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A Review on the Mechanism and Influencing Factors of Heavy Metal Removal by Biosorption Method

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Abstract

Heavy metal pollutants have become a key research topic in the field of environmental science and engineering due to their strong ecotoxicity, significant bioconcentration and difficult degradation. Compared with traditional physical treatment technology, which has the defects of high cost, limited applicability and easily causes secondary pollution, the biosorption technology based on microbial metabolism has emerged as an essential research direction in the field of heavy metal wastewater treatment due to its advantages of strong economy, remarkable removal efficiency and ecological friendliness. In this paper, based on the integration of the previous research, we systematically summarized the effects of typical microorganisms (bacteria, fungi and algae) on Pb^{2+}, As^{3+}/As^{5+}, Cd^{2+}, Cr^{3+}/Cr^{+}, and Cd^{2+}/Cr^{+}. $/Cr^{6+}$, Cu^{2+}, Zn^{2+}, and other heavy metal ions, focusing on their three-dimensional adsorption modes (complexation of extracellular matrix, binding of functional groups on the surface of the cell wall, and active intracellular transport) and their corresponding differences in removal efficacy. By analyzing the mechanism of microbial-heavy metal interactions (including but not limited to extracellular polymer precipitation, ion substitution reaction, ligand complexation, van der Waals force adsorption, redox transformation, inorganic microcrystalline deposition and intracellular bioaccumulation, etc.), we elucidated the influence of key factors, such as strain characteristics, initial concentration of heavy metals, contact time, coexisting ions and environmental parameters (pH, temperature, etc.), on the adsorption efficacy. The study further reveals the potential of this technology to be used in the adsorption of heavy metals. The study further revealed the technical bottlenecks faced by the technology in practical application: limited specific surface area of microorganisms, long screening period of advantageous strains, environmental sensitivity, difficult solid-liquid separation and insufficient selective adsorption. To address these challenges, innovative strategies such as surface modification (acid-base/oxidation treatment), genetic engineering regulation and immobilization technology (embedding method/carrier immobilization) are proposed to enhance microbial adsorption performance. This review systematically constructs the theoretical framework and technological pathway of microbial adsorption of heavy metals, which provides theoretical support and methodological reference for promoting the practical application of this technology in pollution control engineering.

Keywords

Heavy Metals, Water Treatment, Biosorption, Physicochemical Modification, Biomodification

1. Introduction

Heavy metal pollutants pose a serious threat to ecosystems and human health because of their strong toxicity, difficult degradation and significant bioconcentration effects, which can be amplified step by step through the food chain (Boyd, 2010; Timothy & Williams, 2019). Industrial activities such as electroplating, tanning and other production processes continuously discharge heavy metal-containing wastewater, and monitoring data show that about 82% of China's surface water bodies are polluted by heavy metals to varying degrees, with mining, metallurgy and other areas of water pollution being particularly prominent (Zhang & Wang, 2020). Typical pollutants include Pb, As, Cd and other metal elements, and their environmental trends are characterized by multi-media migration (Chen, Zhang, Zhang, Zhu, & Zhuo, 2022). For heavy metal wastewater treatment, the traditional physicochemical technologies (including electrochemical deposition, membrane separation, chemical precipitation, etc.) can achieve a certain degree of treatment effect, but they generally face technical bottlenecks such as high operational energy consumption, significant risk of secondary pollution, and difficulties in selective recycling (Briffa, Sinagra, & Blundell, 2020).

Microbial adsorption, that is, through the chemical composition or structural properties of the microorganisms themselves or products adsorption to remove heavy metals in the medium, and then through the solid-liquid phase separation, thus reducing the metal ion concentration in water (Goyal, Jain, & Banerjee, 2003; Tsuruta, 2004). Microbial adsorption has low cost, good effect, short time, reusable, strong specificity and low risk of secondary pollution, so it is feasible and economical. At present, there are many reports on microbial adsorption of heavy metals in water at home and abroad, and this technology is superior in cost, operation, operation and management, and has good development value and application prospects (Klein & Ziehr, 1990).

