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SOILS, SEC 3 • REMEDIATION AND MANAGEMENT OF CONTAMINATED OR DEGRADED LANDS • RESEARCH ARTICLE

Bioremediation of oil-contaminated soil by combination of soil conditioner and microorganism

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Abstract

Purpose Oil hydrocarbons are widespread pollutants in soil which pose serious threats to ecological environment. Thus, this study carried out the bioremediation of oil-contaminated soil by using the efficient petroleum-degrading bacteria and soil conditioner, to investigate the changes of physicochemical properties of contaminated soil during bioremediation, reveal the relationship among the exogenous degradation strains and indigenous microbe, and finally illuminate the effects of soil conditioner and microbe on the bioremediation of oil-contaminated soil.

Materials and methods A PAH-degrading strain named *Stenotrophomonas maltophilia* was used in this study, which was isolated from an e-waste dismantling area. The soil conditioner in this present study was developed previously by using agricultural wastes, which was in a powdered form and rich in N, P, and K. The simulated experiments were conducted under the control environmental conditions of greenhouse, to study the effects of inoculation and soil conditioner on bioremediation of oil-contaminated soil. Then, the physicochemical properties of soil and the degradation rates of oil were measured at different set times to evaluate the bioremediation effect.

Results and discussion Adding 1% soil conditioner could significantly improve the soil conditions and offer microorganism enough N, P, and K, which would promote microbial growth and played a key role on bioremediation of oil-contaminated soil. Although in polluted soil, the microorganism could maintain metabolic activity and use the petroleum as carbon source. The soil indigenous microbe was more easily to adapt to the contaminated surrounding. However, when both of them co-existed in soil, they would restrain each other, and go against the oil decomposition. Thus, making two types of microorganisms work to achieve synergy was the key to gain much better remediation effect. Because the indigenous microbe was good at decomposing low molecular compounds and saturated hydrocarbons, while the oil-degrading strains can effectively decompose high molecular weight aromatics.

Conclusions The soil nutrient and microorganism, including the exogenous oil-degrading strains and the soil indigenous microbe, had an important effect on degradation of petroleum. The addition of soil conditioner, presence of indigenous microbe, and inoculation of oil-degrading strains all were conducive to bioremediation of oil-contaminated site, but the key was to control the proportion and relationship of the three.

Keywords Oil-contaminated · Bioremediation · Soil conditioner · Microorganism · Physicochemical properties · Biodegradation

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1 Introduction

As an important source of energy in modern society, petroleum occupies a very important position in the national economy. However, with the rapid development of oil drilling, petrochemical industry, and the wide use of petroleum products, the soil pollution of petroleum has become a serious environmental problem. Long-term petroleum pollution, especially the contamination of polycyclic aromatic hydrocarbons (PAHs) that are one kind of persistent organic pollutants, not only led to damage of soil structure and inhibition of plant growth but also threatened human health. Because the PAHs were carcinogenic, mutagenic, and teratogenic (Khamehchiyan et al. 2007; Nie et al. 2011; Singh et al. 2012; Varjani et al. 2017), the pollution of petroleum caused serious damage to soil ecological environment, and led to great economic losses to the country and society (Boopathy 2004; Onwurah et al. 2007). Thus, the oil contamination has become an urgent environmental issue, which we had to face in the sustainable developing society.

The bioremediation technology developed from the 1980s, and it has become one of the most popular environmental remediation technologies because of its low cost, high efficiency, easy operation, and environmental friendliness (Chaineau et al. 2005). There were some natural bioremediation methods in nature, mainly through a process called natural attenuation, which depended on the inherent biodegradation potential of indigenous microbial populations in the polluted soil to degrade and remove pollutants in the soil (Abena et al. 2019). Sometimes in order to promote the degradation and removal of the organic pollutants, adding the specific exogenous high-efficiency degradation bacteria was a good way. Many reports had manifested that microbial degradation serves potentially considerable applications. There were many microorganisms in environment, which had ability to decompose petroleum hydrocarbon. Isolating and inoculating this kind of degrading bacteria had been proved to improve the removal of petroleum hydrocarbon in polluted sites (Wentzel et al. 2007; Kuyukina et al. 2013; Xu et al. 2014; Liu et al. 2016; Polyak et al. 2018). However, the biodegradation of petroleum in soil was a very complex biological process. It was still not clear that exogenous strains could promote or inhibit the degradation when there were co-existing indigenous microorganisms. Therefore, in order to effectively improve the degradation efficiency, exploring the interaction between indigenous microorganisms and exogenous microorganisms was quite important. Furthermore, the activity of microbe was affected by various environmental factors, which would affect the efficiency of bioremediation. And this was also the reason for the limitation of wide application of this technology (Varjani and Upasani 2017). Therefore, it was important to study the effect of soil conditioner and microbe on soil bioremediation, especially making clear the changes of physicochemical properties of polluted soil and the microbial metabolic activity during bioremediation, which helped to promote the degradation of petroleum and improve the efficiency of bioremediation.

