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Microbial Biosorption as a Green Technology for Bioremediation of Heavy Metals.

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ABSTRACT

The exponential growth of Industrialization with the increase in human population has led to a heavy metal pollution problem, which has become ubiquitous from air to soil. Heavy metal pollution has become a more serious environmental problem in the last several decades as a result of its toxicity and insusceptibility to the environment. There are many bioremediation technologies including biosorption process to decontaminate the heavy metal polluted sites. Biosorption is a technique that can be used for the removal of pollutants from waters, especially those that are not easily biodegradable such as metals and dyes. A variety of biomaterials are known to bind these pollutants, including bacteria, fungi, algae, and industrial and agricultural wastes. In this review, the biosorption abilities of bacterial and fungal biomass towards metal ions are emphasized. This review attempts to present a brief summary of the role of biosorption in heavy metal removal from wastewater. Undoubtedly, the biosorption process is a potential technique for heavy metal decontamination.

Keywords: Heavy metals; Biosorption; Tannery; Dyes; Treatment

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INTRODUCTION

Worldwide, the persistent growth of human population has resulted into more demand for the life supporting commodities. Hence, industrialization processes to meet these requirements are growing continuously [1-2]. One of the most severe guarantee damages of the industrial enhancement is the pollution of the environment with many toxic substances including heavy metals above their permissible limits as mention in Table.1 [3]. Contamination of soils, groundwater, sediments, surface water and air with hazardous and toxic chemicals poses significant problems for both human health and the environment. Their presence in the wastewater of several industrial processes, such as electroplating, metal finishing, metallurgical work, tanning, chemical manufacturing, mining and battery manufacturing, has brought about more environmental concerns due to their toxicity even at low concentrations [3–5]. Generally, both quality and quantity of these wastes materials and toxic heavy metals depends upon the types of industries and the raw materials used for the production processes [5]. Among the non-biodegradable heavy metals present into industrial wastes, copper, chromium, cadmium and nickel are reported to be the most common heavy metals and therefore, they are more widespread as contaminants into soils and water bodies [6]. Heavy metal pollution occurs directly by effluent drains from industries and waste treatment plants and indirectly by the contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rain water [7].

Table 1: Types of heavy metals and their effect on human health

Pollutants	Major sources	Effect on human health	Permissible limits(ppm)
Arsenic	Pesticides, fungicides	Bronchitis, dermatitis	0.02
Cadmium	Welding, electroplating, pesticides	Kidney damage, cancer, gastrointestinal disorder	0.06
Lead	Paint, pesticides, smoking, News Papers	Mental retardation in children, lung, kidney damage	0.10
Manganese	Welding, fuel addition	Damage to central nervous system	0.26
Mercury	Batteries, paper industry	Damage to central nervous system, protoplasm poisoning	0.01
Zinc	Refineries, metal plating	Damage to nervous membrane, corrosive effect on skin	15.0

Normally, heavy metals are essential elements in life cycle of prokaryotes and eukaryotes [4]. For example, zinc is the part of an array of enzymes like, dehydrogenases, proteinases, and peptidases and also involved in the metabolism of carbohydrates, proteins, phosphate, and auxins [7]. Similarly, copper plays an important role in numerous physiological processes in plants e.g. nitrogen and cell wall metabolism, seed production, photosynthesis, respiration, and carbohydrate distribution [4-7].

Conversely, the high concentration of these metal species might be a great threat to all organisms including human. For example, in the case of minor zinc exposure, irritability, muscular stiffness, loss of appetite and nausea are common [6]. Chromium on the other hand, can cause cancer in the digestive tract and lungs, while manganese triggers neurotoxicity, low hemoglobin levels, and gastrointestinal accumulation [8]. As another example, copper consumption in high doses brings about serious toxicological concerns since it can be deposited in the brain, skin, liver, pancreas, and myocardium and initiates intestinal distress, kidney damage and anemia [9]. Lead has been cited as one of the three most toxic heavy metals that have latent long-term negative impacts on health, causing anemia, encephalopathy, hepatitis and nephritic syndrome [10]. Cadmium also exposes human health to severe risks, as it can provoke cancer, kidney damage, mucous membrane destruction, vomiting, diarrhea, bone damage, and itai-itai disease, as well as affect the production of progesterone and testosterone [11]. Moreover, the presence of nickel exceeding its critical level might bring about serious lung and kidney problems aside from gastrointestinal distress, pulmonary fibrosis and skin dermatitis [12]. Finally, mercury has been identified for its nervous system deterioration, including protoplasm poisoning [2]. Heavy metals at higher concentrations obstruct the functioning of microbes which are beneficial for the soil and their associated biological activities which results into the decreased soil fertility [7].

