



A Study of the Suitability of Microbial Cells for the Biosorption and Bioaccumulation of Heavy Metal Removal

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ABSTRACT: Heavy metal contamination in the natural environment can occur as long-term site pollution or as surges of pollutants from wastewater discharge. It is well recognized that heavy metal discharge from the metal processing industries has a negative impact on the environment. Conventional methods of heavy metal removal from aqueous solutions are not cost-effective and produce large amounts of harmful chemical sludge. A novel and alternative approach to removing heavy metals from aqueous solutions involve the biosorption of these contaminants by non-living, metabolically inert biomass that is either derived from microorganisms or plants. One of the key elements of environmental and bioresource technologies today is biosorption. Due to their high surface-to-volume ratio, wide availability, quick kinetics of adsorption and desorption, and low cost, microorganisms—more specifically, bacteria, algae, yeasts, and fungi—have attracted increasing attention as biosorbents for the removal of heavy metals. Analyzing the removal of heavy metals from aqueous solutions utilizing diverse biological components, such as fungi, algae, yeast, and bacterial biomass, is the goal of the current study. This article discusses the advantages of heavy metal removal from waste streams, gives a brief overview of the technology's potential for biosorption and bioaccumulation, and emphasizes the undelaying features of biosorption as well as operational factors like pH, the dose required to be given, the initial concentration, temperature, the efficiency of the treatment, and its economic significance.

KEYWORDS: Biosorbents, Contamination, Desorption, Sludge.

INTRODUCTION

Water use has increased over the past 20 years at a rate that is twice as fast as population growth. Contaminants including heavy metals, pesticides, fertilizers, hydrocarbons, organic waste, pathogens, and new pollutants are contaminating freshwater supplies. Climate change is estimated to result in an additional 10% decrease in freshwater supply to 650 million people residing in over 570 cities by 2050 [1]. The wastewater is composed of 99% water and 1% suspended and dissolved particles. Due to increased municipal and industrial waste discharge, decreased runoff, lower water dilution capacity, and industrial intensification, there is an increase in organic matter in water. The use of water and wastewater is responsible for 3-7% of greenhouse gas emissions. Only 8% of industrial and municipal wastewater is treated globally, and more than 80% of wastewater is neither collected nor treated before even being released into the environment. The worldwide ecology is being affected by industrialization. When improperly treated water is discharged into the environment, it directly affects biodiversity, toxin bioaccumulation, greenhouse gas emissions, the condition of aquatic ecosystems, water temperature, and economic productivity (such as decreased industrial and agricultural production, lower market values for harvested crops, etc.). The majority of the wastewater comes from the residential and commercial sectors, with the rest coming from energy production, mining activities, urban runoff, agricultural runoff, and landfill leachates. These wastewater sources contain hazardous organic substances like hydrocarbons, chlorinated solvents, PCBs, volatile organic compounds, and persistent organic pollutants. The capacity for metal adsorption can be increased by the living cells as they grow and produce new biomass. They are significantly more capable of absorbing heavy metals than dead materials because they use both active and passive absorption mechanisms. Therefore, it is important to first learn about the parameters influencing live algae's growth and physiology before using them for bioremediation. Among these, it is crucial to evaluate the metal's inhibitory effects. By preventing their numerous physiological processes, heavy metals can have a variety of effects on algae. Since algae are photoautotrophic organisms, their effects on photosynthesis are of primary concern as this process is often the most sensitive to environmental stress.