In fact, petroleum hydrocarbon in soil could provide a large amount of available carbon sources for microbe, but the absence of nutrients, such as nitrogen and phosphorus, would limit the activity of microorganism, then inhibit the biodegradation of petroleum hydrocarbon. Numerous researches have shown that addition of plenteous nitrogen and phosphorus nutrient in oil-contaminated sites could improve the metabolic activity of soil microbe and promote them to degrade petroleum hydrocarbons efficiently (Dias et al. 2012; Hussain et al. 2018; Lindsey et al. 2018; Roy et al. 2018). Therefore, this study carried out the bioremediation of petroleum-contaminated soil by using the efficient petroleum-degrading bacteria isolated in laboratory, and adding the self-developed soil conditioner. The aim was to investigate the changes of physicochemical properties of contaminated soil during bioremediation, and to find out the relationship among the soil conditioner, exogenous degradation bacteria and indigenous microbe, and their effect on the bioremediation of oil-contaminated soil. Finally, the results could serve as a theoretical basis for the practical application of bioremediation technology, and promote its development.

2 Materials and methods

2.1 Preparation of oil-contaminated soil

The oil sample used in this study was obtained from Guangzhou Petrochemical Complex. The blank soil sample (as the control soil) was collected from an un-contaminated arboretum in the South China Agricultural University, located in Guangzhou, China. The soil was passed through a 2-mm sieve to remove stones, concrete, large paint residues, and any other impurities. Then, the sieved soil was divided in two fractions; one was autoclave sterilized and the other one was being air-dried before use. To prepare oil-contaminated soil, the blank soil was spiked with 20 g kg^{-1} oil, stirring well until blended. The physical and chemical properties of different experimental soils are given in Table 1.

2.2 Inoculation and soil conditioner

2.2.1 Effective strain and microbial culture

The effective strain was isolated from an e-waste dismantling area in the town of Guiyu in Guangdong Province, China. It had been identified as *Stenotrophomonas maltophilia* and proved to be a potential strain for PAH biodegradation (Chen et al. 2012). *S. maltophilia* was incubated in 500 mL

Table 1 The physicochemical properties of testing soils

Soil type	Moisture content (%)	pH	Organic matter (g kg^{-1})	TN (g kg^{-1})	Available N (g kg^{-1})	TP (g kg^{-1})	Available P (g kg^{-1})	Available K (g kg^{-1})	Petroleum (g kg^{-1})
Blank soil	15.2	4.64	83.5	2.46	0.13	0.17	0.0016	0.031	–
Polluted soil	14.5	4.29	110.7	2.26	0.11	0.14	0.0019	0.038	20
Add soil conditioner	13.3	4.87	110.6	4.07	0.31	2.05	0.29	0.072	

of nutrient broth (NB) (the composition of NB is as follows: peptone, 10 g L^{-1}; sodium chloride, 5 g L^{-1}; and beef extract, 3 g L^{-1}) and grown 24 h with shaking at 30 °C. After growth, the bacterial culture was centrifuged at a relative speed of 6000 r min^{-1} for 10 min at 25 °C. The obtained pellet was washed twice using sterile water and then suspended in sterile water for the bioremediation experiments. The inoculation was 5% (volume ratio) each group.

2.2.2 Soil conditioner

The soil conditioner in this study was developed previously by using agricultural wastes, such as deadwood and defoliation. It was in a powdered form and rich in N, P, and K. The additions of soil conditioner in each experiment group were 1% (mass ratio).

2.3 Experimental design of soil remediation

The simulated experiments were conducted to study the effects of inoculation and soil conditioner on bioremediation of oil-contaminated soil. The experiments were carried out in a greenhouse with natural sunlight and 10 h of light every day from July 2016 to September 2016, totally 60 days. The room temperature was kept at 25~30 °C at daytime and 15~20 °C at night. Each set weighted 1000 g of contaminated soil and kept a certain percent of soil moisture (10~20%) by daily watering. Soil sampling at 0, 1, 3, 5, 7, 9, 14, 18, 21, 25, 28, 31, 35, 42, 49, 56, and 60 days respectively, then analyzed and measured its physicochemical properties and removal rates of petroleum.