Microorganisms which are constantly exposed to heavy metal stress evolve adapting mechanisms to the metal contaminants [10]. They respond to these molecules by diverse biological processes like, transportation across the cell membrane, biosorption to the cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation-reduction [3]. The microbial response to a specific heavy metal is of great significance in manipulating them in the remediation of metal contaminated sites [11]. Techniques presently in existence for heavy metal removal from contaminated waters include: reverse osmosis, electro dialysis, ultrafiltration, ion-exchange, chemical precipitation, phytoremediation etc. However, all these methods have disadvantages like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge or other waste products that require careful disposal [2]. Among these strategies adopted, biosorption process has a distinct role in bioremediation of heavy metals because it efficiently sequesters the dissolved heavy metals even from very dilute complex solutions with high competence. Hence, biosorption is considered perfect for the metal removal from the polluted sites [12].

The present review highlights the fundamental concepts of biosorption, underlying mechanisms of biosorption process, development of effective biosorbents, and possible aspects of biosorbent regeneration.

Biosorption

Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake. Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents [13]. The biosorption process involves two phases. One phase is a solid phase (biomass/ sorbent/ biosorbent/ biological material) and another is liquid phase (solvent, usually water) containing a dissolved species to be sorbed (sorbate/ metal ion). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases [2].

Mechanisms involved in biosorption can be classified taking into account various criteria that are based on cell metabolism, they are classified as metabolism dependent and non-metabolism dependent while based on location of the sorbate species it is classified as extra cellular accumulation/precipitation, cell surface sorption /precipitation and intra-cellular accumulation.

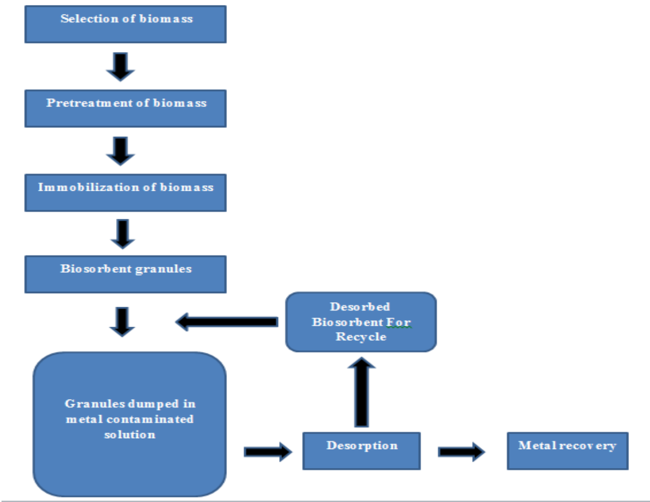

Figure 1: General schematic representation of biosorptionprocess

Principally, the biosorption process which non-metabolism dependent is often rapid as compared to the metabolism dependent biosorption process [14]. Moreover, lower temperature, the absence of energy source and the presence of metabolic inhibitors depresses the efficiency of biosorption process. The application of dead biomass as biosorbent instead of living microbial cells removes constraint of toxicity of heavy metals. Thus, the biosorption processes are more practically applicable compared to the bioaccumulation processes since living system (i.e. active uptake of heavy metals) commonly needs the nutrients supply to carry out the metabolic activities and consequently raise biological oxygen demand (BOD) or chemical oxygen demand (COD) of the effluent/ solution [11]. Figure.1 shows a generalized schematic process of biosorption for heavy metal removal. A successful biosorption process requires preparation of good biosorbent. The process starts with selecting various types of biomass. Pretreatment and immobilization are done to increase the efficiency of the metal uptake. The adsorbed metal is removed by desorption process and the biosorbent can be reused for further treatments.

