Biostimulation-phytoremediation of petroleum hydrocarbon contaminated soil: Response surface methodology (RSM)

Jing Li (2283050052@qq.com)
UPC: China University of Petroleum Huadong
Nian Ma
China University of Petroleum Huadong
Boyu Hao
China University of Petroleum Huadong
Feifei Qin
China University of Petroleum Huadong
Xiuxia Zhang
China University of Petroleum Huadong https://orcid.org/0000-0002-3606-5952

Research Article

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Abstract

In view of the analysis and remediation of petroleum hydrocarbon contaminated soil, the study on ecological technology of oilfield well site was carried out. The response surface was used to optimize the single-factor biological stimulation experiment of moisture content, the leavening agent content and the compound fertilizer content, and get the best experimental plan of biological stimulation. The artificial stimulation-plant combined remediation experiment was designed. Through screening 20 kinds of plant seeds suitable for growth, selected 5 plants with high tolerance to petroleum hydrocarbons. The artificial biostimulation-phytoremediation combined degradation experiment of petroleum hydrocarbons in contaminated soil was designed, and the degradation rate of petroleum hydrocarbons in soil under the optimal ecological remediation scheme was obtained. It was found that petroleum hydrocarbons degradation rate in soil after 70 days artificial stimulation experiment was 28.6%. Through the screening of 20 plants, peanut had the highest tolerance to petroleum hydrocarbons in soil, and the degradation rate in 70 days soil reached 31.1%. After 70 days of biostimulation-phytoremediation, the degradation rate in soil reached 38.9%.

1. Introduction

As one of the important energy sources, petroleum has been widely used in the world. Total petroleum hydrocarbons (TPH) are one of the broadly existing organic pollutants in the environment. It is a mixture of various hydrocarbons (n-alkanes, branched alkanes, cycloalkanes, aromatics)(Rodriguez-Campos et al. 2019) and a small amount of other organic substances (sulfide, nitride, naphthenic acid, etc). Due to industrial activities such as the process of petroleum exploitation, storage, transportation, processing and petrochemical production, a large amount of petroleum enters into the environment and causes an increasing number of sites are severely contaminated(Kost et al. 2017; Prince et al. 2013; Varjani et al. 2017).

Petroleum hydrocarbon pollution has caused different degrees of impact on soil and groundwater environment. It is mainly manifested in the destruction of the structure and function of soil ecosystem. For example, it affects the permeability and the enzyme activity of the soil, resulting in soil hardening and fertility decline (Dindar et al. 2015). Further, the microbial biomass and bacterial population density decreased (Huang et al. 2019; Wu et al. 2020). In addition, petroleum hydrocarbon has lower density than water, so they have a tendency to float on the surface of surface water and groundwater and migrate or move easily with them flow to cause the groundwater pollution (Yang et al. 2020). Some teratogenic and carcinogenic substances in petroleum can also directly harm human health through bioaccumulation of food chain, and thus, effective measures should be taken immediately.

The treatment of contamination was caused by petroleum hydrocarbons is a huge and laborious work. In recent years, lots of in-situ and ex-situ remediation treatment methods and techniques have been researched and applied in the laboratory and on-site. These methods and techniques play a significant role in complete cleaning, containment, removal, reclamation and restoration of contaminated environment(Ossai et al. 2020). Petroleum contaminated soil treatment technology mainly includes physical remediation technology, chemical remediation technology and bioremediation technology, among which physical remediation technology is costly and difficult to operate; chemical repair technology is easy to damage soil structure, causing secondary soil pollution. It is imperative that we must find an effective technology to repair the contaminated soil. Bioremediation technology is considered an economical, efficient and green treatment method. They can remediate or degrade petroleum hydrocarbons and various organic contaminants to simpler and non-toxic substances without secondary pollution on the impacted environments(Lim et al. 2016).

There are many various ways of bioremediation technologies, including biostimulation, bioaugmentation, phytoremediation, animal repaired and microbe-assisted phytoremediation (Khoshkholgh Sima et al. 2019; Roy et al. 2018). Using bioaugmentation technology to increase the degradation rate of pollutants by introducing exogenous strains, this method tends to form a strong competition and predation relationship with indigenous microorganisms(Bidja Abena et al.

2019). Therefore, the selection of indigenous microorganisms has been regarded as the first choice because they can easily acclimatize in the same environment(Suja et al. 2014). In order to overcome the limitations of local microorganisms by environmental factors such as temperature, oxygen, moisture content, pH and nutrients, many investigators have reported that biostimulation could be carried out by adding appropriate nutrients to increase the metabolic activity of microorganisms and accelerate the degradation of petroleum hydrocarbon contaminate(Gong 2012; Koshlaf et al. 2019; Margesin et al. 2003; Naidu et al. 2015; Wu et al. 2019). However, due to the complexity of oil contaminated sites, a single method cannot achieve the ideal treatment effect for petroleum hydrocarbon contaminated soil, so the integration of one remediation approach with another approach either simultaneously or sequentially may result in enhance the combined effect and degradation effect (Rippen 1999). Employing microbial-phytoremediation systems from soil is a novel, effective, and low-cost prospective biotechnological approach (Ni et al. 2018; Sarma et al. 2019). In the microbial-phytoremediation system, microorganisms convert pollutants such as petroleum hydrocarbons into substances that are easy for plants to directly absorb and utilize, and plant roots can provide suitable places for microorganisms to survive (Kiamarsi et al. 2020). In this system, the two can complement each other's advantages, thereby improving the repair ability. There are many examples of application of microbial-phytoremediation system at home and abroad(Fan et al. 2014; Kiamarsi et al. 2020; Wojtera-Kwiczor et al. 2014).

