EFFECT OF DIFFERENT REMEDIATION METHODS ON THE DEGRADATION RATE OF PETROLEUM HYDROCARBON AND ENZYME ACTIVITY IN PETROLEUM CONTAMINATED SOIL

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Abstract. In order to explore the effects of different remediation methods on the degradation rate of total petroleum hydrocarbons and enzyme activity in oil-contaminated soil, a study was conducted using six different treatments, including adding rhamnolipid (S), organic fertilizer (F), degradation bacteria (J), rhamnolipid + degrading bacteria (SJ), organic fertilizer + rhamnolipid (SF), and organic fertilizer + degradation bacteria (FJ), to remediate the oil-contaminated soil. The study examined the changes in the degradation rate of total petroleum hydrocarbons and the activity of four soil enzymes (urease, peroxidase, dehydrogenase, and lipase) at different cultivation times. The results showed that after 60 days of remediation, all treatments improved the degradation rate of total petroleum hydrocarbons in the contaminated soil. The best result was achieved with the FJ treatment, with a degradation rate of 31.72%. The enzyme activity in all treatments was significantly higher than that of the control at different cultivation periods. Statistical analysis showed that the activity of urease, peroxidase, and lipase was significantly negatively correlated with the residual rate of total petroleum hydrocarbons in the contaminated soil. The activity of dehydrogenase was highly significantly negatively correlated with the residual rate of total petroleum hydrocarbons in the contaminated soil.
Keywords: *total petroleum hydrocarbons, urease, dehydrogenase, peroxidase, lipase*

Introduction

Soil petroleum pollution is one of the global environmental problems. The efficient, low-cost, and pollution-free technology for remediation of petroleum-contaminated soil is gradually becoming an important direction for future research on petroleum-polluted soil treatment (Xu et al., 2020). Restoring the original ecological function and status of polluted soil is a long-term and complex systemic ecological process (Shintani et al., 2019). Soil is one of the most important carriers of petroleum hydrocarbon pollutants, and the pollutants are mostly concentrated in the soil surface layer of about 0-20 cm, which is also the area where plant roots are most developed. Therefore, the degree of soil petroleum pollution directly affects the growth of plants. Petroleum pollutants have the characteristics of high carbon content, diverse organic matter, strong hydrophobicity, high viscosity coefficient, density less than water, and certain volatility, which can adhere to soil particles, block soil pores, and affect the soil's ventilation and water permeability, directly affecting the soil's physical state (Wei et al., 2020). Under the conditions of organic pollution, increased hydrophobicity is another feature of its degradation. Biodegradation is the main way for petroleum hydrocarbon pollutants to be reduced in soil, and petroleum-degrading microorganisms are the main bearers of this process. Adding degradation microorganisms is a key factor in strengthening the remediation effect (Szulc et al., 2014; Li et al., 2016; Wu et al., 2018; Shintani et al., 2019; Meng et

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al., 2021). However, the hydrophobicity of petroleum hydrocarbon pollutants makes them easily adsorbed by soil particles, which hinders microbial degradation and leads to long-term retention in the soil. Higher plants, indicator microorganisms, earthworms, and soil enzyme activities (Al-Mutairi et al., 2008; Geissen et al., 2008; Li et al., 2009; Hua et al., 2018; Zhang et al., 2020; Wang et al., 2023) are frequently used teswide, evaluate, and monitor the soil remediation process of contaminated soil (Zhang et al., 2023). Among them, soil enzymes play an important role in material transformation, energy metabolism, and remediation of contaminated soil, and are known as the center of soil ecosystems (Tabatabai et al., 2002). Due to the convenience, speed, and accuracy of soil enzyme activity determination, and its activity being jointly affected by the soil pollution status and physical and chemical properties, it has obvious advantages as a monitoring indicator and has gradually become an important research direction for soil environmental quality monitoring (Olga et al., 2006). Soil enzyme activity has been used to monitor the remediation process of oil-contaminated soil (Baran et al., 2004). Especially, soil enzymes play a catalytic role in important metabolic processes such as organic matter decomposition and toxic substance degradation and can be used as potential indicators to evaluate and monitor the remediation process of oil-contaminated soil. In this paper, the apparent degradation rate of petroleum hydrocarbons in oil-contaminated soil at different remediation stages and the changes in four soil enzyme activities, namely urease, peroxidase, dehydrogenase, and lipase, during the remediation process were studied through cultivation experiments. Furthermore, the relationship between soil enzyme activity and petroleum pollutant removal was analyzed, providing a theoretical basis and technical support for the remediation of oil-contaminated soil.

