

# Application of Microbial Degradation Technology in Oil Pollution Control

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## ABSTRACT

Oil pollution is still a problem that is very difficult to control. To this end, this study investigates the application effect of microbial degradation technology in oil pollution control. First, the degradation microbial strains and substrates were determined according to the type of microorganisms. Then, ultraviolet spectrophotometry was used to determine the concentration of crude oil in the culture medium. After that, the authors designed the flow chart of crude oil standard curve, extracted organic phase, determined the residual oil composition in the culture medium, and calculated the degradation rate of mixed bacteria to crude oil. By studying the degradation mechanism of aerobic bacteria, anaerobic bacteria, and fungi, the degradation products were confirmed and the treatment of oil pollution on the water surface was carried out. It can be seen from the experimental results that the microbial degradation technology is effective in solving the problem of oil pollution.

## KEYWORDS

Microbial Degradation, Oil Pollution, Pollution Control, Surface Oil

## INTRODUCTION

The petroleum industry is of great importance to the economic development in contemporary society. However, in the process of oilfield development and exploitation, the landing crude oil, oil-bearing sewage may cause different degrees of environment pollution, thus threatening human health. In the production process of oil field, the landing oil and oily sewage pose a serious threat to the surrounding environment. The best way to solve this problem is to reduce the pollution source. However, in the development and construction of oil and gas field, it is impossible to completely avoid the generation of landing oil and oily sewage (Gharib et al. 2018). Therefore, it is of great significance to study the migration and transformation of petroleum pollutants in soil. In recent years, with the exploitation and utilization of offshore oil resources, offshore oil spill accidents occur frequently in the production and transportation of petroleum processing products (Yu et al. 2018). Every year, about 3.2 million tons of oil in the world pollutes the ocean due to oil leakage. The oil film covers the surface of the sea water to block the air sea exchange, affecting the growth and reproduction of aquatic organisms, and destroying the balance of the entire marine ecosystem (Barringer 2019).

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Petroleum is a complex mixture formed by the continuous evolution of ancient organisms, mainly composed of hydrocarbons composed of carbon and hydrogen, followed by non-hydrocarbon compounds containing oxygen, sulfur, nitrogen and other heteroatoms. The components of petroleum include saturated hydrocarbon, aromatic hydrocarbon, asphaltene compound and resin (Feldman et al. 2018). Heating distillation can decompose crude oil into fractions with different boiling points, including LPG, gasoline, kerosene, diesel oil and lubricating oil. Liquefied petroleum gas (LPG) is a kind of petroleum gas fuel which is mainly composed of alkanes. The main component of gasoline is liquid petroleum fraction with boiling point lower than 200°C, so it is volatile under normal temperature and pressure. In order to improve octane number of gasoline, a small amount of tetraethyl lead is often added (Hao et al. 2019). The petroleum fraction below 250°C accounts for 95% of kerosene, which is slightly volatile. Diesel oil is mainly used as the fuel of compression ignition internal combustion engines. The ignition temperature of diesel oil is above 50°C, and the petroleum fraction with boiling point below 350°C accounts for 90%. Under normal temperature and pressure, diesel oil has stable properties and low volatility (Gharib et al. 2018). The lubricating oil has a high ignition point, which can retain its properties for a long time when heated, and it is not easy to be volatilized or precipitated. Therefore, in addition to the low-carbon liquefied petroleum gas, other oil products are mainly liquid under room temperature and atmospheric pressure. From gasoline with low boiling point to lubricating oil with high boiling point, the ignition point, viscosity, density and chemical stability gradually increase (Yu et al. 2018). Polycyclic aromatic hydrocarbons (PAHs) and other toxic substances in petroleum only account for about 0.1% in crude oil, but they have strong carcinogenesis, teratogenesis and mutagenicity, and they will accumulate in the food chain step by step, which will pose a huge threat to human health. Absorption to PAHs can cause cataract, kidney problems, and hepatitis in the longer term. Contact with skin with the PAH propylene glycol on a regular basis can cause irritation and swelling. Large doses of naphthalene inhaled or swallowed can induce red cell disintegration. In the influence of uv radiation, they are often more poisonous. PAHs are hazardous to marine life including birds at relatively high doses. Marine oil spills are gradually becoming a major pollution to the marine environment, destroying the living environment of human beings and seriously threatening people's health (Yang et al. 2018).

At present, there are three main oil treatment methods, the first one is to surround the oil slick, Solvent extraction, on the other hand, is the way of removing a solvent ingredient from either a solid that used a supercritical fluid. Finally, the aqueous enzyme oil extraction process is a well-developed method for extracting oil through plant sources (P. soupy et al. 2018). If there is a large amount of oil leaking, the first step is to enclose the oil with a floating fence. Some of these floating fences are inflatable, some are made of materials lighter than water, and both of them are generally about 1 meter above the water surface. However, some are "sitting" on the waves, and some are stretching 1 meter underwater. Method 2: physicochemical method. For the oil slick on the water surface, the "oil tanker" can be used to separate the oil and water (Wang et al. 2018). Some tankers suck the oil-water mixture into the tanks on board, separate it with centrifuges, and then drain the water into the sea. You can also use things like sponges to suck oil into it. This kind of thing can be made of chemical fiber or natural fiber like hair. In order to collect oil, sometimes you can add "coagulant" to it, which can change the oil in water from a liquid state to a semi-solid state, or even a solid state like rubber. This kind of object is easy to be fished, and it can also prevent benzene, formaldehyde, acetone and other substances from volatilizing into the air. The harm of this curing agent to the environment is relatively small. Some hardener manufacturers claim that the solidified oil can be reused to produce asphalt or artificial rubber, or even used as a fuel with little ash. Another way, on the contrary, is not to allow oil to solidify, but to allow them to better combine with water. "Dispersants" break the oil film and make them disperse into small oil drops in seawater.