Selection of Biomass

While choosing the biomass for metal biosorption, its origin is a major factor to be taken into account. Biomass can be taken from, activated sludge or fermentation waste from industries like food, diary and starch. Also, organisms (e.g., bacteria, yeast, fungi and algae) are good sources of biomass. Fast growing organisms that are specifically cultivated for biosorption purposes (e.g., crab shells, seaweeds) can be used as biosorbents. Apart from the microbial sources agricultural products such as wool, rice, straw, coconut husks, peat moss, exhausted coffee, waste tea, walnut skin, coconut fibre, cork biomass, seeds of *Ocimum basilicum* are also proven as good biomass sources. Whereas, sea weeds, molds, yeasts, bacteria owing to their superior performance, little cost and large availability is best biomass sources [15-18].

Recent biosorption experiments have focused attention on waste materials, which are by-products or the waste materials from large-scale industrial operations. Like the waste mycelia available from fermentation processes, olive mill solid residues, activated sludge from sewage treatment plants, biosolids, aquatic macrophytes, etc. Although abundant natural materials of cellulosic nature have been suggested as biosorbents, very less work has been actually done in that respect.

In addition, the high surface to volume ratio of microorganisms and their capability to detoxify heavy metals are the main basis that these microorganisms are selected as potential alternative to the biosorbents to remediate the heavy metal contaminated sites [11].

Bacteria

Bacteria are the most abundant and flexible of microorganisms and constitute a significant portion of an entire living earthly biomass. Bacteria are used as biosorbents on account of their small size, and capability to grow under controlled conditions, and their resistance against a wide range of varying environmental conditions. The metal uptake capacities of bacteria generally range between 568 to 0.70 mg g^{-1} [16].

A great deal of heterogenecity exists among different bacterial species in relation to their number of surface binding sites, binding strength for different ions and the binding mechanisms.

Cell walls of bacteria and cyanobacteria are principally composed of peptidoglycans which consist of linear chains of the disaccharide N-acetyl glucosamine, β-1,4-N-acetyl muramic acid with peptide chains. Gram positive cell walls and surfaces have a negative charge density due to peptidoglycan network, a macromolecule consisting of strands of alternating glucosamine and muramic acid residues, which are often N-acetylated. Carboxylate groups at the carboxyl terminus of individual strands provide anionic character to the cell wall. The phosphodiesters of teichoic acid and the carboxyl groups of teichuronic acid contribute to the ion exchange capacity of cell walls [17-20].

Fungi and Yeast

Fungi basically show filamentous or hyphal growth 90% of dry mass of the cell wall consists of amino or non-amino polysaccharides. The fungal cell walls can be considered as a two phase system consisting of chitin framework embedded on an amorphous polysaccharide matrix [21].

The cell walls are rich in polysaccharides and glycoprotein's such as glycans (β-1-6 and β-1- 3 linked D-glucose residues), chitin (β-1-4 linked N-acetyl-D- glucosamine), chitosan (β-1-4 linked D-glucosamine), mannans (β-1-4 linked mannose) and phosphormannans (phosphorylated mannans). Various metal binding groups, viz amine, imidazole, phosphate, sulphate, sulfhydryl and hydroxyl are present in the polymers. *Aspergillus niger* is a filamentous ascomycete fungus and one of the most common species in the genus Aspergillus. This fungus is commercially and economically essential in fermentation for citric acid production due to its efficiency and high yield outcome. This fungus can also be used to sequester toxic heavy metals from solution. The pre-treatment of *Aspergillus niger* with sodium hydroxide improves its biosorption capacity. The biosorption capacity values of the biomass increases with increasing initial metal ion concentration and temperature. The use of dead microbial cells in biosorption is regarded as more advantageous for water treatment since they are not affected by toxic waste, and therefore do not require a continuous supply of nutrients but can be regenerated and reused for many cycles. An effort has been made recently to determine the sorption capacity of the dead fungal biomass of species *Aspergillus niger*, *Aspergillus sydoni* and *Penicillium janthinellum* for Cr (VI) from aqueous and electroplating wastewater [22].