Materials and methods

Site description

The tested soil was crude oil-contaminated soil collected from Ganguyi town, Baota District, Yan'an City, Shaanxi Province, China. After the soil was collected, a portion was stored in a refrigerator at 4°C for soil enzyme activity determination, while another portion was air-dried after removing large particles and sieved through a 2 mm mesh for further use. The basic physical and chemical properties of the soil under test are shown in *Table 1*.

Table 1. *Soil properties of the tested soil*

Properties	pH	TC (%)	Water content (%)	TN (%)	TP (%)	TK (%)	TPH (%)
Value	7.45	6.65	18.93	0.103	0.050	0.456	2.444

Note: TC is total carbon; TN is total nitrogen; TP is total phosphorus; TK is total kalium; TPH is total petroleum hydrocarbon

Experimental treatments and design

The petroleum-contaminated soil remediation experiment was conducted in plastic pots with a diameter of 20 cm and a depth of 10 cm, each containing 1 kg of dry soil and mixed with the modifier, the field capacity was adjusted to 60%. The soil was then placed in a constant temperature incubator (30 ℃) for petroleum-contaminated soil remediation

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experiments, with each treatment replicated three times. During the experiment, soil samples were collected on the 7th, 15th, 30th, and 60th days, and the concentrations of petroleum hydrocarbons and the activity of various soil enzymes (including urease, dehydrogenase, peroxidase, and lipase activity) were determined. The experimental design is shown in *Table 2*.

Table 2. *Petroleum contaminated soil culture remediation program*

Number	Test plan
CK	1kg of tested soil
S	1kg of tested soil + rhamnolipid 150 mg
J	1kg of tested soil + degradation bacteria 10^9 CFU
F	1kg of tested soil + 150 g organic fertilizer
SJ	1kg of tested soil + rhamnolipid 75 mg+ degradation bacteria 5×10^8 CFU
FJ	1kg of tested soil + organic fertilizer 75 g+ degradation bacteria 5×10^8 CFU
FS	1kg of tested soil + organic fertilizer 75 g+ rhamnolipid 75 mg

Note: degradation bacteria is Bacillus megaterium

Determination method

Determination of total petroleum hydrocarbon content (TPH)

Debris such as sand, rocks, and plant roots should be removed from the soil sample and mixed thoroughly. Dry the sample in a 50℃ oven for two days. 1.0 g soil sample was weighed and placed in a 100 ml conical flask. There was 20 ml carbon tetrachloride was added into the sealed flask and placed into the ultrasonic water bath with a power output of 200W for 10 minutes. The solution was then placed in an oscillator and extracted for 30 min by shaking at the rate of 200 times /min. After standing for 10 minutes, filter the solution into another 100 mL conical flask using a glass sand core funnel (with a layer of anhydrous sodium sulfate on top). After repeated extraction and filtration, the soil was washed and filtered by carbon tetrachloride, and the filtrate was combined. Then 5.0 g MgSiO4 was added to the filtrate, extraction was carried out for 30 min at a rate of 200 times /min by shaking, filtered into a 50 ml colorimetric tube with a glass sand core funnel, cleaned the funnel with an appropriate amount of carbon tetrachloride, and fixed volume to the marked line, to be measured. Oil content analysis was determined by infrared spectrophotometer infrared oil measuring machine (Changchun Jida Cygnet Company, instrument model: MAI-50, *Figure 1*).

Figure 1. *Infrared oil measuring machine (MAI-50)*

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Urease

5.00 g of soil sample was weighed into a 100 ml Erlenmeyer flasks and 1 mL toluene was added to shake evenly, 10 mL of 100 g/L urea solution and 20 mL of pH 6.7 citrate buffer solution were added after 15 min, mix well, and put into a 37 ℃ incubator for 24 h. After filtration, 1.00 mL filtrate was absorbed into a 50 mL volumetric bottle, and then 4 mL sodium phenol solution of 1.35 mol/L and 3 mL sodium hypochlorite solution of 0.9% active chlorine concentration were added successively. Shaked well while added. After 20 min for color development, it was fixed to 50 mL with distilled water. It was colorimetric by respectrophotometer at 578 nm wavelength within 1 hour.