Microbial adsorption realizes heavy metal immobilization through mechanisms such as binding of functional groups on the cell wall surface, complexation of extracellular polymers and transmembrane transport, and is characterized by high adsorption capacity, fast reaction kinetics and selective adsorption (Yin, Wang, Lv, & Chen, 2019). Studies have shown that bacteria (e.g., *Bacillus subtilis*), fungi (e.g., *Aspergillus niger*), and algae (e.g., *Chlorella vulgaris*) exhibit different adsorption characteristics for heavy metals such as Pb^{2+}, Cr^{6+}, etc., and their efficacies are regulated by multiple factors such as metabolic state of the species, solution pH, temperature, and coexisting ions (Lin, Zhou, Li, & Dong, 2023; Yin et al., 2015).

However, microbial adsorption still has certain limitations in practical application, such as susceptible to the influence of the external environment, and the microorganisms are not easy to be separated and recovered after the adsorption of heavy metals, etc. Therefore, this paper summarizes the efficiency and mechanism of heavy metal adsorption by different microorganisms (bacteria, fungi and algae, etc.), the influencing factors of adsorption, describes the limiting factors affecting the application of microorganisms, as well as the techniques and methods to improve the adsorption efficiency in practical application, providing theoretical support and technical reference for improving the efficiency of microorganisms' adsorption of heavy metal ions for their removal and their practical application.

2. Microbe Species

Ruchhoft (1949) successfully removed the radioactive element plutonium from water by using activated sludge, and because microorganisms multiply to form "colloidal matrix materials with a large surface area capable of absorbing the radioactive element", it was assumed that the microorganisms had the ability to adsorb Pu, and the concept of microbial adsorption was then proposed. The concept of microbial adsorption was then proposed. Since then, scholars at home and abroad have begun the adsorption of different heavy metals by different microorganisms. Strandberg et al. (1981) found that *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa* could adsorb uranium (U^{6+}). The study of heavy metal adsorption by microorganisms developed rapidly in the late 1990s, with the development of biosorbent materials and the understanding of the biosorption mechanism. Gloab et al. (1991) found the adsorption of Pb^{2+} by *Streptomyces*; Volesky and Prasetyo (1994) found that Cd^{2+} could be adsorbed by seaweed; and Volesky and May-Phillips (1995) found that the adsorption of Cd^{2+} by Brewer's bacillus could be adsorbed by *Saccharomyces cerevisiae*. In 2008, Teclu et al. (2008) found that *sulfate-reducing bacteria* adsorbed both As^{3+} and As^{5+} in water. Microbial adsorption for heavy metal removal has become an emerging technology that is environmentally friendly and does not introduce secondary pollution, and many microorganisms have been found that can adsorb heavy metals, mainly consisting of bacteria, fungi, algae and actinomycetes (**Table 1**).

Table 1. Microorganisms commonly used for biosorption of heavy metals.

Category	Microbe species
Bacteria	*Bacillus subtilis*, *Pseudomonas*, *Micrococcaceae*, *Zoogloea*
Fungi	*Penicillium*, *Aspergillus*, *Rhizopus Saccharomyces cerevisiae*, *Saccharomyces cerevisiae*
Algae	*Phaeophyceae*, *Aspergillus*, *Rhizopus*, *Saccharomyces cerevisiae*
Actinomyces	*Streptomyces*

3. Mechanism and Efficiency of Heavy Metal Adsorption by Microorganisms

Adsorption of heavy metals by microorganisms is a complex process. The different affinities of the same species of microorganisms for different metal ions and the different tolerances of different species of microorganisms for the same metal ions lead to the diversity of the mechanism and efficiency of microbial adsorption of metals. According to the differences in the distribution of adsorbed ions in microbial cells, they are categorized into three types: intracellular adsorption, extracellular adsorption and surface adsorption (**Figure 1**) (Pavel, Martina, & Tomas, 2011). Surface adsorption is found in active and inactive microorganisms, while extracellular and intracellular adsorption is mainly found in active microorganisms. In an adsorption system, one or more mechanisms may exist simultaneously. Extracellular adsorption refers to the removal of heavy metal ions by adsorption, precipitation or complexation using extracellular polymeric substances (EPS) secreted by microorganisms, such as glycoproteins, lipopolysaccharides, polysaccharides, and soluble amino acids (Carpio, Machado-Santelli, Sakata, Ferreira Filho, & Rodrigues, 2014). Surface adsorption refers to the process of adsorption of metal ions on the cell surface, especially on the cell wall components (proteins, polysaccharides and lipids, etc.) when chemical functional groups (carboxyl, hydroxyl, phosphoryl, amide, sulfate, amino, and sulfhydryl groups, etc.) interact with the metal ions. Due to the existence of conformational, spatial and other barriers, functional groups do not guarantee the successful adsorption of all heavy metals.