Only a few papers have reported a combination of biostimulation-phytoremediation. Accordingly, the objectives of this work as follows: (1) optimizing the single-factor (moisture content, the leavening agent content and the compound fertilizer content) biological stimulation experiment, (2) selecting plants with high tolerance to petroleum hydrocarbons, (3) designing the artificial biostimulation-phytoremediation combined remediation experiment. Therefore, the result of this study would provide valuable information for increasing the efficiency of TPH degradation rate.

2. Materials And Methods

2.1 Materials

The test soil used in this study was obtained from an oil field in Dongying, China. Prior to use, the soil was air-dried and stones, large soil particles and other debris were removed. The homogenized soil was then passed through a 4 mm sieve and the physicochemical properties of the contaminated soil was analyzed.The water used in the biological stimulation experiment was tap water, the leavening agent was broken corncobs with a particle size of about 1~2 mm, and the compound fertilizer was agricultural fertilizer. The flowers and crop seeds used in the phytoremediation experiment were purchased from Dongying Farmers' Market. Beef cream agar medium (used to calculate the total number of bacteria): beef extract 5.0 g, peptone 10.0 g, sodium chloride 5.0 g, agar 20.0g, deionized water 1.0 L, pH 7.0-7.5. The reagents and drugs used in the experiment were analytical grade.

2.2 Characteristics of soil

The specific characteristics of the soil are presented in Table 1, the Petroleum content was 14.6 mg/kg. The soil had a neutral pH (7.35). And they met the clean soil conditions used in the experiment. Also, the saturated content, aromatic content, colloid and asphaltene in oil were 42.89%, 23.91%, 20.42% and 1.55%, respectively. The saturated and aromatic content is easy to degrade(Liu et al. 2020). There was a large saturated and aromatic fraction in the oil component, and it met the requirements of the biodegradation experiment. The soil used in subsequent experiments were all uniformly configured soils with a petroleum hydrocarbon content of 9000 mg/kg. In addition, added the right amount of $NaNO_3$ and $NaHPO_4$ to make the ratio of C, N and P in the soil close to 100:10:1(Wu et al. 2014). By adjusting the CNP ratio, the degradation rate of petroleum hydrocarbons in the contaminated soil reached 9.1% and the total number of bacteria reached 9.1×10^3 CFU/g after 70 days experiment. The microorganisms in subsequent experiments were indigenous microorganisms. Due to the small particle size, poor nutrition and low water content of the tested soil, the three factors of

moisture content, the leavening agent and the compound fertilizer were selected to provide the indigenous microorganisms with sufficient oxygen and nutrients required for growth and to optimize subsequent organisms Stimulus experiment.

Table 1 The physical and chemical properties of test soil

	Petroleum content (mg/kg)	moisture content (%)	pH	C (g/kg)	N (g/kg)	P (g/kg)	Saturate (%)	Aromatic (%)	Resin (%)	Asphaltenic (%)
sample	14.6	9.29	7.34	47.43	4.26	0.3127	42.89	23.91	20.42	1.55

2.3 Experimental instruments

Laboratory special ultra pure water machine, BCD-539WE refrigerator, UV-6000PC UV-Vis spectrophotometer, XS-204 electronic balance, LDZM vertical steam sterilization pot, HZQ-D water bath constant temperature oscillator,101-1A type electric heating blast drying oven, HSX-250 constant temperature and humidity incubator, intelligent electric heating constant temperature blast drying oven.

3 Experimental Methods

3.1 Biological stimulation single factor experiment

The experiments were conducted to investigate the effects of several factors (moisture content, the leavening agent content and the compound fertilizer content) on TPH degradation. As mentioned before, the addition of $NaNO_3$ and $NaHPO_4$ to soil with 9000 mg/kg in petroleum hydrocarbon content brings the proportion of C: N: P in the soil close to 100:10:1. One kilogram of soil were taken and placed in a flower pot. The amount of moisture content was 10%, 15%, 20%, 25% and 30%. The influence of the leavening agent content to TPH biodegradation was investigated by using different corn cob powder content set at 10g, 30g, 50g, 70g, 90g, respectively. And the compound fertilizer content was set at 10g, 15g, 20 g, 25g, 30g, respectively. After fully stirring, the flower pots were placed in a thermostat and set the temperature to 25°C. After 70 days, the petroleum hydrocarbon content in the soil was measured. All experiments were conducted in triplicate, and the average values are reported. The experiment set up a set of control groups.