The fast expansion of industrialization, urbanization and contemporary agricultural development has made environmental destruction a significant global concern. Energy production that relies on depleting natural resources to satisfy human needs and requirements disturbs the ecological balance that maintains the environment's quality. Industries have developed new products and pollutants at abundant levels above the capacity of the environment to self-clean due to technological developments and advancements in their processes and products. The industrial revolution triggered a rise in environmental pollution. When toxins are released into an environment, the ecosystem is harmed, disrupted, and uncomfortably unstable. Because toxic heavy metals are non-degradable and persistent, the increased use of metals and chemicals in processing industries has led to the production of significant amounts of effluents that are highly poisonous and contain toxic heavy metals. Toxic liquid waste is produced during mining, mineral processing, and extractive metallurgical techniques. The development of low-cost technologies for the treatment of effluents presents a challenging task for scientists and environmental engineers. Metal removal from aqueous solutions can be performed using a variety of conventional and biological techniques. The conventional approach includes reverse osmosis, membrane technology, ion exchange, filtration, electrochemical treatment, chemical oxidation or reduction, and evaporation recovery. These procedures could be very costly or ineffective. Another disadvantage of this method of treatment is the expensive and unsustainable generation of harmful chemical sludge and the subsequent treatment and disposal of it. The biological method of heavy metal removal, which is very effective and economical, includes bioremediation, phycoremediation, phytoremediation, mycoremediation, and bacterial remediation. Therefore, it is crucial to get rid of hazardous heavy metals in a manner that is both economical and beneficial to the environment. Heavy metals are effectively interacted with and by biomaterials of microbial and plant origin. The metabolically inactive dead biomass sequesters metal ions and metal complexes from the solution because of its unique chemical composition, which eliminates the need to maintain particular growth-supporting conditions. The removal of heavy metals from solutions and their recovery can both benefit greatly from metal sorption by biomaterials. The emergence of creative mass production techniques and the rising demand for novel environmental technologies have increased attention in microalgal biotechnology. The inexpensive growth requirements and the advantage of being utilized simultaneously for multiple technologies (e.g., carbon mitigation, biofuel production, and bioremediation) make microalgae suitable candidates for several eco-friendly technologies. Microalgae have created a complex defense system to deal with the toxicity of heavy metals. Their ubiquitous occurrence and ability to accumulate and concentrate heavy metals ensure their usefulness in real-world wastewater bioremediation applications. The best method for removing heavy metal ions from wastewater is determined by a variety of important criteria, including the cost of operation, the initial concentration of the metal ions, the environmental impact, the pH levels, the chemicals applied, the removal efficiency, and the economic feasibility.

CONVENTIONAL AND BIOLOGICAL METHODS OF HEAVY METALS REMOVAL

The conventional method of removal of heavy metals includes chemical precipitation (hydroxide precipitation, carbonate precipitation, and sulfide precipitation), chemical oxidation or reduction, lime coagulation, ion exchange, reverse osmosis, solvent extraction, evaporation recovery, adsorption, electrodeposition, reverse osmosis and electrodialysis [15, 22, 27]. However, these conventional methods are ineffective or expensive. The most conventional techniques involved in heavy metal removal provide incomplete removal of heavy metals [41], require a large amount of reagent and energy [22], possess a minimal tolerance for pH changes [15], possess a low or negligible metal selectivity [23], need a very high or low level of working of metals [23] generate hazardous sludge or other waste items and suffer high regeneration and investment expenses [15, 22]. Therefore, the scientific community is under pressure to develop new, innovative, cost-effective, efficient, and sustainable methods for the removal of toxic substances the aquatic waterbodies. Hence, there is a growing urge for the production of cheaper adsorbents to replace costly wastewater treatment methods. Hence, biological methods of heavy metal remediation are gaining immense popularity.

Bioremediation technologies have a promising potential to achieve this goal in an eco-friendly manner. This technology comprises low-cost, high-efficiency techniques for the removal of heavy metals from dilute solutions and may also involve regeneration; in addition, they could provide metals recovery. The accumulation and concentration of pollutants from the aqueous solution by the use of biological materials, facilitating the recovery and environmentally acceptable disposal of the pollutant is termed "bio removal". However, the capacity of removal can be affected by the factors such as i) characteristics of metal ions, ii) environmental conditions (such as pH, temperature, ionic strength, contact time, biomass concentration) and, iii) the nature of biosorbent, which determines the difference in selectivity and affinity to metal ions. In order to remove heavy metals from sewage sludge, a number

of methods have been researched, including chemical leaching, bioleaching, electro-kinetic application, and supercritical fluid extraction [17]. Due to its simplicity of use, low chemical requirement, and lack of pollutant production, bioleaching has recently attracted more attention than other procedures. [12, 17] The dissociation of bonds between heavy metals and sewage sludge that occurs during this process happens either as a result of microbial metabolism or as a result of the metabolic byproducts of such microorganisms [33]. It has recently been suggested to remove heavy metals from sewage sludge using an anaerobic bioleaching technique based on fermentation. This process has the potential to function as a self-sufficient system that does not require oxygen or additional chemical addition [6]. Under anaerobic conditions, sewage sludge is hydrolyzed to its monomers; the monomers are then fermented to yield volatile fatty acids (VFAs). The hydrolyzed sewage sludge is supposed to be complexed with either dissolved organic matter (DOMs) or volatile fatty acids (VFAs) in the bulk solution, in which the bonded heavy metals are then recovered.

