Research on the treatment of heavy metal ions by immobilized microbial technology

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Abstract. With the development of human industry, the mining and utilization of heavy metals has become more and more extensive, especially in the past 200 years. However, the excessive discharge of industrial and domestic sewage, overuse of pesticides and fertilizers in the production process, as well as electronic waste and medical waste in human life have led to many heavy metal ions entering the environment, exceeding the environmental capacity and entering organisms in large quantities, threatening the ecosystem and human health. Therefore, how to economically and efficiently deal with the heavy metal ion pollution generated in the process of production and life has become a hot issue. Currently, the main methods for treating heavy metal ions are chemical precipitation, ion exchange, adsorption, membrane filtration, electrochemical treatment technology, etc. However, the traditional methods for heavy metal treatment are usually faced with the problems of high cost, high complexity and high energy consumption. In recent years, microbial immobilization technology has received wide attention for its advantages of improved microbial stability, high heavy metal ion removal rate, etc. The corresponding microorganisms are encapsulated and aggregated by immobilized carriers, which enhances the cellular bioburden capacity and environmental resistance and improves the microbial removal efficiency of heavy metals.

Keywords: Biotechnology, heavy metal ions, cells.

1. Background

Heavy metal ion pollution is a phenomenon in which heavy metal elements exist in ionic form in the environment, such as water, soil and the atmosphere, and cause harm to human health and the ecological environment; Minamata disease and bone pain among the world's eight major public health hazards, are caused by Hg(II) and Cr(VI). Heavy metal ions have become one of the main factors polluting the environment, and since they cannot be degraded through other natural pathways, they tend to enter the food chain and accumulate in living organisms through bioconcentration, and the amount of accumulation increases with the increase of trophic level, which destroys the normal metabolic activities of living organisms and the stability of the ecosystem. From the distribution of heavy metal ion pollution in China, arable land is more seriously contaminated by heavy metal ions, and the area has been close to 2×10^5 km^2 , which reduces the quality and safety of the crops, and directly causes 20 billion yuan of economic losses (Du et al. 2020) .

The sources of heavy metal ions can be divided into two categories: human activities and natural processes, of which human activities account for the main part, especially wastewater and exhaust gas generated in industrial areas, domestic garbage generated in human settlements, and agricultural sewage irrigation, which will cause heavy metal ions to combine with particles or aerogels in nature, and then diffuse to other areas and living organisms along with the migration; or react with other compounds to cause secondary pollution. Unlike organic pollutants, heavy metals are not directly biodegradable, and are easily absorbed by plants into the food chain, with accumulation positively correlated with trophic level and causing different degrees of harm to each trophic level at the same time, or directly into organisms, destroying the cells of organisms at the molecular level. For example, Fe, Mn, Cu, Zn, and Mo are beneficial to metabolism in trace amounts, but tend to do more harm than good in excessive amounts, while elements such as As, Hg, Pb, and Cd, which are a major threat to human and animal life at low concentrations, have a lesser impact on the growth and developmental status of plants (Zwolak et al. 2019). Cr(VI) is mutagenic, genotoxic and carcinogenic and can be absorbed into the respiratory and digestive tracts of humans, thereby damaging DNA and proteins. Therefore, it is classified as a class A carcinogen by the International Atomic Energy Agency

(Pushkar et al. 2021). Heavy metals are found in nature mainly in rocks and soil matrices. In rocks, heavy metal ions are usually found in the form of ores, e.g., Cd(II) is often accompanied by sphalerite (ZnS) in nature; the natural sources of Hg and Pb are mainly the parent rocks and matrices of natural soils; and As exists in nature mostly in the form of arsenate compounds in soils, which are transported to other layers of the earth through geo-biochemical cycles.