The remaining petroleum hydrocarbons in the soil were used by ultrasonic extraction, the absorbance was measured at a wavelength of 225nm using ultraviolet spectrophotometry, and the petroleum hydrocarbon content was calculated according to the standard curve. The specific steps were as follows: accurately weigh 0.1g oily soil sample into a 50mL centrifuge tube and add 10mL petroleum ether (distillation range 60-90°C). Extracted in ultrasonic for 15min, ultrasonic intensity: 360w (minimum 300w). Ultrasonic water bath temperature: select room temperature and control it below 40°C. Centrifuge at 4000r/min for 10min. The supernatant extract was collected into a 50mL colorimetric tube through a glass long-diameter funnel containing 1cm thick anhydrous sodium sulfate (baked at 500°C for 2h). These operations were repeated three times. Finally, the degradation rate calculation formula is as shown in formula (1).

$$\text{Degradation rate } (\%) = \frac{W0 - W1}{W0} \times 100\% \qquad (1)$$

W_0-initial petroleum content in soil, g

W_1-petroleum residue in soil after 70 days of degradation, g

3.2 Biostimulation response surface optimization experiment design

In this study, the single-factor biostimulation experiments was studied and optimized by using three factors, namely: moisture content (A), the leavening agent content (B) and the compound fertilizer content (C). A rotatable 3^k Box-Behnken design (BBD) was adopted for the TPH degradation rate in 70 days are shown in table 2. The experimental results were analyzed using Design Expert 8.0.6 and a regression model was proposed using the diagnostic checking tests.

Table 2 Response surface experiment results

Run order	A	B	C	Degradation (%)	
				Actual	Predicted
1	10	30	20	15.3	15.2
2	20	30	20	16.6	16.3
3	10	70	20	17.8	18.2
4	20	70	20	18.3	18.5
5	10	50	15	17.7	17.8
6	20	50	15	17.1	17.4
7	10	50	25	17.3	17.0
8	20	50	25	18.9	18.8
9	15	30	15	17.5	17.5
10	15	70	15	22.8	22.3
11	15	30	25	19.5	20.0
12	15	70	25	20.4	20.4
13	15	50	20	29.6	28.9
14	15	50	20	28.1	28.9
15	15	50	20	27.6	28.9
16	15	50	20	29.5	28.9
17	15	50	20	29.9	28.9

3.3 Phytoremediation experiment design

The glass petri dishes with a diameter of 9 cm were chose, they were washed and sterilized at 110°C and put a layer of cotton inside, moistened it with distilled water and set aside. There were 20 plant seeds with 30 seeds each and put them in a petri dish covered with cotton. Then, added distilled water to make the water layer deep was 0.1cm. The test seeds were placed in a 25°C incubator for 14 days to observe the germination of the seeds. The germination rate of seeds was calculated by formula. After the plant seed germination rate experiment was completed, selected the plants with the highest germination rate for the tolerance experiment of petroleum contaminated soil. A petroleum contaminated soil with a concentration of 9000 mg/kg was configured, and 1 kg of soil was placed in a flower pot, and 3 seeds with a germination rate ≥90% were sow in each flower pot. The TPH degradation rate, the number of bacteria, the growth of test plants and the amount of catalase in the soil in the flowerpot were determined and calculated after 70 days of pot tests. All experiments were conducted in triplicate, and the average values were reported.

3.4 Biostimulation-phytoremediation joint restoration experiment design

Selecting the best biostimulation plan optimized by response surface methodology and the most dominant plants for joint restoration. The petroleum hydrocarbon content and the number of bacteria under the joint treatment soil every 10 days were measured.

4 Results And Discussion

4.1 Factors affecting the biodegradation of the TPH degradation rate

After 70 days of degradation, the degradation rate of petroleum hydrocarbons and the number of bacteria in the flowerpot soil were shown in Figure 1a. After 70 days of experimentation, it was found that when the moisture content was 15% into the soil, the degradation rate of petroleum hydrocarbons on the 70th day was 9.4%, it hardly improved the degradation effect. At the same time, the number of bacteria in the soil was 6.1×10^3 CFU/g. Figure 1b showed that the content of leavening agent had effect on the degradation of petroleum hydrocarbons by microorganisms, the degradation rate reached 18.4%, a relative increase of 9.3%. However, it had a greater impact on the total number of soil bacteria. The best amount of leavening agent was 50g. It could be explained with the following viewpoint, if the content of leavening agent was too small, the soil ventilation would also be small, and the normal respiration of microorganisms would be affected; if the content of leavening agent was too much, the living conditions of anaerobic organisms involved in the degradation of petroleum hydrocarbons would be affected. Figure 1c. showed that the degradation rate of petroleum hydrocarbons and the number of bacteria in the flowerpot soil after 70 days. It was concluded that when 20g of compound fertilizer was added to the soil sample, the degradation rate of petroleum hydrocarbons in the soil was the largest, and the maximum degradation rate was 21.2%. When 20g of compound fertilizer was added to the soil sample, the number of bacteria in the soil was the highest, and the number of bacteria was at most 6.4×10^6 CFU/g, relatively improved by three orders of magnitude.

