ECOLOGICAL REMEDIATION OF PETROLEUM-CONTAMINATED SOIL BASED ON MICROBIAL DEGRADATION

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Abstract. In the process of oil exploitation, refining, storage, transportation and use, due to the limitations of technological level and treatment technology, a large amount of petroleum-containing wastewater and waste residue are inevitably discharged into the soil, thus affecting the whole soil ecosystem. In this paper, an ecological remediation method for petroleum contaminated soil based on microbial degradation is proposed. The natural biodegradation of petroleum contaminants in soil is discussed. The types of degrading microorganisms and their degradation mechanisms for saturated hydrocarbons and aromatic hydrocarbons are introduced, and the chemical composition of petroleum and the number of microorganisms in the soil are analyzed. The effects of nutrients, oxygen supply, temperature, humidity and pH on the degradation efficiency of petroleum are studied, and the in-situ soil ecological remediation is carried out after the degradation of pollutants. Experiments show that the ecological remediation of petroleum contaminated soil based on microbial degradation can be more thorough in essence. This method has the advantages of requiring only a low time investment, being, low cost, simple and effective, and having a good remediation effect and no secondary pollution.

Keywords: ecological remediation method, natural biodegradation, soil ecological remediation, degradation efficiency, chemical composition

Introduction

Petroleum is a complex mixture of thousands of substances with different chemical properties, including saturated hydrocarbons, aromatic hydrocarbons, asphaltenes, resins and so on. Pollution and omission accidents in the process of oil exploitation, smelting, use and transportation, as well as the discharge of oily wastewater, sewage irrigation, volatilization of various petroleum products and falling of incomplete combustion materials, have caused a series of soil pollution problems. Especially the crude oil produced in the process of oil exploitation has become a significant source of mineral oil pollution in soil.

With the rapid development of the petroleum industry, the contamination of soil by petroleum pollutants is becoming more and more serious. Many studies have shown that some petroleum hydrocarbons have carcinogenic, teratogenic and mutagenic effects on humans and other mammals after they enter the animal body. It is difficult to remove petroleum hydrocarbons when they enter the soil, which will lead to changes in soil properties, even groundwater pollution and other major ecological crises. The serious pollution of soil will lead to the accumulation of some petroleum hydrocarbons in grain, affect its quality, and endanger human health through the food chain. Finding out how to effectively remediate petroleum contaminated soil has become a global environmental problem.
(1) Rapid pyrolysis technology will be used to remediate the soil. The effects of remediation related to pyrolysis parameters, oil recovery rate and its possible formation pathway, as well as the physical and chemical properties of the remediated soil and its suitability for planting are systematically studied. However, this method cannot degrade petroleum pollutants and remediate soil very well, and there are some shortcomings such as troublesome process and time-consuming (Li et al., 2018).

(2) The technology of supersulphate bioaugmentation foam spray is put forward, and used to accomplish the purpose of removing total petroleum hydrocarbon (TPHS) from diesel petroleum contaminated soil. The penetration/unsaturated hydraulic conductivity of unsaturated soils increased with the use of foam remedies. The mixture of sulfate and surfactant solution penetrates the soil faster than peroxide, but it does not make the soil moisture and dryness to an average effect (Bajagain et al., 2018).

(3) Based on the distribution mechanism of cell temperature in soil, the energy efficiency and distribution mechanism of EKR process are proposed. To some extent, the transformation mechanism of Cr6+ and Cr3+ in EKR process is discussed. Through this transformation mechanism, the regularity of chromium fractionation in soil is obtained, so that the degradation of soil is optimized. Although it had the function of post-transformation distillation, it cannot make remediation of soil well (Fu et al., 2017).

In this paper, an ecological remediation method for petroleum contaminated soil based on microbial degradation is proposed. According to the way of microbial degradation, the essential remediation of petroleum contaminated soil is fundamentally carried out. This method has the advantages of simple, effective, time-consuming and good soil remediation.

Material and methods

Materials

Chemical reagents: MgSO4·7H2O, NH4NO3, CaCl2, FeCl3, KH2PO4, KCl, HCl, CaH2KNaO6, CHCl3, etc., which are all analytical pure.

Additives: chicken manure, chicken manure soil, etc.

The test site is located about 20 kilometers northwest of Daqing oil field, Heilongjiang Province, China (45°46′-46°55′N, 124°19′-125°12′E). For loess, the test depth is 0-15 cm, and the maximum thickness is 50 cm. There are 2-10 mm gravels in the soil. The wet bulk density of the soil is 1.821 g/cm3. The natural water content is 9.18%. The pH is 8.4; the nitrate content is 55.3 mg/kg, and the oil background content is 1.3-4.6 mg/kg. The experimental water is local shallow groundwater with pH of 8.2 and TDS of 420.5 mg/L. The crude oil produced in the test site is 2400 m underground.

Testing glassware: 150 mL, 250 mL triangle bottle with plug, 125 mL, 1000 mL grinding fine-mouth reagent bottle, various types of bacteria culture tube, Petri dish, rubber plug, 2SL plastic barrel.

Main instruments: QZD-1 electromagnetic oscillator, KQ218 ultrasonic cleaner, biothermostat incubator, high-speed centrifuge, high-pressure steam sterilizer, sterile laboratory, biochemical incubator, shaker incubator, Leica biomicroscopy, 752N ultraviolet-visible grating spectrophotometer, pH meter (pH-3), DDB-303A conductivity meter, electrothermally drying chamber and various glass instruments for chemical analysis, etc.
Chemical analysis test methods: ultraviolet spectrophotometry is used for analysis of petroleum and NO₃, reagent colorimetry for NH₄, pHB-3 type of pH meter for pH testing, DDB-303A type of conductivity meter for calculation of TDS. Using the above experimental materials, chemical reagents were used to sample the oil-contaminated soil. Figure 1 shows a sample of oil-contaminated soil.

Figure 1. Oil contaminated soil samples

The relevant data obtained are processed by SPASS18.0 statistical software, and the measured data are represented by ($\bar{x}$ ± s), and the average values of multiple samples are compared by variance analysis method. When there are differences between groups, the average values of multiple samples between groups are pairwise. The average values of the corresponding samples in each comparison group are equal. The test is carried out by LSD-t. If the average value of the corresponding samples in each comparison group is not equal, the Dunnett-T3 test is used. P > 0.05 indicated that there was no significant difference.