Pretreatment of Biomass

Biosorbents are initially prepared by pretreating the biomass with different methods. The biosorption of metals by biosorbent depends on different factors like, the number, accessibility and availability of the site and affinity between the biomass and the biosorbents [23]. Pretreatment is basically done to increase the interaction between the biosorbent and the metal species. Biomass can be pretreated directly however, if it is larger in size, they are sized into fine particles or granules and they are further treated in several ways. Pretreatment involves heat treatment, washing with detergent/ acids/ alkalies, enzymatic treatment etc. Among these methods, heat treatment and detergent washing increases the efficiency of biosorption by exposing additional metal binding groups on biomass while enzymes destroy unwanted components to enable biosorption [24]. In case of alkali pretreatment, bioadsorption capacity of *Mucor rouxii* biomass was significantly enhanced in comparison with autoclaving, while pretreatment of biomass with acid resulted in decreased bioadsorption of heavy metals [25, 21].

Comparison of Biosorbents

To evaluate the practice of a specific biosorbent for the removal of heavy metal ion from environment, it is important to assess its maximum capacity to sequester metals to its surface.

Advantage of using bacteria as biosorbents is that they have polysaccharide slime layers which provide chemical groups for metals biosorption and they can be specifically propagated in large scale. Algae have additional gain over fungal and bacterial biosorbents as they require very few nutrients, being autotrophic produce large biomass and do not produce toxic substances [26, 27].

Immobilization of Biomass

Microbial biomass consists of small particles with low density, poor mechanical strength and little rigidity. However, biosorbents are hard enough to withstand the pressures, water retention capacity, porous and/or "transparent" to metal ion sorbate species, and have high and fast sorption uptake even after repeated regeneration cycles [22].

Immobilization helps biosorbents to have a better shelf life and offers easy usage compared to free biomass, which is easily biodegradable. Therefore the biomass should be immobilized before being subjected to biosorption. The techniques available for application of biosorption are based on:

- Adsorption on inert supports e.g., activated carbon for *Enterobacter aerogens* biofilm.
- Entrapment in polymeric matrix e.g., polymers like calcium alginate, polyacrylamide etc.
- Covalent bonds to vector compounds.
- Cell cross–linking.

The last two techniques are majorly employed for algal immobilization [28].

Regeneration of Biosorbent

The regeneration of the biosorbent is important for keeping the process of biosorption cost down. For this, it is required to desorb the sorbed metals and to regenerate the biosorbent material for another process. It is essential that the desorption should be applied in such a way that the process not only should recover the maximum metals, but also restore the biosorbent close to the original state without any kind of damage and change in the physical state so that it can efficiently be reused with the original metal binding efficiency [15]. Dilute mineral acids (HCl, H_2SO_4, HNO_3) can be used for the removal of metals from biomass, also organic acids (Citric, acetic, lactic) and complexing agents (EDTA, thio-sulphate etc.) can be used for metal elution without affecting the biosorbent [29].

Mechanisms of Biosorption

Microbes mediated remediation of heavy metal contaminated site may involve the following pathways [30]:

- Metal cations may bind on cell surfaces (biosorption) within the cell wall (bioaccumulation) and in turn, metal uptake is augmented through microprecipitation.
- Metal precipitation may occur when heavy metals react with extracellular polymers or with anions (e.g. sulphide or phosphate) produced by microbes.
- Metal volatilization through enzymes mediated biotransformation.
- Metal ions may be actively translocated inside the cell through metal binding proteins (Figure.2).

