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Article

The application of microbial immobilization technology in the cleanup of soil contaminated with petroleum hydrocarbons

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Abstract: Petroleum hydrocarbons represent a challenging global pollution issue, with their degradation presenting a significant for environmental scientists. Microbial immobilization technology (MIT) offers a promising solution due to its efficiency, stability, cost-effectiveness, and environmental benefits, making it a highly promising approach for soil cleanup. Over recent years, the research on using MIT to remediate petroleum hydrocarbon-contaminated soil has surged in popularity. This technology has emerged as a potent means to enhance the microbial breakdown of petroleum hydrocarbons in soil. The paper reviews the advancements in microbial immobilization technology, outlines the distinct attributes of carrier materials, microorganisms, immobilization techniques, and factors affecting the process, along with their influence on the immobilization outcome. It also discusses the current state and future directions of this technology in the remediation of petroleum hydrocarbon-contaminated soil.

Keywords: immobilized microorganism; carrier materials; petroleum hydrocarbons; soil remediation

Improper management or sudden leakage accidents in the process of oil exploration, exploitation, smelting, transportation, use and storage will inevitably cause pollution of petroleum hydrocarbons (PHS) in the surrounding environment, which has attracted extensive attention of scholars at home and abroad [1,2]. It is reported that at least 342,000 sites in Western Europe are polluted by PHS; in the United States, 90% of the contaminated sites contain petroleum pollutants [1]. More than 400 oil fields have been developed in China, about 600,000 tons of oil pollutants enter the environment every year, and the area of soil polluted by PHS reaches 48,000 [3,4]. It is reported that the main pollutants in the soil of oil production areas in China are PHS and polycyclic aromatic hydrocarbons, and PHS contaminated sites have become one of the most important types of contaminated sites at home and abroad [5,6].

PHS, which accounts for more than 95% of petroleum composition, is a mixture of hydrocarbon compounds contained in petroleum, mainly composed of carbon, oxygen, nitrogen, hydrogen and sulfur, including saturated hydrocarbon, aromatic hydrocarbon, resin and asphaltene [2]. It can be divided into short chain hydrocarbons, gasoline, kerosene and diesel oil according to its composition, structure and element ratio [7]. Once PHS is introduced into the soil environment, due to its hydrophobicity, semi volatility and difficulty in degradation, it can not only change the physical and chemical properties of the soil (imbalance in the proportion of soil carbon, nitrogen and phosphorus, pore blockage and decline in water content) [8], but also endanger the growth of soil animals, plants and microorganisms, and even form human health stress through the food chain, thus seriously threatening the safety of the ecological environment and the sustainable development of social economy [7,9]. Therefore, it is urgent to comprehensively carry out the remediation of PHS contaminated soil.

For soil PHS pollution, the current main remediation technologies include physical remediation, chemical remediation and biological remediation [10]. Although the physical and chemical remediation methods can achieve good remediation effect, they have serious damage to soil structure and physical and chemical properties, and are easy to cause secondary pollution; in contrast, bioremediation technology has the advantages of environmental friendliness, low cost, stable effect and no secondary pollution. It has become a widely used bioremediation method [11].

In practical engineering applications, free PHS degrading bacteria are vulnerable to the competition of indigenous microorganisms and adverse environmental conditions, resulting in poor microbial activity and remediation effect [12,13]. In order to solve this problem, many scholars have carried out research on microbial immobilization technology (MIT), and made rich research achievements in the degradation of organic pollutants by using the advantages of immobilized microorganisms [8,14–19]. However, up to now, there are few reports on MIT remediation of PHS contaminated soil. Therefore, this paper systematically summarizes the research progress of MIT at home and abroad, lists the latest research results of its remediation of PHS contaminated soil, and deeply discusses the immobilization methods and related influencing factors, in order to provide some reference for the future scientific research and engineering application of MIT.

1. Overview of immobilized microorganism technology

Immobilized microorganism technology originated in the early 1960s and developed from immobilized enzyme technology [20], which means that microorganisms are fixed in a specific small area or attached to a carrier material by physical or chemical means, so as to improve their concentration and recycling capacity [21]. Compared with free microorganisms, immobilized microorganisms have higher activity and are expected to achieve efficient degradation of pollutants [14,22].

Compared with free microorganisms, the advantages of immobilized microorganisms are mainly reflected in: carrier materials with high mechanical strength can provide a good “shelter” for immobilized microorganisms, the storage time of immobilized microorganisms is long, and the tolerance to external adverse environmental conditions is enhanced [21,23]; the immobilized microorganism has high density, high activity and stable performance, which can significantly improve the pollutant degradation efficiency [24–26]; the carrier material can adsorb soil pollutants, and the selectivity of immobilized microorganisms to pollutants is lower, so as to remove pollutants more thoroughly; the product can be prepared by using a small amount of microorganisms. There are various carrier materials to choose from, which can be recycled and the later maintenance cost is low [27,28].

The possible mechanism of enhanced degradation of PHS by immobilized microorganism technology is shown in **Figure 1**. On the one hand, most of the carrier materials for immobilized microorganisms have adsorptivity, which can act as adsorbents to concentrate the target pollutants in the environment on the carrier surface, increasing the probability of contact between microorganisms and pollutants; at the same time, the loose pores of the carrier also provide a gathering place for microbial extracellular enzymes and strengthen the degradation of pollutants [19]; on the other hand, the pores of the carrier material also provide a buffer place for the proliferation of microorganisms, so that microorganisms can be protected from the direct impact of complex factors in the external soil [29]; finally, some carriers also provide nutrients such as carbon source for the growth of microorganisms. Their pores also facilitate the inward transportation of air, water and other substances, facilitate the material exchange of microorganisms, and enhance their activity [30]. Therefore, the immobilized microorganism and its carrier play a complementary role, providing relatively favorable conditions for the solution of pollutants [31].