As early as the early 19th century, it was discovered that certain microbial cells have a natural tendency to adsorb to the surface of solid materials and are immobilized in this way. From the middle of the 20th century onwards, the water pollution situation became more and more serious, people need a kind of high efficiency, fast, can continuously treat the wastewater technology, so the immobilized microbial technology came into being. Immobilized microbial technology is a bioremediation technology that uses physical or chemical methods to confine free microbial cells to an insoluble, water-soluble, specific carrier, limiting their ability to migrate and maintaining their activity for continuous reaction (Giese et al. 2020) The immobilization of microorganisms is a technique that has the ability to maintain their biological activity while increasing the tolerance of immobilized cells and intracellular enzymes to the external environment. Immobilization techniques are commonly used in environmental protection, energy development, food processing, and pharmaceutical industries, and are characterized by continuous, economical, and efficient production compared to cells in the free state. Microbial immobilization technology has the characteristic of repeated recycling, overcoming the difficulty of multiple recycling in traditional microbial technology.

2. Fixed carriers

The choice of carrier material is a crucial step in the process of microbial immobilization as it controls the metabolic activity of the cells, provides operational stability, reduces or protects from aggressive and hazardous external environments, and helps to achieve more efficient biodegradation (Das and Adholeya 2015) . The best carrier matrix should be lightweight, flexible and cost-effective; it should also be mechanically and chemically stable, inert, non-biodegradable, non-contaminating, non-toxic, insoluble in water, easy to immobilize with high biomass retention (Mehrotra et al. 2021)

2.1 Agar

Agar, scientific name agar, polysaccharides extracted from seaweed, is one of the most widely used seaweed gum in the world, containing complex carbohydrates, calcium and sulfate, and is often used in the chemical industry to make culture media and ointments. It is insoluble in cold water and easily soluble in boiling water. It has the advantages of strong elasticity, high stability and good biophilicity.

Due to the low mechanical strength and small specific surface area of agar, agar is usually applied as a composite carrier together with other materials (Lu et al. 2020) . Zhang et al. (2017) M-HAP/Agar composite microbeads were prepared by emulsification method with large specific surface area, moderate pore size, and high adsorption of both Co(II) and Pb(II), 90.6 mg/g and 806.7 mg/g, respectively. Four different agar-based hybrid biosorbents were synthesized on agar skeleton using different ratios of acrylamide and NN′-methylenebisacrylamide to study the removal of Fe(III), Cr(III), Ni(III), and Mn(III), and they found that the radius of Fe(III) was smaller than Ni(III) and Mn(III), and the shells had a higher reactive activity is higher (2018). Although Cr(III) also has a smaller radius, it has a half-filled electronic configuration at the t2g energy level and low reactivity with the biosorbent functional groups.

2.2 Nanoparticles

A nanoparticle is any particle with a size in the range of 1×10^{-9} to 1×10^{-7} (Vert et al. 2012) that have the following characteristics in heavy metal ion treatment:

Compared with conventional materials, nanostructures are characterized by small size, large specific surface area and high surface curvature (Jia et al. 2021);

Nanoparticles act as carriers, serving to reduce solid-phase impediments to substance transport with lower matrix diffusion limitations (Jia et al. 2021);

During the adsorption of heavy metal ions, magnetic nanoparticles are also characterized by high chemical stability, superparamagnetism, and high colloidal stability, and play an important role in the detoxification of various toxic compounds (Giese et al. 2020).

Stability, Agglomeration, Reactivity and Hydrophobicity (Kumar A and Dixit 2017).

The unique physicochemical properties of nanoparticles enhance their ability to adsorb heavy metal ions or process the ions by cells immobilized on the particles. Bai et al. (2006) incubated synthesized ZnS nanoparticles with immobilized rhizobacterial hydrate at 30°C using R. vulnificus, sterile $ZnSO_4 \cdot 7H_2O$ and immobilized rhizobacterial hydrate. The precipitate at the end of the biotransformation reaction was rinsed several times with distilled water and then dried for 3 hr in a drying oven. The synthesized ZnS nanoparticles had three distinct diffraction peaks in the XRD pattern and showed spherical particles with an average diameter of 12 nm in TEM, with an atomic ratio of 0.98:1.00, thus completing the clean biotransformation reaction. However, due to the high cost of synthesis of the nanoparticles in question and the complexity of the internal transformation reaction, industrialization on a large scale is not possible at present.

