table 5).

Considering from the composition point of view, 8 OTUs has been domesticated at 25 ˚C. Analyzed and retrieved in GeneBank Database, *Bacillus*, *Sinorhizobium*, *Rhizobium*, *Chryseobacterium* and *Bartonella* were homologous and the dominant bacteria were *Rhizobium* and *Bacillus*. Meanwhile, 11 OTUs has been domesticated at 10˚C, which were *Arthrobacter*, *Micrococcus*, *Rhodobacter*, *Bacterium*, *Paracoccus*, *Rhizobium* and *Halomonas* and the dominant bacteria were Arthrobacter and Halomonas. Some studies had showed that parts of Rhizobium strains could survive by using organic sulfur instead of carbon [12] for energy. It was reported that Bacillus had the ability of degrading petroleum hydrocarbon. It was also

OTU	Species with most similar sequence (Accession number)	Similarity /%	Number of Strains
OTU 1-C1	Bacillus sp. KDNB4(EU835566)	99	5
OTU2- C3	Sinorhizobium sp. CCBAU 05296(EU571251)	98	1
OTU3 -C4	Rhizobium sp. SL-1(EU556969)	98	8
OTU4 -C6	Bacillus sp. cp-h46(EU584545)	99	1
OTU5- C10	Chryseobacterium sp. LDVH 42(AY468475)	99	1
OTU6- C13	Bacillus sp. cp-h45(EU584544)	99	1
OTU7- C14	Bacillus megaterium(EU931553)	99	2
OTU8- C15	Bartonella elizabethae(AB246807)	98	1

Table 4. The cultured bacteria and their most closely matched species in Gene Bank(25°C).

OTU	Species with most similar sequence (Accession number)	Similarity /%	Number of Strains
OTU1′-D2	*Arthrobacter bergerei*(AJ609633.2)	98	7
OTU2′-D8	*Micrococcus* sp. BSs20065(EU330348)	95	2
OTU3′- D9	*Rhodobacter changlensis*(AM399030.1)	97	1
OTU4′-D10	*Bacterium* Ips_3(DQ836709)	98	1
OTU5′- D11	*Micrococcus* sp. PB7-11B(EU394442)	97	3
OTU6′- D14	*Paracoccus* sp. WPCB175(FJ006918)	98	5
OTU7′- D17	*Paracoccus* sp. 428(EU841535)	98	1
OTU8′-D18	*Rhizobium* sp.28(DQ310471)	98	1
OTU9′-D19	*Halomonas* sp. Claire(AJ969933)	98	8
OTU10′-D22	*Paracoccus* sp. B-1018(DQ270725)	98	1
OTU11′-D24	*Halomonas* sp. ice-oil-302(DQ533958)	98	1

Table 5. The cultured bacteria and their most closely matched species in Gene Bank (10°C).

reported that lots of *Arthrobacter bergerei* could degrade oil effectively [13, 14]. Some studies suggested that degradation rates could reach 50% after 4d while others found a 90.8% degradation rates after 20d [13, 14]. *Arthrobacter bergerei* were likely to formed biological emulsifier when they used petroleum as carbon sources. The results showed that *Halomonas* was also feasible for high-sodium soil contaminated by petroleum and could be used for bioremediation in future.

It was obvious that the community composition was more diverse under the conditions of 10°C than of 25°C (Fig. 1). The microbial community diversity was significantly higher

under the conditions of 10 °C. The dominant bacteria were different in different temperature conditions and Rhizobium was the only same genus. 40% of the total community composition was Rhizobium at 25°C and the proportion at 10°C was 3%.On the other hand, the total bacterial concentration at 10°C was 6.2 × 10^5 CFU/mL and which under the conditions of 25°C was 4×10^8 CFU/mL. The bacteria abundances was higher at 25°C.Low temperature condition would inhibit growth of microbes. As a result, the temperature had a strong impact on the composition of soil microbial communities.

