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THE ROLE OF IMMOBILIZED MICROBIAL TECHNOLOGY IN REMEDIATING HEAVY METAL CONTAMINATED SOIL

Estella Glennie
Department of Civil and Environmental Engineering, University of Houston, United States.

Abstract: Immobilized microbial technology is an effective way to remediate heavy metal pollution in soil. The main content of immobilized microbial technology is reviewed, including the repair mechanism, methods of immobilized microorganisms, requirements and types of immobilized carriers, and from three aspects: improver-assisted microbial remediation, new material composite immobilized microorganisms, and co-immobilized microbial remediation. The application and potential of this technology to remediate heavy metal pollution in soil were expounded, and the research trends and existing problems of immobilized microbial technology were reviewed.
Keywords: Immobilized microorganisms; Soil; Soil pollution; Heavy metals; Environmental remediation

1. REPAIR MECHANISM OF IMMOBILIZED MICROORGANISM TECHNOLOGY

The rapid development of industrialization and science and technology has caused soil to be contaminated by a large number of heavy metals and metalloids. Heavy metals in contaminated soil mainly include elements with significant biological toxicity such as mercury, cadmium, lead, chromium and metalloid arsenic, as well as zinc with certain toxicity., copper, nickel and other elements. Heavy metals are difficult to degrade in soil. They are pollutants that exist for a long time once they appear. They can easily enter the food chain and have become a major threat to humans and the environment [1]. Based on research progress at home and abroad, remediation methods for soil heavy metal pollution mainly include physical remediation, chemical remediation and biological remediation. Physical remediation refers to the use of electrical or thermal energy to fix or convert pollutants into non-toxic or less toxic substances. Although physical remediation has a short cycle, its application range is small and energy consumption is high; chemical remediation uses reducing agents or other agents to convert toxic substances into non-toxic substances. Heavy metals or metalloids with high mobility and poor stability are reduced to valence states or compounds with low toxicity and strong stability. The repair cycle is short and cost-effective. However, the introduction of other agents can easily cause secondary pollution and be unstable., if some studies show that Cr (III) may be oxidized to Cr (VI) again after a period of chemical reduction [2-3]. Immobilized microbial technology is a type of bioremediation technology. Compared with physical and chemical methods, immobilized microbial technology makes up for many problems existing in traditional remediation methods. Immobilized microbial technology targets microorganisms and focuses on the reuse of resources., has the advantages of low cost, high efficiency and stability, easy later maintenance, and greatly reduces secondary pollution, so it can become an important method to remediate heavy metal pollution in soil [4].

In areas contaminated by heavy metals, there are often microorganisms that are extremely resistant to heavy metals, and many natural or genetically modified microorganisms have the ability to degrade, transform or chelate various toxic chemicals. Immobilized microbial technology is a technology that uses physical or chemical means to immobilize microorganisms with specific functions inside or on the surface of carrier materials and effectively utilize them to treat pollutants. In 1959, Hattori[5] immobilized E. coli on a resin carrier, realizing the immobilization of E. coli for the first time. Since then, he has opened the chapter of immobilized microorganism technology. This review describes the research progress of immobilized microbial technology in the remediation of heavy metal pollution in soil.

The different oxidation states of metals or metalloids determine their mobility and toxicity, so changes in redox potential are an important factor affecting the repair effect [6]. Microorganisms cannot degrade and destroy heavy metals, but microorganisms are metal fixatives that can change the physical and chemical properties or valence state of heavy metals through biosorption, bioaccumulation and biomineralization to affect the migration, transformation or redox precipitation reaction of heavy metals, thus Reduce its toxicity[7]. The biosorption of metals by living microorganisms is divided into two steps: the binding of metals to the cell wall and the transport of metal ions through the membrane [8]. Microorganisms and their metabolites can adsorb and transform heavy metals to a certain extent. Not only living cells can degrade heavy metals, but dead strains can also be used as biosorbents for metal cations, except for mobile alkali metals (such as K+) [9]. In dead cells, cell accumulation is a passive process. Metals may be attached to surface molecules. The cell walls of dead cells may have been broken. There are more binding sites available when metals bind to surface molecules [10], which makes death possible. The reduction efficiency of heavy metals by cells may be faster than that of living cells [11]. This mechanism has nothing to do with metabolism and can occur in both living cells and dead cells; but in bioaccumulation, because of this The mechanism relies on metabolism and energy, so dead cells cannot participate in this process [12]. Although dead cells are easy to preserve and use, biosorption and the status of many heavy metal ions are very sensitive to pH.

Dead cells lack active metabolism and pH adjustment, which will reduce the adsorption effect. This also exposes the role of dead cells in Defects in contaminant repair relative to living cells.