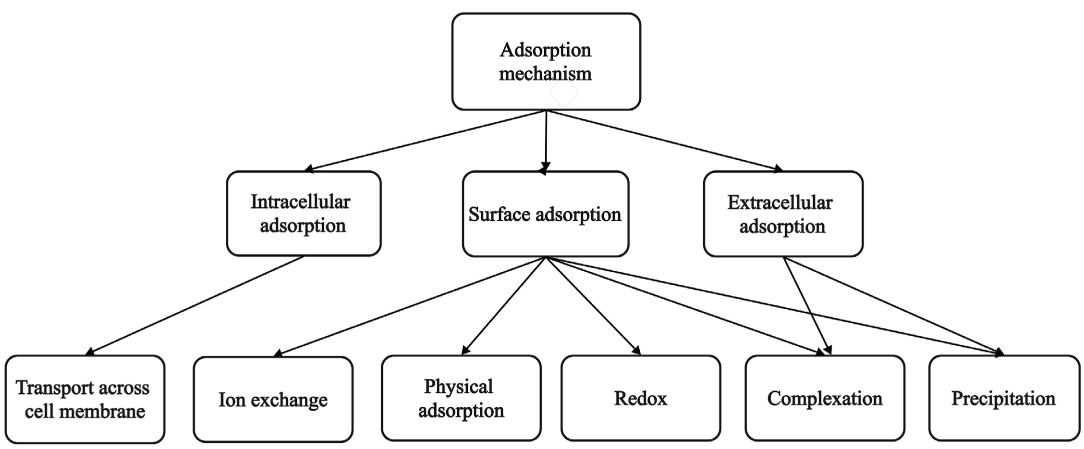

Figure 1. Mechanisms of heavy metals by microbial sorption.

At present, the main mechanisms of surface adsorption of metal ions include ion exchange, surface complexation, physical adsorption (van der Waals force, electrostatic effect), redox or inorganic microprecipitation. Ion exchange refers to the existence of microorganisms on the surface of hydroxyl, carboxyl and amino groups that can be combined with heavy metal ions, and the exchange process of heavy metal ions and functional groups is the ion exchange process. Surface complexation means that heavy metal ions can form complexes with the lone pair of electrons provided by nitrogen, sulfur and oxygen in the functional groups of microbial cell wall components. Electrostatic adsorption is due to the presence of van der Waals forces between the negative charges on the outer surface of microbial cells and the positively charged heavy metal ions. Redox means that some microorganisms with redox ability can change the valence state of heavy metal ions into low toxicity substances by changing the valence state of heavy metal ions. Inorganic microprecipitation refers to the process of forming inorganic precipitates of heavy metal ions on or inside the cell walls of microorganisms through chemical and physical effects. In addition, the differences in the composition of the cell walls of different microorganisms lead to differences in their adsorption mechanisms. For example, Lei et al. (2014) suggested that the ion exchange of chemical groups in the cell wall is the main mechanism for the adsorption and removal of heavy metals (Cu^{2+}, Pb^{2+}, etc.) from water by Bacillus sp. Intracellular adsorption means that when the concentration of extracellular metal ions is higher than that of intracellular ions, the metal ions can pass through the cell wall and the cell membrane to enter the intracellular through free diffusion. After entering the cell, microorganisms distribute the metal ions to metabolically inactive regions (e.g., vesicles) through compartmentalization, or bind the metal ions to heat-stable proteins to transform them into less active and less toxic forms.

The adsorption of heavy metals by microorganisms is related to the nature of the microorganisms themselves and the nature of the heavy metal species. Different species of microorganisms have different cell structures, and their adsorption mechanisms and adsorption capacities for the same heavy metals are different. For example, in the adsorption of Pb^{2+}, the adsorption rate of Bacillus licheniformis was as high as 96.5% by chemical adsorption; when As was adsorbed by Aspergillus niger, its adsorption and removal of As^{5+} could reach more than 90%, and its adsorption and removal of As^{3+} could reach 75%, and the adsorption mechanism was oxidization and co-precipitation; in the adsorption of Cd^{2+}, the adsorption rate of *S. aeruginosa* was as high as 99%, and its adsorption sites were mainly carbonyl, carboxyl and carboxylic groups. In the adsorption of Cd^{2+}, the adsorption rate of *Saccharomyces cerevisiae* was as high as 99%, and the adsorption sites were mainly carbonyl, carboxyl and amino groups; the adsorption rate of *Penicillium microbialis* was as high as 87% when adsorbing Cr^{6+}, and the main sites were amino, carboxyl, carbonyl and hydroxyl groups; the highest adsorption rate of *Bacillus thuringiensis* adsorption of Cu^{2+} could be up to 91.8%, and the adsorption rate was mainly on the surface of the cells; the adsorption rate of *Aspergillus tama-*