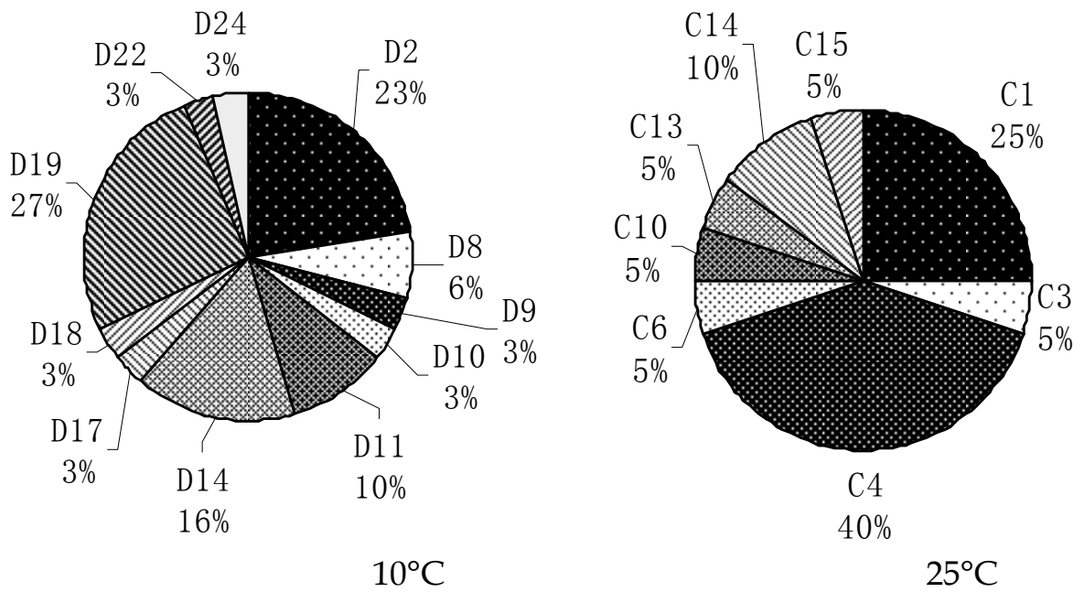

Fig. 1. Comparison of microbial community structure of 10°C and 25°C.

3.2 The bacteria growth situation in liquid medium at 10°C

31 strains were domesticated and purified at 10°C and 11 genotypes were obtained. The growth situation of the 11 kinds of bacteria cultured in a liquid medium supplemented with petroleum oil as the sole source of carbon and energy at 10°C was researched. Bacteria density was detected after being cultivated for 4d, 11d and 20d respectively (Table 6). After being cultivated for 20d, crude oil was emulsified by some of the bacteria while D17 and D24 could produce emulsification to a greater degree. Some studies showed that a lot of strains which used petroleum hydrocarbons as the sole source of carbon and energy could secrete various biological emulsifier and biosurfactant [15]. The increment of D2, D9, D17 and D24 was clear in liquid medium while which of D17 and D24 was obvious especially. Therefore, D17 and D24 were selected and their ability of degradation of petroleum was researched then.

Representative strains	Bacteria density after 4d (A_{600})	Bacteria density after 11d (A_{600})	Bacteria density after 20d (A_{600})
blank	0.0018	0.0152	0.0189
D2	0.0351	0.0838	0.5309
D8	0.0362	0.0402	0.0377
D9	0.0236	0.0473	0.4652
D10	0.0282	0.0143	0.3822
D11	0.0907	0.017	0.324
D14	0.0095	0.0335	0.3463
D17	0.8591	1.3843	1.6063
D18	0.0051	0.0715	0.6154
D19	0.132	0.0299	0.3453
D22	0.0125	0.0635	0.3249
D24	0.1064	1.1922	1.5301

Table 6. The bacteria growth situation in liquid medium at 10°C.

3.3 The biodegradation of petroleum by cold-adapted bacteria

As showed in Figure 2 and 3, both of the strains had ability of degrading petroleum at different temperature. The degradation rates were analyzed at 0d, 25d, 50d, 75d, 100d respectively. The degradation rate was 25.8% by D17, 25.3% by D24 and 21.9% by D17+D24 at 25°C while which was 11.3%, 10.2% and 10.3% respectively at 100d. Low temperature would affect the growth and enzyme activity of bacteria .However, it was suggested that the degrading of petroleum by cold-adapted bacteria was feasible at lower temperature.