Before measuring the absorbance value of the sample, 0.00, 1.00, 3.00, 5.00, 7.00, 9.00, 11.00, 13.00 ml solutions containing 0.01 mg/mL ammonia were extracted into a 50 mL volumetric flask, respectively, and then 20 mL of distilled water were added. Then, 4 ml of 1.35 mol/L sodium phenol solution and 3 mL of 0.9% sodium hypochlorite solution with active chloride concentration were successively added and shaked well. After 20 min the color was developed, the volume was fixed, A standard solution of 0.0, 0.2, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6 ug/mL was prepared. It was colorimetric by respectrophotometer at 578 nm wavelength within 1 hour. Then a standard curve with ammonia concentration as the horizontal coordinate and light absorption value as the vertical coordinate was drawn.

For each sample, prepare a blank without matrix by replacing the matrix (100 g/L urea solution) with an equal volume of distilled water.

$$\text{Urease activity (mg/g·24h)} = (C1-C2)*N/(m*F*1000) \quad \text{(Eq.1)}$$

where,
C1: ammonia amount obtained from the sample plus matrix absorbance value by standard curve (μg/mL)
C2: ammonia amount obtained from the sample without matrix absorbance value by standard curve (μg/mL)
V: visible colorimetric constant volume (mL)
N: dilution factor = leaching solution volume (mL) / sample filtrate volume (mL)
M: dry soil weight (g)
F: proportion of dry soil to fresh soil.

Catalase

2 g of soil samples were placed in a triangular flask, added 40 mL of distilled water and 5 mL of 0.3% H_2O_2 solution, and immediately sealed. After shaked for 20 minutes, 1mL of saturated potassium aluminum sulfate was added and the solution was immediately filtered into a triangular flask containing 5 mL of 1.5 mol sulfuric acid. After drying, pipetted 25 mL of the filtrate and titrate with 0.02 mol/L potassium permanganate solution to purple red, with a control sample without soil.

Result calculation: $KMnO_4$ calibration: 0.13-0.16 g of sodium oxalate were accurately weighted and placed in a 250 mL Erlenmeyer flask, then 30 mL of distilled water and 20 mL of 1.5 mol/L H_2SO_4 were added, heated to around 75℃ and titrated with $KMnO_4$ until a purple-red color appeared. The solution will turn purple-red at the beginning of the titration, but this is not the endpoint. Wait for the initial purple-red color to fade, then continue titrated until the solution turns purple-red again, and continue titrated until the purple-red color did not fade for 30s. H_2O_2 calibration: 5 mL of H_2O_2 was added to 10 mL

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of 1.5 mol/L H_2SO_4, and titrated with $KMnO_4$ until a purple-red color appears. The activity of peroxidase is expressed in milligrams of hydrogen peroxide decomposed per gram of soil within 20 minutes:

$$\text{Catalase activity} = (V\text{-}Vs)^* C^*51 / V_0^*17 / W/F \quad \text{(Eq.2)}$$

where,
V: the volume of $KMnO_4$ used for blank titration
Vs: the volume of $KMnO_4$ used for sample titration
C: the concentration of $KMnO_4$
V0: the titration volume is 25 mL
W: soil weight
F: the proportion of dry soil to fresh soil.

Dehydrogenase

4 g of fresh soil sample sieved through a 2 mm mesh were placed in a test tube, and 2 mL of 1% 2, 3, 5-triphenyltetrazolium chloride (TTC) solution and 2 mL of 1% glucose solution were added, shaked well. At the same time, set up each treatment with 2 mL of tromethamine (Tris) buffer instead of TTC as a control. The test tubes were incubated at 37℃ for 24 h, added 20 mL of methanol and then were transferred to a 50 mL Erlenmeyer flask, shaked on a shaker (180-220 r/min) for 1 hour, and filtrated. The volume of filtrate was measured with a cylinder, and the absorbance value of filtrate at 485 nm was measured with a spectrophotometer, rinse with distilled water and the test solution, and directly measure the next sample. The entire experiment is set up with no soil control (2 mL of TTC solution and 2 mL of glucose solution). Standard curve preparation: 50 mg of triphenyl fomazan (TF) were accurately weighed in 250 mL of methanol, with a concentration of 200 mg/L; 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 mL of standard solution were placed in a 25 mL volumetric flask and make up to volume with methanol. The TF concentrations were 0, 2, 4, 8, 12, 16, and 20 mg/L.

$$\text{Dehydrogenase activity (mg/g/d)} = (cv) / (mt^*F) \quad \text{(Eq.3)}$$

where,
c: the concentration of TF in the filtrate
v: the volume of the filtrate
m: dry soil mass
t: incubation time at constant temperature
F: the proportion of dry soil to fresh soil.