4.2 Optimization for biological stimulation experiment

The experimental design and responses values of biological stimulation experiment were shown in Table 3. As P is less than 0.05, the index has a significant impact, and if P is less than 0.01, the index is very significant. It can be seen from Table 3 that the P of the model is less than 0.0001 and the coefficient of the lack-of-fit term is greater than 0.05, indicating that the model is of great significance for TPH bio-degradation. Use response surface software to perform binary regression fitting, and the regression equation obtained is as follows:

$$Y = 28.94 + 0.35A + 1.30B + 0.12C - 0.20AB + 0.55AC - 1.10BC - 7.12A^2 - 4.82B^2 - 4.07C^2 \quad (2)$$

where Y represents the response variable (TPH degradation rate); A, B, C are the actual values of moisture content, the leavening agent content and the compound fertilizer content, respectively. The experimental results were subjected to analysis of variance (ANOVA) (Table 3). The model with calculated F value and a very low P value (less than 0.05) showed that the model was highly significant (Karthic et al. 2013). In this experiment, the regression equation coefficient R^2=0.9885, the calibration determination coefficient Adj R^2=0.9737>0.80, the standard deviation Std. Dev.=0.86, and the coefficient of variation C.V.%=4.01, indicating that the model fits well and the accuracy is high. Therefore, this empirical model can be applied to predict the TPH degradation rate. Also, the significant coefficients of the first-order coefficients B and the quadratic coefficients A^2, B^2, C^2 are all less than 0.01, and their influence were very significant, while the significant coefficients of the quadratic coefficients BC was less than 0.05, but greater than 0.01, its impact was also significant. Comprehensive comparison shows that the significant impact on the degradation rate of petroleum hydrocarbons was: the leavening agent content (B)<moisture content (A)<the compound fertilizer content (C).

It can see from Fig. 2a, the model has a good fitting capability between the experimental values and the responses. The normal probability distribution of the residuals is on a straight line (Fig. 2b), indicating that the model has good adaptability. In addition, random dispersion of the residues and a distribution between -2 and +2 can also be seen in Fig.2c. To sum up, it was reasonable and acceptable for the regression model adequacy.

Table 3 Analysis of variance (ANOVA) for response surface quadratic model

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value prob > F	
Model	443.11	9	49.23	66.83	< 0.0001	significant
A	0.98	1	0.98	1.33	0.2866	
B	13.52	1	13.52	18.35	0.0036	
C	0.13	1	0.13	0.17	0.6927	
AB	0.16	1	0.16	0.22	0.6554	
AC	1.21	1	1.21	1.64	0.2408	
BC	4.84	1	4.84	6.57	0.0374	
A^2	213.45	1	213.45	289.73	< 0.0001	
B^2	97.82	1	97.82	132.78	< 0.0001	
C^2	69.75	1	69.75	94.67	< 0.0001	
Residual	5.16	7	0.74			
Lack of fit	0.98	3	0.33	0.31	0.8152	not significant
Pure	4.17	4	1.04			
Cor Total	448.27	16				

4.3 Response surface plotting and optimization of bio-degradation process

According to the quadratic equation model, the three-dimensional response surface and the contour map for the interaction between experimental factors were respectively made, and the interaction of the other two factors affects the degradation rate of TPH in a factor fixed in the same core value study, as shown in Figures 3.

When the model diagram is ellipse, indicating the interaction of factors is obvious, otherwise, it means that the interaction between the two factors is not significant(Sun et al. 2019). The contour map and 3D surface map of moisture content and the leavening agent content on the degradation rate of petroleum hydrocarbons are shown in Fig.3a below. It can be seen that the contour map of the interaction between moisture content and the leavening agent content on the degradation rate of petroleum hydrocarbons was almost circular. At this time, the degradation rate of petroleum hydrocarbons in the three-dimensional surface map shows a trend of first increasing and then decreasing with the increase of leavening agent content, and the effect of water content on it is similar, indicating that there is no significant interaction between the two factors. Therefore, it shows that in the experimental soil at this time, there is no synergistic effect between moisture content and the leavening agent content.

Figure 3b shows the effect of moisture content and the compound fertilizer content on the degradation rate of petroleum hydrocarbons, and there is an optimum point for moisture content at around 15%. The degradation rate of petroleum hydrocarbons in the 3D surface graph first increases and then decreases with the amount of moisture content and the

compound fertilizer content, indicating that there is no significant interaction between the amount of water sprayed and the amount of compound fertilizer.

In Fig.3c, the 3D pattern of leavening agent content was steeper than the contour line of the compound fertilizer content, indicating that the leavening agent content affected degradation rate more significantly(Antonopoulou et al. 2017). Under the leavening agent content of 30–50g, the degradation rate increased with the increase of addition. On the one hand, the function of leavening agent is to adjust the moisture and material porosity, increase soil oxygen content(Adhikari et al. 2009). On the other hand, it can be used as the regulation of soil nutrient composition to adjust the nutrient balance for the microbial community, so that the soil properties meet the needs of microbial growth and metabolism(Barrington et al. 2002). We also found that when the leavening agent content was about 50 g, the compound fertilizer content was about 20 g slightly, the degradation rate reached the highest. It can be seen from Figure. 1c that the contour map is nearly oval, suggesting that there is a significant interaction between the two factors.