In the fourth group of experiments, the effect of D17 and D24 on the biodegradation of crude oil was studied. Throughout the course of the experiment, D17 had a better effect not

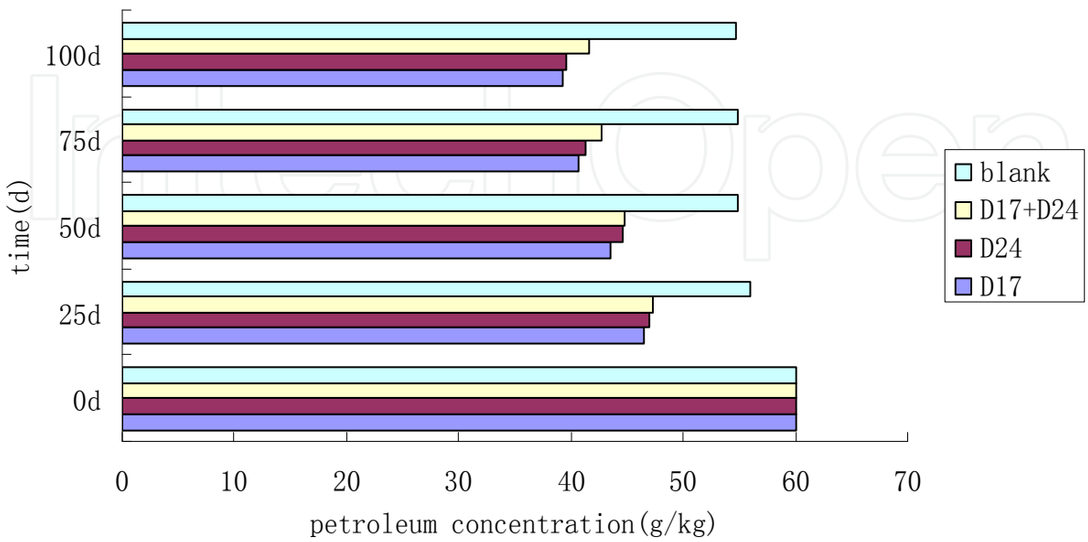

Fig. 2. The biodegradation of petroleum by cold-adapted bacteria at 25°C.

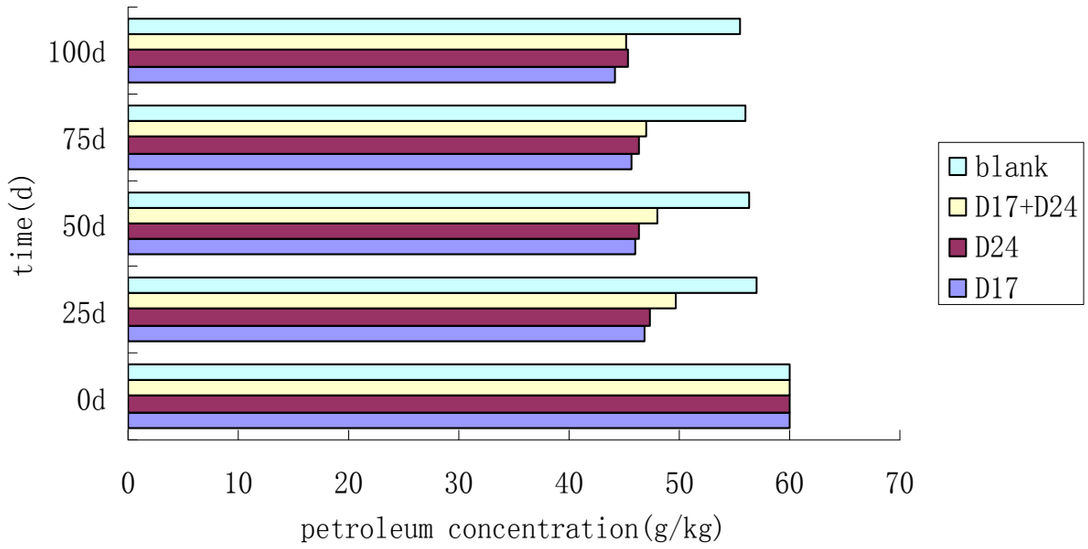

Fig. 3. The biodegradation of petroleum by cold-adapted bacteria at 10°C.

only at 25°C but also at 10°C. As showed in Figure 3, the trend of degradation rate by the strains D17, D24 and D17+D24 respectively was also similar with which showed in Figure 2. However, the general degradation rate by D17+D24 was lower than which by D17 and D24 respectively. It was concluded that the degradation may be inhibited when the strains existed at the same time.