## MATERIAL METHOD

### Microbial Species

Microbial degradation is the most important way to remove oil from water surface. Bacteria, actinomycetes, fungi and algae can be degraded, among which *Rhodococcus*, *Pseudomonas*, *Corynebacterium*, *Micrococcus*, *Alcaligenes*, *Mycobacterium* and sphingolipid bacteria are common bacteria (Zhang *et al.* 2018). In the atmosphere or even in the lab, microbial degradation relates to just the microbiological transformation of biomass chemicals, usually those who are harmful to health, with less harmful or even more beneficial counterparts. The material is transformed by symbiotic strains using metabolism or enzyme mechanisms. Development and cometabolism are the two methods on which it is founded. An organic contaminant is employed as the only carbon and energy source during development. Organic pollutants are completely degraded (mineralized) as a result of these processes. The petroleum polycyclic aromatic hydrocarbon degrading fungi can be divided into lignin degrading fungi and non-lignin degrading fungi. Furthermore, the lignin degrading fungi usually include gram-negative fungus, *Pleurotus ostreatus*, *Coriolus versicolor*, etc.; The non-lignin degrading fungi usually include *Penicillium*, *Aspergillus* and *Klebsiella minor* (Kamelia *et al.* 2018). Some of the algae also have the ability to degrade petroleum polycyclic aromatic hydrocarbons, such as *agrimony*, *Chaetomium*, *Scenedesmus* and *lunata*. Only fungus have the ability to degrade or drastically alter lignin. They're even far stronger than most of the other creatures at breaking carbohydrates. Fungi breakdown lignin through cells secrete enzymes known as "ligninases," which are divided into two types: phenol oxidases and heme peroxidases. Lignocellulose accessory catalysts are still unable to digest lignin under their own, necessitating the participation of further enzymes to utter annihilation. The mechanism of lignin removal is facilitated by the simultaneous activity of numerous proteins, that might include oxidant H<sub>2</sub>O<sub>2</sub>.

Table 1. Degradable microorganism strain and substrate

Generic name	Strain	Substrate
<i>Rhodococcus</i>	P14, UW1	Phenanthrene, pyrene, benzopyrene, anthracene, fluoranthene, triphenyl, fluorene
<i>Pseudomonas</i>	PB1, P2, VUN10, 003	Cai, Qu, pyrene, phenanthrene, benzopyrene, benzoanthracene
<i>Alcaligenes</i>	AFS-5, PPH	Acenaphthene, Qu, Fei
Sphingolipids	PNB, KK22, C100, FB3, LD29	Naphthalene, 2-methylnaphthalene, phenanthrene, pyrene, fluorene, acenaphthene, anthracene, fluoranthene
<i>Nocardia</i>	TSH1, TRH1	Naphthalene, phenanthrene, pyrene, anthracene
<i>Penicillium</i>	SFU403	Anthracene, benzopyrene, pyrene
<i>Sinoderma Xiaoke</i>	IM1785/21Gp	Phenanthrene, 7-methylbenzoanthracene, 7-hydroxybenzoanthracene, acenaphthene
<i>Pleurotus</i>	ATCC38540, HP-1	Naphthalene, acenaphthene, phenanthrene, fluoranthene, triphenyl, pyrene, benzoanthracene, benzofluorescein

## REAGENTS AND INSTRUMENTS

Reagents and instruments are shown in Table 2 and Table 3 respectively.

Table 2. Reagents

Chemical Reagents	Name	Chemical formula
1	Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$
2	Ammonium nitrate	$NH_4NO_3$
3	Calcium chloride	$CaCl_2$
4	Ferric chloride	$FeCl_3$
5	Potassium dihydrogen phosphate	$KH_2PO_4$
6	Potassium chloride	$KCl$
7	Calcium carbonate	$CaCO_3$
8	Sodium chloride	$NaCl$
9	Other materials	Fresh potatoes - for media Soil samples of oil polluted loess in oil field area - strain selection Groundwater samples - Tests