In summary of the three response surfaces, contour maps and regression equations, the best values of the three factors are 15 mL of moisture content, 50 g of leavening agent content, and 20 g of compound fertilizer content. The degradation rate of petroleum hydrocarbons predicted by the model under the optimal conditions of the three factors is 28.9%. Under these optimal conditions, the experiment was conducted again to verify that the degradation rate of petroleum hydrocarbons within 70 days was 28.6%, which is close to the model prediction results, and the accuracy of the model is better. Thus, it could be used to predict the effect of artificial stimulation to repair oil-contaminated soil. Studies have shown that bio-stimulation is the most successful and effective bioremediation method in simulated soil contaminated by petroleum hydrocarbons(Simpanen et al. 2016). The researchers used a mixture of bacterial consortia and nutrients to biodegrade petroleum hydrocarbon-contaminated soil within 18 months to achieve a 99.9% removal rate(Singh et al. 2012) . In contrast, the degradation effect of this study was not obvious, so the following joint plant restoration experiment was carried out.

4.4 Dominant plant selection

In order to explore phytoremediation of petroleum-contaminated soil, selected oil-tolerant plants, and improved the effect of on-site remediation of petroleum-contaminated soil. Twenty kinds of plants were selected for germination rate experiment. The germination rate of 8 kinds of seeds is ≥90% (Table 4), such as peanuts, shepherd's purse, zinnia, cotton, chives, beans, morning glory and Helianthus. The germination rate of the remaining 12 kinds of plant seeds is less than 80%. It might be there are many factors that affect seed germination, and different seeds have different germination rates.

Table 4 Table of growth of tested plants

	Plant species	Number of tested seeds	Number of sprouted seeds	Germination rate/%
1	Cotton	30	28	93.3
3	Soy	30	22	73.3
4	Spinach	30	12	40.0
5	Carob	30	27	90.0
6	Lettuce	30	0	0
7	Rape	30	11	36.7
8	Chives	30	28	93.3
9	Cabbage	30	22	73.3
10	Shepherd's purse	30	29	96.7
11	Strawberry	30	23	76.7
12	Common China-aster	30	0	0
13	Morning glory	30	27	90.0
14	Bluebonnet	30	5	16.7
15	Spearmint	30	14	46.7
16	Clover	30	0	0
17	Helianthus	30	27	90.0
18	Callipsis	30	16	53.3
19	Zinnia	30	29	96.7
20	Eustoma	30	0	0

Five kinds of test seeds were selected and cultured in petroleum-contaminated soil for 70 days. The petroleum hydrocarbon content of the soil in the flowerpot was determined. After 70 days of exploration, the degradation rate of petroleum hydrocarbons in the soil by plants as shown in Fig. 4. Among the 5 tested plants, peanuts and cotton could germinate normally in the soil, and the degradation rates of TPH were 31.1% and 20.3%, respectively. It might be that petroleum hydrocarbon pollution had not invaded the seed epidermis before seed germination and harmed the seed germ, so it showed that petroleum hydrocarbon pollution had little effect on seed germination and emergence. Because the experimental groups of shepherd's purse, zinnia and chives failed to germinate normally, their petroleum degradation was manifested as natural degradation. Plants can transform pollutants into environmentally less toxic and persistent substances through mechanisms such as phytoaccumulation, phytotransformation, rhizodegradation, phytostabilisation(Wang and Dai 2011). Peanut plants can remove organic pollutants in the soil through adsorption and accumulation, and the root exudates can complex and degrade organic pollutants, and the enzymes released by the roots into the soil also promote the degradation of pollutants(He et al. 2021).

The colony count could be considered as a method for the quantification of bacteria in soil. It might provide us with a useful insight regarding their growth in comparative studies. In the flowerpot where peanuts grew, the number of bacteria was the most, reaching 4×10^7 CFU/g. In phytoremediation, peanuts plants affect the activities of microorganisms by changing the living environment of soil microorganisms, including changing water content, pH, and releasing organic matter, transporting more nutrients for rhizosphere microorganisms, etc (Doornbos et al. 2012). In addition, peanuts have a larger root system, which provides a suitable habitat and sufficient oxygen for microorganisms.

Enzyme activity is widely used to monitor soil pollution and remediation processes(A et al. 2020; Topac et al. 2009). The enzymes are used to monitor hydrocarbon degradation include lipase, dehydrogenase, catalase and urease, etc(Ueno et al. 2007).With the progress of restoration, soil catalase activity had a greater increasing trend. In the cultivation of peanut biological flowerpots, the content of catalase was the most, reaching 2.94 $mL·h^{-1}·g^{-1}$. Catalase activity could reflect the ability of the soil to remove the hydrogen peroxide produced during respiration. Catalase degrades hydrogen peroxide (H_2O_2) into oxygen and water. If hydrogen peroxide has been accumulated but never decomposed, it will have a toxic effect on the microorganisms in the soil(Cristaldi et al. 2017; Hoang et al. 2021; Hussain et al. 2017).