2.4 Analysis of soil nutrient index

In this study, one portion of soil was taken out from each pot for analyses of physicochemical properties, including pH, moisture, organic matter, total nitrogen (TN), total phosphorus (TP), available nitrogen (AN), and available phosphorus (AP). Soil pH value was measured using a pH meter (1:10 s/w). Organic matter was determined by potassium dichromate oxidation method. TN was estimated using semi-micro Kjeldahl distillation. Available N was measured by alkali hydrolysis method using 1.0 mol L^{-1} NaOH. TP was analyzed with the alkali fusion-molybdenum-antimony colorimetric method. And the available P was determined by molybdenum-antimony colorimetric method using 0.5 mol L^{-1} $NaHCO_3$ extraction (Bao 2000).

2.5 Extraction of petroleum hydrocarbon

Soil samples (5 g) that were already freeze-dried, grinded, and sieved, were taken to be dissolved using 20 mL dichloromethane in a 50-mL conical flask, and were extracted by ultrasonic extraction for 10 min. Then, the suspension was centrifuged for 10 min in a centrifugal velocity of 4000 r min^{-1}. Each soil sample was extracted four times, finally merged the supernatant and evaporated it to constant weight.

2.6 Determination of petroleum hydrocarbon

The residual petroleum hydrocarbon in soil was measured by gravimetric method (Villalobos et al. 2008). The calculation formula is shown below:

$$M = \frac{(M_1 - M_2) \times 10^6}{m} \quad (1)$$

In the formula, M was the content of petroleum hydrocarbon (mg kg^{-1}), M_1 was the total mass of the beaker and the oil (g), M_2 was the mass of beaker (g), and m was the mass of oil-contaminated soil.

The removal rate of petroleum hydrocarbon was determined using the following formula:

$$T = \left[\frac{(W - M)}{W}\right] \times 100\% \quad (2)$$

In the formula, T was the removal rate of petroleum hydrocarbon, M was the content of petroleum (mg·kg^{-1}), and W was the original content of petroleum hydrocarbon in soil (mg·kg^{-1}).

3 Results and discussion

3.1 Changes of physicochemical properties of soil during bioremediation

3.1.1 The changes of soil pH values

The soil used in the experiment was highly acidic, of which the pH value was lower than 5.0 before bioremediation. The soil pH value has great effect on the soil fertility and the growth of plants, as well as the availability of soil nutrients. Therefore, this study investigated the effect on soil pH changes caused by microbe and soil conditioner during bioremediation. The results are shown in Fig. 1.

It could be seen that the soil pH values increased gradually with time. Especially, in the experimental groups 3, 4, 5, and 8 that were added with soil conditioner, the pH value of the soil was significantly higher than other groups, and was close to 6.0 after 8th day, then tended to neutrality gradually. However, the soil pH value in controls 1 and 2 was almost unchanged, maintaining a range from 4.0 to 5.5, and the similar changed trends could be found in test groups without soil conditioner. The results showed that soil conditioner had more obvious effect on soil pH than microorganism. It could improve soil pH value and keep the soil slightly acid to neutral. This result was similar as the results of field research by Gu et al.; they found that adding soil amendment could improve soil pH (Gu et al. 2018). This not only conduced the growth of crops but also helped to retain the activity of animals and microbe in soil, which was a key impact factor on biodegradation of organic pollutants. Overall, the changes of soil pH values were obvious in the early stage (1~21 days) of remediation, but tending toward stabilization in the late period.

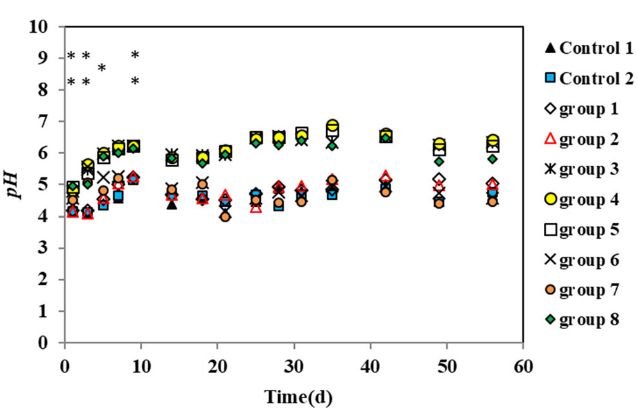

Fig. 1 Changes of soil pH during remediation. The experimental groups 3, 4, 5, and 8 contained soil conditioner, and the groups 1, 2, 3, and 4 were sterilized to make indigenous microorganism inactivated. All groups had exogenous degradation bacteria added except the 5th group

3.1.2 The changes of soil organic matter content

The content of soil organic matter is an important indicator that affects the fertility and quality of soil. And its main source included residues of plants, animals, and microbe as well as excreta and secretions. The waste water and waste residues also can increase the soil organic matter. Soil organic matter is an important component of solid phase of soil, providing nutrition for plants, which promoted their growth and development, and improved the physical properties of soil, and it also could be regarded as the main source of nutrient and energy for soil microbe. According to the analysis results in this study, the content of organic matter in blank soil was 83.5 g kg^{-1}. But in contaminated soil, it increased significantly and reached 110.7 g kg^{-1}. Thus, it could be seen that the organic pollution resulted in the increase of soil organic matter content.