The main repair mechanisms of heavy metals by immobilized microbial technology are:

(1) Biosorption of heavy metals by microorganisms. The electrostatic adsorption and ion exchange between the negative charges on the cell membrane and the positive charges on the free ions of heavy metals, the complexation on the outer surface of the cells, and the formation of an environment around the cells that is conducive to microprecipitation, resulting in chemical interactions between heavy metals and the surface of microbial cells. As a result, metal cations and microorganisms form insoluble aggregates;

(2) Reduction of heavy metals by microorganisms. Reductase produced by bacteria reduces heavy metals;

(3) The adsorption, reduction and ion exchange effects of carrier materials on heavy metal ions [13-15]. In general, heavy metals are repaired by a combination of immobilized carriers and microorganisms.

2 METHODS OF IMMOBILIZING MICROORGANISMS

According to the binding method between microorganisms and carriers, the current main immobilization methods include adsorption method, encapsulation method, cross-linking method, surface binding method, flocculation method, and composite immobilization method [16-18].

2.1 Adsorption Method

The adsorption method mainly relies on the physical properties of the carrier material or the force between the microorganism and the carrier (such as hydrogen bonding, van der Waals forces, etc.). This method is fast, simple, environmentally friendly and economical, and is a commonly used method in the bioremediation process. However, the adsorption on the carrier surface is achieved through the formation of weak bonds, which results in weak stability and makes it easier for cells to leak from the carrier into the environment.

2.2 Surface Bonding Method

Surface binding methods are divided into surface electrostatic adsorption methods and covalent binding methods. Surface electrostatic adsorption is very similar to physical adsorption. The carrier surface needs to be cleaned with a buffer solution to obtain a hydrophilic surface to attract negatively charged cells or enzymes. This method has a higher probability of microbial leakage. The advantage of covalent bonding is that they are strong enough to reduce the probability of microorganisms leaking into the environment [19], but the binding agents used in this method are usually toxic to cells, and the viability and activity of microorganisms are often affected by the binding agents. reduce.

2.3 Flocculation Method

The flocculation method uses the characteristics of certain microorganisms to self-flocculate to form particles, so that the microorganisms can self-fix and become a carrier-free immobilization technology. Artificial flocculation, or cross-linking method, uses a cross-linking agent to cross-link microbial cells with a non-water-soluble cross-linking agent with two or more multifunctional groups, which can be used to enhance the aggregation of naturally non-flocculated cells. The flocculation method has good stability, but the reaction is violent, which will reduce cell activity.

2.4 Embedding Method

The embedding method is a commonly used method for immobilizing microorganisms. The encapsulation method is an immobilization method in which microorganisms are coated in a polymer carrier. After embedding, microbial cells can only move within the carrier, which prevents cells from leaking into the environment, but also limits the exchange of nutrients and metabolites, making it unsuitable for the removal of macromolecule-contaminated substrates. The microorganisms in the encapsulated carrier are physiologically diverse. Compared with the starved cells inside the carrier, the cells located near the surface have high metabolic activity. The most important parameters in the bioembedding process are the carrier pore size and cell diameter. When the ratio is large, they may leak into the environment [20].

2.5 Encapsulation Method

Encapsulation is very similar to encapsulation. In this method, the immobilized particles are separated from the external environment through a semipermeable membrane. The biggest advantage of this method is its better protection and interception of microorganisms. However, the membrane used by this method has limited permeability and may be damaged by internal microorganisms. This method is rarely used in in situ bioremediation.

2.6 Composite Immobilization Method

The composite immobilization method is a combination of different immobilization methods. This method can not only make microorganisms have higher activity, but also improve the performance of treating pollutants. For example, some studies have shown that while embedding microorganisms, using cross-linking agents to cross-link them not only connects microbial cells to each other, but also improves the stability of the system and its ability to handle pollutants [twenty one]. This combined approach can often overcome the limitations caused by a single approach, allowing each approach to synergize and complement each other to bring greater economic and environmental benefits.

3 CARRIERS FOR IMMOBILIZED MICROORGANISMS

3. 1 Carrier Requirements

The carrier is the basis for the implementation of immobilized microbial technology. It provides a place for microorganisms to grow, attach and react. When selecting exogenous microorganisms, it is necessary to consider the competition between them and native microorganisms for nutritional elements [22]. Some carrier materials can also provide nutrients for microorganisms, and some carrier materials themselves also have certain adsorption and repair capabilities for heavy metals. The structure of the carrier material Performance is an important factor affecting the implementation effect of immobilized microbial technology, and it is particularly critical to select a suitable high-performance carrier.