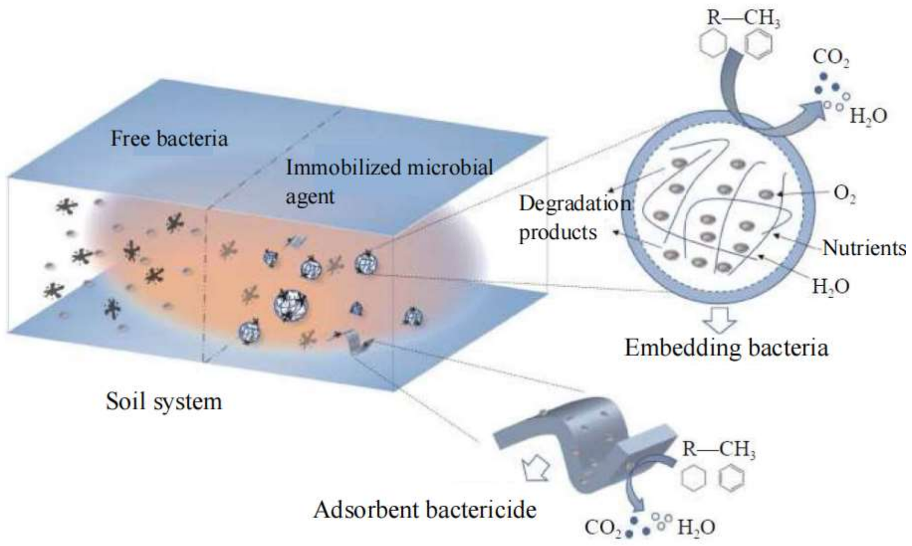

Figure 1. Mechanism of enhanced degradation of PHS by MIT.

2. Immobilized carrier material

Microbial immobilized carrier materials provide a microenvironment for microbial survival and reproduction. The source, specific surface area, porosity, stability, mechanical strength and other properties of carrier materials can affect microbial activity, and then affect the repair effect and engineering application of immobilized products [32,33]. In view of the complexity of the soil system, the selection of immobilized carrier materials should focus on the following factors: a) The immobilized carrier materials have good mechanical strength, thermal stability and chemical stability, and can provide a good shelter for microorganisms in the complex soil environment [34]; b) the immobilized carrier material has a high specific surface area, which can provide more adsorption sites and reaction sites for pollutants and degradation products [24]; c) the immobilized carrier material has good compatibility with microorganisms, has no toxic effect on fixed microorganisms, has good mass transfer to substrates and oxygen, and can provide necessary carbon sources and nutrients for microorganisms [27]; d) the availability and low cost of the immobilized carrier materials, and the carrier materials into the soil environment will not cause secondary pollution [35]. Microbial immobilized carrier materials can be divided into inorganic carrier materials, organic carrier materials, composite carrier materials and new carrier materials according to their properties [32].

2.1. Inorganic carrier materials

Inorganic carrier materials have the characteristics of high mechanical strength, good physical and chemical stability, wide cost sources and good mass transfer performance. Common inorganic carrier materials for microbial immobilization include quartz sand, diatomite, zeolite, activated carbon, volcanic rock, perlite, biochar, porous ceramics, etc. [13,27]. Li et al. [36] fixed Citrobacter sp. Strains on corncob biochar by adsorption method to prepare immobilized microorganisms. After 60 days of remediation of PHS heavy metal cadmium compound polluted soil by immobilized microorganisms, the degradation rate of PHS by immobilized microorganisms reached 51.25%, significantly higher than that of free bacteria group (40.44%), biochar group (31.11%) and blank group (15.18%). Ren et al. [37] confirmed that biochar immobilization of polycyclic aromatic hydrocarbon degrading bacterium ad-3 not only maintained the efficient degradation ability of bacteria to phenanthrene, but also prolonged the storage time of degrading microorganisms, providing good bioremediation functional materials for the remediation of polycyclic aromatic hydrocarbon contaminated soil. Ren et al. [38] found that after immobilization of oil degrading bacteria F-3, r-7 and their mixture by Corncob and straw biochar, the oil removal rate was significantly improved, which were 417% and 295%, 525% and 428%, 638% and 532% respectively. The oil removal rate of corn cob biochar immobilized bacteria was 106% higher than that of straw biochar immobilized bacteria. In recent years, in order to improve the immobilization effect and repair efficiency, inorganic carrier materials are often combined with existing materials to prepare organic-inorganic composite carrier materials [39,40].

2.2. Organic carrier materials

Organic carrier materials include natural organic polymer carrier materials and synthetic organic carrier materials. Natural organic carrier materials include agar, alginate and chitosan; among the synthetic organic carrier materials, polyvinyl alcohol (PVA), polyurethane and polyacrylamide have been widely studied [27,32]. Organic carrier materials have good affinity and adsorption properties for microorganisms, and have been widely used in microbial immobilization and adsorption of toxic and harmful pollutants. Natural organic materials are cheap and easy to obtain. Compared with inorganic materials, they have more functional groups and stronger affinity on the surface, but their overall mechanical strength is relatively poor and their stability is poor [41]. Synthetic organic materials have good mechanical strength through a series of chemical and photochemical reactions. They can artificially control the porosity, shape and other characteristics of the materials. They have superior surface properties and can be used to prepare carrier materials more suitable for degradation targets [42]. However, some synthetic organic carrier materials have been found to have certain biological toxicity, which limits their engineering application to a certain extent [40]. At present, the organic carrier materials commonly used to immobilize PHS degrading microorganisms mainly include PVA, alginate and chitosan [13]. Liu et al. [43] used sodium alginate and chitosan to embed and immobilize marine PHS degrading bacteria. The degradation results of PHS contaminated soil showed that the degradation rates of free bacteria and immobilized bacteria agents were 45.9% and 70.6% respectively, indicating that the immobilized microorganisms of this organic material carrier had a good repair effect on PHS.