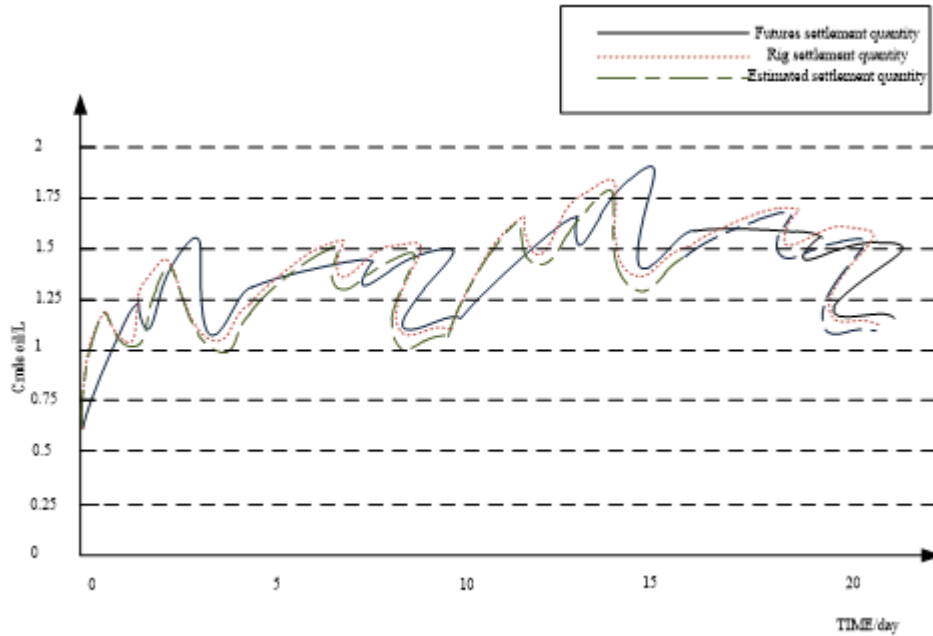
Table 3. Instruments

Instrument	Model	Production company
Electromagnetic oscillator	ZDQ50	Anyang ande Electronic Machinery Co., Ltd
Ultrasonic cleaner	KQ218	Kunshan Shumei Ultrasonic Instrument Co., Ltd
Biological constant temperature incubator	DHP-9012	Shanghai Xinyi Instrument Co., Ltd
High speed centrifuge	TGL-16B	Shanghai Anting Scientific Instrument Factory
Autoclave	DSX-280B	Shanghai Anting Scientific Instrument Factory
Aseptic Laboratory	Stainless steel filter	Haining Zhengyi filtration equipment Co., Ltd
Biochemical incubator	LRH-70	Shanghai Jingruo Scientific Instrument Co., Ltd
Shaker incubator	SHA-C	Changzhou Zhongbei Instrument Co., Ltd
Biomicroscope	Lycra	Leica microsystem (Shanghai) Trading Co., Ltd
Ultraviolet visible grating spectrophotometer	756S	Qingdao Mingbo Environmental Protection Technology Co., Ltd
Electric drying box	DHG-9030A	Shanghai Xinyi Instrument Co., Ltd

## DETERMINATION METHOD OF OIL DEGRADATION RATE

The concentration of crude oil in the medium was determined by ultraviolet spectrophotometry, and the standard curve of crude oil is shown in Figure 1.

Figure 1. Crude oil standard curve



Shaking is the process of combining, cooling, and reducing a drink, usually one with “overcast” components. Through with a systematic way, minimizing pipe shaking to optimize safety standards will resulting in cost-effective, effective, secure, and time-bound benefits. During the shaking process, after a certain period of culturing, 40 ml of petroleum ether was added to 100 ml of culture medium twice, and the upper organic phase was taken after extraction for 1 h. The residual oil component in the culture medium was determined by ultraviolet spectrophotometry (Wilson *et al.* 2018). Ultraviolet spectrophotometry is much more expensive than VISS because it needs quartz glass sample containers, quartz or fluorine optical elements, and a separate source of light, and it has become similarly available and simple to use. The method is used to determine materials which may include aromatic chemicals like benzene derivatives, as well as a broad range of UV absorption chemicals used it to preserve painting surfaces, human flesh, and textiles. The degradation rate of crude oil by the mixed flora is calculated according to formula (1):

$$\lambda = \frac{W_0 - W_s}{W_0} \times 100\% \quad (1)$$

Where,  $W_0$  is the absorbance of the original oil component in the blank bottle,  $W_s$  is the absorbance of the oil component in the experimental bottle. Under aerobic conditions, many industrial effluents degrade; nevertheless, a few chemicals contained in petroleum products, such as resin, right to petition, charged particles, and pentachlorophenol, degrade at speeds that are nearly unnoticeable. Because crude oil is composed of a variety of chemicals, and single organisms can only metabolize a limited number of hydrocarbons substrate, microbial degradation of oil products necessitates a combination of microbial species or consortium that can digest a wider range of compounds.