3.4 The effect of petroleum pollution of soil on growth of the winter wheat

When petroleum pollutant leaked into the soil environment, it would damage the plant ecosystem structure and function of the microbial, soil, the plant growth, seed and fruit quality, etc [16]. The higher the petroleum concentration became，the more rapidly the germination, growth and yield of crops declined[17].

The height of winter wheat was similar when transplanting. After transplanting, the winter wheat grew in the same environment. With the increase of culture time, the height and stem width of wheat varied. The stem width decreased in accordance with the increase of the TPH content. Figure 4 showed the average height of wheat when harvested in different contaminated concentration soil. The figure showed that with the increase of the initial concentration of oil, the height of wheat decreased. All theses indicated that the petroleum pollution had certain inhibition effect on the growth of the winter wheat.

3.5 Changes of soil catalase activities with time

The responses of soil enzymes activities to pollutant exposure could be used to evaluate the soil microbial properties. Enzyme activity was widely used to monitor soil pollution and remediation process [18]. Catalase in the soil can undermine the hydrogen peroxide which was toxic to the organisms. Catalase could be induced by environmental harmful factors, and the activity status reflects the stress situation of the environment to some extent [19].

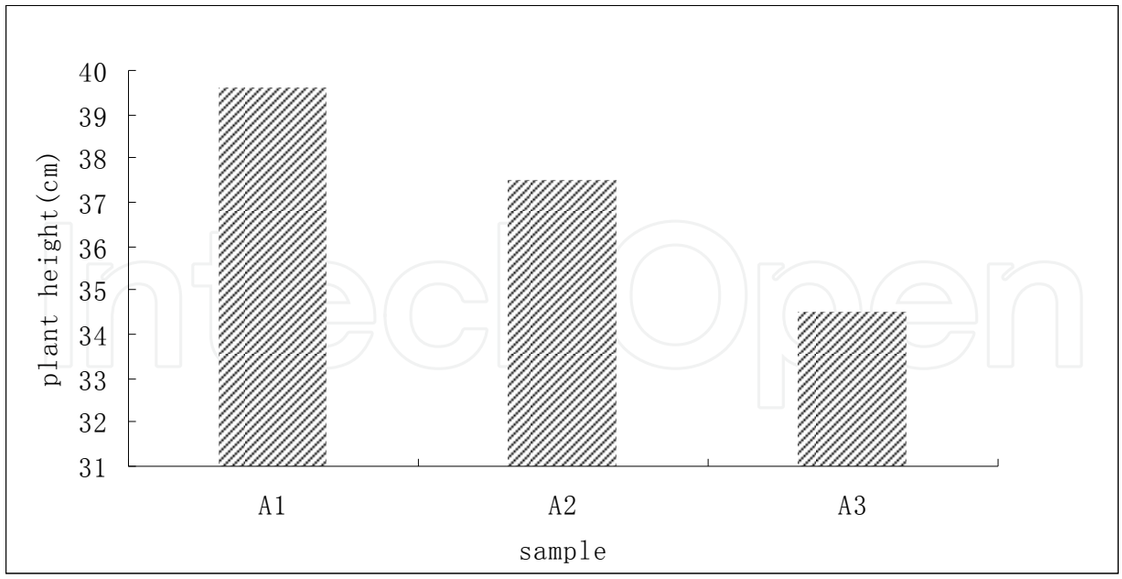

Fig. 4. Height of winter wheat when harvested.

Figure 5 showed the catalase activity of each soil sample at different incubation time in the combined remediation. The figure showed that the catalase activity increased after the combined remediation, however there was a decrease in catalase activity in April. The catalase activity was closely related to the aerobic microorganism quantity and the soil fertility. So the reason for this phenomenon might be related to the decline of the microbe quantity or the shortage of soil fertility. The decline of the microbe quantity might be caused by the competition of the inoculating microbes and the indigenous microorganisms.