2.3. Composite carrier materials

The synthesis of composite carrier materials is a new hot technology in recent years. Common composite carrier materials include polyacrylonitrile and montmorillonite, sodium alginate and metal ions, chitosan and sodium alginate [35]. According to the actual situation and specific needs, the composite of such materials can be the composite of inorganic materials and inorganic materials, and the composite of organic materials and inorganic materials. The composite carrier material can be the combination of organic materials and organic materials, or the properties of the composite can be further extended by modifying a certain component [35]. Composite carrier materials usually have the advantages of both organic and inorganic materials, and have better comprehensive effects. However, the preparation cost is high, the process is complex, and the ability of recycling will be limited, so more in-depth research is still needed [30,32]. Li et al. [44] prepared the composite carrier material with tomato straw, sodium alginate and PVA, immobilized Bacillus and degraded the monomer fluorene in the soil. The results showed that the degradation rate of the immobilized bacteria prepared by the composite carrier material was up to 95.25%, significantly higher than that of the free bacteria.

2.4. New carrier materials

In recent years, aiming at the problems in the application of traditional carrier materials, researchers at home and abroad are committed to developing new carriers with low cost, high stability, high efficiency and environmental friendliness [6,23,45–47], mainly including modified materials, metal organic frame materials (mofs), magnetic nano materials, biodegradable polymer materials and biological slow-release materials [22,34,47]. Mofs is an organic-inorganic hybrid material composed of metal nodes and organic connectors. Compared with other immobilized carriers of most enzymes, mofs as porous framework materials have larger specific surface area and pore volume, which is very conducive to the adsorption and diffusion of molecules. At the same time, it can be adjusted according to the size of bacteria and enzymes. In addition, the functional groups on the framework of mofs can actively promote the catalytic process, and the regulation of organic ligands can optimize the pore size as required [47].

At present, many researches on biochar and its modified materials have been carried out. The modification methods mainly include acid modification, alkali modification and salt modification [40,48]. The loading capacity and affinity of modified materials to microorganisms have been enhanced, which can also promote soil fertility and soil structure. Zhang et al. [49] used the immobilized microbial agent prepared with nano porous SiO_2 synthesized by biomacromolecules as the carrier to degrade petroleum pollutants. The results showed that the degradation rate of the microbial agent reached 96.2% in 50 h. After 8 repetitions, the degradation rate still reached more than 85%, which was 30% higher than that of free bacteria. Wang et al. [50] used humic acid modified biofuel ash to prepare immobilized microbial agents. The degradation rate of PHS in soil reached 47.8% within 60 days, twice that of free bacteria. Zheng et al. [51] used biomass power plant ash as a carrier, modified it with humic acid, loaded with PHS degrading bacteria to form immobilized bacteria for remediation of crude oil contaminated soil. After 60 days of remediation, the degradation rate of PHS in contaminated soil by immobilized bacteria reached 51.9%, 25.0% higher than that of free bacteria, and the degradation rates of long-chain normal alkanes, aromatic hydrocarbons and colloids increased by 9.6%, 31.7% and 37.5% respectively. Tian et al. [52] used acetic acid modified ramie fiber as a carrier to adsorb and fix petroleum degrading bacteria. The results showed that the immobilized bacteria showed better environmental tolerance than the free bacteria. The degradation rate of short chain alkanes (C12~C20) by the immobilized bacteria was as high as 94.85%.

3. Immobilized microorganisms and PHS degrading bacteria

3.1. Immobilized microorganisms

In MIT, microorganisms are the main body involved in the remediation of pollutants, and their properties have an important impact on the effect of soil remediation [30]. At present, microorganisms used for microbial immobilization include bacteria, fungi and algae [22]. For target pollutants, it is very important to select specific microorganisms [53]. Previous studies have shown that the removal rate of pyrene in soil by bacteria is poor. The removal rate of zoogloea sp. No. 9 is 52.7% [54], while the removal rate of pyrene by fungi is higher than that by single bacteria. The removal rate of Fusarium sp. Reaches 69% [55]. Even if the same carrier material and the same immobilization method are used, and different microorganisms are selected for immobilization, the degradation of the same pollutant by the prepared products is quite different [30]. Intraspecific or interspecific synergistic degradation of strains is usually better than that of a single strain [56]. Hu et al. [57] found that the degradation rate of phenanthrene, anthracene and pyrene in soil polluted by polycyclic aromatic hydrocarbons (PAHs) was 3–4 times higher than that in the control group after treatment with immobilized mixed microbial agents. In addition, compared with the cultivation of exogenous bacteria, screening and domesticating indigenous high-efficiency bacteria have certain advantages in the degradation of organic pollutants. It is an environmentally friendly choice to apply them to immobilized microorganisms to repair organic polluted soil [30].

3.2. PHS degrading bacteria

It is reported that there are about 100 genera and more than 200 kinds of microorganisms that can degrade PHS pollutants, including bacteria, fungi, actinomycetes and algae [58]. The most common PHS degrading bacteria in soil mainly include *Pseudomonas*, *arthrobacter*, *alcaligeues*, *corynebacterium*, *flavobacterium* and *Achromobacter*. Common PHS degrading fungi mainly include *Trichoderma*, *peuicillium*, *aspergillus* sp. and *Mortierella* [2].

Generally, the microorganisms degrading PHS are screened from the oil polluted soil, separated and purified from the culture medium added with oil, and domesticated. Due to the co metabolism caused by the lack of synergy, the degradation effect of mixed strains on specific pollutants is often not as good as that of a single strain [41], and PHS contains a variety of components, which is a mixture composed of a variety of alkanes and aromatic hydrocarbons. The degradation effect of a single microorganism may not be as good as that of mixed strains. Ren et al. [38] isolated two highly efficient PHS degrading bacteria F-3 and F-7 from oil contaminated soil, using corncob biochar and wheat straw biochar as carriers, F-3, F-7, F-3 and F-7 mixed bacteria were immobilized respectively. After degradation of PHS, the results showed that the degradation rates of PHS were 417%, 525%, 638%, 295%, 428% and 532% respectively, indicating that for the two carrier materials, the degradation effect of the mixed bacteria immobilized was higher than that of the other single bacteria immobilized.