ricus for the adsorption of Zn^{2+} could be up to 99.6%, and the adsorption rate of amines, alcohols and conjugated C=C groups formed covalent bonds with the adsorbent and the matrix layer of the crosslinking network was the main reason for the removal of Zn^{2+} (Abas, Ismail, Kamal, & Izhar, 2013; Goyal et al., 2003).

3.1. Mechanism and Efficiency of Heavy Metal Adsorption by Bacteria

Bacteria are the most abundant and important microbial resources, as they are widely distributed and multiply rapidly in soil and water. Bacteria can adsorb heavy metals in many ways, such as surface adsorption, intracellular accumulation and extracellular precipitation, thus reducing the activity and toxicity of heavy metals in soil or water. The complex structure of bacterial surface contains a large number of metal ion binding sites (carboxyl, amino and phosphate groups, etc.), which provides a variety of ways for metal ion adsorption. For example, the cell wall of Gram-positive bacteria contains a large number of peptidoglycan (N-acetylglucosamine, N-acetylcytidylic acid, and amino acids) and phosphoglycolic acid, which are electronegative after the loss of a proton, and adsorb heavy metal ions by electrostatic forces (Javanbakht, Alavi, & Zilouei, 2014). Gram-negative bacteria adsorb heavy metal ions using lipopolysaccharides (lipids, core polysaccharides, and specific polysaccharides with a thickness of 8 - 10 nm) on the outside of peptidoglycan. Currently, many bacteria have been found to possess heavy (analogous) metal adsorption, among which Clostridium difficile is the most studied one, which has outstanding adsorption of Pb^{2+}, Cd^{2+} and Cr^{6+} with adsorption capacities as high as 164, 132 and 257 mg/g (Aryal, 2021; Gupta & Diwan, 2017).

3.2. Mechanism and Efficiency of Heavy Metal Adsorption by Fungi

There are about 120,000 species of fungi, including yeasts, molds and macrofungi. Fungi are widely available and suitable for expanded culture, with large adsorption capacity and easy separation of metal ions from the fungus after adsorption, which can greatly reduce the production cost of heavy metal biosorbents. The cell wall of fungi is rich in negatively charged polysaccharides and chitin, which is favorable for the adsorption of heavy metals. There are two main ways of adsorption of heavy metals by fungi, one is active metal ion adsorption for metabolic purpose, i.e., metal ions enter into the cell through the cell membrane, and the process is living cell adsorption, such as intracellular enrichment of white rakedentine fungus (*Irpex lacteus*) in adsorption of Cu^{2+}; the other is passive adsorption and binding caused by the cell and its constituent components of the chemical compensation, i.e., through complexation, ion exchange, ion exchange, and chemical compensation, i.e., through the cell and its constituent components, i.e., through the cell and its constituents of the chemical compensation, i.e., through the cell and its components of the chemical compensation. The other is passive adsorption binding caused by chemical compensation of cells and their constituents, i.e., removal of heavy metals by complexation, ion exchange, chelation, synergism, precipitation, and physical adsorption, which occurs in both living and dead cells, e.g., Cu^{2+} adsorption by surface adsorption in yeast (*Saccharomyces*); *Saccharomyces cerevisiae*, and *Beauveria bassiana* adsorbed Cd^{2+} by ion exchange (Lu et al., 2020; Shakya, Sharma, Meryem, Mahmood, & Kumar, 2016).