Different treatment combinations were carried out to assess the changes of soil organic matter content during remediation of oil-polluted soil. The results in Fig. 2 showed that at the beginning, there was little difference in soil organic matter content between different experimental groups; then, it decreased gradually in all 10 experimental groups over time. But because of different experimental conditions, the loss of soil organic matter content was different, which reduced to 83.1~101.8 g kg^{-1} at the 60th day, respectively. Among them, the loss of organic matter content in group 2 that only contained exogenous degradation bacteria was the most, which lost 24.8 g kg^{-1} at the end. The reason for this was that the organic matter in soil was decomposed by microbe. However, the reduction of soil organic matter in group 7 that without sterilization and with inoculation of degradation bacteria simultaneously was least. This indicated that the indigenous microorganism could not use the petroleum hydrocarbon effectively.

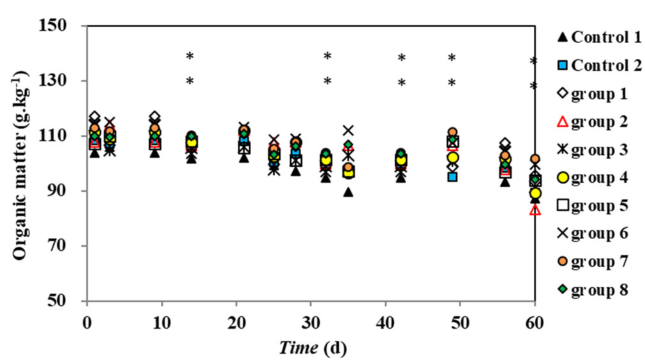

Fig. 2 Changes of soil organic matter content during remediation. The experimental groups 3, 4, 5, and 8 contained soil conditioner, and the groups 1, 2, 3, and 4 were sterilized to make indigenous microorganism inactivated. All groups had exogenous degradation bacteria added except the 5th group

3.1.3 The changes of soil total nitrogen

Total nitrogen (TN) was including organic nitrogen and inorganic nitrogen, which can affect soil fertility quality and the growth of plants. In order to investigate the changes of TN content in petroleum-contaminated soil in the process of remediation, ten experimental groups were set up in this study. The specific treatments are shown in Table 2.

It could be seen from Fig. 3 that the addition of soil conditioner increased the content of TN in soil significantly. Before the remediation experiment begins, the TN value of soil with conditioner (4.08~4.57 g kg^{-1}) was twice as much as the soil without conditioner (2.26 g kg^{-1}). With the time extended, the TN contents of experimental groups 3, 4, 5, and 8 (adding soil conditioner) not only were unchanged significantly but also were almost the same, and the value of TN remained between 4.08~4.57 g kg^{-1} from first day to the 60th day.

Studies had shown that the soil TN content had a positive correlation with the growth of soil microorganisms, and it could promote the biodegradation of petroleum hydrocarbons (Auti et al. 2019). The results of this study also found that soil conditioner that is rich in nitrogen could increase the TN content of oil-polluted soil, promote the growth of soil microorganisms, and maintain their metabolic activity, which finally would benefit the biodegradation of oil. Petroleum is a mixture, which contains various hydrocarbons and other organic compounds containing nitrogen or sulfur. Because of the biodegradation, various intermediate products of petroleum were released to soil; some were degraded further by microbe, but some were left in soil. Thus, although the nitrogen in soil was used by microbe, the degradation of organic nitrogen compound still could make the TN content in soil keeping stable. The results in Fig. 3 proved this point, especially the experimental groups 2 and 7. The TN content in soil of these two groups increased rapidly from the beginning of bioremediation, although there was no soil conditioner added. Finally, the TN content remained between 3.05~3.75 g kg^{-1} at the 10th day. This result also illustrated that even if without adding soil conditioner and the level of nitrogen in polluted soil was low, the microbe in soil still could get nitrogen through degrading petroleum to keep their own metabolic activity, so that the petroleum in soil not only was decomposed, but the soil fertility was also improved because of the supplement of nitrogen.