4. Immobilization method and its influencing factors

4.1. Immobilization method

Immobilization method is an important factor connecting immobilized carrier and immobilized microorganism, which affects the growth and development of microorganism [30]. Only selecting appropriate immobilization method can maximize the role of carrier material and microorganism. At present, the most used and studied methods can be divided into four categories according to their physical and chemical mechanisms, including adsorption, embedding, cross-linking and covalent bonding (see **Figure 2**) [23,41]. However, in the actual immobilization process, a single immobilization method will have limitations. It is often a combination of two or more immobilization methods to enhance the immobilization of microorganisms.

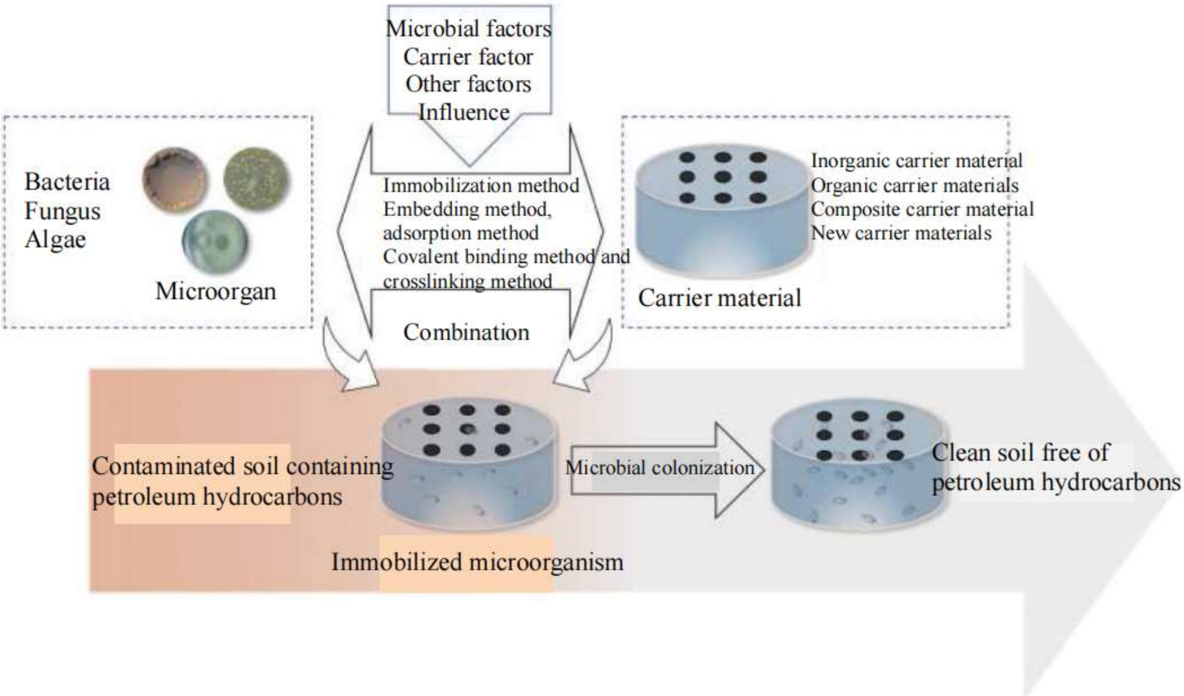

Figure 2. Technical points and classification of MIT.

4.1.1. Adsorption method

The adsorption method relies on the weak forces (hydrogen bond, van der Waals force, charge force, etc.) On the surface of microorganisms and carrier materials through physical adsorption. Generally, the carrier materials and bacterial solution after sterilization are mixed in proportion for a period of time, and then the immobilized microorganisms are centrifuged and separated for use [48]. The adsorption method is simple and convenient to operate, the exchange of microbial substances is not blocked, and the carrier can be reused. However, the adsorption depends on the combination of weak forces, which is a reversible effect. Therefore, the fixation of microorganisms is unstable, and it is easy to leak due to desorption [41]. The factors affecting adsorption are: a) Physiological conditions of bacteria, cell age, bacterial surface appendages, cell membrane charge, hydrophobicity and other characteristics; b) characteristics of culture medium, such as composition, pH, etc.; c) the size and structure of the adsorbent used and the surface properties of the supporting matrix composed of the pores on the adsorbent [41].

4.1.2. Embedding method

The embedding method is to entrap microorganisms in the fixed space in the carrier in the form of capsules, microspheres or membranes to complete immobilization [33]. According to the different restricted materials, it can be divided into two types: one is to intercept microorganisms in porous granular materials and polymer gel. Microorganisms can move freely between compartments formed by materials, and normal material exchange can also be carried out between compartments. Now, they are mostly embedded in PVA and sodium alginate; another method, also known as encapsulation method, is to limit microbial cells and macromolecules to the semi permeable membrane, while small molecules such as oxygen, nutrients and degradation products can pass through freely. This method can not only fix individual cells in the membrane, but also fix multiple cells together [59]. Different from adsorption, embedding is an irreversible method, which can not only limit microorganisms in the carrier to reduce leakage, but also protect them from the interference of external substances, so as to better preserve the vitality of microorganisms. The method is simple and low-cost, which is more suitable for large-scale production. However, when the cell density of fixed microorganisms is high, the shell of microspheres will affect the material exchange of microorganisms and reduce the mass transfer efficiency, the shear force during the embedding process is also easy to inactivate the cells [22]. The most important factors affecting the embedding method are the carrier pore size and microbial diameter, which determine the stability and fixation efficiency of immobilized microorganisms [60].