3.3. Mechanisms and Efficiency of Heavy Metal Adsorption by Algae

Algae is a kind of natural biomass which can be regenerated, and the known algae in the world are about 40,000 species. As a kind of photosynthetic autotrophic organisms, algae show good adsorption capacity for many kinds of heavy metals, with the advantages of strong adsorption capacity, high removal rate, easy-to-obtain raw materials and low price. The cell wall composed of cellulose, gliadin and polysaccharides has a certain charge and viscosity, which plays an important role in the adsorption of heavy metals. Various chemical groups such as hydroxyl, carboxyl, sulfhydryl, amino, carbonyl, sulfur, aldehyde, phosphate and sulfate on the surface of algae can adsorb heavy metal ions through ion exchange, complexation and electrostatic adsorption (Bayramoglu & Arica, 2011). In addition, algal cell membranes are natural semipermeable membranes with high selectivity, and these characteristics determine their good adsorption effects on heavy metal ions. For example, Mata et al. (2010) found that the cell wall of *Fucus vesiculosus* contained, alginate, etc., which could adsorb Pb^{2+}, Cu^{2+} and Cd^{2+} through surface complexation. Sheng et al. (2004) found that the high adsorption capacity of *Scagassum* and *Padina* for Pb^{2+}, Zn^{2+}, Cu^{2+} and Cd^{2+} was mainly due to the complexation of hydroxyl, ethanol and amino groups on the surface of the cell wall.

4. Microbial Adsorption Influencing Factors

4.1. Microbial Species

During the adsorption process, the adsorption capacity of different microorganisms for the same metal ion was different, for example, the adsorption capacity of *Ascophyllum nodosum* for Zn^{2+} was 7.42 times higher than that of *Saccharomyces cerevisiae*, and that of *Streptomyces rimosus* for Cu^{2+} was 1.85 times higher than that of *Streptomyces rimosus*, *Streptomyces rimosus* adsorbed 1.85 times more Cu^{2+} than *Saccharomyces cerevisiae*, and *Phanerochaete chrysosporium* adsorbed 1.85 times more Cu^{2+} than *Saccharomyces cerevisiae* and *Phanerochaete chrysosporium* adsorbed 3.45 mg/g. The adsorption of Pb^{2+} by *Streptomyces clavuligerus* was nearly three times higher than that by *Streptomyces clavuligerus*.

4.2. Microbiological Status

Microbial status is also an important influence on the adsorption process. Active microorganisms can accumulate intracellularly through the energy provided by metabolism, while inactive microorganisms do not have the ability to accumulate intracellularly, but their inactivated state can reduce the toxicity effect of heavy metals, thus promoting adsorption. In addition, when the microorganisms exist in different states (free state, carrier-immobilized state), their adsorption effects are also different. In general, the immobilization of microorganisms can increase the cell concentration of microorganisms, so that the microorganisms maintain a high biological activity, thus improving their adsorption performance. For example, Bartucca et al. (2022) immobilized inactive *Oscillatoria* using silymarin, and found that the adsorption amount of immobilized and free *Oscillatoria* reached the maximum at pH = 5, the initial concentration of Pb^{2+} was 70 mg/L and 60 mg/L, and the contact time was 90 min and 60 min, respectively. The adsorption amount of immobilized and free *Trichoderma reesei* reached the maximum at the contact time of 90 min and 60 min, and the adsorption amount of the former increased by about 20.6%.

4.3. Initial Concentration of Heavy Metal Ions

In a certain concentration range, the adsorption capacity and efficiency increased with the increase of the initial concentration of heavy metal ions, and the adsorption capacity reached the peak value when a certain concentration was reached, and then the adsorption capacity fluctuated in a fixed range with the adsorption efficiency gradually decreased when the concentration continued to increase. Basci et al. (2004) found that when *Irpex lacteus* adsorbed Cu^{2+}, the adsorption capacity of *Irpex lacteus* increased with the increase of the initial Cu^{2+} concentration (20 - 80 mg/L), and the adsorption rate increased within the range of the initial Cu^{2+} concentration 20 - 40 mg/L, and reached a maximum at 40 mg/L, after which it began to decrease.

4.4. Adsorption Time

The efficiency of microbial adsorption increased with time. The process of heavy metal adsorption by microorganisms can be divided into two stages, the fast adsorption stage and the slow adsorption stage. The former is a rapid surface adsorption process, usually reaching about 70% of the saturated adsorption capacity in a few minutes, which usually occurs on the cell surface and is mainly physical adsorption. The latter is a slow intracellular accumulation process, in which heavy metal ions are gradually transferred to the cells and reach the adsorption equilibrium after a few hours, which is related to chemisorption or microbial metabolism. In addition, the adsorption of heavy metals by active strains of bacteria is related to their growth rate and period, and studies have shown that the removal rate of active strains of bacteria increases fastest during the logarithmic growth period and reaches the maximum in the stagnation period. For example, when *Irpex lacteus* adsorbed Cu^{2+}, the adsorption capacity of the strain increased with the increase of time in the first 1 h, and then the adsorption capacity decreased slightly with the increase of time, and finally the adsorption reached saturation and stabilized (Hansda & Kumar, 2015).