Data analysis

The value of each indicator was the mean of three replicates per treatment, and the SPSS statistics v.22 (IBM, Inc, Chicago, IL, USA) was used to perform analysis of variance and Pearson correlation analysis. All pair-wise comparisons of the treatment means were performed using the least significant difference (LSD) test with significance determined at the 5% level.

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Results

Total petroleum hydrocarbon degradation rate

In the process of soil bioremediation, petroleum is gradually degraded, and the total amount of petroleum hydrocarbons is generally used as a routine indicator to measure the restoration process of petroleum-contaminated soil (Liu et al., 2007). As shown in *Figure 2*, compared with the CK treatment, the total petroleum hydrocarbon degradation rates of the other treatments all decreased significantly over time after the addition of different substances and combinations. After 60 days of cultivation, compared with the CK treatment, the total petroleum hydrocarbon degradation rates of the other treatments were significantly increased, with degradation rates increasing from 2.68% to 10.77%, 22.71%, 18.02%, 26.21%, 19.52%, and 31.72%, respectively, and the degradation efficiency increased by 4.01-11.84 times. There was no significant difference in the degradation rates among the J, SJ, FS, and FJ treatments after 7 days of cultivation ($P>0.05$), but there was a significant difference among all treatments after 15 days of cultivation ($P<0.05$). After 30 days of cultivation, the degradation rate of the FS treatment was higher than that of the F treatment, but the difference was not significant, while the degradation rates of the other treatments were significantly different ($P<0.05$). The trend of the results after 60 days of cultivation was the same as that after 30 days of cultivation. As shown in *Figure 2*, the total petroleum hydrocarbon degradation rate was highest during the 0–15-day period of cultivation, and the degradation rate slowed down after 15 days. This is because in the early stage of restoration, the carbon source is abundant, which promotes the growth and reproduction of microorganisms and accelerates the degradation of petroleum hydrocarbon components. With the passage of time, the easily degradable petroleum hydrocarbon components are consumed in large quantities, and the remaining components are mainly difficult to degrade aromatic hydrocarbons, resulting in a decrease in the degradation rate (Wang et al., 2013). Except for the treatment at 7 days of cultivation, the total petroleum hydrocarbon degradation rates of the treatments that added organic fertilizer and degradation bacteria were significantly higher than those of the other treatments in all other cultivation periods, among which the FJ treatment had the best overall petroleum hydrocarbon degradation effect. Therefore, the combination of organic fertilizer and degradation bacteria treatment is the best choice.

Urease activity

Urease is an amidase that catalyzes the hydrolysis of carbon-nitrogen bonds in organic substances and plays a unique role in soil nitrogen cycling. Its increased activity is beneficial for the conversion of stable organic nitrogen into available nitrogen in soil, thereby improving soil nitrogen supply (Wu et al., 2010). As shown in *Figure 3*, the urease activity in polluted soils under various treatments was significantly higher than that in the CK treatment during different cultivation periods. From days 0 to 30, the urease activity in polluted soils under all treatments showed an increasing trend, with a higher rate of increase from days 0 to 7 than from days 7 to 30. This indicates that the biological conversion of nitrogen in soil was more active after the addition of different additives. From days 30 to 60, the urease activity showed a decreasing trend but remained significantly higher than that in the CK treatment. At the same time under different treatment conditions, the urease activity of all treatments was higher than that of CK treatment, among which J and FJ treatment had the highest urease activity. The urease activity of each treatment showed the trend of J and FJ > F > SJ > FS > S > CK at different

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time. Treatments that included degradation bacteria and organic fertilizers had better repair results. Which may be attributed to the ability of the added degrading bacteria to quickly adapt to the soil environment and use petroleum hydrocarbons and organic fertilizers as carbon sources, thereby enhancing microbial activity.