4.1.3. Cross linking method

The cross-linking method is a method that uses high molecular cross-linking agents (glutaraldehyde, hexamethylene diisocyanate, double azobenzidine, etc.) To immobilize microorganisms through the formation of covalent bonds and intermolecular cross-linking [48]. In the preparation process, the cross-linking method is similar to the embedding method. The embedding method is to mix the embedding agent with the microorganism for a period of time and then drip it into a ball, while the cross-linking method also needs to add a cross-linking agent during the mixing and cross-linking for a period of time [48]. The cross-linking method has strong binding property, but generally the cross-linking agent has cytotoxicity, which will reduce the microbial activity. It is difficult to determine when the cells can be immobilized without causing any damage [41], and the cross-linking agent is relatively expensive, which may cause higher costs in actual use, so it is not as common as the adsorption method and the embedding method in practical application.

4.1.4. Covalent binding method

Covalent binding method is to form a chemical covalent bond connection between the active groups on the microbial surface and the groups on the carrier surface, so as to form the immobilization of microorganisms [14]. Both covalent binding method and adsorption method are methods to fix microorganisms on the carrier surface, but they are different from the weak binding force of adsorption method. Covalent binding method uses the strong force to form covalent bonds to fix microorganisms. Its binding effect and stability are stronger than that of adsorption method, which is an irreversible binding. Like the cross-linking method, some cross-linking agents will reduce the activity of microbial cells in the covalent binding operation, and the functional groups on the microbial surface are not suitable for covalent bond [59]. Therefore, the covalent binding method is commonly used for enzyme immobilization, and is rarely used in cell immobilization.

4.2. Influencing factors of immobilization

For MIT, in addition to the selection of carrier materials, microorganisms and immobilization methods, there are also some important factors that affect the immobilization effect and the degradation effect of target pollutants, such as the surface properties of microorganisms and carriers, immobilization time, carrier dosage, microbial immobilization amount, rotating speed during immobilization, pH, etc. [4,15]. The influencing factors of immobilization can be specifically divided into microbial factors, carrier factors and other factors, as shown in **Figure 2**.

4.2.1. Microbial factors

Microbial factors mainly include microbial growth period and physiological state, microbial cell surface properties, cell inoculation amount and cell density during fixation [61]. Generally, immobilized microorganisms will be cultured in the culture medium until the late logarithmic growth period, and then fixed, because the microorganisms have the specific growth rate, the highest activity and the strongest resistance to harmful factors during the whole growth period, which is conducive to immobilization, the maintenance of microbial cell activity and the efficient degradation of pollutants. It is also an effective way to control the immobilization effect by indirectly changing the cell density by changing the inoculation amount of microorganisms. The research shows that after exceeding a certain range, the cell density fixed in the carrier increases, and the material transmission efficiency will be reduced [6]. The possible mechanism is that the presence of a large number of microbial cells will increase the mass transfer resistance, thus affecting the uptake of nutrients by microorganisms and reducing the cell activity; at the same time, the growth of cells in the carrier will also block the pores of the carrier and reduce the mass transfer efficiency. Lu et al. [12] showed that when the microbial inoculation amount increased from 5 g/L to 35 g/L, the degradation rate of crude oil by immobilized microspheres was also increasing with the increase of inoculation amount; when the inoculation amount exceeds 35 g/L, the degradation rate of crude oil decreases with the continuous increase of inoculation amount.

4.2.2. Carrier factors

The selection of carrier materials is an important part of immobilization technology. The shape and structure of materials, surface properties, immobilized particle size and carrier concentration will have an important impact on immobilization [62]. The structure and surface properties of carrier materials, such as specific surface area, surface roughness, porosity, hydrophilicity and hydrophobicity, will affect the growth and immobilization of microorganisms. Carrier materials with large specific surface area, rough surface, loose and porous can bring favorable effects on immobilization, and can bring a good growth and reproduction environment and more adsorption sites for microorganisms [13]. Generally, the diameter of immobilized microspheres is 0.5~5 mm, which will limit the efficiency of material transfer and affect the diffusion of substrates and nutrients. Generally, the larger the particle size, the lower the mass transfer efficiency; at the same time, it will also affect the adsorbed microbial biomass and further affect the degradation effect [61]. Zhang et al. [63] found that with the decrease of carrier particle size, the oil removal rate of the bacterial agent increased from 33.9% to 48.4%, which may be due to the increase of specific surface area, which led to the increase of adsorption sites and enhanced the immobilization and degradation effects. In addition, Zhang et al. [63] and Zhang et al. [64] found that the degradation of substrate by immobilized particles increased first and then decreased with the increase of carrier dosage. The change of carrier dosage affected the adsorption capacity and intensity of degradation bacteria by unit mass carrier, or the collision between carriers led to the fall off of degradation bacteria, which ultimately affected the immobilization effect.

4.2.3. Other factors

Some environmental factors and immobilization process factors also have a certain impact on immobilization [18]. On the one hand, environmental factors such as pH and temperature control the cultivation and screening of microorganisms; on the other hand, they determine the adsorption performance of **Table 1**.

The carrier to microorganisms and affect the immobilization effect by affecting the form of pollutants and the physical and chemical properties of the carrier and microorganisms, such as zeta potential [61]. The influence of immobilization time is mainly related to the growth curve of microorganism [63]. If the immobilization time is too long, the microorganism may have reached the stable period or the decay period, thus reducing the activity; if the immobilization speed is too fast, the cells will be broken due to too strong oscillation and the activity will decrease. The addition of some additives during immobilization will also have a positive or negative impact on the survival rate and immobilization effect of microorganisms. These factors have strong uncertainty, and they should also be analyzed according to the body conditions to determine the best immobilization conditions.

5. Case study on degradation of PHS in soil by immobilized microorganisms

At present, soil PHS pollution has become a hot spot at home and abroad [65]. Compared with traditional repair technology, it shows good repair potential. Most of the existing studies on immobilized microbial technology focus on water treatment [19,53], while the research on degradation of soil pollutants started late and mainly focused on the laboratory scale, and there are relatively few degradation studies in the actual site [60]. Some domestic and foreign research cases on immobilized microbial remediation technology are shown in **Table 1**.