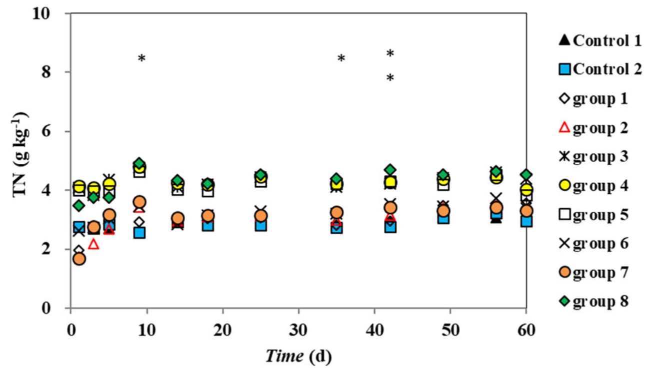

Fig. 3 Changes of TN content in soil in process of remediation. The experimental groups 3, 4, 5, and 8 contained soil conditioner, and the groups 1, 2, 3, and 4 were sterilized to make indigenous microorganism inactivated. All groups had exogenous degradation bacteria added except the 5th group

3.1.4 The changes of soil available nitrogen

Available nitrogen (available N) is an important indicator of the soil nitrogen content supplying, and its changes can reflect the utilization of nitrogen by microbe in soil. Therefore, it was necessary to study the changes of available N content in soil in the process of remediation.

The results in Fig. 4 showed that the contents of available N in experimental groups 3, 4, 5, and 8 that had soil conditioner added were obviously higher than those in the other groups. This indicated that the chemical forms of nitrogen in soil conditioner were what were easy to hydrolyze, such as ammonium nitrogen, amino acids, or amides. Thus, the addition of soil conditioner could effectively improve the level of available N content in oil-contaminated soil. From Fig. 4 we can know

Table 2 Experimentation scheme and conditions

Serial number	Groups	Sterilized	Inoculation	Soil conditioner	Turn the soil
1	Control 1	Yes	–	–	–
2	Control 2	No	–	–	–
3	Group 1	Yes	5%	–	Once a day
4	Group 2	Yes	5%	–	–
5	Group 3	Yes	5%	1%	Once a day
6	Group 4	Yes	5%	1%	–
7	Group 5	No	–	1%	Once a day
8	Group 6	No	5%	–	Once a day
9	Group 7	No	5%	–	–
10	Group 8	No	5%	1%	Once a day

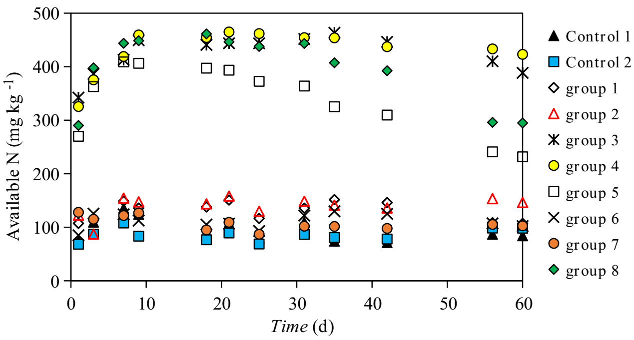

Fig. 4 Changes of soil available N content over time. The experimental groups 3, 4, 5, and 8 contained soil conditioner, and the groups 1, 2, 3, and 4 were sterilized to make indigenous microorganism inactivated. All groups had exogenous degradation bacteria added except the 5th group

that the contents of soil available N in control groups and experimental groups 1, 2, 6, and 7 were almost unchanged over time. And it kept in a level lower than 100 mg kg^{-1} in control groups, while remained between 98~153 g kg^{-1} in experimental groups (1, 2, 6, and 7) from day 1 to day 60. However, the contents of available N in soil of experimental groups 3, 4, 5, and 8 changed significantly. In the early stage of remediation, the content of available N in these four groups increased rapidly and reached the maximum at the 9th day. Then, the available N in group 5 decreased continuously, and that in group 8 also decreased gradually at the 30th day. Finally, the contents of soil available N in both groups reached to the initial level and stabilized. Even so, the changes of available N content of groups 3 and 4 were not evident during 10th day to 30th day, but decreasing slightly in later period.