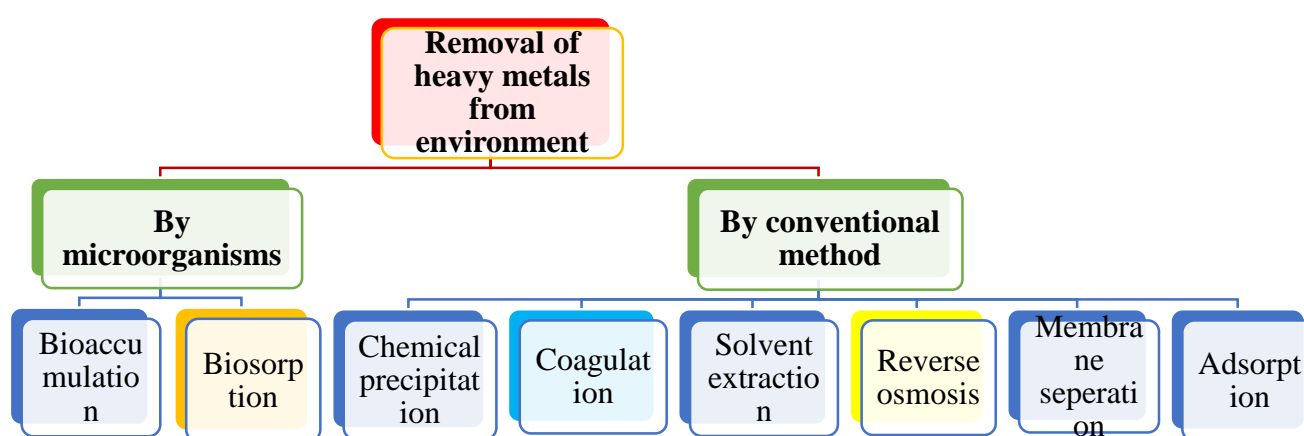


Figure 1. Showing different methods for removing heavy metals from the environment [3]

MICROALGAE AND THEIR ROLE IN METAL REMEDIATION

Microalgae are photosynthetic microscopic organisms found in both marine and freshwater environments. It possesses photosynthetic mechanisms that are fairly similar to land plants. They form the world’s largest group of primary producers in terms of biomass which is responsible for at least 32% of global photosynthesis. Most of them are autotrophs, but they are also capable of producing energy in a heterotrophic or mixotrophic manner. They are aquatic organisms possessing molecular mechanisms that allow them to discriminate, non-essential HMs from essential ones, for their growth [14]. The benefits of using microalgae in metal biosorption include- rapid metal uptake capability, time and energy saving, eco-friendly and user-friendly, year-round occurrence, ease of handling, recyclable or reusable, low cost, faster growth rate (as compared to higher plants), high efficiency, large surface to volume ratio, ability to bind up to 10% of their biomass, with high selectivity, no toxic waste generation, no synthesis required, useful in both batch and continuous systems, and, applicability to waters containing high metal concentrations or relatively low contaminant levels [10]. Recently, the interest in microalgae has increased due to their very high rate of growth of biomass and the possibilities of using it in industrial fields. In addition, its cultivation can be carried out in bioreactors on soil fallow that do not meet the criteria in the valuation. The utilization of algal biomass benefits the environment by reducing the emission of greenhouse gases as well as water and wastewater treatment. It also benefits the economy by producing biofuels, and benefits society by producing food, cosmetics, pharmaceuticals, fertilizers, and feed for animals. They can be seen as a potential solution to the problem of the demand for liquid fuels.