Sources and hazards of petroleum contaminated soil

(1) Mode of production

Petroleum pollutants (Varjani et al., 2017) are products of oilfield development and petroleum processing. Every link of the petroleum industry may produce petroleum pollutants, of which drilling, oil recovery, transportation, emissions and accidents are the main links. Drilling wastewater and oil-bearing mud containing petroleum pollutants are produced during drilling in oilfields, mainly from oil pollution on flushing surface equipment, loss of drilling mud and leakage of mud circulation system. During the period of oil production, oil production wastewater will be discharged from normal production operation, well washing wastewater will be discharged from well washing, dewatering treatment of crude oil will produce wastewater, and overhaul of equipment will also produce oily wastewater. Accident pollution includes natural and human factors. Natural accidents include blowout and equipment failure. When vehicles are used for transportation, crude oil leakage is caused by traffic accidents caused by landslides, avalanches, floods and gales. Man-made accidents refer to the destruction of oil production equipment and pipelines caused by various man-made factors, and the pollution caused by the rollover of crude oil transport vehicles caused by man-made traffic accidents, etc. Investigations show that the oil content of most soils collected in the 100 m range around oil wells in Shengli and Daqing oilfields is higher than the national standard threshold (500 μg/g). In addition, oil sludge from oil tanks, sedimentation tanks, sewage tanks and sediment from oil transfer stations and combined stations in oilfields can also pollute the soil, causing the oil content in the soil to exceed the standard seriously.
Harmful to soil ecological environment

Petroleum pollutants rank eighth among 48 hazardous wastes in China (Sharma et al., 2018). After petroleum pollutants are discharged into soil, soil structure is destroyed, soil permeability is affected, soil organic matter composition and structure are changed, and soil quality is reduced. Petroleum hydrocarbons accumulated in soil, mostly macromolecule compounds, form a layer of mucosa on plant roots, hinder root respiration and absorption, and even cause root decay. Petroleum pollutants often accumulate in the soil surface, and the soil surface is the most developed area of crop root system, so the degree of soil pollution directly affects the growth of crops. As oil contaminates the soil, it also causes pollutants to enter the grain, causing the accumulation and amplification of pollutants. It not only affects the quality of food, but also makes oil pollutants enter the food chain, endangering human health. In the process of infiltration into the soil, oil also diffuses along the surface, erodes the soil layer, makes it saline-alkali, asphalt, hardening, and migrates to the deep part of the soil under the action of gravity. Because of the strong viscosity of petroleum pollutants, they will form a small range of high concentration pollution in a short time.

Ecological remediation of petroleum contaminated soil based on microbial degradation

Bioremediation technology (Fruchter, 2017) is a new clean technology developed on the basis of biodegradation. It is the development of traditional biological treatment methods. Compared with physical and chemical remediation of contaminated soil, it has the advantages of low cost, no damage to the soil environment needed by plant growth, safe oxidation of pollutants, no secondary pollution, good treatment effect and simple operation. Bioremediation can accelerate the rate of natural biodegradation by optimizing environmental factors. It is an efficient, economical and ecologically affordable clean technology. At present, there are two main types of bioremediation technologies for petroleum hydrocarbon contaminated soil: one is microbial remediation technology, which can be divided into in situ bioremediation and heterotrophic bioremediation according to the location of remediation; the other is phytoremediation.

In situ bioremediation and ectopic bioremediation technology

In situ treatment method is to treat contaminated soil in situ. During the treatment period, the soil is basically undisturbed. The most common method of in-situ treatment is to biodegrade the water saturated area of the soil. In addition to adding nutrients and oxygen sources (mostly hydrogen peroxide), microorganisms should be introduced to improve the biodegradability. Sometimes, a group of wells are dug in the contaminated area and injected directly into the appropriate solution, so that microorganisms in the water can be introduced into the soil. After some treatment, groundwater can be recovered and recycled, and soil amendment can be added before groundwater is recycled. It is impossible to remove most PAHs (polycyclic aromatic hydrocarbons) effectively by in situ treatment within a certain period of time, and this method has some limitations due to the influence of temperature and soil type. Ectopic bioremediation mainly includes field treatment, prefabricated bed treatment, composting treatment, bioreactor and anaerobic biological treatment.
Field treatment method

In recent years, there have been many studies on Bio-treatment of petroleum hydrocarbon contamination (Jupp et al., 2017; Wu et al., 2017), among which soil tillage treatment is a common method for field treatment of soil contamination. Polluted wastes are applied to the soil, and the optimum values of oxygen, water and pH are maintained through fertilization, irrigation and lime addition. Tillage is carried out to improve soil ventilation and ensure the degradation of pollutants in polluted wastes and underlying soil layers. Most of the microorganisms used in the degradation process are indigenous microorganisms, but domesticated microorganisms need to be introduced to improve the effect.

Prefabricated bed method

The biggest drawback of soil tillage treatment in field treatment is that pollutants may migrate from the treatment area. The design of prefabricated bed can minimize the amount of pollutants migration because of its filtrate collection and emission control system. The bottom of the prefabricated bed is low permeability material, such as high-density polyethylene or clay. Contaminated soil is transferred to prefabricated bed, pH is adjusted through fertilization, irrigation, and sometimes microorganisms and surfactants are added to make it most suitable for pollutant degradation.

Piling treatment method

Soil piling treatment (Selvi et al., 2017) is to excavate contaminated soil from contaminated areas, prevent contaminants from spreading to groundwater or larger areas, transport it to a treated site (layout of seepage prevention bottom, ventilation pipelines, etc.) and pile it up to form a rising slope, and carry out biological treatment. Piling is a new alternative technology in bioremediation technology.