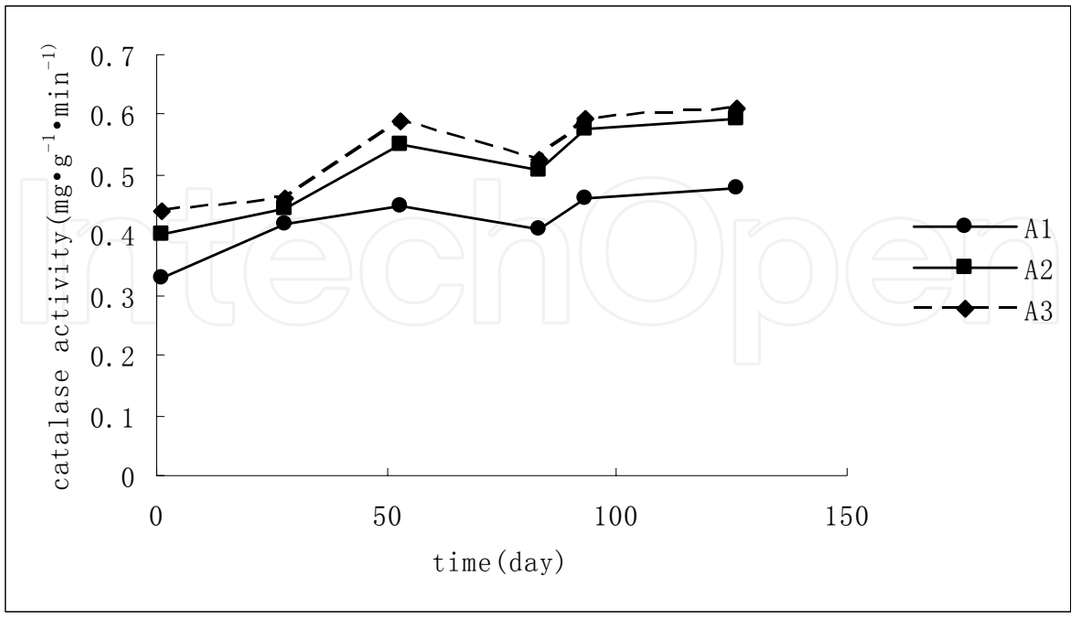

Fig. 5. Soil catalase activities in different soil samples of combined remediation at each incubation time.

3.6 Comparison of degration between combined remediation and microbial remediation

The degradation of petroleum hydrocarbon in soil was the combined effect of physical, chemical and biological processes. In general, the effect of natural process was limited [20]. In this study，the natural degradation was limited too.

There were indigenous microorganisms in the soil, the species and quantity of which were larger than the inoculating microbes. In these conditions, the inoculating microbes needed to adapt the complex environment and compete with the indigenous microorganisms [21].

The TPH removal after remediation in each treatment was shown in Figure 6. The figure showed that the TPH removal of combined remediation was higher than that of microbial remediation, which indicated that the winter wheat promoted the TPH degradation by microorganism.