## MICROBIAL DEGRADATION TECHNOLOGY

Under the action of gravity, inertia force, friction force and surface tension, the offshore oil rapidly expands into thin films on the ocean surface, and then divided into block or strip oil films of different sizes under the action of wind waves and currents, drifting and diffusing with the wind. Diffusion is the main process to eliminate oil pollution in some sea areas. Wind is the most important factor affecting the oil drift on the sea surface, and the oil drifting speed is about 3% of the wind speed. The floating oil found along the coast of Shandong Peninsula, China is more in the North Bank of the peninsula in winter and more in the South Bank of the peninsula in spring, which is mainly due to the influence of wind. Non hydrocarbon components such as nitrogen, sulfur and oxygen in petroleum are surfactants, which can promote the diffusion of petroleum. In the process of diffusion and drifting of oil, light components escape into the atmosphere by evaporation, and their rates vary with molecular weight, boiling point, oil film surface area, thickness and sea state. Most of the hydrocarbons with carbon atom number less than 12 evaporate away within a few hours after entering the sea. In contrast, the evaporation of hydrocarbons with carbon atom number between 12 and 20 will take several weeks, and the hydrocarbons with carbon atom number more than 20 are not easy to evaporate. Evaporation is an important factor related to the natural disappearance of marine oil pollution. About  $1/4 - 1/3$  of the total amount of oil discharged into the sea is eliminated by evaporation. The self-oxidation and photochemical oxidation of the oil film on the sea surface are catalyzed by light and trace elements. Photo-oxidation of subsurface oil has major consequences for both the strategic approach, disaster management, and ecological regeneration in the following of an oil spill, and petroleum fate simulations must take this into account. Oxidation is the main way of petrochemical degradation, and the oxidation rate depends on the chemical characteristics of petroleum hydrocarbons. The processes of diffusion, evaporation and oxidation play an important role in the disappearance of oil in water within a few days after the oil enters the sea, and the diffusion rate is normally higher than the natural decomposition rate. The rate of such a dissemination is determined by the monomer's absorption in the emulsion's continual liquid state. Emulsions may become destabilized as a result of this. It causes oil to dissolve, the solution to diffuse via the liquid, and the oil to re-precipitate. Oil oxidation is a sequence of unwanted organic compounds containing oxygen that cause an oil's grade to deteriorate. Oil becomes sour as a result of oxidation, which is accompanied by foul odors and flavors. All oil oxidizes; you can't entirely prevent it, but you could still slow it down. Whenever the lightest elements in the lubricating oil turn into vapors and depart the ocean surface, this is called evaporation. The heavy elements of the oil are left behind after this procedure, but they may deteriorate further or fall towards the sea floor [17].

### (1) Aerobic bacterial degradation

According to the oxygen content of degradation environment, aerobic bacteria can be divided into aerobic degradation and anaerobic degradation. Through the introduction of ortho or interposition, trans dihydro dibasic compounds are formed to improve the activity of aromatic ring, and then continue to oxidize until the aromatic ring breaks to form unsaturated straight chain fatty acids. The subsequent degradation enters into the central degradation pathway of polycyclic aromatic hydrocarbons and connects with the intermediate products of tricarboxylic acid cycle. Taking phenanthrene as an example, phenanthrene is one of the most abundant polycyclic aromatic hydrocarbons in the environment after petroleum pollution. The most well-known contaminated solvents breakdown mechanisms are those which happen biotically beneath anaerobic conditions as well as those who occur aerobically for fewer chlorine molecules. The major degradation process includes the combination of polyenes and the removal of acetic acid. These could then go through more oxidative alterations. Preservatives may temporarily reduce that mechanism of acetic acid clearance, although they can diminish its autocatalytic action. There are many ways to open the ring of phenanthrene in aerobic

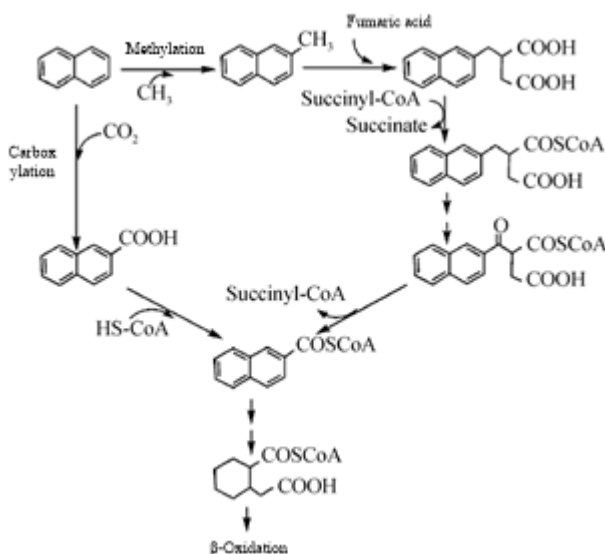
degradation of bacteria. According to the different action sites of oxygenase, phenanthrene generally opens the ring at 1, 2, 3, 4 and 9, 10 positions. Phenanthrene is a polyaromatic hydroxyl group that is made up of three fused carbon atoms and gets its power from the word's phenol and phenanthrene. It's a pollutant in the atmosphere and a human pathogen. Non-benzenoid aromatic compounds, on either hand, need not include a benzene ring instead and include highly unsaturated compounds with fragrant characteristics[18].

A strain of bacteria has one or more metabolic pathways. There are three ways to reduce phenanthrene from 1, 2, 3, 4 and 9, 10, respectively. There are two ways for *Burkholderia* sp.c3 to reduce phenanthrene, one is to open the ring at 1, 2 and the other is to open the ring at 3, 4. *Mycobacterium* sp. strains pyr-1 and *martellella* sp. ad-3 have two ways to reduce phenanthrene, that is open the ring at 3, 4 or at 9, 10, respectively. In *Pseudomonas putida* NCIB 9816 and *Sphingomonas* sp. pheb4, phenanthrene has only 3, 4-site ring opening. Phenanthrene at 1, 2 and 3, 4 sites have a common intermediate product, 1, 2-naphthalene, which has two degradation pathways. For the bacteria that can use naphthalene, 1, 2-bis-naphthalene is transformed into salicylic acid by naphthalene and then degraded by gentilic acid pathway; While for the bacteria that cannot use naphthalene, 1, 2-dicarboxynaphthalene is degraded by protocatechuic acid pathway. Phenanthrene can also form dihydroxy group at 9, 10, and then form 2, 2'biphenyldiacid. Low molecular hydrocarbons and some polar compounds also dissolve in seawater. The solubility of n-alkane in water is inversely proportional to its molecular weight, and the solubility of aromatics is higher than that of alkane. Although low molecular hydrocarbons are prone to dissolution and evaporation, their effects on water environment are different. Petroleum hydrocarbon dissolves in seawater, and is easily absorbed by marine organisms, but some harmful consequences may be caused.