Bioreactor method

Bioreactor method (Ming et al., 2017) is to treat contaminated soil in a special reactor. Bioreactor is usually built on site or in a specific treatment area, usually in horizontal drum and elevator shape, with two types of gap and continuous. Because the reactor can completely mix the soil with microorganisms and other additives such as nutrients, surfactants and so on, and can control the degradation conditions well, the treatment speed is fast and the effect is good. The process of bioreactor treatment is as follows: firstly, the soil is dug out and mixed with water to form mud, and then transferred into the reactor. In order to improve the degradation rate, domesticated microorganisms are often isolated from the soil previously treated by the reactor and added to the soil prepared for treatment.

Anaerobic bioremediation method

Aerobic remediation technologies such as bio-layer, composting and soil slurry reactor have been developed for the remediation of petroleum hydrocarbon-contaminated soils. However, when some degrading bacteria are isolated, some degrading bacteria are accompanied by products with high ecological risk. Recent studies have shown that anaerobic microbial remediation technology characterized by anaerobic reductive dechlorination has great potential.
Biodegradation of petroleum pollutants in soil

After petroleum pollutants enter the soil, they can be transformed and degraded through three natural ways: volatilization, autoxidation and degradation. The latter mainly includes biodegradation (Chandrangsu et al., 2017), photolysis and mechanical degradation, which is a very slow process. The ultimate fate of petroleum pollutants in the environment is that they are degraded by microorganisms, and the photolysis is very small. When petroleum pollutants enter the environment, various microorganisms will participate in their biodegradation process. As nutrients, pollutants are absorbed and transformed into organic components in microorganisms or reproduced into new microorganisms. The rest is oxidized and decomposed into simple organic or inorganic substances such as methane, carbon dioxide and water by microorganisms. Petroleum pollutants are biodegraded by aerobic respiration, anaerobic respiration and fermentation after they enter the cell membrane of degrading microorganisms. In aerobic respiration, organic matter is oxidized to carbon dioxide, water and other final products, and the electron acceptor is atomic oxygen; in anaerobic respiration, other inorganic substances are used as electron acceptors, and organic matter is oxidized to methane, sulfate is reduced to sulfide, nitrate is reduced to N$_2$ or ammonia; the fermentation process does not depend on oxygen, but on the presence of oxygen. As electron acceptors, the final products are carbon dioxide, acetic acid, alcohol, propionate and so on. Generally, biodegradation of petroleum substances is mainly accomplished by aerobic degradation.

In soil, bacteria (Papale et al., 2017) are dominant decomposers, followed by fungi. Different petroleum degrading bacteria have different degrading ability for different hydrocarbons in pollutants. Most petroleum degrading bacteria can degrade only one or several hydrocarbons. Among them, long-chain n-alkanes in petroleum hydrocarbons can be utilized by most petroleum-degrading bacteria, while short-chain alkanes and aromatics can only be degraded by a few bacteria. The proportion of hydrocarbon-degrading bacteria in the total heterotrophic population varies greatly. So far, more than 200 kinds of hydrocarbon-degrading microorganisms have been known.

Biochemical principle of petroleum degradation by microorganisms

It is a very complicated process that petroleum pollutants are degraded by assimilation after they enter the cells of degrading microorganism (Barp et al., 2017). Under aerobic conditions, mainly under the catalysis of oxygenase, molecular oxygen is combined into the matrix to form oxygen-containing intermediates, and then converted into other substances. In the degradation of alkanes, one oxygen atom in O$_2$ is bound, while in the degradation of aromatics, two oxygen atoms are bound. Since petroleum is a mixture of hydrocarbons, the degradation of petroleum can only be accomplished by the interaction of various microorganisms.

When the degradation of petroleum pollutants by microorganisms are caused by intracellular enzymes, the whole process of microbial degradation can be divided into the following three steps:

1. Compounds are adsorbed on the surface of microbial cell membrane, which is a dynamic equilibrium process.
2. Compounds adsorbed on the surface of cell membrane enter the cell membrane (when the biomass is constant, the penetration rate of the compound to the cell membrane determines the amount of the compound penetrating the cell membrane).
(3) Enzymatic reactions occur when compounds entering microbial cell membranes bind to degrading enzymes, which is a fast process. The metabolic pathways and mechanisms of microorganisms to different hydrocarbons in petroleum pollutants are different. The following are mainly discussed.

**Biodegradation of saturated hydrocarbons in petroleum**

Straight-chain alkanes: it is generally believed that saturated hydrocarbons (Pattison et al., 2018) are first oxidized to alcohols under the action of microorganisms, alcohols are oxidized to corresponding aldehydes under the action of dehydrogenase, and then oxidized to fatty acids through the action of aldehyde dehydrogenase. The oxidation pathways include single-end oxidation, double-end oxidation and sub-end oxidation. After converting to the corresponding fatty acids, one form of conversion is to directly undergo the subsequent β-oxidation sequence, i.e. to form carboxyl groups and to shed two carbon atoms; the other is to undergo the ω-hydroxylation of fatty acids to form ω-hydroxy fatty acids, which are then oxidized to dicarboxylic acids with the participation of non-specific hydroxylase, and finally experienced the final β-oxidation sequence. Fatty acids are degraded into acetyl coenzyme A by β-oxidation, which enters the tricarboxylic acid cycle, decomposes into CO₂ and H₂O and releases energy, or enters other biochemical processes. Alkanes can also be dehydrogenated directly to form olefins, which can be further oxidized to alcohols and aldehydes through enzymatic catalysis, and finally to fatty acids, which can be further decomposed according to the sequence of β-oxidation; or alkanes can be oxidized to alkyl hydrogen peroxide and then directly converted to fatty acids.

Branched alkanes: the degradation mechanism of branched alkanes by microorganisms is basically the same as that of straight alkanes. Compared with straight alkanes, the presence of branched chains increases the resistance of microbial oxidation and degradation. The main part of oxidation and decomposition occurs on the straight chain, and it is difficult to oxidize near the end of the side chain. The degradation of branched alkanes can be carried out through α-oxidation, ω-oxidation or β-base removal pathway. Generally speaking, the degradation rate of hydrocarbons with branched chain structure is slower than that of straight chain hydrocarbons with the same carbon number, because the branched chain of alkanes reduces the decomposition rate.