2.3 Chitosan

Chitosan is a linear polysaccharide made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance such as sodium hydroxide, and consists of a new polymer formed by deacetylation of N-acetyl-D-glucosamine polymers, i.e., β-1,4-D-glucosamine polymers (Kou et al. 2021). Chitosan is a stable natural carrier with potentially reactive amino and hydroxyl functional groups, which not only improves the adhesion of bacteria to the overlying substrate (Mehrotra et al. 2021). It has also been widely used for the treatment of heavy metals and dyes(Feng et al. 2009).

The adsorption capacity and functional group activity of chitosan are largely dependent on the pH of the treatment environment. When the pH is lower than 5.5, chitosan is unable to form gels, especially in acidic wastewater, where chitosan is highly soluble (Chiou et al. 2004). Moreover, the properties of the polymer limit its application in large packed columns due to its hydrodynamic restriction and clogging, which makes it prone to breakage (Crini and Badot 2008). In order to overcome the above problems, many polymers based on shells have been developed. In order to overcome these problems, many composite matrices based on chitosan and formed by chemical cross-linking have been invented. For example, Boddu et al. (2008) studied the adsorption effect of chitosan-alumina composites on As(III) and As(VI), they found that when the packed bed volume reaches about 40 ml of As(III) and 110 ml of As(V), the corresponding As(III), As(V) uptake is about 90%, 60%. Popuri et al. (2009) studied the adsorption of Cu(II) and Ni(II) by chitosan-coated PVC beads: the maximum removal was observed at doses up to 0.5 g. The removal was 94% for Cu(II) and 96% for Ni(II). Kumar et al. (2009) Removal and recovery of Cd(II) from wastewater using chitosan coated PVA beads. The adsorption capacity of crosslinked chitosan-coated bentonite beads on tartaric acid was explored under different conditions, and at pH values below the pH_{ZPC} value of the beads (<8.88), positively charged substances would dominate on the adsorbent surface (Wan Ngah and Hanafiah 2008) , which can thus be used as Cu(II), Co(II), Ni(II) (Wan Ngah et al. 2011) , Cr(VI) (Turkan, 2020) adsorption materials.

Chitosan composites, which are easy to prepare and inexpensive, are economically feasible, but chitosan itself has low mechanical strength and small specific surface area, and it needs to be formed into a polymer by cross-linking, covalent binding and other mmeishi ethods. At the same time, factors such as the high or low removal rate of heavy metals, the size of the cost, and reusability determine the effect of the composite material in the process of practical application. There are more types of chitosan composite materials, with different methods of production, which need to take into account the cost factor as well as the type of heavy metal ions. In the future production and processing of the development direction may be a variety of chitosan composite materials and even a variety of crosslinking technology applications.

2.4 Sodium alginate

Sodium Alginate is a natural polysaccharide extracted from brown algae, usually in white or brownish yellow powder. The structure of sodium alginate itself is rich in hydroxyl and carboxyl groups, and has a high adsorption capacity for heavy metal ions (Gao et al. 2020). The structure of sodium alginate is rich in hydroxyl and carboxyl groups. However, the mechanical strength, physicochemical stability and thermal stability of sodium alginate are relatively low, therefore, physical or chemical cross-linking modification is mostly used to improve the ability of alginate composite carriers for heavy metal ions (Gokila et al. 2017). Sodium alginate derivatives treat heavy metal ions in four different ways: ion exchange, chelation, electrical interactions, and photoreduction (Gao et al. 2020) .

Ion exchange refers to the exchange of ions between two electrolytes or between an electrolyte solution and a complex, and is also considered an adsorption mechanism. Most commonly, polymers or minerals are used to purify, separate, or purify ionic solutions, and the adsorbent can be regenerated or loaded with the desired ions by excess ion washing (Gao X. et al. 2020) . The order of selectivity of ions by ion exchange is dominated by the activity of heavy metals and is a reversible process. It can be used to selectively remove Pb^{2+} , Hg^{2+} , Cd^{2+} , Ni^{2+} , Cr(VI) and Cr(III) (Dabrowski et al. 2004) .