Table 1. Researches on PHS degradation by immobilized microorganisms.

Immobilized microorganism	**Carrier**	**Immobilization method**	**Repair media**	**Removal rate%**	**Literature**
Citrobacter sp. W3~W9	Corncob biochar	Adsorption method	Combined pollution of soil by PHS and heavy metal cadmium	51.25	[36]
Serratia marcescens BZ-L	Sodium alginate and activated carbon	Embedding method	Oil contaminated saline soil	61.70	[12]
Oil degrading strain H3	Mushroom based biochar	Adsorption method	Oil contaminated soil in Shengli Oilfield	58.08	[66]
Microbacterium foliorum, gordonia alkanivorans and *mesorhizobiu*	Sodium alginate and sodium alginate diatomite	Adsorption embedding method	Oil contaminated soil in the Yellow River Delta	29.8	[25]
Efficient PHS degrading bacterium M3	Humic acid modified biofuel ash	Adsorption method	Oil contaminated soil in Xinjiang	47.8	[50]
Citrobacter sp.	Corncob biochar	Adsorption method	PHS nickel contaminated soil	45.52	[67]
Bacillus subtilis ZF3-1 Co culture	Water hyacinth straw	Adsorption method	Simulated PHS contaminated soil	51.7	[68]
Bacillus alcalophilus SG	Straw	Adsorption method	Crude oil medium	73.88	[69]
Pseudomonas, *Acinetobacter*, *Sphinpobacterium*	Wheat bran biochar	Adsorption method	Oil contaminated soil in Dagang Oilfield	58.31	[56]
Sphinpobacterium multivorum	Pine needles, corncob biochar	Adsorption method	Oil contaminated soil	47.6~50.7	[63]
High efficient petroleum degrading bacteria h, f	Nano porous SiO_2	Adsorption method	Oil contaminated soil	96.2	[49]

6. Conclusion and prospect

MIT has a broad application prospect in the field of environmental remediation because of its high efficiency, high stability, low cost and environment-friendly advantages. In particular, it shows good application value in the field of remediation of PHS contaminated soil. In addition, during the application of MIT, the selection of immobilized carrier materials, immobilized microbial biomass and activity, and immobilized technology can affect the remediation effect of soil pollutants. It is worth noting that there are still shortcomings in the decomposition of carrier materials and high preparation costs in microbial immobilization. Stable, efficient, low-cost and environment-friendly immobilized carrier materials will be the focus of future research. At present, most of the immobilized microbial technology is still limited to the stage of laboratory research, and only a few are used in bioreactors. In the future, it is urgent to carry out the research on the application of MIT in oil contaminated sites. In addition, the combined Phyto microbial remediation of soil pollution has shown high potential. The collaboration between MIT and phytoremediation technology is expected to improve the removal rate of soil oil pollution. It has green, environment-friendly and aesthetic values, and is easy to be accepted by the public.

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References

1. Hoang SA, Sarkar B, Seshadri B, et al. Mitigation of petroleum-hydrocarbon-contaminated hazardous soils using organic amendments: A review. Journal of Hazardous Materials. 2021; 416: 125702. doi: 10.1016/j.jhazmat.2021.125702
2. Fu B, Liu S, Yan Y, et al. Research Progress on microbial remediation of oil contaminated soil. Environmental protection and circular economy. 2021; 41(3): 54-60.
3. Wei X. Hazard and current situation analysis of soil oil pollution. Comprehensive utilization of resources in China. 2020; 38(4): 120-122.
4. Kong L, Wang X, Gu M, et al. Analysis on Influencing Factors of biochar enhanced biodegradation of PHS. Environmental Science and technology. 2021; 44(3): 8-15.
5. Liu D. Current situation and trend of soil PHS environmental quality standard system in polluted sites in China. China environmental monitoring. 2020; 36(1): 138-146.
6. Partovinia A, Rasekh B. Review of the immobilized microbial cell systems for bioremediation of petroleum hydrocarbons polluted environments. Critical Reviews in Environmental Science and Technology. 2018; 48(1): 1-38. doi: 10.1080/10643389.2018.1439652
7. Haider FU, Ejaz M, Cheema SA, et al. Phytotoxicity of petroleum hydrocarbons: Sources, impacts and remediation strategies. Environmental Research. 2021; 197: 111031. doi: 10.1016/j.envres.2021.111031
8. Egret, Wu C, Gu F. Study on Optimization of remediation conditions for oil contaminated soil by immobilized microorganisms. Nonferrous Metals (smelting). 2021; (3): 51-56.
9. Ossai IC, Ahmed A, Hassan A, et al. Remediation of soil and water contaminated with petroleum hydrocarbon: A review. Environmental Technology & Innovation. 2020; 17: 100526. doi: 10.1016/j.eti.2019.100526
10. Zhang Z, Chen Y, Chen D, et al. Research status of bioremediation of oil contaminated soil. Soil bulletin. 2019; 50(1): 246-252.