4.5. Co-Existing Ions

Co-existing ions reduce the adsorption of target metal ions by competing for adsorption sites. On the contrary, some coexisting ions promote the adsorption of target metal ions. For example, the addition of K^+, Na^+, Ca^{2+} and Mg^{2+} during the adsorption of Pb^{2+} by *Gordona amarae* inhibited adsorption because of the competition for electronegative adsorption sites on the cell surface with Pb^{2+}, whereas the addition of NH_4^+ facilitated adsorption because of the disruption of the cell membrane by NH_4^+, leading to an increase in the pathway, and hence an increase in the amount of cell membrane transport.

4.6. Environmental Factors

1) pH

It is generally believed that pH is the most significant factor affecting the adsorption of microorganisms. pH has an effect on the chemical properties of metal ions, the activity of functional groups on the surface of the cell wall and the competition between metal ions. It directly affects the complexation of organic or inorganic ligands, hydrolysis of ammonia ions, redox reactions and precipitation reactions. When the environmental pH is low, the concentration of H_3O^+ in the solution increases, and the surface of microorganisms can be occupied by H_3O^+, and it is difficult for metal ions to bind to the adsorption sites on the surface of microorganisms due to electrostatic repulsion. With the increase of pH, more electronegative groups such as carboxyl group, phosphate group and amino group were exposed on the surface of the microorganisms, which could bind to the positively charged metal ions, and the adsorption efficiency gradually increased. With the increase of pH, the metal ions in the solution combined with OH^- ionized from water to form hydroxide, which led to the decrease of microbial adsorption. When the pH exceeded the upper limit of the precipitation reaction of metal ions, the heavy metals were hydrolyzed to form insoluble/insoluble precipitates, which led to the decrease of their biosorption efficiency.

2) Temperature.

Temperature can affect the physicochemical properties of the solution and the physiological metabolic activities of microorganisms, when the temperature is too high, it can slow down the bacterial metabolism and inactivation of metabolic products; when the temperature is too low, it can inhibit the activity of metabolic products, and at the same time the metabolism is slowed down, which affects its adsorption of heavy metal ions. In the appropriate temperature range (20˚C - 35˚C), the microbial metabolic activities were enhanced with the increase of temperature, and the adsorption capacity was increased. However, high temperature operation is not recommended because of the increase in adsorption cost. It has been shown that the microorganisms that can be used for the adsorption of metal ions are mainly mesophilic microorganisms. It has also been shown that temperature has no significant effect on the adsorption process when the temperature is within the range suitable for bacterial colonization.

5. Desorption of Heavy Metal Ions after Adsorption

After the adsorption of heavy metals by microorganisms, it is necessary to desorb the adsorbed heavy metals to avoid secondary pollution and at the same time to realize the recovery of precious metals. There are many methods for desorption, and washing with chemical reagents is one of the common methods. Commonly used desorbing agents are hydrochloric acid, sulfuric acid, nitric acid, acetic acid, EDTA, thiourea and carbonate and so on. The desorption efficiency of different desorbing agents for different heavy metals varies, for example, the desorption rate of nitric acid on Pb^{2+} is higher than that of Na_2EDTA on Pb^{2+}, while the desorption rate of Na_2EDTA on Ni^{2+} is only 21.1%, and the desorption rate of sodium nitrate on Hg^{2+} is only 3.65%; hydrochloric acid on Mn^{2+} is 92%, while oxalic acid is 92% on Cu^{2+}. The desorption rate of hydrochloric acid for Mn^{2+} was 92%, and that of oxalic acid for Cu^{2+} was 42.2%. Therefore, it is necessary to choose desorbing agents with fast desorption speed and high efficiency according to different adsorption systems in the process of practical application.