## (2) Anaerobic bacterial degradation

The initial steps of anaerobic bacteria degradation of oil on sea surface mainly involve carboxylation and methylation, as shown in Figure 2.

Figure 2. Anaerobic degradation process of naphthalene



In the case of naphthalene, there are two degradation pathways in anaerobic environment, one is to add methyl group to 2-methylnaphthalene through methylation, and the other is to add carboxyl group to 2-methylnaphthalene through carboxylation. The further degradation of methylated and carboxylated products requires the participation of coenzyme (succinyl coenzyme A or coenzyme A), and then enoyl coenzyme A can be formed through the action of reductase. The aromatic ring of enoyl coenzyme A connected with coenzyme A is opened under the action of hydratase, and the latter is further completely degraded to acetyl coenzyme A and  $\text{CO}_2$  through the similar  $\beta$ -oxidation step. It was found that different anaerobic bacteria play different roles in the degradation of surface oil.

The sulfate degrading bacteria will degrade the drug and phenanthrene into the common intermediate phenol, while the desulfurizing enterobacteria and *Clostridium* will convert the phenol into benzoate under anaerobic conditions. *Clostridium perfringens* is a microbe bacterium that can be applied to a variety of environments, and in people and mammals' intestine belongs to the Clostridiaceae group and is a species of gram-positive, spore-forming bacterium. Rod-shaped proliferative activities are connected in pairings or shorter strings. The variety of animals are facultative anaerobes, although some were aerotolerant or may survive in aerobic circumstances. The heterotrophic bacteria can further degrade the benzoate into acetate,  $\text{H}_2$  and  $\text{CO}_2$ , and the methanogenic bacteria will finally convert acetate,  $\text{H}_2$  and  $\text{CO}_2$  into methane. Aerobic degradation is more significant than Anaerobic degradation for the production of severe quantities of sewage fluids organic matter. Aerobic bacteria identify the approval of oxygen to survive, whereas anaerobic bacteria do not.

### (3) Fungal degradation

In nature, there are a kind of fungi that can secrete lignin degrading enzymes (lignin peroxidase, manganese peroxidase and laccase). These non-specific enzymes secreted have a wide range of action substrates, which can degrade a variety of organic pollutants including polycyclic aromatic compounds. They are the unique mechanism of fungi to degrade polycyclic aromatic compounds. The presence of organic matter can induce the activation of peroxidase and laccase to degrade polycyclic aromatic hydrocarbons. For example, after adding oxalic acid and malonic acid to white rot fungi, it was found that the content of lignin peroxidase increased. Moreover, manganese containing organic matter was found to stimulate the activity of manganese peroxidase. Lignin degrading enzymes can introduce hydroxyl groups into the specific sites of polycyclic aromatic compounds. The degradation of phenanthrene by *Pleurotus ostreatus* occurs from 9, 10 site to dihydrodiol, then to 2, 2'-biphenyldiacid, and finally to  $\text{CO}_2$ . This process is very similar to that of 9, 10 site in the degradation of phenanthrene by aerobic bacteria. After evaporation and dissolution, the oil on the sea surface forms dense dispersed ions, which are then polymerized into asphalt blocks, or adsorbed on other particles, settling on the sea floor or floating on the beach. Asphalt Blocks are one-of-a-kind blocks that greatly boost the mobility of teams running through these. Additional speed-boosting goods, like the Hermes Boots and its variant accoutrements, combine alongside their speed. It works well in both industrial and leisure settings. This can be utilized for commercial floor that is subjected to continuous forklifts activity. They could be utilized on an outdoor delivery truck or even as an inside flooring. Under the action of current and wave, the oil or oil oxidation products that sink into the sea bottom can also float to the sea surface again, causing secondary pollution. Microorganisms play an important role in the degradation of petroleum hydrocarbons. Hydrocarbon oxidizing bacteria are widely distributed in seawater and sea mud (see biodegradation of petroleum hydrocarbons). Marine plants and animals can also degrade some petroleum hydrocarbons. Phytoplankton and sedentary algae can directly absorb Dissolved Petroleum Hydrocarbons from seawater. Marine animals will feed on the particulate matter with oil adsorbed, so that oil can enter their bodies through the digestive tract or gills. Since petroleum hydrocarbon is fat soluble, the content of petroleum hydrocarbon in marine organisms generally increases with the increase of fat content. In clean seawater, the oil accumulated



in marine animals can be discharged relatively quickly. So far, there is no evidence that petroleum hydrocarbons can expand along the food chain.

Using aerobic bacteria, anaerobic bacteria and fungi to control oil pollution on water surface. Many distinct hazardous chemicals can be found in oil. Cardiac disease, developmental delays, immune response impacts, and even deaths can all be caused by these hazardous substances. Drilling disturbs wildlife populations, making oil and gas production a threat to biodiversity. Oil and chemical leaks can lead to cancer, immune suppression, and reproductive problems in species, as well as induce long-term environmental change by destroying nesting or reproductive habitats. People are now burning ever more fossil fuels since before the industrialization, putting increasing greenhouse gases into the atmosphere. Facilities designed for oil extraction can have drastic environmental consequences.