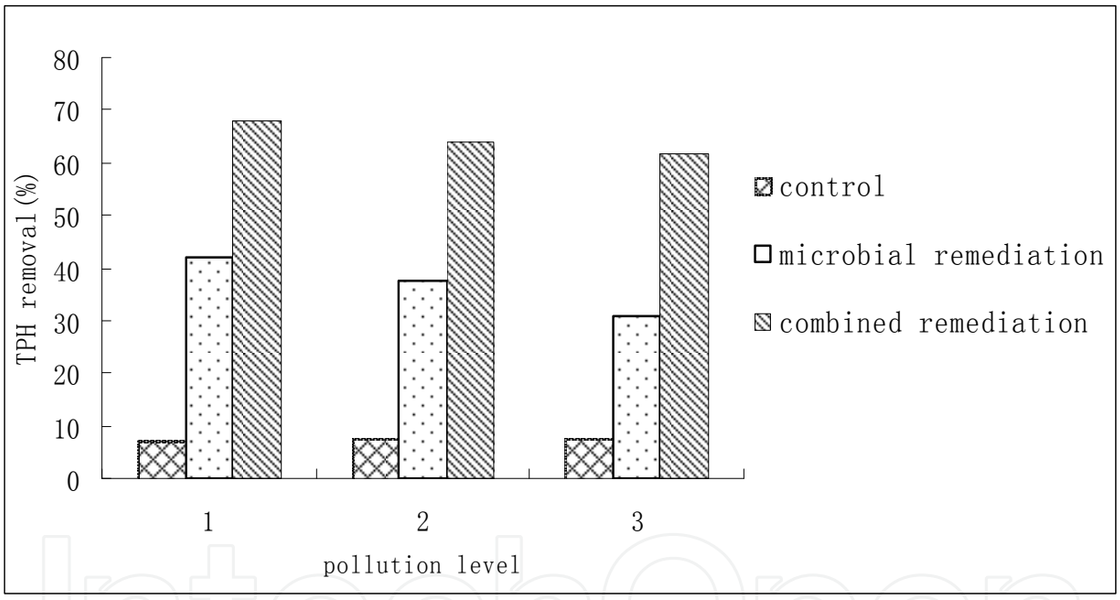

Fig. 6. TPH removal in different treatment after remediation.

3.7 Combined remediation of the petroleum polluted soil

Figure 7 showed the oil content of each soil sample at different incubation times. From the figure, it could be concluded that the degradation trend of petroleum is similar. And there was positive correlation between the catalase activity and the TPH removal. Figure 8 showed the removal rate of TPH by combined remediation at different time. In the initial month, the ground temperature was low when the enzyme activity and the degradation ability of microorganisms were at a low level. Thus the TPH removal rate was slow. With the increase of the ground temperature, the wheat began to turn green when the wheat root began to active. In the same time the TPH removal rate of the oil increased.

Some studies indicated that root quantity was the minimal in earlier winter stage, latter with the winter wheat grew, to flowering stage, reached the largest. Then the root quantity continuously reduced, and to the ripe stage, achieved roughly the same level with that in the over wintering stage. And there were two times that the root quantity changed fastest. One was the rapid rising period from the reviving stage to the flowering stage, the other was the rapid decline period from the flowering stage to the ripe stage[22].

Figure 8 showed a sharp decrease in the TPH removal rate in April when the winter wheat was in the jointing stage. According to the variation of root quantity and TPH removal rate, it could be concluded that the TPH removal rate increased in the reviving stage, reduced in the jointing stage, recovered in the flowering stage, and then reduced again in the ripe stage.

The reason for the decline in the jointing stage might be related to the reducing of microorganisms quantity caused by the added of inoculating microorganisms, or the shortage of soil fertility which would influence the degradation of TPH by the microorganisms. The specific reasons couldn't be determined, so a further study was needed to analyze the influencing factors on the TPH removal in the jointing stage of the winter wheat. After the florescence stage, the TPH removal rate was at a low level. It might be related to the reducing of root quantity. From the study, it could be inferred that the main period for the TPH removal was from the turning green stage to the flowering stage.