11. Pan Y, Tang Z, Peng X, et al. Research status and progress of microbial remediation technology for PHS contaminated soil. Progress in chemical industry. 2021; 40(8): 4562-4572.
12. Lu F, Li R, Zhang L, et al. Study on immobilization and degradation characteristics of a PHS degrading bacterium. Chemical and biological engineering. 2015; 32(11): 44-47.
13. Zhang W, Dong C, Ji Y. Research progress of immobilized materials for petroleum degrading bacteria. Material guide. 2017; 31(S2): 214-218.
14. Si Y, Peng J. Immobilized microorganism technology and its application in remediation of contaminated soil. Soil. 2007; 39(5): 673-676.
15. Zhang X, Bai X, Xu N, et al. Study on Influencing Factors of oil contaminated soil remediation by immobilized microorganisms. Journal of environmental engineering. 2013; 7(3): 1156-1162.
16. Cubitto MA, Gentili AR. Bioremediation of Crude Oil–Contaminated Soil By Immobilized Bacteria on an Agroindustrial Waste—Sunflower Seed Husks. Bioremediation Journal. 2015; 19(4): 277-286. doi: 10.1080/10889868.2014.995376
17. Lin M, Liu Y, Chen W, et al. Use of bacteria-immobilized cotton fibers to absorb and degrade crude oil. International Biodeterioration & Biodegradation. 2014; 88: 8-12. doi: 10.1016/j.ibiod.2013.11.015
18. Shen T, Pi Y, Bao M, et al. Biodegradation of different petroleum hydrocarbons by free and immobilized microbial consortia. Environmental Science: Processes & Impacts. 2015; 17(12): 2022-2033. doi: 10.1039/c5em00318k
19. Nhi-Cong LT, Lien DT, Mai CTN, et al. Advanced materials for immobilization of purple phototrophic bacteria in bioremediation of oil-polluted wastewater. Chemosphere. 2021; 278: 130464. doi: 10.1016/j.chemosphere.2021.130464
20. Chibata I, Tosa T. Immobilized microbial cells and their applications. Trends in 1980. 5(4): 88-90.
21. Hu J, Su X, Han H, Et al. Application prospect of immobilized microorganism technology in remediation of PCB contaminated soil. Journal of Applied Ecology, 2014; 25(6): 1806-1814.
22. Yang Z, Xu W, Wu Z, et al. Research progress of microbial immobilization and its application in environmental pollution control. Microbiology bulletin. 2020; 47(12): 4278-4292.
23. Daronch NA, Kelbert M, Pereira CS, et al. Elucidating the choice for a precise matrix for laccase immobilization: A review. Chemical Engineering Journal. 2020; 397: 125506. doi: 10.1016/j.cej.2020.125506
24. Zhang X, Wu H, Han Y, et al. Remediation of oil contaminated soil by immobilized microorganisms on modified straw carriers. Journal of Petroleum (petroleum processing). 2014; 30(5): 915-920.
25. Wang ZY, Xu Y, Wang HY, et al. Biodegradation of crude oil in contaminated soils by free and immobilized microorganisms. Pedosphere. 2012; 22(5): 717-725.
26. Song L, Niu X, Zhang N, et al. Effect of biochar-immobilized Sphingomonas sp. PJ2 on bioremediation of PAHs and bacterial community composition in saline soil. Chemosphere. 2021; 279: 130427. doi: 10.1016/j.chemosphere.2021.130427
27. Li H, Chen L, Xiao C, et al. Research progress of microbial immobilized carrier materials. Modern chemical industry. 2020; 40(8): 58-61.
28. Dike CC, Shahsavari E, Surapaneni A, et al. Can biochar be an effective and reliable biostimulating agent for the remediation of hydrocarbon-contaminated soils? Environment International. 2021; 154: 106553. doi: 10.1016/j.envint.2021.106553
29. Galitskaya P, Akhmetzyanova L, Selivanovskaya S. Biochar-carrying hydrocarbon decomposers promote degradation during the early stage of bioremediation. Biogeosciences. 2016; 13(20): 5739-5752. doi: 10.5194/bg-13-5739-2016
30. Qian L, Yuan M, Chen B. Research Progress on remediation of pahs contaminated soil by immobilized microorganism technology. Environmental Science. 2012; 33(5): 1767-1776.
31. Dzionek A, Wojcieszyńska D, Guzik U. Natural carriers in bioremediation: A review. Electronic Journal of Biotechnology. 2016; 23: 28-36. doi: 10.1016/j.ejbt.2016.07.003
32. Peng C, Liu T, Gao Y, et al. The latest research progress of microbial immobilized carrier materials. Modern chemical industry. 2021; 41(6): 55-59+64.
33. Valdivia-Rivera S, Ayora-Talavera T, Lizardi-Jiménez MA, et al. Encapsulation of microorganisms for bioremediation: Techniques and carriers. Reviews in Environmental Science and Bio/Technology. 2021; 20(3): 815-838. doi: 10.1007/s11157-021-09577-x
34. Yang Y, Li Y, Bai Y, et al. Research progress of carrier materials for enzyme immobilization technology. Chemical bulletin. 2007; (4): 257-263.