## RESULTS

In view of the application of microbial degradation technology in the treatment of oil pollution on the water surface, experimental verification and analysis were carried out.

## OVERVIEW OF EXPERIMENTAL AREA

Topographic and contour maps formations on the Earth's surface are known as geomorphic. High-definition altitude information is constantly acquired utilizing light detection and ranging methods to simulate features. The study area is in the north of Shaanxi and some parts of Gansu loess areas. In terms of geomorphic characteristics, the study area is mostly loess residual source and beam screen, with vertical and horizontal valleys, strong terrain cutting, and more concentrated rainfall, forming a unique water hole and groundwater skylight in the loess area, so that the groundwater supply path is short, and precipitation can directly supply groundwater. Although the high content of silty clay in loess has a strong adsorption capacity for oil pollution, which can act as a barrier, local crude oil is easy to enter shallow groundwater due to the particularity of loess structure. In addition, since the oil is buried shallowly and has been exploited for a long time, there are some problems in the early well completion technology. Well Completion Technology is a global management and research firm for the oil and gas sector. Expert advice as well as on monitoring are provided by skilled, trained professional researchers and technicians which provide tailored training and boost your abilities. It specializes in organizing general industry and business education that is realistic and palms. The oil pipeline runs, overflows, drips, leaks and infiltrates into the groundwater, causing pollution.

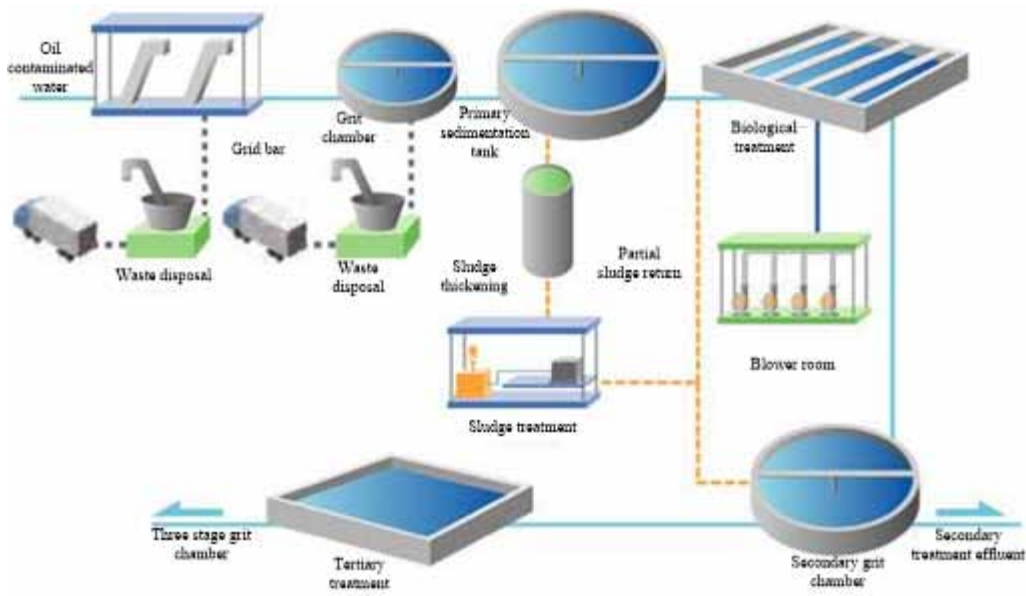
Seven groups of underground water samples were collected in the key oil exploitation areas of the study area. Wastewater soils polluted with oil - based effluents such as oil and grease, chemical products and solid particles from lubricating oil, forming wastewater generated together with oil products, and exhaust fumes containing CO, SO<sub>2</sub>, NO<sub>x</sub>, liquid fuels, and fine particles from gas flare are the significant environmental sources of pollution in oil and gas development and expropriation [19]. The oil content was between 0.061-0.243 mg/l, which was detected by the geoscience experiment center of China University of Geosciences (Beijing). It shows that the groundwater has been polluted by oil, and the degree of pollution varies from place to place, and the way of pollution remains to be studied, but it is of great significance to research and develop the treatment and remediation technology suitable for groundwater oil pollution in this area.

## FIELD OPERATION

Mix the microbial repair agent and zeolite after fermentation according to 2:1 (weight ratio), add 25% flocculent to assist adsorption, mix well, and then stand for more than 1 hour to make the agent adsorbed on the zeolite, then suck off the supernatant of the microbial agent fermentation liquid,

and load the zeolite into a bag to be put on the sea floor in the experimental area. The experimental process is shown in Figure 3.

Figure 3. Experimental processing



Resolved activation sludge gathered in the settling tank or filtration basin and transferred to the aeration basin to combine with raw or primary settling sewage. Oxygen transfer is a term that refers to a mixture of waste and organic matter. Residual blended liquid is emptied into settle chambers in all activated sludge facilities after the wastewater has been sufficiently cleaned, and the handled precipitate is run off to undertake additional processing until release [20]. Set up a sea mining site, from the 10th day, considering the impact of tidal current erosion, the added bacteria will drift with the current, increasing the experimental area.