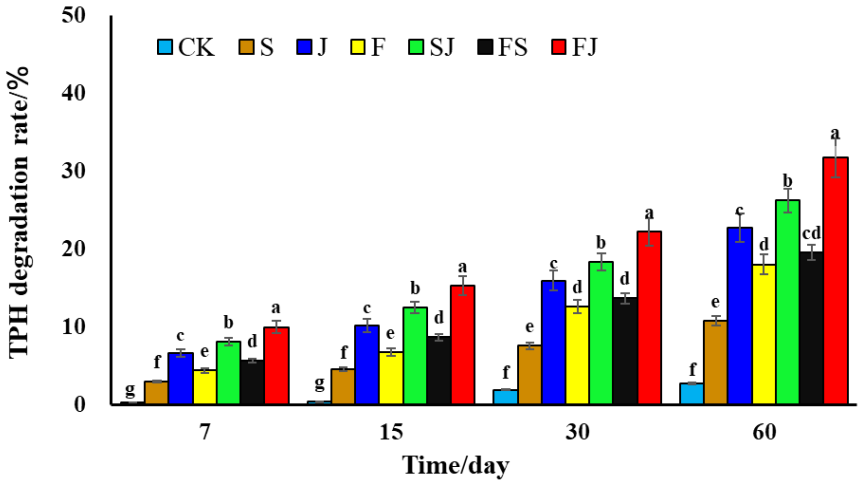

Figure 2. *Effect of different treatments on degradation rate of total petroleum hydrocarbons in petroleum-contaminated soil. CK: control; S: rhamnolipid; J: degradation bacteria; F: organic fertilizer; SJ: rhamnolipid+degradation bacteria FJ: degradation bacteria +organic fertilizer FS: organic fertilizer+rhamnolipid. Errors dars mean standard errors. Different letters within a column indicate significant differences at $p < 0.05$*

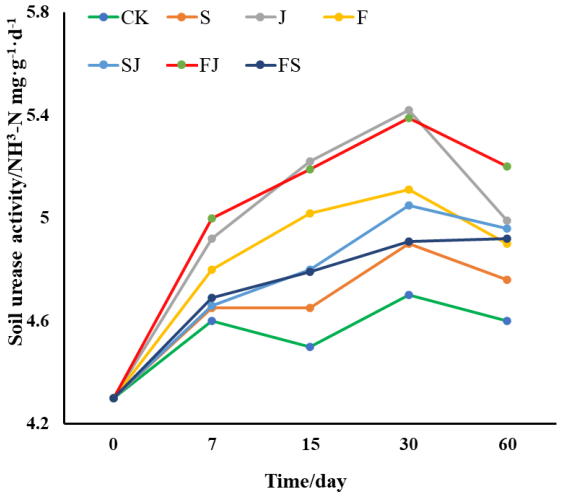

Figure 3. *Effect of different treatments on soil urease activity. CK: control; S: rhamnolipid; J: degradation bacteria; F: organic fertilizer; SJ: rhamnolipid+degradation bacteria FJ: degradation bacteria +organic fertilizer FS: organic fertilizer+rhamnolipid*

Catalase activity

Catalase is widely present in microorganisms and plant cells and plays a crucial role in the conversion of soil nutrients. It can promote the decomposition of H_2O_2, reduce the toxic effects of H_2O_2 on microorganisms, and thus provide a good soil environment for

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soil microbial activity (Wang et al., 2013). From *Figure 4*, it can be seen that the peroxidase activity in the polluted soil sample without the addition of bacterial agents is significantly lower than that in the uncontaminated soil sample and remains at a low level, indicating that petroleum has an inhibitory effect on its activity. Peroxidase activity in polluted soils under different treatment conditions can be significantly improved, with the greatest increase observed in the first 7 days of incubation, followed by a slower increase from 7-30 days. Enzyme activity decreased during the 30–60-day incubation period, but all treatments were still significantly higher than the CK treatment. As shown in *Figure 4*, at the same time under different treatment conditions, the catalase activity of all treatments was higher than that of CK treatment, among which J and FJ treatment had the highest catalase activity. The catalase activity of each treatment at different time showed a trend of J and FJ > F > SJ > FS > S > CK, which was consistent with the results of catalase activity. Treatments that included degrading bacteria and organic fertilizers had better repair results, the catalase activity in the J and FJ treatments was significantly higher than that in the other treatments.

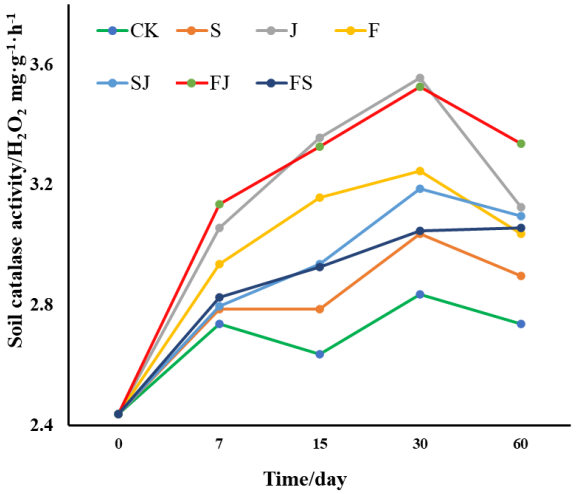

Figure 4. *Effect of different treatments on soil catalase activity. CK: control; S: rhamnolipid; J: degradation bacteria; F: organic fertilizer; SJ: rhamnolipid+degradation bacteria FJ: degradation bacteria +organic fertilizer FS: organic fertilizer+rhamnolipid*