35. Zdarta J, Meyer AS, Jesionowski T, et al. Multi-faceted strategy based on enzyme immobilization with reactant adsorption and membrane technology for biocatalytic removal of pollutants: A critical review. Biotechnology Advances. 2019; 37(7): 107401. doi: 10.1016/j.biotechadv.2019.05.007
36. Li L, Wang Y, Luo, et al. Bioremediation of PHS cadmium contaminated soil by biochar loaded microorganisms. Journal of environmental engineering. 2021; 15(2): 677-687.
37. Ren J, Shen J, Zhang L, et al. Preparation and stability of biochar immobilized polycyclic aromatic hydrocarbons degrading bacteria. Journal of environmental science. 2020; 40(12): 4517-4523.
38. Ren H, Ma L, Wang B, et al. Efficient degradation of petroleum pollutants by biochar based immobilized bacteria. Journal of environmental engineering. 2017; 11(11): 397-403.
39. Lu J, Liu L, Wang X, et al. Research Progress on degradation of pesticides in soil by immobilized microorganism technology. Anhui Agricultural Science. 2013; 41(20): 8540-8542.
40. Wang X. Research progress of immobilized technology in improving the degradation of PHS in soil by microorganisms. Anhui Agricultural Science. 2018; (30): 13-17.
41. Mehrotra T, Dev S, Banerjee A, et al. Use of immobilized bacteria for environmental bioremediation: A review. Journal of Environmental Chemical Engineering. 2021; 9(5): 105920. doi: 10.1016/j.jece.2021.105920
42. Bayat Z, Hassanshahian M, Cappello S. Immobilization of microbes for bioremediation of crude oil polluted environments: A mini review. Open Microbiol J. 2015; 9: 48-54.
43. Liu Zhi, Zhao Q, Wang A, et al. Immobilization of marine PHS degrading bacteria and its petroleum degradation performance. Environmental pollution and prevention. 2021; 43(6): 691-696.
44. Li Y, Li C, Zhang X, et al. Effect of immobilized Bacillus M1 from tomato straw on remediation of aged soil contaminated by three ring pahs. Agricultural environment and development. 2019; 36(6): 806-813.
45. Alvarado-Ramírez L, Rostro-Alanis M, Rodríguez-Rodríguez J, et al. Exploring current tendencies in techniques and materials for immobilization of laccases – A review. International Journal of Biological Macromolecules. 2021; 181: 683-696. doi: 10.1016/j.ijbiomac.2021.03.175
46. Bouabidi ZB, El-Naas MH, Zhang Z. Immobilization of microbial cells for the biotreatment of wastewater: A review. Environmental Chemistry Letters. 2018; 17(1): 241-257. doi: 10.1007/s10311-018-0795-7
47. Huang W, Zhang W, Gan Y, et al. Laccase immobilization with metal-organic frameworks: Current status, remaining challenges and future perspectives. Critical Reviews in Environmental Science and Technology. 2020; 52(8): 1282-1324. doi: 10.1080/10643389.2020.1854565
48. Wang X, Chen H, Zheng J, et al. Research progress of immobilized technology in improving the degradation of PHS in soil by microorganisms. Anhui Agricultural Science. 2018; 46(30): 13-17.
49. Zhang X, Geng C, Fang M, et al. Application of immobilized microorganisms in bioremediation of oil contaminated soil. Journal of petroleum: petroleum processing. 2008; (4): 48-53.
50. Wang X, Zheng J, Han Z, et al. Bioremediation of crude oil-contaminated soil by hydrocarbon-degrading microorganisms immobilized on humic acid-modified biofuel ash. Journal of Chemical Technology & Biotechnology. 2019; 94(6): 1904-1912. doi: 10.1002/jctb.5969
51. ZHENG J, Wang X, Li J, et al. Remediation of PHS contaminated soil by immobilized microorganisms from biomass power plant ash modified with humic acid. Environmental engineering. 2020; 38(8): 34-40.
52. Tian X, Wang X, Peng S, et al. Study on remediation of oil pollution by immobilized microorganisms on acetic acid modified ramie fiber. Applied chemical industry. 2019; 48(9): 2045-2049.
53. Hasanzadeh R, Abbasi Souraki B, Pendashteh A, et al. Application of isolated halophilic microorganisms suspended and immobilized on walnut shell as biocarrier for treatment of oilfield produced water. Journal of Hazardous Materials. 2020; 400: 123197. doi: 10.1016/j.jhazmat.2020.123197
54. Wang X, Li P, Song S, et al. Degradation of polycyclic aromatic hydrocarbons in soil by immobilized microorganisms. Journal of Northeastern University. 2006; (10): 1154-1156.
55. Wang X, Gong Z, Li P, et al. Degradation of Pyrene and Benzo(a)pyrene in Contaminated Soil by Immobilized Fungi. Environmental Engineering Science. 2008; 25(5): 677-684. doi: 10.1089/ees.2007.0075
56. Guo S, Liu X, Tang J. Enhanced degradation of petroleum hydrocarbons by immobilizing multiple bacteria on wheat bran biochar and its effect on greenhouse gas emission in saline-alkali soil. Chemosphere. 2022; 286: 131663. doi: 10.1016/j.chemosphere.2021.131663

57. Hu G, Liang C, Li P, et al. Degradation of polycyclic aromatic hydrocarbons contaminated soil by immobilized microorganisms. Journal of ecology. 2008; 5: 745-750.
58. Li Y, li F. Microbial remediation of oil contaminated soil. Chemical environmental protection. 2017; 37(6): 605-610.
59. Ge X, Yang L, Xu J. Cell Immobilization: Fundamentals, Technologies, and Applications. Industrial Biotechnology. Published online November 25, 2016: 205-235. doi: 10.1002/9783527807833.ch7
60. Jin J, Sun Y. Research progress of immobilized microorganism technology in remediation of heavy metal contaminated soil. Hunan Journal of Ecological Sciences. 2021; 8(2): 90-96.
61. Varjani SJ, Upasani VN. A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants. International Biodeterioration & Biodegradation. 2017; 120: 71-83.
62. Zeng Y, Gao D. Research Progress on immobilization technology of white rot fungi and its influencing factors. Journal of Harbin Institute of technology. 2008; 40(1): 141-146.
63. Zhang W, Zhao Y, Wang J, et al. Preparation of biochar immobilized fossil oil degrading bacteria. Jiangsu agricultural science. 2015; 43(6): 341-345.
64. Zhang X, Qin L, Huang C, Et al. Selection of microbial immobilization carriers and their properties. Progress in chemical industry. 2011; 30(12): 2781-2786.
65. Wang S, Wang D, Yu Z, et al. Advances in research on petroleum biodegradability in soil. Environmental Science: Processes & Impacts. 2021; 23(1): 9-27.
66. Zhang B, Zhang L, Zhang X. Bioremediation of petroleum hydrocarbon-contaminated soil by petroleumdegrading bacteria immobilized on biochar. RSC Advances. 2019; 9(60): 35304-35311.
67. LI X, Wang Y, Luo T, et al. Remediation potential of immobilized bacterial strain with biochar as carrier in petroleum hydrocarbon and Ni co-contaminated soil. Environmental Technology. 2018; 40(18): 2345-2353.
68. Tao K, Zhang X, Chen X, et al. Response of soil bacterial community to bioaugmentation with a plant residue-immobilized bacterial consortium for crude oil removal. Chemosphere. 2019; 222: 831-838. doi: 10.1016/j.chemosphere.2019.01.133
69. Shao J, Yin H, Peng H, et al. Preliminary study on degradation of crude oil by straw immobilized fossil oil degrading bacteria. Environmental pollution and prevention. 2006; 28(8): 565-568.