The metals accumulative bioprocesses generally fall into one of the two categories based on the extent of metabolic dependence [40]. In particular, the mechanisms by which microorganisms remove metals from solutions include: (i) Cell-surface sorption or complexation occurs with both living and dead microorganisms, (ii) extracellular accumulation or precipitation that can be aided by the use of viable microorganisms, and (iii) intracellular accumulation that necessitates microbial activity [24]. Although both living and dead cells can accumulate metal, the mechanisms involved differ. Generally, HM ions are entrapped in the cellular structure, and, subsequently absorbed into the binding sites present in the cellular structure. This method of uptake is independent of the

biological metabolic cycle and is known as “biosorption” or “passive uptake” [21]. Additionally, HMs could enter the cell through the metabolic cycle of the cell and cross the cell membrane; this mechanism of metal uptake is known as "active uptake." "Bioaccumulation" refers to the metal absorption in both passive and active forms Two main categories could also be employed to describe the mechanism of microalgal remediation: (i) bioaccumulation by living cells, and (ii) biosorption by non-living, non-growing biomass or biomass products. This first process (comprising bio accumulative uptake) forms the principle for waste detoxification processes (e.g., biological fluidized beds employing continually growing biofilms).

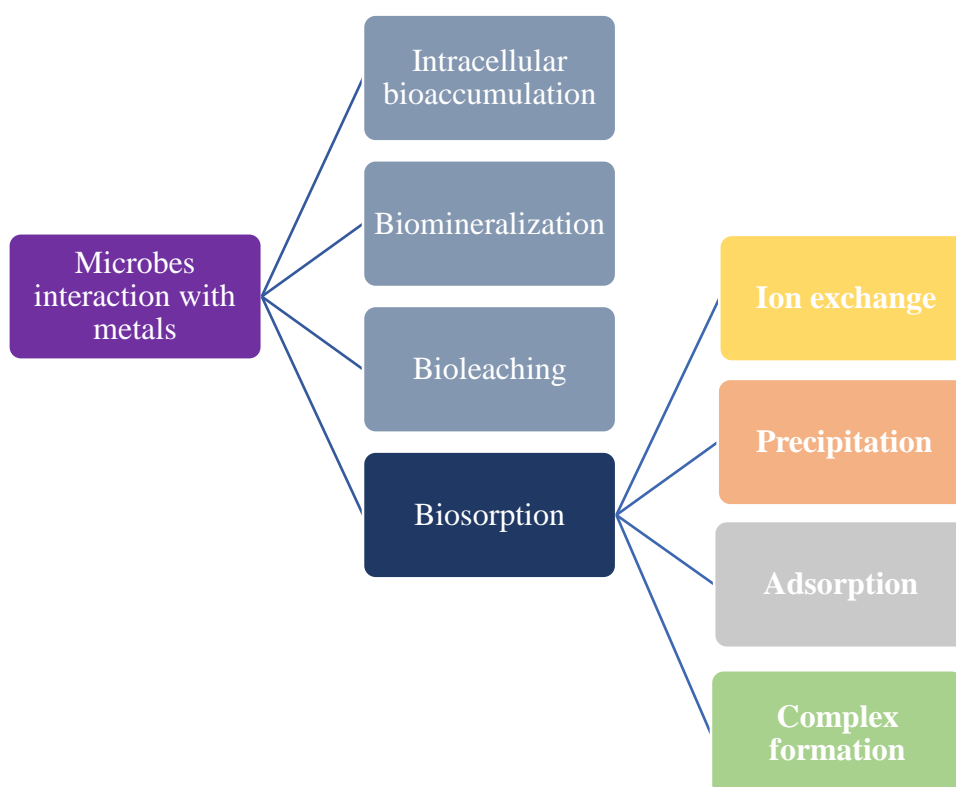


Figure 2. Different ways that microorganisms interact with heavy metals [2]

Table 1. Removal of metals by non-living biomass of microbial and plant origin

Category	Examples	References
Bacteria	Gram-positive bacteria- e.g., Bacillus sp., Corynebacterium sp., etc. Gram-negative bacteria- e.g., Escherichia sp., Pseudomonas sp., etc. Cyanobacteria- e.g., Anabaena sp., Synechocystis sp., etc.	[7, 34, 41]
Fungi	Molds- e.g., Aspergillus sp., Rhizopus sp., etc. Mushrooms- e.g., Agaricus sp., Trichaptum sp., etc. Yeast- e.g., Saccharomyces sp., Candida sp., etc.	[18, 20, 37]
Algae	Micro-algae- e.g., chlorella sp. Chlamydomonas sp., etc. Macro-algae- green seaweed, e.g., Enteromorpha sp., Codium sp., etc. Brown seaweed (e.g., Sargassum sp., Ecklonia sp., etc., and red seaweed (e.g., geildium sp., Porphyra sp., etc.	[4, 32, 35]
Agricultural wastes	Fruit/Vegetable wastes, rice straws, wheat bran, soybean halts, etc.	[25, 26]
Natural residues	Plant residues, sawdust, tree barks, weeds, etc.	[29, 30]