In this study, the groups 5 and 8 were all not sterilized but the group 8 had inoculation of degrading bacteria, while the groups 3 and 4 were all sterilized to remove indigenous microbes, and both had soil conditioner and exogenous degrading bacteria added. Thus, it can be known that the demand and utilization of soil available N by indigenous microbes were more obvious than that of exogenous degrading bacteria. Because of the addition of soil conditioner, the content of soil available N was higher at the beginning, and as the biodegradation of petroleum which led to the continuous increase of soil available N content. But at the same time, the indigenous microbes in soil, in order to maintain their self-metabolism, must continually use the soil available N, so resulting in the level of soil available N decreasing gradually, which also prompted petroleum in soil to decompose. Nevertheless, from the results of groups 3 and 4, the utilization of soil available N by exogenous degrading bacteria was not significant. This was because the degrading bacteria first need to adapt the complex soil conditions, and could not use the nitrogen in soil effectively, which inhibited the biodegradation of petroleum in soil. And the analysis of removal rates of petroleum also could demonstrate this. Besides, the experimental group 8 contains both indigenous microbe and exogenous degrading bacteria, but the available N content was nearly change from 10th to 30th day. This result indicated that there was a competitive antagonism between indigenous microbe and exogenous degrading bacteria. Thus, the activity of indigenous microbes was repressed, leading to the declining utilization of soil available N and declining removal rate of petroleum hydrocarbon.

3.1.5 The changes of soil phosphorus during remediation

Total phosphorus (TP) in soil was including organic phosphorus and inorganic phosphorus, but in the form of available phosphorus (available P) only accounts for a small fraction. There was no significant linear correlation between the contents of TP and available P, so the quantity of soil TP could not be the exact index to evaluate supply capacity of soil phosphorus but the soil available P can. Available P could be directly absorbed and used by plants, and was generally including all water-soluble phosphorus, some adsorbed phosphorus, and organic phosphorus. Phosphorus is a key nutrient in soil; thus, it was important to detect the amount of phosphorus especially available P in the process of soil remediation.

The results are showed in Fig. 5. It could be found that at the beginning of experiment, the content of soil TP was with an average of 140 mg kg^{-1}, but the average content of available P was only 2 mg kg^{-1}. Although the addition of soil conditioner could significantly increase the level of TP and available P, the amount of available P only accounted for 12% of the TP. The analysis of results in Fig. 5a and c revealed that the level of available P in oil-contaminated soil was low, and most of the soil TP was unable to be absorbed and used by microbe, so the content of TP was nearly unchanged with time in the process of remediation. The microbial biomass and its efficiency of organic pollutant decomposition in soil were inhibited, because of lack of available P, which was not good to the biodegradation of petroleum. However, in the group adding soil conditioner, the content of TP and available P decreased with time, and kept stabilize in the later phase (see Fig. 5b, d). This meant that soil conditioner could effectively improve the soil environment and help to increase the biomass and metabolic activity of microorganisms, which would promote the biodegradation of petroleum hydrocarbon in soil. Therefore, adding appropriate amount of nitrogen and phosphorus was an effective strategy for bioremediation of petroleum contaminated soil (Wu et al. 2019).

3.1.6 The changes of soil available potassium content during remediation

The presence forms of potassium (K) in soil mainly include water-soluble potassium, exchangeable potassium, and fixed potassium in clay minerals, of which the first two categories can be absorbed by crop directly, so they are collectively