In order to meet the needs of microorganisms and technology, carrier materials should have the following characteristics:

(1) Have certain physical, chemical and mechanical stability;

(2) It has certain biocompatibility, is non-toxic to microorganisms, is not easily decomposed by microorganisms, and has a long shelf life;

(3) Low price, easy to obtain or produce in large quantities, easy to handle and regenerate;

(4) It has excellent mass transfer performance, has a suitable diffusion distance from the flow medium to the center of the carrier, and is easy to diffuse microorganisms and metabolites;

(5) It has a large specific surface area and high cell mass loading capacity, resulting in a high density of immobilized cells.

3.2 Types of Carriers

Commonly used immobilized microbial carriers can be divided into organic and inorganic carriers according to their properties, and can be divided into natural or synthetic carriers according to their synthesis methods.

3.2.1 Natural organic carrier

Natural organic carriers have many functional groups that stabilize biocatalysts. Common ones include sodium alginate, corn cobs, diatomaceous earth, etc. Most of them are wastes from the food industry. Generally, these carriers are hydrophilic, biodegradable, and have good biological properties. Features such as compatibility and cheapness. However, this type of carrier has low resistance to biodegradation, is sensitive to organic solvents, and is generally only stable within a narrow pH range, resulting in limited application in the bioremediation process [16,23].

3.2.2 Synthesis of organic carriers

Synthetic organic carriers generally have many functional groups and have diverse characteristics. This type of carrier includes polypropylene, polyvinyl alcohol, etc. During the synthesis process, the porosity, pore size, polarity and hydrophobicity of the carrier can be controlled. In addition, the synthetic carriers can be formed into various shapes (such as spheres, ellipses, etc.), and they are easy to obtain and relatively cheap [16,24].

3.2.3 Inorganic carrier

Inorganic carriers are divided into natural inorganic carriers and synthetic carriers. These carriers have high chemical, physical and biological resistance, including volcanic rocks, porous glass, ceramics, nanoparticles, etc. An important disadvantage of this type of carrier is that it has fewer functional groups and weak binding ability to microorganisms. And cannot provide nutrients for microorganisms [16,25].

Since different microorganisms and degrading compounds have different requirements for the carrier used, the choice of carrier is a critical step that affects the success of the bioremediation process. Based on the different characteristics of the materials, different types of carrier materials can be combined into composite carriers. If studies have shown that reed biochar is combined with sodium alginate (SA) and polyvinyl alcohol (PVA) into a composite carrier, the biochar contains a large number of pore structures and more oxygen-containing functional groups, amine groups, sulfonic acid groups, and carboxyl groups. and amide groups, which makes this type of carrier not only have good adsorption performance and strong acid-base buffering capacity, thereby reducing the particle damage rate, but also conducive to the adhesion and proliferation of microorganisms [26]. The research on new composite carriers is an important direction for future research on immobilized microorganism technology.

4 APPLICATION OF IMMOBILIZED MICROBIAL TECHNOLOGY IN REMEDIATION OF HEAVY METAL CONTAMINATED SOIL

Immobilized microbial technology is relatively mature in the field of water treatment engineering, but its research in the remediation of contaminated soil has just started. Research on immobilized microbial technology in the remediation of heavy metal-contaminated soil is mostly laboratory research. This article mainly reviews the application of immobilized microbial technology in the remediation of heavy metal pollution in soil in the following three aspects: amendment-assisted immobilized microbial remediation, immobilization of new material composite immobilized microorganisms, and co-immobilization remediation of multiple strains.

4. 1 Modifier-Assisted Repair

Microorganisms are relatively sensitive to environmental stresses such as heavy metals. When using immobilized microbial technology, it is usually necessary to add amendments to assist in the treatment of pollutants. Some strains can only tolerate heavy metal stress at low concentrations [27], which requires the addition of some improvers to assist the strains. Biochar, humus or composting methods as mild amendments and repair methods are making up for this shortcoming [28-30]. Humic acid can catalyze certain reduction processes and adsorb to microorganisms and soil minerals. Moreover, humic acid can promote microbial extracellular electron transfer and play a key role in reducing Cr(VI) [31]. Hou [32] studied the chromium fixation effect of humic acid combined with the new chromium-resistant bacteria QY-1. The study confirmed that humic acid has no obvious negative impact on the soil itself. Taking into account various soil biochemical indicators, the new bacteria and humic acid synergistically The degree of improvement in soil biochemical properties is high, and the passivation rate of heavy metals reaches 82.83%. The larger surface and nutrients of humic acid can provide better protection for the survival of microorganisms.

Many studies have proven that biochar not only has the ability to adsorb heavy metals, but also can increase the input of carbon sources, thereby reducing the mobility, bioavailability and resulting toxicity of heavy metals in soil [28,33-35]. Heavy metals can inhibit the growth and enzyme activity of soil microorganisms and thus affect the ability to fix heavy metals. However, biochar can restore the microbial activity in heavy metal-contaminated soil and improve the carbon fixation efficiency of microorganisms, thereby reducing the biological toxicity to soil microorganisms [36,37]. Liu[38] et al. used chemical activation and microwave-assisted activation to modify biochar. After treatment with activated biochar, the Cd concentration in the upper water and pore water decreased by 71% and 49% respectively. The stress of heavy metals and the concentration of cadmium Reduced bioavailability. These studies have shown that activated biochar is a cost-effective in-situ repair material for immobilizing heavy metals. Based on these advantages, biochar provides an effective carrier for immobilized microbial technology.