Dehydrogenase activity

Dehydrogenase is an important participating enzyme in the process of petroleum hydrocarbon degradation. It can activate the hydrogen atoms of oxidized organic compounds and transfer their specific hydrogen acceptors. Microbial degradation or transformation of petroleum pollutants begins with dehydrogenation. Therefore, the activity of dehydrogenase can reflect the activity and degradation activity of microorganisms in the treatment system, and the activity of dehydrogenase can be used to reflect the activity of petroleum-degrading microorganisms and to evaluate degradation performance (Li et al., 2002; Chen et al., 2017). As shown in *Figure 5*, with the passage of time, the dehydrogenase activity of the polluted soil samples in each treatment gradually increased, reaching a maximum at 30 days of cultivation, followed by a decreasing trend. At the same time under different treatment conditions, dehydrogenase activities of all treatments were higher than CK treatment, 0-60 days, among which the

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dehydrogenase activities of J and FJ treatment were higher than F treatment, F treatment was higher than other treatments. The enhancement of soil dehydrogenase activity indicates that the petroleum contains components that are easily oxidized by soil microorganisms.

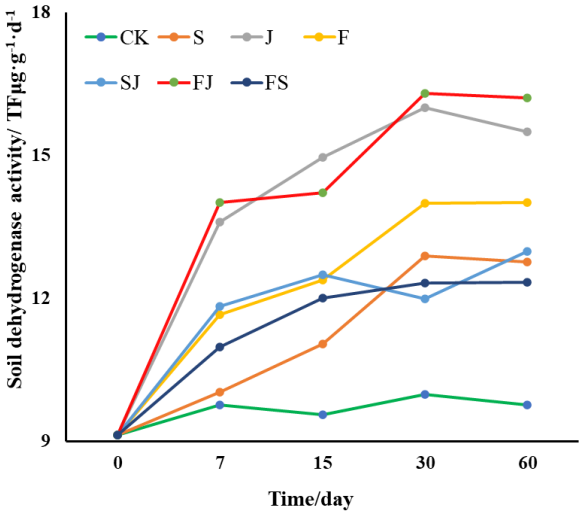

Figure 5. *Effect of different treatments on soil dehydrogenase activity. CK: control; S: rhamnolipid; J: degradation bacteria; F: organic fertilizer; SJ: rhamnolipid+degradation bacteria FJ: degradation bacteria +organic fertilizer FS: organic fertilizer+rhamnolipid*

Lipase activity

Lipase is widely present in animals, plants, and microorganisms. It can hydrolyze triglycerides to form diglycerides and fatty acids and catalyze the reverse reaction of hydrolysis - esterification. Under the action of lipase, carboxylic acid lipids in soil organic compounds are hydrolyzed into soluble substances, which plays an important role in soil biodynamics (Geissen et al., 2008). From *Figure 6*, it can be seen that the lipase activity of each treatment showed a trend of first increasing and then decreasing and was higher than that of the CK treatment. Among them, the degradation bacteria + organic fertilizer treatment had the highest lipase activity, and the treatment with added degradation bacteria was higher than the treatment without added degradation bacteria. The lipase activity of each treatment on contaminated soil samples increased slightly in the early stage, and then did not change much, and its activity remained at a low level. At the same time conditions, the lipase activity of each treatment was higher than that of CK treatment, and the lipase activity of FJ treatment was higher than that of other treatments, followed by J treatment.

Correlation between soil enzyme activity and total petroleum hydrocarbon residue

The correlation analysis between soil enzyme activity and total petroleum hydrocarbon residue during the remediation of petroleum-contaminated soil indicates that the four soil enzyme activities are negatively correlated with the petroleum residue. Among them, the urease activity and total petroleum hydrocarbon residue show a significant negative correlation, with a correlation coefficient of -0.754 (P=0.020); the catalase activity and total petroleum hydrocarbon residue show a significant negative correlation, with a

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correlation coefficient of -0.715 (P=0.049); the dehydrogenase activity and total petroleum hydrocarbon residue show a significant negative correlation, with a correlation coefficient of -0.896 (P=0.0073); the lipase activity and total petroleum hydrocarbon residue show a significant negative correlation, with a correlation coefficient of -0.674 (P=0.022).