## OIL REMOVAL RATE IN WATER

The first degradation experiment was carried out from March 30 to April 27, 2018. The temperature was set to 30°C, 25°C, 10°C, respectively. The oil mass concentration was analyzed at different time  $T$  was different. The experimental results are shown in Table 4.

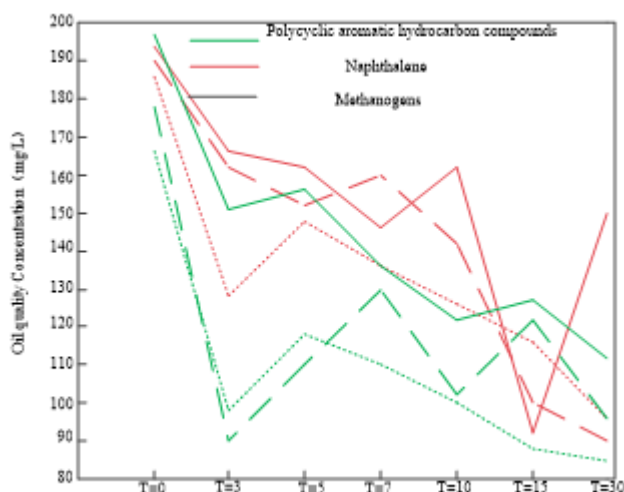
Table 4. Test results of microbial degradation of oil on the first oil polluted water surface

Temperature/°C	Oil mass concentration, mg/L						
	T=0	T=3	T=5	T=7	T=10	T=15	T=30
30	190.0	160.5	150.2	150.0	159.5	85.5	83.2
25	185.0	145.5	125.5	165.5	154.0	115.2	102.5
10	181.0	145.0	158.5	154.5	151.2	142.5	134.8

Microbial degradation had certain degradation effect on oil on water surface, and the degradation effect varied with temperature. Among the three selected temperatures, the experimental effect under 25°C was relatively stable. Each sample was an independent experimental device, and there were many factors affecting the growth of microorganisms in the experimental process, such as the number of microorganisms added, the operation error of the experimental process, etc.; Secondly, the vibration amplitude might be different in the different experimental process, and the oil might be absorbed by the cylinder wall without being degraded.

In order to better verify the application effect of microbial degradation technology in the treatment of oil pollution on water surface, the first test results were verified and analyzed, as shown in Figure 4.

Figure 4. Effect of microbial degradation on different oil compounds on water surface (first test)



The degradation of organic matter under the catalysis of microorganism is basically called the biochemical degradation of organic matter. Organisms in water, especially microorganisms, can make many substances undergo biochemical reactions, and most of the organics are degraded into simpler compounds. For example, alkanes in petroleum are generally degraded into carbon dioxide and water through biochemical oxidation substances such as alcohol, aldehyde, ketone and fatty acid. The main way of methane degradation is as follows:  $\text{CH}_4 \rightarrow \text{CH}_3\text{OH} \rightarrow \text{HCHO} \rightarrow \text{HCOOH} \rightarrow \text{CO}_2 + \text{H}_2\text{O}$ . Methane does have a flame when it ignites throughout the atmosphere. Methane melts in the presence of enough oxygen to produce  $\text{CO}_2$  from the atmosphere and water. It creates a lot of temperature as it burns, therefore renders it an excellent fuel supply. Methane is an extremely strong greenhouse gas.

There are three main ways for the degradation of higher alkanes: single end oxidation, double end oxidation or sub end oxidation to fatty acids; Whenever the polymer chains are too lengthy for the mitochondrial to manage, single end oxidation process in peroxisomes. Peroxisomes manufacture acetyl-CoA that use the same enzymes as are employed in the mitochondrial membrane. The metabolic designed to break up fatty acid chains is known as double end oxidation. A chemical process is a type of response that occurs when a substance reacts with another substance. When a sub stoichiometric fuel-air mixture is partly vaporized in a reformer, a hydrogen-rich syngas is produced, that can subsequently be used in other applications, such as fuel cells [21]. Fatty acids are decomposed into carbon dioxide and water through other biochemical reactions. The microorganisms

that can cause alkane degradation include trichoderma anolyticus, actinobacterium and bizarre bacilli. Saponibacterium, Nocardia opaque, Nocardia red, etc. The basic reactions of biodegradation of organic compounds can be divided into two categories: hydrolysis reaction and oxidation reaction. For organic pesticides, in addition to the above two basic reactions, dechlorination and dealkylation can also occur. It can be seen that the microbial degradation method is more accurate for the first test results of microbial degradation of oil on water surface.

The second degradation experiment was carried out from June 21 to August 6, 2018. According to the results of the first experiment, the temperature was set to 40°C, 35°C, 30°C, which were higher than the first degradation experiment. In the second experiment, the experiment time was extended to increase the effect of bacteria. At each temperature, two groups of parallel experiments of oil content were carried out with the same time length. The experimental results are shown in Table 5.