Naphthalene: Naphthalene accounts for a large proportion of petroleum fractions. Its biodegradation principle is similar to that of sub-terminal oxidation of alkanes. First, naphthenols are oxidized by mixed-function oxidase (hydroxylase), then dehydroketones are oxidized to esters, or fatty acids are formed by direct ring opening. Taking cyclohexane as an example, its biodegradation mechanism is as follows: cyclohexane is hydroxylated by oxidase to form cyclohexanol, which dehydrogenates to ketone, then oxidizes further. An oxygen atom inserts into the ring to form ester, ester opens, and hydroxyl at one end is oxidized to form aldehyde group, then oxidized to form carboxyl group, finally, dicarboxylic acid is further metabolized by β-oxidation.

Monocyclic aromatic benzene and short-chain alkylbenzene are metabolized to catechol and substituted catechol through the intermediate process of diol under the action of dehydrogenase and reductase enzyme. The aerobic degradation of benzene is the formation of cis-dioxyl compounds by introducing two hydroxyl groups into the benzene ring, and then deoxidized to form catechol. Catechol (Du et al., 2017) can be cleaved in two ways: between two hydroxyl groups; between hydroxyl carbon atoms and non-hydroxyl carbon atoms.
There are two ways to degrade toluene, ethylbenzene and xylene: oxidation of methyl or ethyl groups on benzene rings to form carboxyl groups, then removal of carboxyl groups, and introduction of two hydroxyl groups to form catechol under the action of hydrogenase; direct oxidation of benzene rings to connect two hydroxyl groups, and further oxidation.

Polycyclic aromatic hydrocarbons (PAHs) are very difficult to degrade. The degree of degradation is related to the solubility of PAHs, the number of rings, the type of substituents, the position of substituents, the number of substituents and the properties of heterocyclic atoms. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) begins with the progressive degradation of pyruvic acid and CO₂ by hydroxylation of the first ring, followed by the decomposition of the second ring in the same way. Prokaryotic and eukaryotic microorganisms have the ability to oxidize aromatic hydrocarbons, but the mechanisms of aromatic hydrocarbon oxidation by bacteria and fungi are different. Bacteria produce dioxygenase, which combines the two oxygen atoms of molecular oxygen with the substrate by the catalysis of dioxygenase to oxidize aromatic hydrocarbons into cis-structured diols, and cis-diols break the aromatic ring into catechols under the catalysis of another peroxidase. Fungi, on the other hand, can oxidize aromatic hydrocarbons to trans-dihydrodiphenols by catalysis of monooxygenase and cyclohydrolase. Different metabolic pathways have different intermediates, but the common intermediates are catechol, 2,5-dihydroxybenzoic acid and 3,4-dihydroxybenzoic acid. These metabolites are degraded through similar pathways, i.e. the breaking of carbon bonds. On the one hand, the metabolites can be utilized by microorganisms to synthesize cell components, on the other hand, they can also be oxidized to CO₂ and H₂O.

**Influencing factors of microbial degradation of petroleum pollutants**

Chemical composition and environmental factors of petroleum are the most significant factors affecting biodegradation in soil. Environmental factors include microbial species and quantity in soil, nutrient elements in soil, oxygen supply, temperature, humidity, pH value and so on.

**Chemical composition of petroleum**

The degradability of petroleum products varies with their composition. Petroleum compounds are mainly composed of saturated hydrocarbons, aromatics, nitrogen sulfur oxides and asphaltene. Petroleum compounds can be classified into thousands of chemical systems according to the length of carbon chain (Schmidbaur and Schier, 2017) and whether they form straight chain, branched chain, ring or aromatic hydrocarbon structure (Sinsabaugh et al., 2017). The degree of biodegradation of various hydrocarbons is different, and the degradation rate is mainly determined by the number of carbon and its functional groups. Among the components, saturated hydrocarbons are the most easily degraded, followed by aromatic compounds with lower molecular weight, while aromatic compounds, resins and asphaltenes with higher molecular weight are extremely difficult to degrade. Among saturated hydrocarbons, straight-chain alkanes are the most easily degraded, while branched-chain alkanes are more difficult to be degraded by microorganisms. The presence of branched-chain enhances the corrosion resistance of alkanes, and the more and larger the branched-chain is, the more difficult it is to be degraded by microorganisms. In aromatic
hydrocarbons, dicyclic and tricyclic compounds are easily degraded. The degradation order of aromatic compounds generally decreases with the increase of aromatic rings and alkyl substituted side chains. The biodegradability of polycyclic aromatic hydrocarbons decreases in the following order: triaromatic steroids, yield series, phenanthrene series and naphthalene series. Cycloalkanes and aromatic hydrocarbons with more than four rings, or compounds containing tar and asphaltene components, have a slower biodegradation rate. A large number of these substances remain in the final products of biodegradation and become the main residual substances after degradation.

Generally, medium-length alkanes degrade rapidly, while longer-chain alkanes are not easy to degrade. Compounds with relative molecular weight more than 500 cannot be directly used as carbon source for microbial growth (Han and Currell, 2017). Generally speaking, C10-C22 compounds in alkanes and aromatics are the least toxic and most easily degraded. C1-C6 volatilizes rapidly, mainly in the form of gases in the environment, and short-chain hydrocarbons are degraded by a small number of microorganisms because of their relatively stable structure. Hydrocarbons above C22 are poorly water-soluble, usually in the form of gases. In the case of solid, the degradation of microorganisms is very limited; with the increase of relative molecular weight, the biodegradability decreases. Because of the difference of biological stability, the degree of biodegradability of petroleum components is very different, and the environment of microorganisms in the same polluted area is also quite different.

**Microbial species and quantities**

As the executor of petroleum biodegradation, the number and species of microorganisms are the important factors affecting the efficiency of petroleum biodegradation. Environmental conditions affect the biodegradation of organic pollutants (Lee et al., 2017), generally by affecting the activity of microorganisms. Different microorganisms have different ability to degrade petroleum hydrocarbons, and the same strain has different ability to utilize different hydrocarbons. In the petroleum contaminated soil environment, the more the number and species of petroleum-degrading microorganisms are, the faster the degradation rate is. Petroleum pollution can induce the growth of oil-degrading microbial population. The number of oil-degrading microbial population is less than 0.1% in non-oil-polluted areas, but the proportion and quantity of oil-degrading bacteria in the polluted areas have increased significantly. The more serious the pollution is, the more bacteria there are. The results show that the number of microorganisms is an important factor affecting the oil degradation efficiency. Within a certain range of oil concentration, the petroleum degradation efficiency of microorganisms is not affected by other factors.