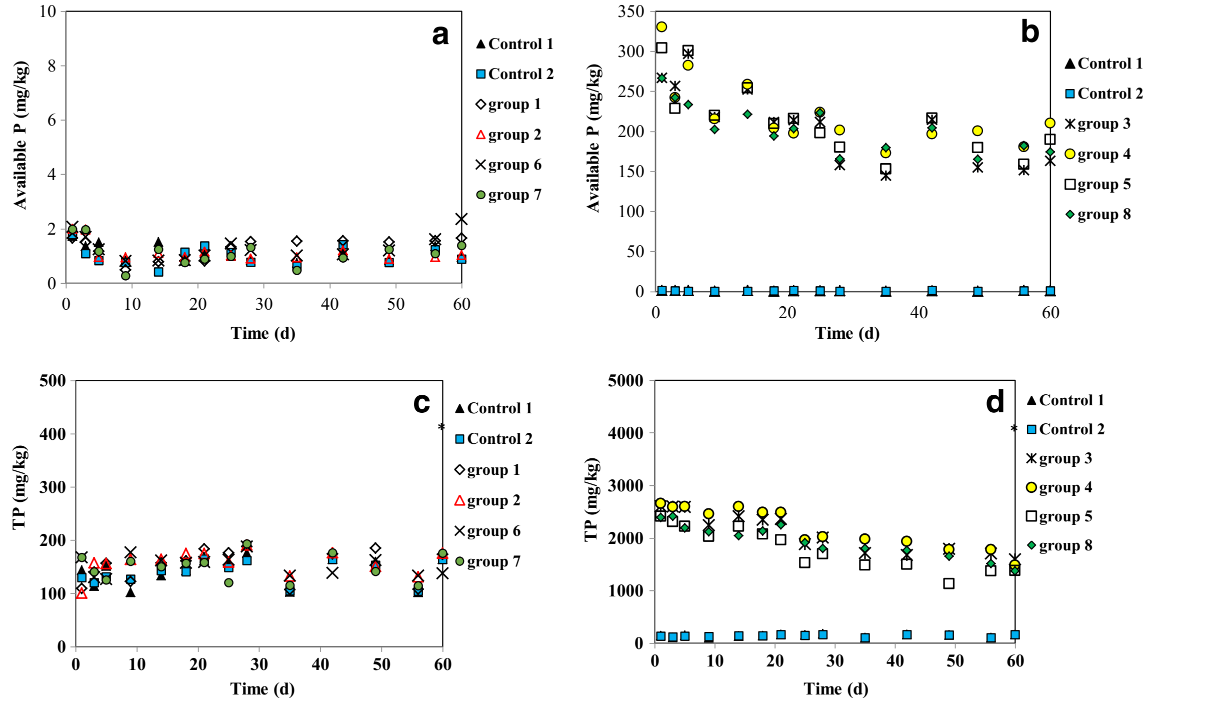

Fig. 5 Changes of phosphorus content in soil. **a** and **b** were the changes of soil available phosphorus content, while **c** and **d** were the changes of soil TP content

called as "quick available K." In general, various forms of K are transformed into each other in soil, maintaining a dynamic balance. Similar to TP in soil, the total potassium (TK) content can only explain the storage capacity of soil potassium instead of the supply capacity. Therefore, the detection and analysis of changes of available K content in the process of soil remediation were necessary, which was helpful to assess the soil fertility and for precise fertilization.

The variations of soil available K content in the process of remediation are showed in Fig. 6. It could be seen that the level of soil available K in the experimental groups with soil conditioner adding were higher. At the beginning of the soil bioremediation

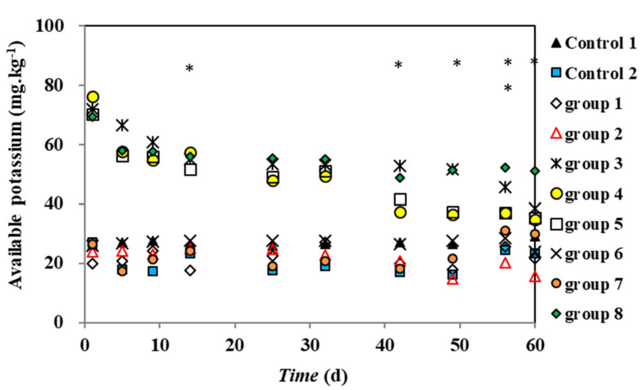

Fig. 6 Changes of soil available potassium content with time. The experimental groups 3, 4, 5, and 8 contained soil conditioner, and the groups 1, 2, 3, and 4 were sterilized to make indigenous microorganism inactivated. All groups had exogenous degradation bacteria added except the 5th group

experiment, the available K contents of groups 3, 4, 5, and 8 were with an average of 74 mg kg^{-1}, while in other experimental groups without soil conditioner adding were only 26 mg kg^{-1}. And this result also proved that the soil conditioner could improve the soil condition, which was important for soil microbial growth and metabolism that can accelerate the decomposition and utilization of petroleum hydrocarbons in soil. From Fig. 6 it can found that the soil available K content in the groups with soil conditioner adding decreased rapidly at the beginning, and then to slowdown. Finally, the amount of available K in group 8 was 51 mg kg^{-1}, while it stabilized around 36 mg kg^{-1} in groups 3, 4, and 5. The reason for this was the utilization and absorption of available K by microbe. K^+ is a major ion in cells, which can regulate cell apoptosis, and can be used as an activator of various intracellular enzymes. It is also crucial to phosphorus metabolism (such as ATP hydrolysis) in bacterial cells (Yu 2003). Thus, in the early stages, soil microbe, in order to adapt to the contaminated surrounding, and to use the petroleum as carbon source, needs an amount of K to activate the related intracellular enzyme. Moreover, comparing the results of group 3 and 8, the level of soil available K content in group 3 was lower after 60 days, although the soil of group 3 had been sterilized to remove indigenous microbe. This indicated that there was antagonistic effect between exogenous degrading bacteria and indigenous microbe, which would inhibit the metabolic activity of each other when both were co-existing. However, the contents of soil available K in other experimental groups without soil conditioner adding maintained between 20 and 25 mg kg^{-1} in the process of bioremediation. This result further indicated that the environmental conditions were the key in remediation of polluted soil and lack of nutrients would inhibit the soil microbial metabolic activity, which might go against the biodegradation of organic pollutants (Liu et al. 2018).