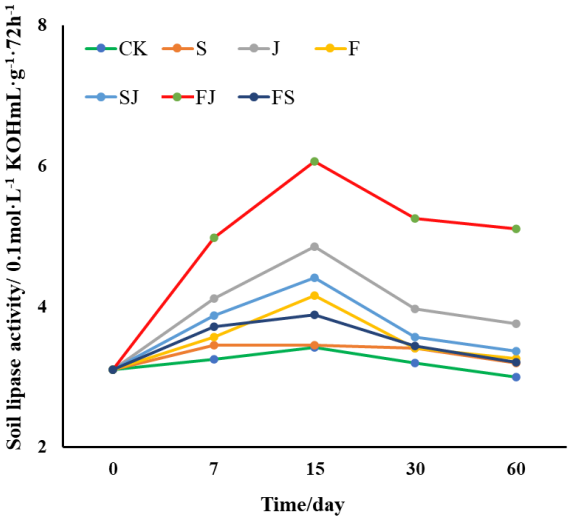

Figure 6. *Effect of different treatments on soil lipase activity. CK: control; S: rhamnolipid; J: degradation bacteria; F: organic fertilizer; SJ: rhamnolipid+degradation bacteria FJ: degradation bacteria +organic fertilizer FS: organic fertilizer+rhamnolipid*

A linear regression equation is placed in *Table 3* by using soil enzyme activity as the independent variable (X) and total petroleum hydrocarbon residue as the dependent variable (Y) for model analysis.

Table 3. *Correlation regression equation between different soil enzyme activities and total petroleum hydrocarbon residue rate*

Enzyme type	Regression equation	R^2
urease	Y=-11.023X+145.55	0.952
catalase	Y=-11.879X+126.43	0.981
dehydrogenase	Y=-1.5968X+109.48	0.966
lisape	Y=-3.5871X+87.65	0.888

Discussion

Studies have shown that restoring the original ecological function and state of contaminated soil is a long-term and complex ecological process (Shintani et al., 2019; Wei et al., 2020). Even if the content of target petroleum pollutants in petroleum-contaminated soil meets environmental standards, the presence of residual refractory components and secondary metabolites or intermediate products can still result in strong ecological toxicity in the soil, which will eventually be manifested in organisms (Al-Mutairi et al., 2008). Changes in ecological toxicity during the process of restoring petroleum-contaminated soil can to some extent affect the activity of soil microorganisms,

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leading to changes in soil enzyme activity. Soil enzyme activity, as a comprehensive indicator of soil environment, is an important factor in the metabolism of soil microorganisms (Wang et al., 2023). Therefore, using it as an indicator of soil ecological toxicity is feasible to some extent.

Adding degradation bacteria effectively reduced TPH (Zhou et al., 2020). Organic fertilizer is added to provide carbon source for microorganisms (Radziemska et al., 2021; Zhou et al., 2023). The increase in urease activity indicates a significant improvement in the nitrogen conversion ability of soil after biological restoration treatment. The increased effective nitrogen involved in urease activity promotes the growth of microorganisms in the soil and provides more abundant nutritional conditions for microorganisms to participate in the degradation of petroleum hydrocarbons (Wu et al., 2010; Zhang et al., 2020). The reason for the decrease in the rate of increase in urease activity during the later stages of remediation may be due to the large consumption of nutrients and a relative increase in the content of difficult-to-degrade components. In contrast, the urease activity in the CK treatment did not show inhibitory effects initially and even showed a certain degree of stimulating effect. This may be due to the adaptability of indigenous microorganisms in soil to petroleum and the fact that some components of petroleum can be used by microorganisms in soil related to urease (Li et al., 2003).