**Oxygen supply and nutrition in soil**

Adequate oxygen content in soil has an important influence on degradation efficiency. The initial process of microbial degradation of petroleum pollutants is the oxidation of matrix by oxidase (Naafs et al., 2017). First of all, oxygen must be involved. That is, under the catalysis of oxygenase, oxygen is added into the matrix to form an intermediate product containing oxygen. In theory, the amount of oxygen required for degradation is about 3.5 times the mass of hydrocarbons. Under anaerobic conditions, although some microorganisms may react to degrade petroleum pollutants, it
is much slower than that under aerobic conditions, and there are few kinds of microorganisms that can degrade hydrocarbons. Experiments show that under anaerobic conditions, the degradation rate of pollutants is less than 5% after 233 days, while under aerobic conditions, the degradation rate of some hydrocarbons is increased, and the degradation rate of some hydrocarbons is more than 20% after 14 days.

The most basic limiting factor for microbial growth is the lack of appropriate energy sources. There are at least 11 macronutrient and micronutrient elements (N, P, K, Na, S, Ca, Mg, Fe, Mn, Zn, Cu) necessary for microorganisms in the soil. These elements must maintain a certain amount, form and proportion in order to maintain the growth of aerobic bacteria. The other micronutrient elements necessary for several anaerobic bacteria include Ni and Co. Lack of nutrients inhibits microbial degradation of petroleum hydrocarbons. Most microorganisms are heterotrophic and use certain organic matter as energy source. In petroleum contaminated soils, organic carbon is usually high, while N and P are relatively deficient. If m(C):m(N):m(P) does not reach the required proportion of bacterial metabolism, the metabolic rate of bacteria will be limited, thus restricting the degradation of organic pollutants. Therefore, adjusting the proportion of essential nutrients is a necessary condition for the smooth degradation process. The results show that close to 100:10:1 is the most suitable ratio for hydrocarbon biodegradation.

**Soil temperature**

Soil temperature also affects degradation efficiency. Temperature can directly affect the growth, reproduction and metabolism of microorganisms, and the biochemical reaction accords with the general law of chemical reaction rate, that is, the higher the temperature is, the faster the reaction rate is. The results show that the effect of soil temperature (T) on the rate constant (k) of biological reaction is in accordance with the following relationship:

\[ k = 3145 \exp(-5233/T) \]  
(Eq.1)

In the aerated zone, the half-lives of petroleum pollutants at 5 ℃, 10 ℃, 20 ℃ and 30 ℃ are 1499 d, 1075 d, 572 d and 317 d, respectively. The results show that microorganisms can degrade at -72 – -2 ℃; the number of microorganisms increases and the degradation rate increases with the increase of temperature at -10-0 ℃; the degradation rate of n-alkanes can be doubled at 20-30 ℃, and the degradation rate of heavy oil and light oil can be decreased by 50-60% and 0-40% respectively, when the temperature decreased from 20 to 10 ℃. Temperature affects the degradation of petroleum organisms mainly in the following two aspects: the effect of temperature on enzymes in microorganisms and the effect of temperature on petroleum properties.

The effect of temperature on enzymes in microorganisms: because microorganisms are accompanied by complex biochemical reactions in the process of degradation of organic substances, the biodegradation of these organic substances actually needs to be carried out under the catalysis of enzymes produced in microorganisms, and temperature is an important dominant factor in the kinetics of enzymatic reaction. The catalytic activity of each enzyme can only be brought into play within a certain temperature range, and beyond a certain temperature range, the activity of the enzyme will be inhibited or even cause the inactivation of the enzyme. The activity of microbial
enzymes can be reduced by too low temperature, and the degradation rate of hydrocarbons can be accelerated and the maximum value can be achieved by increasing temperature. The appropriate temperature is generally 15-35 °C. The toxicity of hydrocarbons will increase when the temperature is higher, which will affect the degradation and biological activity of hydrocarbons.

The influence of temperature on petroleum properties: temperature also affects the physical and chemical properties of petroleum, and ultimately affects the interaction between microorganisms and petroleum molecules, thus changing the process and rate of biodegradation. When the temperature is low, the membrane toxicity of hydrocarbons is small, the oil viscosity is high, the volatility decreases, the start-up of biodegradation lags behind, and the rate decreases. With the increase of temperature, the membrane toxicity of hydrocarbons increases, and the emulsification degree of oil increases. In addition, the microbial activity needs liquid water, so the degradation temperature should be at least above the freezing point of water (Wu et al., 2018).

**Soil moisture and pH value**

Water is an indispensable nutrient for microbial life activities. Microorganisms cannot survive without water, and let alone degrade petroleum pollutants. In addition, organic matter must be water-soluble in order to be utilized by microorganisms. Water is also closely related to redox potential and solubility of compounds. The results are important factors affecting petroleum degradation. Soil moisture varies in depth, soil moisture is too low, microorganisms cannot get enough water supply, cell activity is inhibited, and metabolic rate is reduced; but excessive soil moisture will hinder air permeability and oxygen supply. The results showed that the maximum soil water holding capacity is 25-85%, which is the most suitable time for microorganisms to survive, and the best soil water holding capacity is 30-90% for petroleum pollutants degradation.

Most heterotrophic bacteria and fungi which can degrade petroleum pollutants prefer neutral environment. The suitable pH value of many soil microorganisms is 6-8. Excessive or low pH value will affect the degradation ability of microorganisms. The optimum pH values of hydrocarbons and polynuclear aromatic hydrocarbons are 7.5, 7.5-7.8, respectively. The optimum pH values of most bacteria are slightly greater than 7. The degradation rate of petroleum in neutral soil is twice as fast as that in acidic soil (pH 4.5) or alkaline soil (pH 8.5).