Chelation refers to the formation of a heterocyclic structure containing a metal ion by a chemical reaction in which the metal ion is attached by a coordination bond to two or more non-metallic coordination atoms in the same molecule. Due to the abundance of polar functional groups in the structure of sodium alginate derivatives, heavy metal ions can be chelated with metal cations or anions in solution and depend on the availability of the ion's outermost electron and empty molecular orbitals (Guibal 2004). Chelation of metal cations by ligands in solution leads to the formation of metal anion complexes, thus changing the chelation mechanism to an electrical interaction mechanism (Gao et al. 2020). Under specific environmental conditions, the surface charge of sodium alginate derivatives is opposite to that of the coexisting metal ions, and at the same time, the larger the water and radius of sodium alginate, the greater the adsorption capacity for cations (Gao et al. 2019). In addition, sodium alginate-derived materials have high photoactivity, with the strongest ability to adsorb light at 300-400 nm wavelength, and the photo-reduction process promotes in the adsorption of high valence metal ions such as Au(III) and Cr(VI) ions (Gao et al. 2020).

2.5 Composite carriers

Natural organic polymer carriers, such as chitosan, agar, plant fiber material, sodium alginate and its derivative materials, have the advantages of low cost, good mass transfer performance, and harmlessness to the immobilized cells and microorganisms, but there are limitations such as low mechanical strength, poor environmental resistance, and small specific surface area of some of the carriers. Synthetic polymer carriers, such as chitosan-coated PVA, PVC beads, polyvinyl alcohol and other materials have higher mechanical strength and better physicochemical stability than natural carriers, but some of the polymers have cytotoxicity, which makes it difficult for immobilized microbial cells to survive; inorganic carriers such as dermis, sawdust, eggshells, ceramics, and coffee residue can be obtained in a simple way, easily operated, and economically used, but inorganic carrier Surface chemical properties are not easy to change, low removal rate, microbial adsorption is limited, and some of the peel, dregs waste itself has some pollutants, need to be pre-treated before use. It can be seen that a single type of carrier although each has its own advantages, but also has different shortcomings, while the composite carrier combines the traditional fixed carrier and the new carrier, with the advantages of the new carrier at the same time, make up for the shortcomings of the traditional carrier, making the immobilization technology to a higher level.

The addition of modifiers to traditional immobilization carriers is one of the focuses of modern microbial immobilization research. Fan et al. (2019) carboxymethyl cellulose-Fe_3O_4 nanoparticles were synthesized by impaction spinning technique based on process-enhanced high gravity basis. In the experiment, Fe_3O_4 nanoparticles were modified by carboxymethyl cellulose during in situ growth, which increased the specific surface area of the nanoparticles, and the modified nanoparticles possessed a lower pH_{zpc} value (4.6), and the surface of the particles was negatively charged under a weakly alkaline environment, which was more favorable for the adsorption of Pb(II), with a maximum adsorption capacity of 152.0 mg/g.

Graphene oxide is a composite carrier formed by combining a traditional organic carrier with an inorganic carrier and belongs to a kind of composite material. Graphene oxide has a large specific surface area, abundant carboxyl, hydroxyl, carbonyl, and epoxy functional groups, and strong hydrophilicity, but its inherent shortcomings of easy aggregation and difficult separation limit its application in the environment (Zhang et al. 2020). The Yang (2014) introduced graphene oxide on the surface of cross-linked chitosan and synthesized CS-GO composites with the highest adsorption capacity of 202.5 mg/g for Cu(II), which is an excellent adsorbent for heavy metal ions. Cu(II) and U(VI) were adsorbed using graphene oxide-coated poly(vinyl alcohol)-sodium alginate hydrogel spheres with maximum adsorption capacities of 247.16 mg/g and 403.78 mg/g respectively, and showed good reusability (Yi et al. 2018). Li et al. (2015) synthesized a novel mesoporous silica grafted graphene oxide material with high selectivity and adsorption of Pb(II) with a maximum adsorption capacity of 255.10 mg/g.