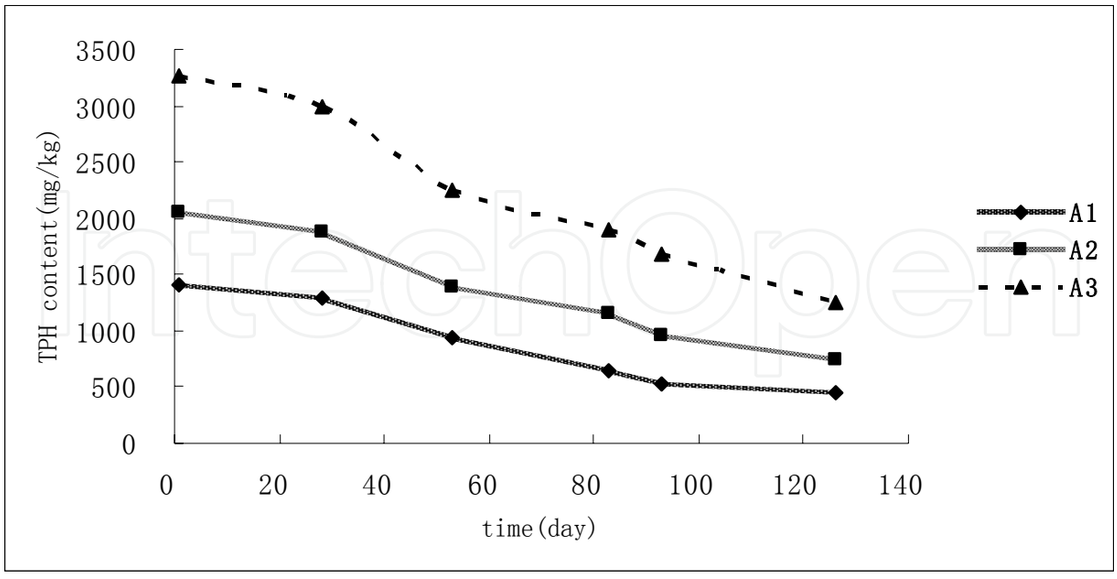

Fig. 7. Changes of TPH content in each treatment at different incubor time.

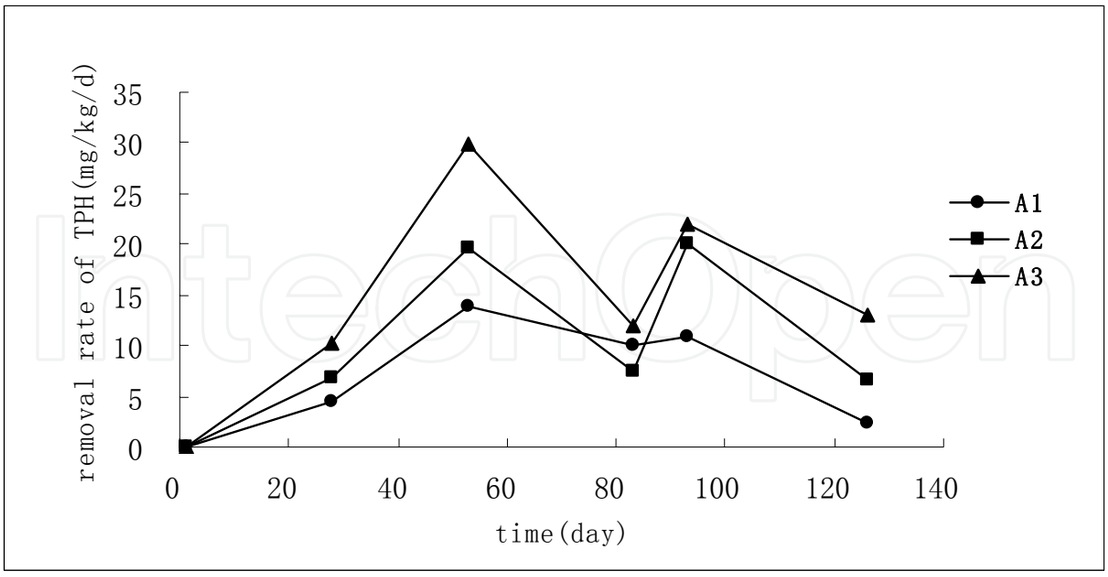

Fig. 8. Changes of TPH removal rate with time in the combined Remediation.