4.5 Biostimulation-phytoremediation of petroleum hydrocarbon contaminated soil

The selected peanuts are combined with the best biological stimulation to treat petroleum-contaminated soil, and the TPH degradation rate in the soil under the combined situation is explored. As shown in Figure 5, the degradation rate of petroleum hydrocarbons in the soil reached 38.9% after 70 days. It contributes to the strong root system of peanuts. The introduction of peanuts provides better living conditions for bacteria and promotes bacterial growth. A large number of bacteria use petroleum hydrocarbons as nutrients, so petroleum hydrocarbons in the soil are degraded(Huang et al. 2014). After repairing 9000 mg/kg, the residual petroleum hydrocarbons in the soil reached 5499 mg/kg, which is far lower than the risk control value of 9000 mg/kg in the second type of construction land. This scheme can be used for on-site site restoration.

5 Conclusions

The present study was designed to explore and optimize the responses of biological stimulation experiment on petroleum-contaminated soil by response surface methodology, and combined with phytoremediation to enhance the effect of degradation and assist in setting up novel and highly efficient rhizoremediation systems. Through the response surface experiment design, under the best biological stimulation conditions, the experiment was conducted again to verify that the degradation rate of petroleum hydrocarbons within 70 days was 28.6%. The degradation rate of individual peanut plants reached 31.3% after 70 days plant degradation experiments under natural conditions. After 70 days of biostimulation-phytoremediation, the degradation rate of petroleum hydrocarbons in the soil reached 38.9% and the residual petroleum hydrocarbons in the soil reached 5499 mg/kg. Exposing the peanut to petroleum pollution soil stimulated the root biomass and enzyme activity, concomitantly induced the biochemical and physiological responses in this plant. It is interesting to mention that the inoculation of bacterial consortium increased plant growth as well as hydrocarbon degradation. Therefore, the utilizing of peanut and specially the interaction of bacteria suggests an alternative strategy for effectual phytoremediation of soils contaminated with petroleum. The exact mechanisms and toxico-logical risk with the remediation process in this interaction and the possible effect of the polar fractions produced during the degradation process need further elucidation and also investigation.

Declarations

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Authors' contributions

Jing Li: conceptualization and writing original draft; Nian Ma: methodology and investigation; Boyu Hao: experiental performance, conceptualization, methodology; Feifei Qin: material preparation, data collection and analysis; Xiuxia Zhang:

project administration and funding acquisition.

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Data availability

All data and materials generated or analysed during this study are included in this published article.

Compliance with ethical standards

Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

The authors confirm that the manuscript has been read and approved by all authors. The authors declare that this manuscript has not been published and not under consideration for publication elsewhere.

Consent to participate

The authors have been personally and actively involved in substantive work leading to the manuscript and will hold themselves jointly and individually responsible for its content.

Consent for publication

The authors consent to publish this research.

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Figures

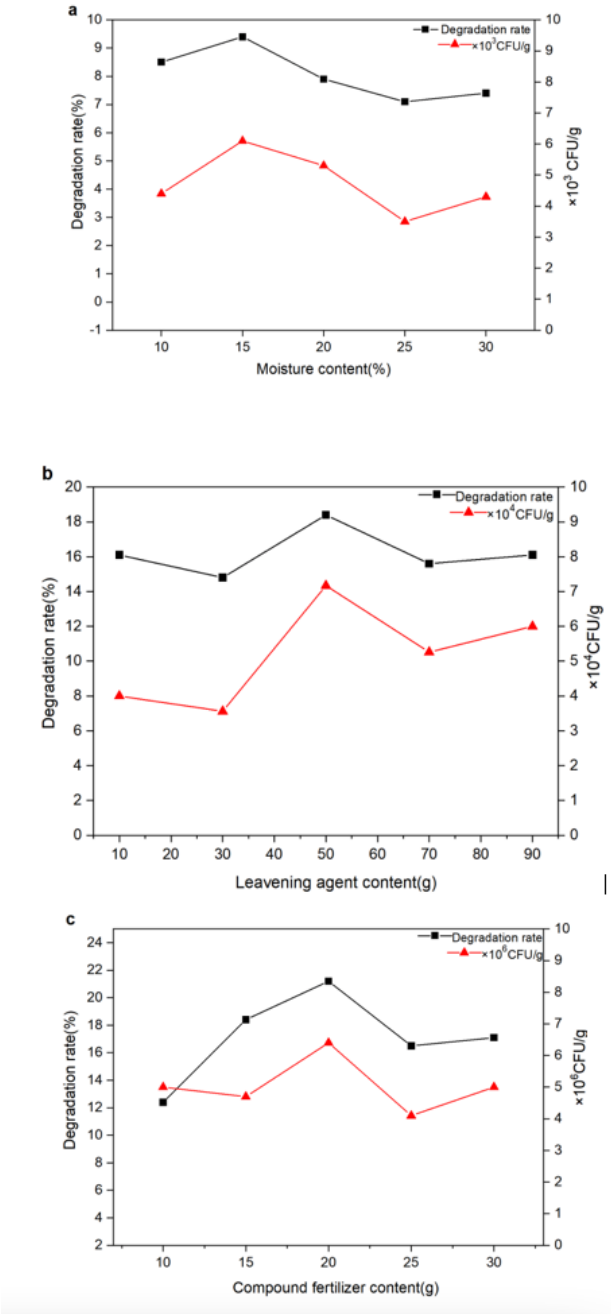

Figure 1

Factors affecting the biodegradation of the TPH degradation rate and the number of bacteria: a. the moisture content; b. the leavening agent content; c. the compound fertilizer content