**Results**

The main mechanism of microbial remediation technology is that petroleum hydrocarbons are directly involved in the biochemical reaction of microorganisms. The development and research of microbial remediation technology for degradation of contaminants in soil by metabolic process has attracted extensive attention of scholars at home and abroad. At present, more than 100 genera and 200 species of microorganisms are known to degrade petroleum pollutants prefer neutral environment. The suitable pH value of many soil microorganisms is 6-8. Excessive or low pH value will affect the degradation ability of microorganisms. The optimum pH values of hydrocarbons and polynuclear aromatic hydrocarbons are 7.5, 7.5-7.8, respectively. The optimum pH values of most bacteria are slightly greater than 7. The degradation rate of petroleum in neutral soil is twice as fast as that in acidic soil (pH 4.5) or alkaline soil (pH 8.5).
and achieves good results, which provides technical support for the popularization and application of this technology, and has important practical significance.

**Experimental steps**

The crude oil obtained in the test area is used as carbon source (pollutant). The strains and flora of petroleum-degrading soil in the test area are selected and cultured in a large scale. According to the inoculum amount used in the test, sufficient bacterial liquid preparations are cultured, and a large number of bacterial flora for the test are grown through microscopic examination. According to the calculation, a certain amount of oil is evenly mixed into the surface layer of soil in the test area at a depth of 15 cm. The microorganisms that degrade petroleum compounds can be divided into bacteria, fungi and algae according to their biological types (as shown in **Table 1**).

**Table 1. Microbial composition of oil contaminated soil**

| Soil sample | Germ | Fungus | Actinomyces |
|-------------|------|--------|-------------|
|             | 10^3 CFU (g) | Percentage composition (%) | 10^3 CFU (g) | Percentage composition (%) | 10^3 CFU (g) | Percentage composition (%) |
| CK          | 33 ± 8b | 94.25 | 3 ± 1c | 0.08 | 176 ± 55a | 5.10 |
| PCS-1       | 297 ± 35a | 98.88 | 27 ± 4a | 0.08 | 9 ± 3b | 0.03 |
| PCS-2       | 193 ± 49a | 99.92 | 12 ± 3b | 0.08 | 3 ± 1b | 0.02 |

According to the soil weight of the test layer in the test area, 4.3% chicken manure and 50% chicken manure are evenly mixed as additives. The amplified culture liquid is evenly inserted into the test area according to the 3% spray tillage, and then the nutrient solution of nitrogen, phosphorus, calcium, magnesium, sulphur and iron is adjusted uniformly according to the proportion of the medium. The water content of the soil layer is about 20% with the local groundwater regulation test. Agricultural plastic film is covered in the test area for heat preservation, moisturizing, rainproof and so on. Within a certain time interval, the sampling method is to take the sample with same depth soil (15 cm) at five different points in the shape of plum blossom in each area, and then mix it thoroughly and test it with 4-point sampling method. After sampling, the experimental layer of the tillage test area is aerated by the storm air and supplemented with a certain amount of water to ensure that the soil's water content in the test area is about 20%. The amount of petroleum added into the control area is basically the same as that in the experimental area, but not in the other areas, which is regarded as natural degradation. There is no substance added to the blank area as a monitoring sample. Three areas are sampled at the same time and tested components are petroleum content, pH, soil soluble salt, water content, NH₄, NO₃ and so on. At the same time, the surface temperature and soil temperature are monitored. After the completion of the test period, samples are taken from the lower part of the test layer in each district. The test results are shown in **Table 2**.

**Experimental result**

In this paper, Matlab simulation tool was used to simulate the oil content and removal rate of soil, the degradation rate of organic matter in oil pollution samples with different water content, the degradation rate of petroleum by microorganisms and the
degradation characteristics of n-alkane in oily soil under the environment of Microsoft Windows XP operating system, Intel (R) Celeron (R) 2.6 GHz processor and 24 GB memory.

**Removal rate of petroleum from soil**

From *Table 2* it can be seen that the implementation of in-situ micro-ecological remediation technology for petroleum contaminated soil has certain effectiveness through field experiments. In the early stage of the experiment (0-7 d), the added optimum bacterial solution does not play a role. That is to say, when indoor optimum bacterial solution is applied in the field, it needs an adaptation period or a lagphase of bacteria. The optimum period of this experiment is about 7 days. Later, the proliferative phase (logarithmicphase) is entered. *Table 1* shows that the removal rate is over 80% after the 11th day of the experiment, that is, after the adaptation period. The sample is slightly higher because of the sampling location and heterogeneity. However, the removal rate is over 68% at the 16th day of the experiment, and 84.3% at the 32nd day of the experiment. The oil content of the soil in the control area does not change much, except that the two abnormal low values are less than 10%, which indicates that the degradation of oil in the soil is slow under natural conditions. The test data of 16 and 21 days of the two abnormal low values may be caused by the uneven oil content in the soil, and also reflects the heterogeneity and complexity of soil material composition. The blank area reflects the oil content in the soil without adding any substance, but in the later stage of the experiment, the oil content increases because of the adjacent of the test area and the control area, as well as the contamination of the area by rainfall and artificial sampling.

*Table 2. Testing results of changes of oil content and removal rate with time in soils of different areas*

| Sampling date (month/day) | 8/19 | 8/23 | 8/26 | 8/30 | 9/4 | 9/9 | 9/14 | 9/20 |
|--------------------------|------|------|------|------|-----|-----|------|------|
| Number of test days      | 0 d  | 3 d  | 7 d  | 11 d | 16 d| 21 d | 26 d | 32 d |
| Test area                | 2302.0 | 1868.0 | 2335.0 | 422.6 | 731.0 | 516.0 | 557.8 | 364.0 |
| Control area             | 2279.0 | 2442.0 | 2245.0 | 2122.0 | 1532.2 | 1855.2 | 2110.6 | 2120.2 |
| Local area               | 4.6  | 18.2 | 4.5  | 0.95 | 5.75 | 29.55 | 45.55 | 159.0 |
| Removal rate of test area/\% | 0    | 0    | 0    | 81.77 | 68.47 | 77.74 | 75.94 | 84.3  |

**Controlling factors of geological microecological remediation technology**

Geological microecology technology is an in-situ remediation technology which changes macro-environment by micro-effect. The key to the application of this technology is the combination and regulation of microorganisms and geological environment. The main controlling factors are improvement of temperature, water, oxygen, nutrient elements and geological environment.