Soil urease activity is not only closely related to nitrogen in the soil but also has a significant positive correlation with total phosphorus and organic matter in the soil (Li et al., 2003; Wang et al., 2010). Wang found that there were differences in urease activity in soils of different types contaminated by petroleum. The enhancement of catalase activity is due to the proliferation of dominant microorganisms during the restoration process, in which microorganisms participate in the degradation process of petroleum hydrocarbons, and a large amount of hydrogen peroxide is produced, increasing the microbial and soil environment, and reducing the ability of respiration to produce hydrogen peroxide (Zhang et al., 2020). The slight decrease in catalase activity after 30 days may be due to severe C/N imbalance in the soil during the late stage of biological restoration, which affects the activity of microorganisms. Studies have shown that the bacterial cell composition of petroleum degradation can be represented by $C_{106}H_{180}O_{45}N_{16}P_1$, and the optimal biologically utilizable component ratio in microbial degradation process is C/N=100/15 (Graham et al., 1999). Some highly toxic intermediate products produced by petroleum hydrocarbons during the biological restoration process can inhibit the growth of microorganisms (Lu et al., 2009). Hydrogen peroxide produced in the process of biological respiration accumulates in the soil, which has a toxic effect on microorganisms. In fact, the catalase activity in the soil characterizes the strength of the biochemical oxidation process in the soil, and it is closely related to the organic matter and nitrogen in the soil. Studies have shown that the catalase activity of the tested soil is highly positively correlated with the organic matter content and total nitrogen content in the soil, and there is no significant correlation with other soil properties (Wang et al., 2013). The increase in dehydrogenase activity may be due to the improved stress environment of different additives or the availability of new carbon sources, which increases microbial activity (Zhang et al., 2020; Devi et al., 2022). The rate of dehydrogenase activity increased the most during the 0–7-day cultivation period, indicating that the oxidation and transformation of petroleum hydrocarbons in the soil were the fastest, and the rate of petroleum hydrocarbon degradation was also the highest, which is consistent with the results reported by Liu et al. (2018) and Zhen et al. (2021). The increase in dehydrogenase activity in the CK treatment also proves that some

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indigenous microorganisms in petroleum-contaminated soil can use petroleum pollutants as a new carbon source. The decrease in dehydrogenase activity after 30-60 days of cultivation may be due to the reduction of easily degradable pollutants in the late stage of restoration, the accumulation of difficult-to-degrade substances, and the increase in toxic metabolites, leading to a decrease in microbial activity (Devi et al., 2022). Studied the effect of crude oil and refined petroleum products on soil dehydrogenase activity and found that the change in dehydrogenase activity in petroleum-contaminated soil varied significantly with the type of oil, where crude oil is a natural product and is more easily oxidized in the soil than refined oil. TPH concentration is significantly positively correlated with dehydrogenase and lipase, and high microbial activity promotes the degradation of petroleum pollutants (Zhang et al., 2019). In the early stages of bioremediation, the significant increase in soil lipase activity may be due to the stimulation of the growth and metabolism of petroleum-degrading microbial communities by the lipid substances in oil-contaminated soil (Wang et al., 2013). Margesin et al. (1999) believed that soil lipase activity is a valuable indicator during the bioremediation process of oil-contaminated soil. Lipase activity has a rapid rise phase of 0-15 days, and its activity reaches its peak on the 15th day and then decreases continuously, which is consistent with the research results of Li et al. (2009). In the later stages of bioremediation, the activity of lipase is not inhibited by the reduction of readily biodegradable components, but instead maintains a relatively high level. Some studies also indicate that the decrease in readily biodegradable components and the accumulation of recalcitrant substances in soil after bioremediation do not have a significant effect on soil lipase activity. In this study, a significant decrease in soil activity was observed in the later stages of bioremediation, which may be due to the production of some toxic intermediate metabolites during the bioremediation process, which are more toxic than the original pollutants and inhibit microbial activity.

Conclusions

Compared with CK, different additives can significantly enhance the total petroleum hydrocarbon degradation rate in petroleum-contaminated soil. After 60 days of cultivation, the combined treatment with FJ showed the highest total petroleum hydrocarbon degradation rate. In this study, four representative soil enzymes, namely urease, peroxidase, dehydrogenase, and lipase, were selected to investigate their changes during soil remediation under different treatment conditions. A regression model coupling soil enzyme activity and total petroleum hydrocarbon residue was established to estimate their relationship in the bioremediation system. The results showed that the activity of the four enzymes in petroleum-contaminated soil under different treatments had a similar variation pattern with cultivation time. The enzyme activity of the four enzymes in each treatment was significantly higher than that in the CK treatment during the cultivation process, and their activity increased rapidly during 0-30 days of cultivation and then tended to decrease for 30-60 days, but still significantly higher than that in the CK treatment. Under the soil conditions tested in this experiment, the activity of the four enzymes was negatively correlated with the total petroleum hydrocarbon residue in the contaminated soil.

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