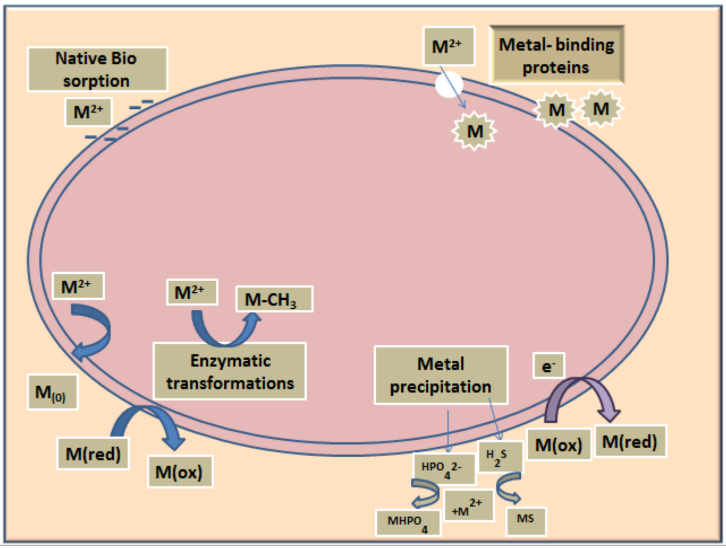

Figure 2: Mechanisms of heavy metal uptake on microbial surface

Factors Affecting Biosorption

Following are the factors that affect the biosorption process:

- Temperature doesn't influence the biosorption process in the range 20 to 35^{0}C [31].
- pH is the most important parameter in the biosorptive performances as it affects the chemistry of the metals in the solution and the activity of the functional groups in the biomass [23, 32].
- Concentration of biomass in the solution affects specific uptake. An increase in biomass concentration leads to interference between the binding sites [33].
- Presence of more than one type of metal ions also affects the biosorption process. For example, cobalt uptake by different microorganisms is inhibited in the presence of mercury, lead, copper and uranium [34, 35].
- Pretreatment of biomass may increase the metal sorption [2].

Future Prospects

Biosorption has been studied extensively in research and small-scaled demonstrations, but in only a few full-scale applications. Biosorption is moving into the realm of commercialization [36].

Given the current effectiveness, biosorption is best for cleanup over a wide area in which contaminants are present at low to medium concentrations. Before biosorption is fully commercialized, further research is needed to assure that biosorbents used for biosorption do not have adverse effects on environment. The important features required for the successful application of biosorption technology to real situations include, but are not limited to [37]:

- Screening and selection of the most promising biomass, with sufficiently high biosorption capacity and selectivity.
- Optimizing the conditions for maximum biosorption, including optimization of pH, temperature, ionic strength and co-ion effects, etc.
- Improving the selectivity and uptake via chemical and/or genetic modification methods.
- Examining the mechanical strength of biomass and if insufficient for reuse, improving rigidity by proper immobilization or other chemical methods.
- Testing the performance of biosorbents under different modes of operation.
- Analyzing the behavior of biosorbent for use with real industrial effluents and simultaneously analyzing the impact of water quality on the biosorption uptake of the specific pollutant of interest.

Conversely, it is no small feat to replace well established conventional techniques. However, in addition to being cost effective, biosorption has huge potential, as many biosorbents are known to perform well, if not better than most conventional methods [38]. Also being aware of the hundreds of biosorbents able to bind various pollutants, sufficient research has been performed on various biomaterials to understand the mechanism responsible for biosorption. Research is also needed to find more efficient biosorbent in terms of requiring less nutrient resources and which can withstand high metal stressed conditions. There is also the need for a commercialized method so that the biomass can be recycled. Biosorption is slower than traditional methods of removing heavy metals from soil but is much less costly. Prevention of soil contamination is far less expensive than any kind of remediation and is much better for the environment.

CONCLUSION

From all the information mentioned we can conclude that nowadays biosorption is considered as a most powerful alternative to conventional methods for removal of heavy metals from industrial effluents. Use of microbial biosorption makes biosorption more ideal approach for the decontamination of heavy metals. Thus, biosorption of heavy metals by using different microbial genera as biosorbents can be used as an environment friendly technique to remediate heavy metals from environment. The development of biosorption process requires more investigation for cheapest biosorbent in terms of requiring less nutrient resources and which can withstand high metal stressed conditions. Due to the extensive research and significant economic benefits of biosorption, some new biosorbent materials are poised for commercial exploitation.

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