(1) Regulation and control of soil temperature

Temperature is one of the important factors affecting the growth and survival of bacteria, and the activity intensity and biochemical function of bacteria are related to it. Excessive or low temperature can inhibit growth or cause bacterial death, and moderate
temperature can accelerate the biochemical reaction rate in bacterial cells. Most of the microorganisms we used are mesophilic microorganisms (13-45 °C), and the optimum growth temperature is 25-38 °C. How to control the temperature is the key to the test effect. Therefore, agricultural plastic film is used for thermal insulation in the test area, and grass curtain is used to cover the night after September because of the obvious drop in temperature.

(2) Analysis of soil pH, water content, soluble salt, NH4 and NO3 content

Firstly, the effect of soil dryness and wetness on the degradation rate of oil pollution. Under different water content conditions, the degradation characteristics of petroleum contaminated soil samples with 100 g/kg petroleum pollutant concentration by microorganisms are shown in Figure 2.

![Figure 2. Degradation rate of organic matter in oil pollution samples with different water contents by microorganisms](image)

Figure 2 shows that the water content of petroleum contaminated samples has a significant effect on the petroleum-degrading ability of oil-loving microorganisms. When the water content is less than 50%, the ability of microorganism to degrade petroleum increases with the increase of water content; however, when the water content is higher than 50%, the ability of microorganism to degrade petroleum begins to weaken gradually with the increase of water content. In fact, microorganisms in soil need water to maintain their basic metabolism. If the soil water content is too low, microorganisms will not get enough water supply, and cell activity will be inhibited, resulting in a decrease in its metabolic rate; while excessive soil moisture will hinder air permeability and oxygen supply. According to the conditions required for microbial activity, the degradation of petroleum hydrocarbons is more advantageous in the range of 30-80% of the maximum water holding capacity of soil. When the water content is less than 30% or more than 90%, the activity of petroleum hydrocarbon-degrading bacteria will be adversely affected. This phenomenon shows that the growth and reproduction of microorganisms need certain water conditions, and water is necessary for some enzymes of microorganisms; however, excessive water will prevent
microorganisms from effectively contacting with oxygen, resulting in microbial hypoxia and inhibiting the ability of microorganisms to degrade petroleum.

Secondly, the effect of adding H$_2$O$_2$ on the degradation of petroleum pollution.

Microorganisms continuously consume dissolved oxygen in petroleum hydrocarbon degradation process, so that the concentration of dissolved oxygen decreases. In this study, H$_2$O$_2$ is added to the sample, which is decomposed to supplement the dissolved oxygen consumed by microorganisms. In order to maintain the dissolved oxygen in the sample, a certain amount of H$_2$O$_2$ is added every day. At the same time, because of the strong oxidation of hydrogen peroxide, if the amount of hydrogen peroxide is too high, it will inhibit the growth of microorganisms. Therefore, this study determines that the upper limit of adding H$_2$O$_2$ is 0.5 ml/d. In the process of experiment, under the best conditions of other factors, for the sample 1-4 containing 100 g/kg oil-polluted soil, 0.1 ml, 0.2 ml, 0.5 ml and 0.6 ml of H$_2$O$_2$ are added. And the degradation characteristics of petroleum contamination by microorganisms are shown in Figure 3.

![Figure 3. Effects of microorganisms on petroleum degradation rate under different conditions of H$_2$O$_2$](attachment:image.png)

It can be seen that the influence of oxygen content on the ability of microorganisms to degrade petroleum is also relatively large. With the addition of H$_2$O$_2$, the activity of microorganisms is gradually strengthened and the degradation ability is gradually improved. When H$_2$O$_2$ is added to 12 ml in 24 days, the degradation efficiency reaches its maximum value. Moreover, it can be seen from the determination of microorganisms in oily soil that the number of living bacteria in test samples increases with the addition of H$_2$O$_2$, and the number of living bacteria in test samples increases gradually. Increase of the number of living bacteria will inevitably accelerate the degradation rate of petroleum pollutants. In addition, the pH value of slurry can be maintained stable without obvious change with the addition of H$_2$O$_2$. In the absence of electron acceptor, microbial oxidation of fatty acids is difficult and hydrocarbon contaminants cannot be completely oxidized, which results in the accumulation of some short-chain fatty acids in the test samples and the decrease of pH value. If sufficient electron acceptors are provided, microorganisms will completely oxidize hydrocarbons to CO$_2$ and H$_2$O, thus keeping the pH value of the sample stable. This is conducive to the growth and reproduction of microorganisms, enhancing their activity, and leading to the degradation of microorganisms more obvious.

Thirdly, the degradation characteristics of alkanes under different N/P ratios.
Under different N/P ratios, the relative content of residual alkanes after degradation of petroleum contaminated samples with 100 g/kg contamination by microorganisms is shown in Figures 4 and 5. Figures 4 and 5 show that the residual content of n-alkanes and iso-alkanes with various carbon numbers is the least when the ratio of N to P is 10:1 under different N/P ratios, while the residual amount of n-alkanes and iso-alkanes is higher under other N/P ratios. Quality can effectively enhance the degradation ability of microorganisms and promote oil-loving microorganisms to effectively degrade alkanes in petroleum. It also reflects that oil-loving microorganisms have a relatively balanced degradation of n-alkanes in the range of C16-C21, however, oil-loving microorganisms are selective for the consumption of isoparaffins in the range of C15-C20. The degradation rates of C16 isoprene, Pr and Ph are significantly lower than those of C15, C18 isoprene, Pr2 and MP isoalkanes. The characteristics of selective degradation show that Pr, Ph are difficult to degrade, and the stability of Pr and Ph can also be utilized, so that Pr and Ph are used as molecular markers for the degradation of petroleum pollutants.