6. Common Problems and Prospects in Practical Application

Microbial adsorption is a new technology arising from the intersection of disciplines, which has good application prospects due to its low cost and technical difficulty and environmental friendliness (Maqsood, Hussain, Mumtaz, Bilal, & Iqbal, 2022). However, due to the limited applicability of laboratory studies, few reports have evaluated its feasibility on an industrial scale. The cost of transferring microbial adsorption from the laboratory to the field is high, and its practical application is still limited. Because heavy metals are very toxic to microorganisms in general, the main problem is the selection of microbial strains. At the same time, due to different environmental conditions (such as temperature, pH, other substances and components in water) and other factors will have an impact on the growth and reproduction of microorganisms, biological activity, and so on, which in turn affects the adsorption effect, once the environmental conditions are changed, the process of microbial adsorption is very easy to be limited. Therefore, screening and finding microorganisms that are resistant to heavy metals and have a high capacity for heavy metal enrichment is a top priority for the application of microbial adsorption to industrial water treatment. In recent years, some scholars have adopted genetic engineering means to change the genetic characteristics of microorganisms, and expressed complex proteins (peptides) with strong adsorption of certain heavy metals on the surface of microbial cells or in vivo, so as to improve the adsorption effect of microorganisms on heavy metal ions (Diep, Mahadevan, & Yakunin, 2018; Ranjbar & Malcata, 2022).

Zhao et al. (2005) successfully propagated an As-resistant metallurgical engineering bacterium by inserting an As-resistant gene fragment into a *Thiobacillus caldus* bacterium by in vitro recombination in DNA technology, and this bacterium was able to treat high As wastewater. In addition, nanobiosorbents can be formed by chemically combining biosorbents with nanoparticles (Boubakri et al., 2017). The combination of the two parent materials (nanoparticles and biosorbent material) results in a novel material with multiple enhanced properties. Currently, this technology has been used more for other materials and less for microorganisms. For example, Arshadi et al. (2017) reported the preparation of zero-valent iron nanoparticles modified with aquatic plant Azolla filiculoides for the removal of Pb^{2+} and Hg^{2+} from water, and Thanh et al. (2011) reported the improved performance of pearlite nanocomposites compared with iron and manganese nanomaterials for the removal of As(V). The application of nano-microbial composites is a new and effective way to remove heavy metals from water. In order to ensure the safety of microbial adsorbents, it is necessary to analyze and test the biosafety of adsorbents, which generally includes acute toxicity test, reproductive toxicity test, and teratogenicity-sensitive toxicity test.

In the analysis of the biosafety of microbial adsorbents, because microbial adsorbents are often used to treat polluted water bodies and have obvious impacts on aquatic ecosystems, the acute toxicity of fish is commonly used for evaluation (Vasylyuk, Shved, Hubrii, Vichko, & Shved, 2022). At the same time, fish are more sensitive to changes in the water environment, when there is a certain concentration of exogenous substances in the water body, it can cause a series of physiological and biochemical reactions, including behavioral abnormalities, physiological dysfunction, tissue cell lesions and even death. Therefore, under certain conditions, the safety of the test organisms can be assessed by the short-term exposure effect by exposing the fish to specific types and concentrations of microbial adsorbents, and observing the reaction phenomenon and mortality of the experimental fish within a certain period. In addition, microorganisms have small specific surface area and their surface active sites are often covered by organic matter (Mohapatra et al., 2019); planktonic microorganisms usually have small particles and poor mechanical strength, and are prone to loss of adsorbent during operation, which may cause problems such as swelling of the bacterial body during solid-liquid separation (Yu, Tong, Sun, & Li, 2007); strain selection and breeding are time-consuming; and microorganisms are not easy to be separated and recovered from the aqueous solution.

The specific selection of microbial adsorbents for heavy metals is also a problem in microbial adsorption, and it is difficult to find a microorganism that is tolerant to a variety of heavy metal ions. It is difficult to find a microorganism that is tolerant to a variety of heavy metal ions, and the adsorption capacity and selectivity are not high enough to be applied in practical water treatment projects. In addition, due to the diversity of functional groups and functional differences of microbial adsorbents, the degree of stabilization and unpredictability of adsorption effect is also a factor limiting its application. Therefore, the development and popularization of microbial adsorption were hindered.

In the follow-up research, we can focus on the following aspects: 1) using molecular biology, genetic engineering and other screening of efficient heavy metal-resistant microorganisms and the construction of genetically engineered bacteria; 2) the development of a new type of high-efficiency immobilized bioreactor to reduce the interference and influence of the environment on the microorganisms;

3) designing microbial adsorbents with high surface areas, such as microbial composites; 4) selective and efficient recovery of precious metals after adsorption.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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