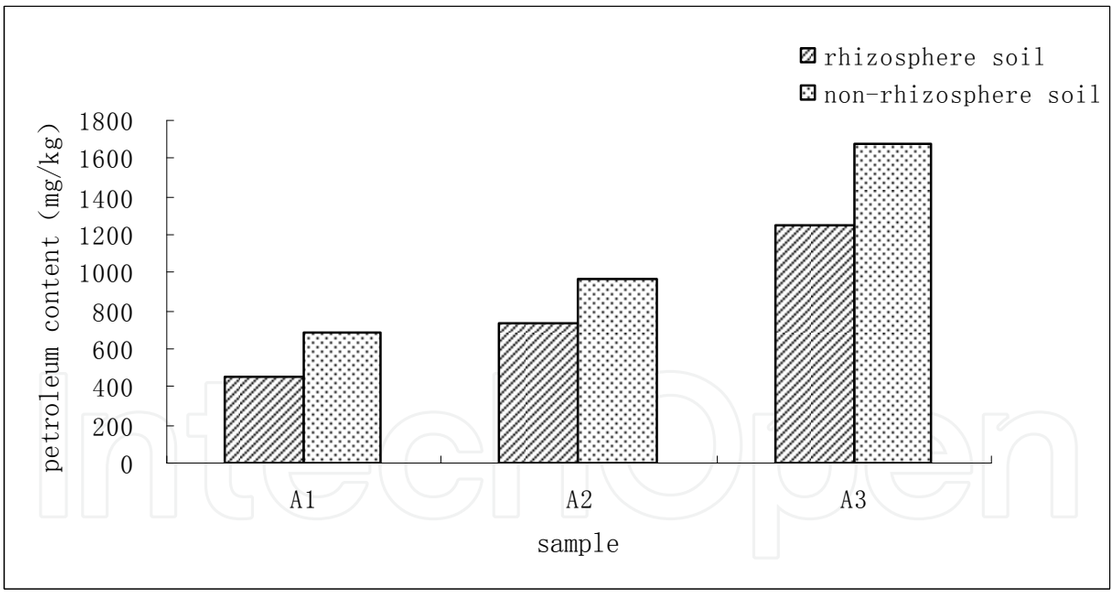

Fig. 9. Petroleum content in non-rhizophere soil and rhizosphere soil of the winter wheat after combined remediation.

The physical and chemical characteristics in rhizosphere soil were significantly different from that in non-rhizosphere soil. After the combined remediation of 126 days, there was a significant difference between the TPH removal in the rhizosphere soils and that in the non-rhizosphere soils, as showed in figure9. The TPH removal in the rhizosphere soil was higher than that in the non-rhizosphere soil.

4. Conclusions

The total bacterial concentration at 10°C was 6.2 × 10^5 CFU/mL and which under the conditions of 25°C was 4×10^8 CFU/mL. It was obvious that the community composition was more diverse under the conditions of 10°C than of 25°C. The microbial community diversity was significantly higher under the conditions of 10 °C. The dominant bacteria were different in different temperature conditions and Rhizobium was the only same genus. 40% of the total community composition was Rhizobium at 25°C and the proportion at 10°C was 3%.As a result, the temperature had a strong impact on the composition of soil microbial communities.

31 strains had been isolated in the low temperature conditions. With preliminary degradation test at 10°C, D17 and D24 had been selected to be used. D17 was identified as *Paracoccus sp.*, and D24 was identified as *Halomonas sp.* The degradation rate was 25.8% by D17, 25.3% by D24 and 21.9% by D17+D24 at 25°C while which was 11.3%, 10.2% and 10.3% respectively. It was suggested that the degrading of petroleum by cold-adapted bacteria was feasible at lower temperature.

The combination of the winter wheat and the cold-adapted bacterial had great potential ability in the remediation of the petroleum contaminated soil. In the rhizosphere soil system, TPH disappeared faster than that in unvegetated pots soil. The main period for the TPH removal was from the turning green stage to the flowering stage. During the period of combined remediation, the TPH removal rate varied in different stages. And a further study was needed in order to study the influencing factors in different stages of the winter wheat, thus we could take different measures in different stages. As incubation proceeded, the catalase activity in rhizosphere soil was improved by planting winter wheat, which indicated that the microbial growth and the metabolic activity were enhanced. And there was positive correlation between the catalase activity and the TPH removal.

5. Acknowledgment

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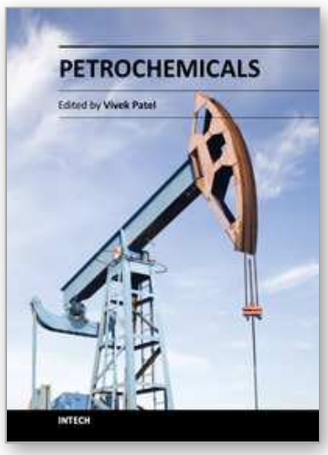

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The petrochemical industry is an important constituent in our pursuit of economic growth, employment generation and basic needs. It is a huge field that encompasses many commercial chemicals and polymers. This book is designed to help the reader, particularly students and researchers of petroleum science and engineering, understand the mechanics and techniques. The selection of topics addressed and the examples, tables and graphs used to illustrate them are governed, to a large extent, by the fact that this book is aimed primarily at the petroleum science and engineering technologist. This book is must-read material for students, engineers, and researchers working in the petrochemical and petroleum area. It gives a valuable and cost-effective insight into the relevant mechanisms and chemical reactions. The book aims to be concise, self-explanatory and informative.

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