4.2 New Material Composite Immobilized Microorganisms

Single physical or chemical repair has many limitations. The characteristics of modern new materials and composite repair can often achieve better repair results. Nanomaterials have become a promising carrier material due to their advantages such as surface effect and small size effect. Nanomaterials also have good immobilization effects on heavy metals [39]. Tan [25] used carboxymethyl cellulose as a stabilizer to prepare humic acid-coated nano-FeS, synthesized a new nanocomposite carrier material, and then immobilized anti-chromium microbial flora to treat chromium in the soil. This treatment group It has high enzyme activity, indicating that humic acid can promote the metabolism of soil microorganisms and improve soil quality. The experimental group of new composite materials combined with bacterial flora has the highest Cr (VI) immobilization efficiency of 99.16%, and this new type of Composite materials can gradually reduce the pH value of the soil and improve the soil's self-purification and repair capabilities. Peng[40] et al. studied the impact of composite iron-based nanoparticles on the availability of lead and iron and microecology in lead-contaminated soil. He found that biochar can improve the crystal form and surface area of iron-based nanoparticles. His research showed that Nano zero-valent iron and biological composite groups have good effects in the remediation of alkaline soil contaminated by high concentrations of lead. Nanomaterials cannot provide sufficient nutrients for microorganisms and are harmful to some soils.

Soil microbial activity has a slight inhibitory effect, but the preparation of composite immobilized carriers reduces the toxicity of nanomaterials, can also prevent nanomaterials from agglomerating, improves the stability of nanomaterials, and has good repair potential.

4.3 Co-Immobilized Microbial Remediation

Mixed strains are able to perform more complex repair tasks and survive in more variable environments than single strains. Kang[41] et al. evaluated the urealytic activity and carbonate precipitation ability of mixed bacteria on lead, cadmium and copper. Compared with a single type of bacteria, the bacterial mixture has a higher growth rate and urease activity. The mixed bacteria are more resistant to heavy metals. The bacterial mixture shows a higher heavy metal bioremediation capacity than a single culture, which may be due to Resulting from synergistic effects between different strains of mixed cultures. The higher remediation efficiency of mixed cultures reflects the ability of microorganisms to better adapt to adverse environmental conditions caused by metal toxicity, suggesting that mixed cultures are a promising approach for remediation because bioaugmentation does not target a single species but rather It can provide more diversity and improve the removal time and efficiency of microbial mixtures. In actual pollution, the contaminated site is often contaminated by more than one heavy metal or pollutant. The immobilization performance of the mixed bacteria finds a way to solve this problem. Possible solutions.

5 CONCLUSION

Immobilized microbial technology is an application that integrates modern biotechnology and the environment in pollution control. It has the characteristics of small secondary pollution, low cost, high treatment efficiency, high microbial activity, strong stability, and strong impact load resistance. It is a kind of It is a relatively suitable and promising method for remediating heavy metal pollution in soil, but there are still many problems that need to be solved:
(1) It is still in the laboratory research stage, and there are almost no reports of field applications. At this stage, most researchers' experiments on heavy metal treatment are in static adsorption conditions. The study of adsorption capacity in dynamic reactors may be future research. Focus, in addition, the experimental scale-up issues faced when moving from the laboratory to actual projects also need to be considered.
(2) Immobilized microorganism technology requires special cultivation and domestication in advance to obtain high-efficiency strains. The strains obtained by this method are very specific. These strains are usually only suitable for small -scale laboratory cultivation, but they may be used in practical applications. A large number of bacterial strains will be required, and the general pollutants are complex. Most of the current studies only focus on a single heavy metal, and there are fewer studies on simultaneous contamination by multiple heavy metals. A single targeted strain cannot treat multiple pollutants at the same time., finding multifunctional strains may be the focus of future research.
(3) Carrier materials and immobilization methods are also key to the efficient implementation of immobilization technology. Developing and improving new composite carriers that can accommodate a variety of bacterial strains based on the actual project environment so that they can jointly treat different pollutants is a research focus that must be faced in the future.
(4) Compared with traditional physical and chemical remediation methods, the immobilized microbial method has a longer cycle. Finding efficient immobilization methods and strains to shorten the remediation cycle as much as possible is also an issue that needs to be considered.
(5) The combined application of traditional restoration technology and immobilized microbial technology may produce better results for practical problems, and this may also be a major trend in future research.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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