3. Microbial immobilization methods

3.1 Adsorption

The adsorption method utilizes the van der Waals forces, chemical bonds and other forces between the immobilization carrier and microorganisms to adsorb the microorganisms onto the carrier, thus achieving immobilization (Huang Z et al. 2015). Common adsorbent materials include clay, chitosan, agricultural wastes, magnetic nanoparticles (Chakraborty et al. 2020). Some fruit peels and sawdust contain cellulose, pectin, and lignin, which are chemically crosslinked by graft copolymers and then interacted with methyl acrylate to synthesize chemically modified peels with good adsorption of Pb(II), Cd(II), and Cu(II) (Feng et al. 2009). Li et al. (2019) investigated the bioreduction capacity of Cr(VI) by adsorbed *Shewanella xiamenensis* in 2-hydroxy-1,4-naphthoquinone-graphene oxide composites. After several consecutive reactions, the catalytic activity of the material was hardly lost, and the presence of 2-hydroxy-1,4-naphthoquinone could act as an electron shuttler to promote extracellular electron transfer and enhance the reduction of Cr(VI). The adsorption method is currently recognized as an effective and economical treatment method for heavy metal wastewater due to its easy operation, flexible process design and good mass transfer performance. However, it also faces problems such as limited number of microorganisms immobilized, limitation of carrier selection, and surface area.

3.2 Covalent binding

When atoms are attracted to each other, the shared electron pairs formed between the atoms are called covalent bonds, at which time the attractive and repulsive forces between the atoms have reached a state of stable equilibrium. In microbial immobilization, the functional groups on the surface of the immobilized cells form chemical covalent bonds with the reactive groups on the surface of the carrier, thus achieving the effect of immobilizing microorganisms (Xia et al. 2020). The Covalent bonding can be characterized by good binding between microorganisms and carriers, high degree of stability, and good selectivity, but it also faces the shortcomings of high cost of use, complex and intense operation and reaction, and high damage to cells (Hashem et al. 2016). The enzyme is commonly immobilized in the laboratory (Wang et al. 2023).

3.3 Crosslinking

Cross-linking is the combination of biological macromolecules or bacterial cells with each other by means of covalent or ionic bonds, and is usually used to improve the physicochemical properties of polymers. Common cross-linking agents include epichlorohydrin, aldehyde-based reagents, and toluene diisocyanate. Zhang et al. (2017) used isophorone diisocyanate as a cross-linking agent to modify graphene oxide nanosheets, a novel graphene oxide-framework membrane was designed to effectively remove dyes and heavy metals by filtration. The cross-linking method can complete the immobilization without relying on the immobilization carrier and only relying on the chemical bonding, which has the characteristics of high binding strength and good stability of the immobilized cells, but the cross-linking reaction is more violent and difficult to control, and the cross-linking agent is more expensive and cytotoxic.

Jeong et al. (2020) used gamma irradiation to induce cross-linking of polypyrrole with polyvinylpyrrolidone to synthesize polypyrrole/polyvinylpyrrolidone hydrogels. The induced hydrogels have good conductivity, electrical conductivity and mechanical properties. In addition, the polymerized hydrogel has a porous structure, which can maintain a high cell survival rate. Compared with the traditional cross-linking method, the process of polymerization, cross-linking, and sterilization can be carried out at the same time because the inducing factor is gamma rays, and the process can be carried out at room temperature without adding any cytotoxic cross-linking agent. It has the potential to be used in the future as a major material for prosthetic limbs, as well as for smart drug delivery systems and bio-conductivity.

3.4 Encapsulation

Commonly used agar , alginates, carbon nanotubes, and other encapsulating materials to limit the active space of immobilized bacteria, increase mass transfer efficiency, and improve heavy metal ion treatment (Hu et al. 2020).

The 4% alginate nanogel encapsulated *B. safensis* KTSMBNL 26 bacteria was used as a biosorbent to investigate the effect of different pH, temperature, and incubation time on the removal of aluminum from aqueous environment (Dhanarani et al. 2016). They found that the removal efficiency of Al(III) by the adsorbent reached a maximum value of 84 mg/L at pH about 6.5, temperature around 35°C, and incubation time of 24 h.