3.2 Degradation of petroleum hydrocarbon by microbe

The oil contamination would change the physicochemical properties of soil and affect the soil fertility. Microorganism was the primary member in soil, and it played a key role on decomposition of organic pollutants, so bioremediation would be an effective technology for petroleum-polluted soil (Vasilyeva et al. 2019). But often due to a lack of essential nutrients in contaminated sites, most microbes were restrained to use and degrade the pollutants. Therefore, this research chose one soil conditioner and the exogenous degrading bacteria as objects, to study their effects on the oil-contaminated soil remediation, and finally revealing the interrelation and mechanism of soil conditions and soil microbe respectively in soil remediation.

It can be seen from Fig. 7, the results showed that the removal rates of petroleum gradually increased over time, and after 60 days, the removal rate of group 5 reached the maximum, which was 57%, while the minimum was only 22.4% which was the removal rate of group 2. Besides, as showed in Fig. 7, it could be found that the removal rates of petroleum in groups 3, 4, 5, and 8 were significantly higher than those of other experimental groups, because of the addition of soil conditioner. The analysis had shown that the major components of soil conditioner were N, P, and K, which were all essential soil nutrients. Thus, the addition of soil conditioner could effectively improve the soil condition in contaminated site, and then promote the microbial degradation of petroleum. Even more, the results revealed that the role of the improvement of soil condition was more important than inoculating oil-degrading bacteria. In this study, there was only soil indigenous microbe in group 5, and only exogenous degrading bacteria in group 3, but group 8 had both. The results showed that the average removal rate of petroleum hydrocarbon in group 3 and group 5 was higher than that of group 8, and the maximum value was group 5. This might be because indigenous microorganisms could adapt to the polluted environment more quickly, so they played a key role in the biodegradation of petroleum (Abena et al. 2019; Zawierucha et al. 2014). In addition, it further revealed that the interrelation between indigenous microbe and exogenous degrading bacteria was antagonism; as a result, the removal rate of petroleum decreased in group 8.

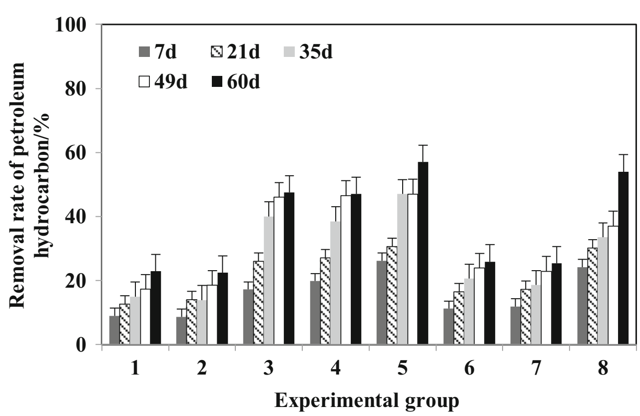

Fig. 7 The removal rates of petroleum on different times. The experimental groups 3, 4, 5, and 8 contained soil conditioner, and the groups 1, 2, 3, and 4 were sterilized to make indigenous microorganism inactivated. All groups had exogenous degradation bacteria added except the 5th group

4 Conclusions

The microbial activity in contaminated soil was inhibited because of a lack of nutrients. Adding 1% soil conditioner could significantly improve the soil conditions, which played a key role on bioremediation of oil-contaminated soil, because it could offer the soil microbe enough N, P, and K that were essential nutrients for microbial growth and metabolism. And the microorganism could maintain metabolic activity and use the petroleum as carbon source. Thus, the addition of soil conditioner was good for biodegradation of petroleum in contaminated site. The results of this study also indicated that the microorganism including the exogenous oil-degrading strains and the soil indigenous microbe, had an important effect on degradation of petroleum. Overall, the addition of soil conditioner, presence of indigenous microbe, and inoculation of oil-degrading strains all were conducive to bioremediation of oil-contaminated site. But how to make indigenous microbe and high-efficiency degrading strains work to achieve synergy was the key to gain much better remediation effect, because the indigenous microbe was only good at decomposing low molecular compounds and saturated hydrocarbons, while the oil-degrading bacteria can effectively decompose the high molecular weight aromatics, such as polycyclic aromatic hydrocarbons.

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Compliance with ethical standards

The authors declare that there is no conflict of interest. The research did not involve human participants and/or animals. All authors agreed with the content and that all gave explicit consent to submit.

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