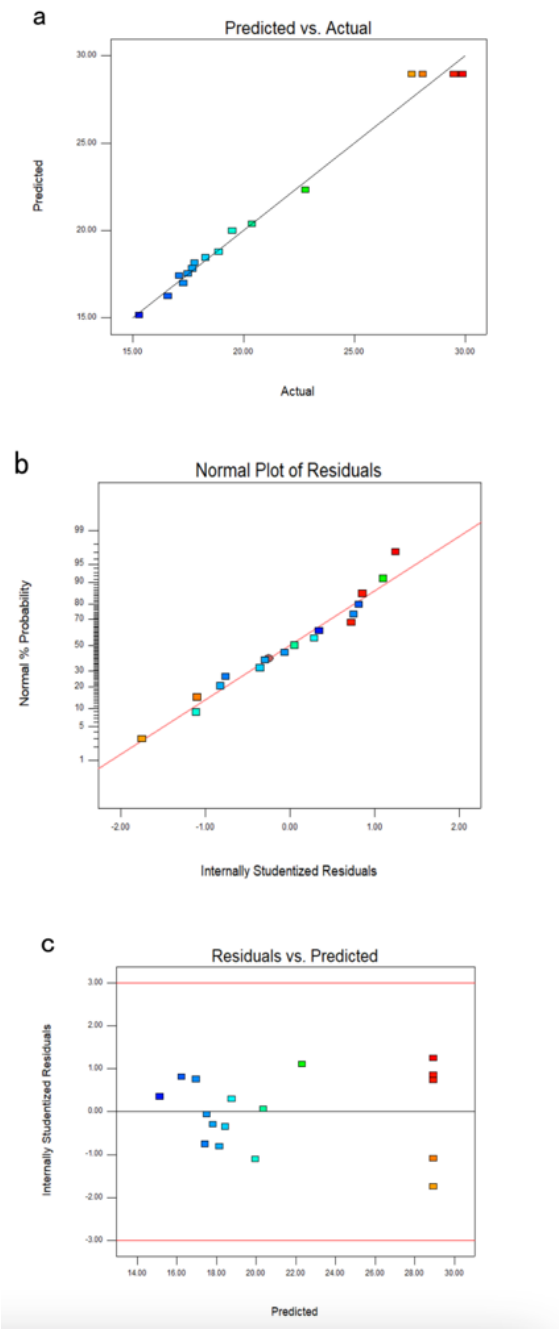

Figure 2

Residual diagnostics of quadratic model: a predicted vs. actual plot, b normal probability plot, and c internally studentized residuals vs. predicted values plot.