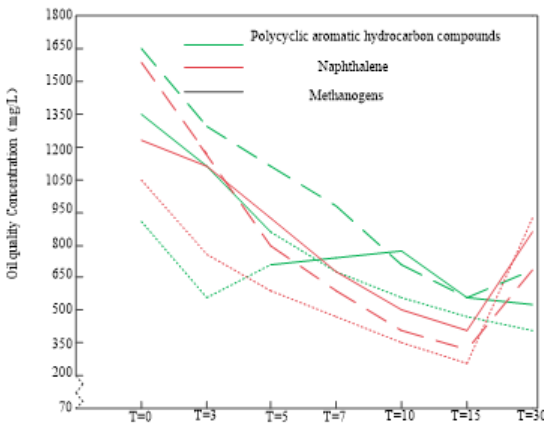
Table 5. Test results of the second degradation experiment

Temperature/°C	Oil mass concentration, mg/L						
	T=0	T=3	T=5	T=7	T=10	T=15	T=30
40	862.0	715.2	617.4	625.2	500.5	433.0	389.0
35	1694. 3	1565. 2	1384.3	1228.9	1128. 5	1024. 3	1694. 3
30	865.4	601.3	684.2	435.5	300.5	275.3	70.5

The second experiment exhibited improved degradation rate of oil, which verifies that microorganisms have a good remediation effect on oil pollution on water surface. The degradation rate at 30°C was better than that under the other three temperatures.

In the same way, the second test results of surface oil were verified and analyzed by microbial degradation method, which supports the degradation effect of microorganisms on polycyclic aromatic compounds, naphthalene and methanogenic bacteria, as shown in Figure 5. PAHs (polycyclic aromatic hydrocarbons) are a broad category of chemical molecules with two or even more bonded benzene ring. The word PAHs denotes to substances that exclusively include carbon and hydrogen atoms.

Figure 5. Degradation effect of microbial degradation on different oil compounds on water surface (second test)



It can be seen from Figure 5 that microbial degradation is realized through catabolism and anabolism, and catabolism is also known as “dissimilation”: Macromolecules are degraded into small molecules, generating energy in this process. There are three stages of catabolic atmosphere. In the first stage macromolecular nutrients (proteins, polysaccharides and lipids) are degraded into small molecules (amino acids, monosaccharides and fatty acids). In the second stage, the first stage products are further degraded into simpler acetyl coenzyme A, pyruvate and some intermediates that can enter the tricarboxylic acid cycle. In this stage, some ATP will be produced NADH and FADH<sub>2</sub>. In the third stage, through the tricarboxylic acid cycle, the second stage products are completely degraded into CO<sub>2</sub>, and ATP, NADH and FADH<sub>2</sub> are produced. ATP, NADH and FADH<sub>2</sub> produced in the second and third stages are oxidized by electron transfer chain, which can produce a large amount of ATP. Anabolism, also known as “assimilation”, refers to the process during which complex macromolecules are synthesized using simple small molecules, and energy is consumed. Absorption is a process in which the organism continuously absorbs various nutrients and energy from the outside. Anabolism uses the absorption of various nutrients, intermediate metabolites and energy into its own components. Catabolism and anabolism are inseparable. Their direction and speed are regulated by various factors so as to adapt to the changing internal and external environment. Complex molecules (organics) generate simple molecules + ATP + [H] (organic or inorganic) through catabolic enzymes and anabolic enzymes.

## DISCUSSION

There are abundant microorganisms in natural soil and groundwater, which have the potential to degrade petroleum pollutants. Moreover, the degradable petroleum microorganisms have been domesticated for many years, which has a strong potential to degrade petroleum pollutants. However, how to activate it to produce high degradation effect is an urgent problem. Using the microorganism selected from the original indigenous system can avoid a series of problems faced by other exogenous bacteria, such as weak viability, possible competition with indigenous microorganisms and so on. In the experiment, the microorganism selected from the original indigenous system was added for biological strengthening. However, since petroleum is a complex mixture of thousands of substances with different chemical properties, it is difficult to degrade it with a single strain. At present, in the biological treatment of petroleum pollution, scholars at home and abroad are paying attention to the use of bacterial flora for biological treatment of petroleum. Therefore, a variety of different microbial flora were selected for comprehensive degradation in the experiment.

## CONCLUSION

Due to the limited time and experimental conditions, there are still many limitations in this paper, which need to be improved in the following experimental work:

- (1) Light and other environmental factors will also have a certain degree of impact on the weathering process of crude oil. In the follow-up research process, the weathering process of crude oil can be investigated more carefully;
- (2) In the part of the research on the migration and transformation of crude oil in the beach sediment, there is a certain gap between the depth of the beach sediment in the experimental process and the depth of the sediment in the actual conditions, and the experimental device can be further improved. In addition, investigations of biological toxicity can be carried out under the same environmental conditions, on this basis, we can make a more accurate judgment on the ecological harm of oil spill pollutants;

- (3) In the future evaluation process of microbial agents, according to the different agents used, each experimental period should be appropriately extended, and the investigation of each process parameter should be more specific, so as to accurately and accurately characterize the whole process of microbial degradation of crude oil;
- (4) In the future, we can introduce gene engineering methods to cultivate multi degradation plasmid strains and improve their degradation performance, which will become a breakthrough in the research of microbial degradation in the future.

The experimental results preliminarily verify that microbial remediation technology is effective and feasible in the remediation of oil pollution. It has many advantages, including, low cost, good remediation effect, and little impact on the environment, no secondary pollution, and in-situ treatment. Moreover, microbial remediation technology is one of effective methods to fundamentally repair and control the oil pollution of groundwater as well as one of effective methods for remediation of other similar polluted groundwater, which shows great practical significance.

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