3.5 Substrate embedding

Substrate embedding method is applied to the immobilization of many enzymes and microorganisms, gel embedding, microcapsule method and freeze-drying method are the three commonly used methods in embedding method, and the commonly used ones are sodium alginate, chitosan and its cross-linked polymers and other porous materials. Advantages of the substrate specificity is strong, the nature of the embedded material can be retained, gel embedding and microcapsule method of high degree of immobilization combined with the relatively low cost of gel embedding; disadvantages are basically impossible to regenerate, the preparation is difficult. Mainly used in the preservation of bacterial and fungal spores, food additives, bioengineering application (Lv et al. 2013). To study the effect of polyvinyl alcohol/sodium alginate-embedded Fe^0-Fe_3O_4 nanocomposites on the removal of Cr(VI). The ratio of 5.0 wt% polyvinyl alcohol to 1.5 wt% sodium alginate removed up to 97.7% of Cr(VI) after acidification and reduction steps. Compared with free Fe^0-Fe_3O_4 nanocomposites, although the removal rate of the immobilized composites is relatively slower, the advantages of immobilization, such as high recovery, easy separability, environmentally benign, and unrestricted under aerobic conditions, are still favored.

4. Treatment of heavy metal ions by immobilized microorganisms

4.1 Bacteria

Bacteria have the advantages of small size, fast growth rate, and high environmental adaptability, which can be utilized to treat heavy metal ions in the environment. Generally, heavy metal cations are bound to the functional functional groups of the cell wall of bacteria and then internalized into the cells (Priyadarshanee and Das 2021). Magnin et al. (2014) studied the effect of Zn(II) uptake by *Rhodobacter capsulatus*. They found that the uptake capacity of the wild-type strain B10 was greater than that of the strain RC220, which lacked the endogenous plasmid, and that the B10 strain had the strongest uptake of Zn(II) up to 164 ± 8 mg/g at pH 5 to 7, compared with 73.9 mg/g for the RC220 strain. Huang et al. (2013) investigated the adsorption capacity of Cd(II) by live and dead cells of *Bacillus cereus* RC-1. Based on the langmuir isotherm, the maximum biosorption capacity was calculated to be 31.95 mg/g for dead cells and 24.01 mg/g for live cells, and it was further found that Cd(II) severely damages the cell surface, resulting in the loss of the binding capacity of Cd(II) by live cells, and that the bioaccumulation of Cd(II) by this strain mainly depends on extracellular biosorption, so dead cells are instead more suitable as Cd(II) biosorbents.

Various strains, including *Rhodococcus opacus* (Calfa and Torem 2008) and *Bacillus subtilis* (Aravindhan et al. 2011) have been employed for the treatment of Cr(III). In other studies, *Bacillus licheniformis* (Zhou et al. 2006) and *Bacillus thuringiensis* strain OSM29 (Oves et al. 2013) demonstrated effectiveness in treating Cr(VI). Additionally, *Pseudomonas* pseudoalcaligenes (WA C. LEUNG et al. 2001) proved valuable in the removal of heavy metals such as Pb(II), Cu(II), and Ni(II). Bacterial treatment of heavy metal ions is a rapid process, with high binding efficiency and without the need for expensive growth media. However, they also face problems such as difficulties in recycling and some bacteria themselves are pathogenic to humans. Moreover, the complexity of mixed pollutant types in the actual treatment environment is a great challenge to minimize the threat of other complex pollutants during the treatment of specific heavy metal ions.