Figure 4. Degradation characteristics of n-alkanes in oily soil under different nutrient conditions

Figure 5. Degradation characteristics of isoalkanes in oily soil under different nutrient conditions
The pH of the environment has a certain influence on the life activities of microorganisms. It can cause the change of cell membrane charge, affect the absorption of nutrients and the activity of enzymes, and change the availability of nutrients and the toxicity of harmful substances in the environment. The survival of each microorganism has a certain range of pH value and the optimum pH value. The optimum pH value of most bacteria is 6.5-7.5, and that of actinomycetes is 7.5-8.0. Fungi can grow and develop in a wide range of pH. For example, fungi can grow at pH below 3 and above 9, and the optimum is 5-6.

Effect of test process on lower soil in test area

Table 3 is to test the contents of oil, pH, water content, soluble salt, NH₄ and NO₃ in different depths of the lower part of the test area after the completion of the test. From the test results, it can be seen that the oil content in the lower part of the test layer does not increase significantly. Compared with the control and blank areas, it shows that the oil in the soil of the experimental layer has not diffused downward or has been degraded. From the contents of pH, water content, soluble salt, NH₄, NO₃, it can be seen that they are different from the control area and blank area. That is to say, some soluble nutrients such as nitrogen and phosphorus enter the lower soil layer with water. In the future, this kind of remediation work is of great significance to the requirements of water quantity and soluble nutrients and the adding methods.

| Different depths | Petroleum (mk/kg) | pH | Water content (%) | Soluble salt (Ms/cm) | NH₄ (mol/L) | NO₃ (g/cm³) |
|------------------|------------------|----|------------------|---------------------|------------|------------|
| Test area 20-25  | 61.9             | 7.8| 14.04            | 821.5               | 70.0       | 246.4      |
| 30-35            | 102.2            | 8.0| 15.27            | 612.0               | 5.0        | 242.7      |
| 45-50            | 87.7             | 8.0| 17.78            | 504.0               | 3.0        | 242.6      |
| Control area 20-25| 30.15            | 8.5| 13.06            | 97.0                | 1.5        | 24.2       |
| 30-35            | 58.31            | 8.4| 13.51            | 96.5                | 1.5        | 22.2       |
| 45-50            | 76.45            | 8.4| 14.67            | 123.0               | 1.5        | 18.4       |
| Blank area 20-25 | 99.42            | 8.7| 10.8             | 68.1                | 3.0        | 24.1       |
| 30-35            | 36.55            | 8.7| 10.58            | 80.2                | 1.5        | 32.7       |
| 45-50            | 36.95            | 8.5| 14.09            | 171.2               | 1.5        | 31.6       |

Discussion

Oxygen supply has become one of the important regulators in the process of bacterial degradation of petroleum pollution. The amount of oxygen supply can affect the activity of many enzymes and cell respiration in microbial cells, and control the growth of microorganisms and the ability to degrade petroleum pollution. Generally, 1.02 g petroleum per 3.5 g of oxidation is required, which can be degraded rapidly only under sufficient oxidation conditions. In this experiment, the supply of soil oxygen is regulated from four aspects. Firstly, the soil is fully tilled and the experimental layer is tilled after each sampling, which is fully mixed with the atmosphere. The second is to ensure that the tested soil has a certain water content and oxygen provided by water. In addition, chicken manure is used as an additive, which is not only cheap and easy to obtain, but also can supplement nutrients, and the soil of the experimental layer is improved to increase the fluffy and permeability, so that oxygen in the air is easy to
enter. Finally, the added nutrients of NH₄O₃, KH₂PO₄, K₂HPO₄, NO₃, etc., not only increase the nitrogen source, but also the sources of oxygen.

Nutrient elements are the active components of microbial cells, enzymes, substance transport systems and the energy required to provide physiological activities. Microbial cells are mainly composed of C, H, O, N, P and so on, in which C and H come from petroleum pollutants. Oxygen comes from water and air and other regulated sources of oxygen. The trace elements of N and P as well as S, K, Ca, Mg and Fe need to be supplemented and regulated as nutrients, and chicken manure from local chicken farms is as additive to supplement other biotin and nutrients. Table 2 shows the changes of soluble salts, NH₄ and NO₃ contents with the test process. It can be seen that the test area is supplemented various nutrient elements on August 21, reflecting the process of utilizing, degrading and transforming petroleum and various elements with the microbial activities in the test process. This process verifies that the nutrient elements added in the regulation and control of this experiment are appropriate. The control area and blank area reflect the natural content change, and the rainfall makes the content decrease.

Conclusions

(1) Based on the preliminary experimental study on in-situ microecological remediation of petroleum contaminated soils, the soil temperature, water, oxygen, nutrients and geological environmental factors in the test area are regulated by optimizing in-situ (indigenous) microbial flora and combining physical and chemical methods with geological environment. The experiment of degradation and remediation of man-made petroleum contaminants in soil shows that the average content of oil in soil is 2318.5 mg kg⁻¹. After 11-32 days of in-situ microecological remediation, the removal rate of petroleum in soil can reach 68.47-84.30%. The oil content of artificial filling in the soil of the control area does not change much, basically less than 10%, indicating that the degradation of oil in the soil is slow under natural conditions. The validity of geological microecological remediation technology for petroleum pollution remediation is verified, and the feasibility of its popularization and application is explored.

(2) It is concluded that the best season for using microecological restoration technology in this area should be from late June to early September every year, and soil temperature can be kept above 25 °C through regulation.

(3) It is proved that the nutrients added in the experiment and the improvement of soil environment are moderate, and the method is feasible.

The experimental process preliminarily verifies that the geo-microecological remediation technology is effective and feasible in the field in situ soil’s oil pollution remediation experiment. It has the advantages of simple treatment method, low cost, good remediation effect, little environmental impact, no secondary pollution, and in situ remediation. Although it is an experimental study and needs to be improved for large-scale field restoration, it can be achieved through continuous efforts. It can not only effectively remediate soil, aeration zone and prevent and control oil pollution of groundwater in situ, but also increase soil fertility and improve soil environment. It is of great significance to remediate contaminated soil and increase crop production. It is also one of the effective methods to remediate and control oil pollution in large areas of soil fundamentally, having certain practical application value. In this paper, the ecological restoration of soil oil pollution is studied, but the application of the technology to the ecological restoration of heavy metal soil pollution is still to be verified.
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