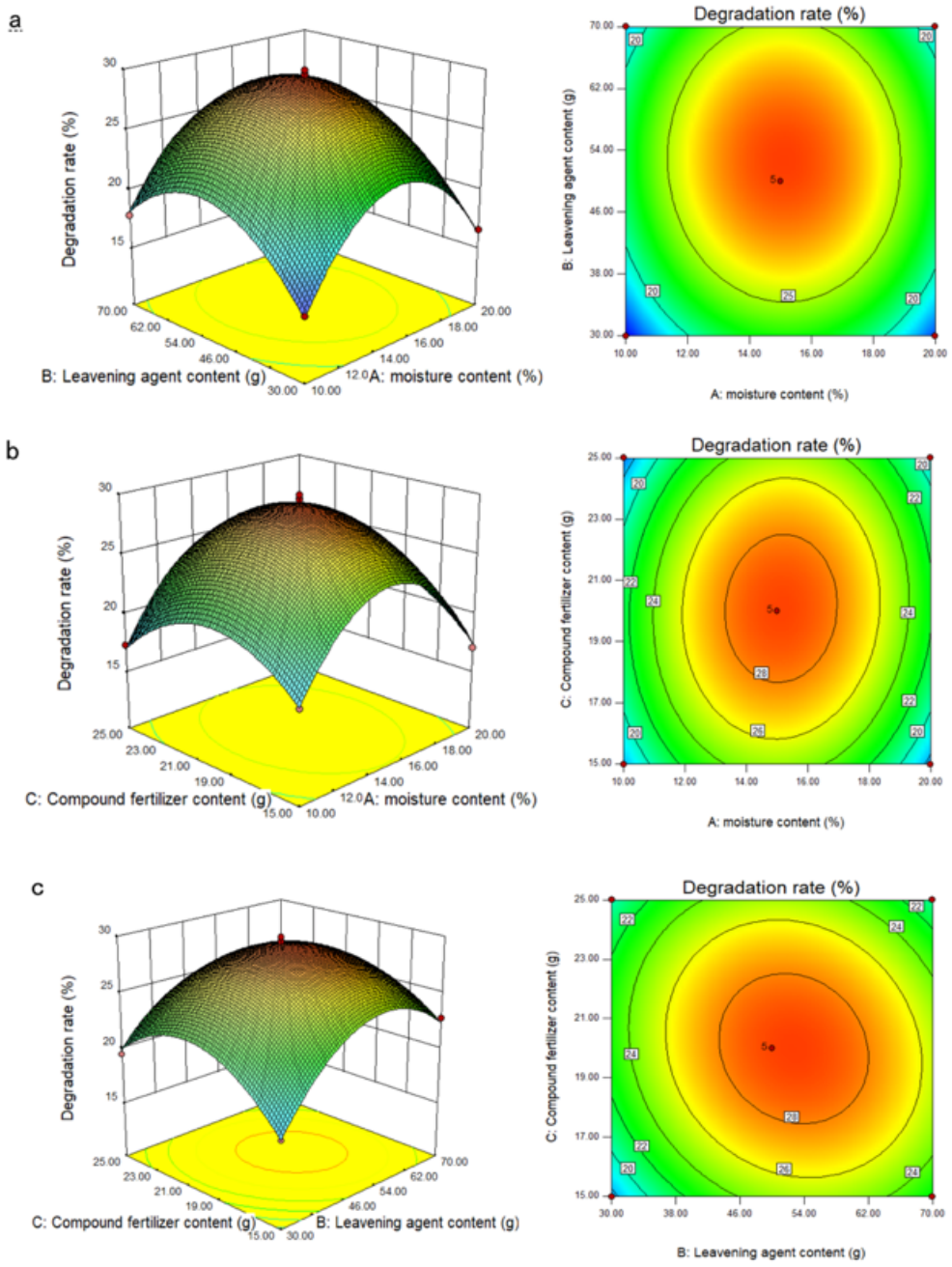

Figure 3

Three-dimensional response surface plots using RSM for TPH degradation rate: a moisture content vs. leavening agent content, b moisture content vs. compound fertilizer content, c compound fertilizer content vs. leavening agent content

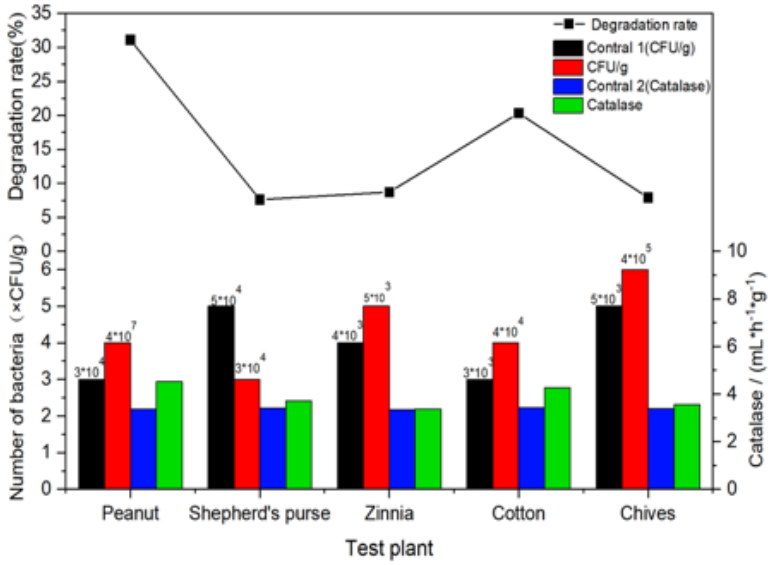

Figure 4

The degradation rate of petroleum hydrocarbons, the number of bacteria and the amount of catalase in 5 kinds of test plant soils

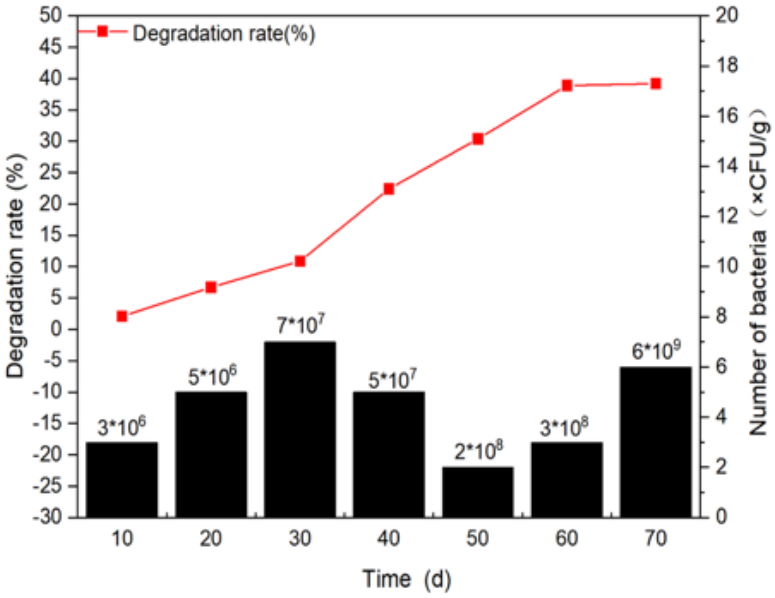

Figure 5

Joint Remediation of Oil Contaminated Soil