4.2 Fungi

The wide distribution of mycelium formed by the fungus and the large cell size can be utilized to treat heavy metal ions over a large area using its mycelial network and the secreted by-products can be used as raw materials for heavy metal ion adsorbents (Shakya et al. 2016). However, it is unable to maintain sufficient activity and biomass in contaminated soils, and therefore is inefficient in treating contamination (Harms et al. 2011). However, immobilized materials such as agar, alginate, glycol, etc. need to be used to provide a stable environment for the plant (Shakya et al. 2016). Ali et al. (2021) investigated the potential of *Penicillium chrysogenum* and *Cephalotheca foveolata* strains immobilized in loofah sponges and calcium alginate beads for Cd(II) treatment. Loofah sponge-immobilized strains of *Penicillium chrysogenum* and *Cephalotheca foveolate* showed Cd(II) removal rates of 52.0% and 46.8% (Sample 1) and 54.2% and 50.0% (Sample 2), respectively. Meanwhile, strains immobilized in calcium alginate removed 48.8% and 44.9% (Sample 1) and 51.7% and 48.3% of Cd(II), respectively. Alothman et al. (2019) conducted a study on the adsorption of Cd(II), Cu(II), and Pb(II) using *Penicillium chrysogenum* and *Aspergillus ustus* derived from fungal biomass. They found that the maximum adsorption efficiency of the strains on heavy metal ions was achieved at a temperature of 60°C. The adsorption efficiencies of *Penicillium chrysogenum* on Cd(II), Cu(II), and Pb(II) were 91%, 53%, and 56%, and the adsorption efficiencies of *Aspergillus ustus* on Cd(II), Cu(II) were 84%, and 52%, respectively. Particularly noteworthy is the adsorption efficiency of *Aspergillus ustus* for Pb(II) at 30°C, reaching a substantial 42%. Fungal biosorbents offer advantages over traditional adsorbents, including cost-effectiveness, ease of regeneration, versatility, selective adsorption, potential for heavy metal recovery, and minimal secondary impact on the water environment. However, in order to ensure the survival of fungal cells, factors such as pH, contact time and temperature need to be taken into account.

4.3 Algae

Mechanisms by which algae adsorb heavy metal ions include ion exchange, chelation, electrostatic attraction, surface adsorption, or binding to enzymes to reduce the amount of heavy metal ions in the environment (Chen et al. 2023). The adsorption capacity of algae mainly depends on the macromolecules in the cell wall with different functional groups such as hydroxyl, carboxyl and carbonyl groups, which can bind heavy metal ions to cellular proteins to form complexes (Anastopoulos and Kyzas 2015) , or by active transport allowing some of the protein-heavy metal complexes to enter the vesicle for storage (Bilal et al. 2018) , thereby reducing the amount of heavy metal ions in the cytoplasm and mitigating the cytotoxicity of heavy metal ions. Wang et al. (2021) immobilized microalgae in fungal hyphae to form a symbiotic system and adsorbed up to 98.89% of Cd(II). Alwared et al. (2019) used calcium alginate to immobilize chlorophyll and cyanobacteria for up to 90% removal of Cu(II) by adsorption. Barquilha et al. (2019) explored the removal of Ni(II) and Cu(II) by immobilizing brown algae using sodium alginate and the actual wastewater showed a reduction of 29.69% for Ni(II) and 26.24% for Cu(II) and the adsorbent could be reused using a small amount of calcium chloride solution with an adsorption capacity of 75% of the initial adsorbent.

Although immobilized algae are very effective in treating heavy metal ions, have low cost, high efficiency, do not produce toxic wastes, and have reusability, they are limited to water bodies only and cannot be applied in soil on a large scale, and most of the industrial wastewater containing heavy metal ions is cytotoxic, which is not conducive to the survival of living algal cells for a long period of time.

5. Conclusion and outlook

Heavy metal ion pollution is one of the pollution that human beings can not ignore because of its wide source, great harm and easy accumulation in organisms. While immobilized microbial technology has achieved good results in the treatment of heavy metal ions, there are still many problems to be faced in order to achieve large-scale application.

There are many kinds of immobilized cells and immobilized carriers, and the external environmental conditions required to achieve the best processing effect are different, so how to choose the most suitable carriers and cells under different contamination conditions to achieve the optimal processing effect with the smallest opportunity cost is the key to the application of this technology.

Some of the polymer carriers may be sticky and clogged during the process, and the contents may overflow, affecting the normal operation of the subsequent related machinery; or hindering the recovery of the carriers and the reuse of the repaired environment. Therefore, it is necessary to modify the carrier accordingly, or use it in combination with other processes and methods.

Some immobilized materials are inherently resistant to degradation, and the immobilized carrier or immobilized cells should be handled appropriately in the process of practical application to prevent other forms of secondary pollution to the environment.

Although the effect of immobilized microbial technology in treating heavy metal ions is greener and safer than traditional physical and chemical effects, traditional means of treatment have been developed for a long time, the process is mature, and there is relevant policy support. Also need the corresponding policy to promote the development of immobilization technology.

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