BIOACCUMULATION

The concept of this heavy metal removal method was oriented around bioremediation applications when bioaccumulation research first began in the early 1990s as environmental stewardship gained importance. The majority of research is concentrated on using genetically engineered bacteria to import and store heavy metals so that their concentration in both stimulated and actual wastewater effluents would fall below regulatory limits. Instead of focusing on improving the overall functionality of genetically engineered microbes, the aim was to increase the bioaccumulative ability to treat heavy metal pollution so that practical implications might be taken into consideration.

The cell wall and lipid membrane are physically or chemically destroyed during the bioaccumulation process, which prevents the cells from being recycled. By utilizing importer complexes, which form a translocation pathway through the lipid bilayer, microorganisms uptake heavy metals into their intracellular space through the metabolically active process known as bioaccumulation. Proteins and peptide ligands may be able to capture heavy metals once they have penetrated the intracellular space [8, 21]. The term "Metabolically active" in this context implies that bioaccumulation necessarily requires the presence of an active host cell, which presents special challenges such as the need for nutrient feeds to sustain and propagate biomass, a level of aeration to fulfill aerobic and anaerobic requirements, and accidental release of genetically modified microorganisms into the environment. Additionally, it suggests that both cytosolic and lipid membrane-embedded proteins are involved in this process. Due to the production of heterologous import-storage proteins, excessive protein aggregation, and phenotypic loss as a result of competition from native microorganisms, this poses additional, special challenges. There are two stages to bioaccumulation. First, the cell's surface is covered in metal ions. Similarly, the biosorption mechanism, this process is metabolically inactive. Following that, the metal ions are carried inside the cell. Only metabolically active cells are capable of the second procedure. The biomass grows if the second stage continues to provide favorable conditions for the growth of organisms. This allows it to bind larger amounts of metal ions than with biosorption. Through precipitation, metals can build up in microbial biomass.

BIOSORPTION

The biosorption process refers to the ability of biological materials to absorb heavy metals from wastewater via metabolically mediated or physicochemical processes [39]. Algae, bacteria, fungi, and yeasts are potential metal biosorbents [44]. It is a process that represents a biotechnological innovation as well as a cost-effective excellent tool for removing heavy metals from the aqueous solutions. In today's environmental and bioresource technology, biosorption is one of the key elements. The application of microorganisms (specifically bacteria, algae, yeasts, and fungi) as biosorbents for the removal of heavy metal have received growing interest due to the high surface-to-volume ratio; large availability, rapid kinetics of adsorption and desorption, and low cost. The main advantage is that the cells no longer exhibit metabolic activity and bind heavy metals. Because maintaining living biomass demands an additional source of nutrients and energy, this makes the process simpler and less expensive by allowing contaminants to be removed by dead organisms.

The sorption process largely depends on the functioning of the cell membranes. Before passing through the cell membrane and cytoplasm, all ions pass through the cell wall, which is made up of many polysaccharides and proteins and has many active sites for binding metal ions. The surface of the cell is often negatively charged mainly due to a lack of carboxylic and phosphate acid residues which allows for the passive binding of cations on the cell surface. Metal ions that are positively charged in the solution are drawn to the cell and absorbed on its negatively charged surface. The overall process is passive and takes place without the cell's metabolic pathways. The following processes are part of the biosorption mechanisms. Heavy metals-containing wastewater is first combined with bio, which enables metal ions to bind to the surface of microbes (biosorption). The regeneration of the biomass (desorption) is then carried out, and the remaining liquid fraction can be used to recover the metals. Ion exchange, chelation, physical force-induced adsorption, and trapping in inter- and intrafibrillar capillaries, and voids of the structural polysaccharide network are the primary components of the complex biosorption mechanism.

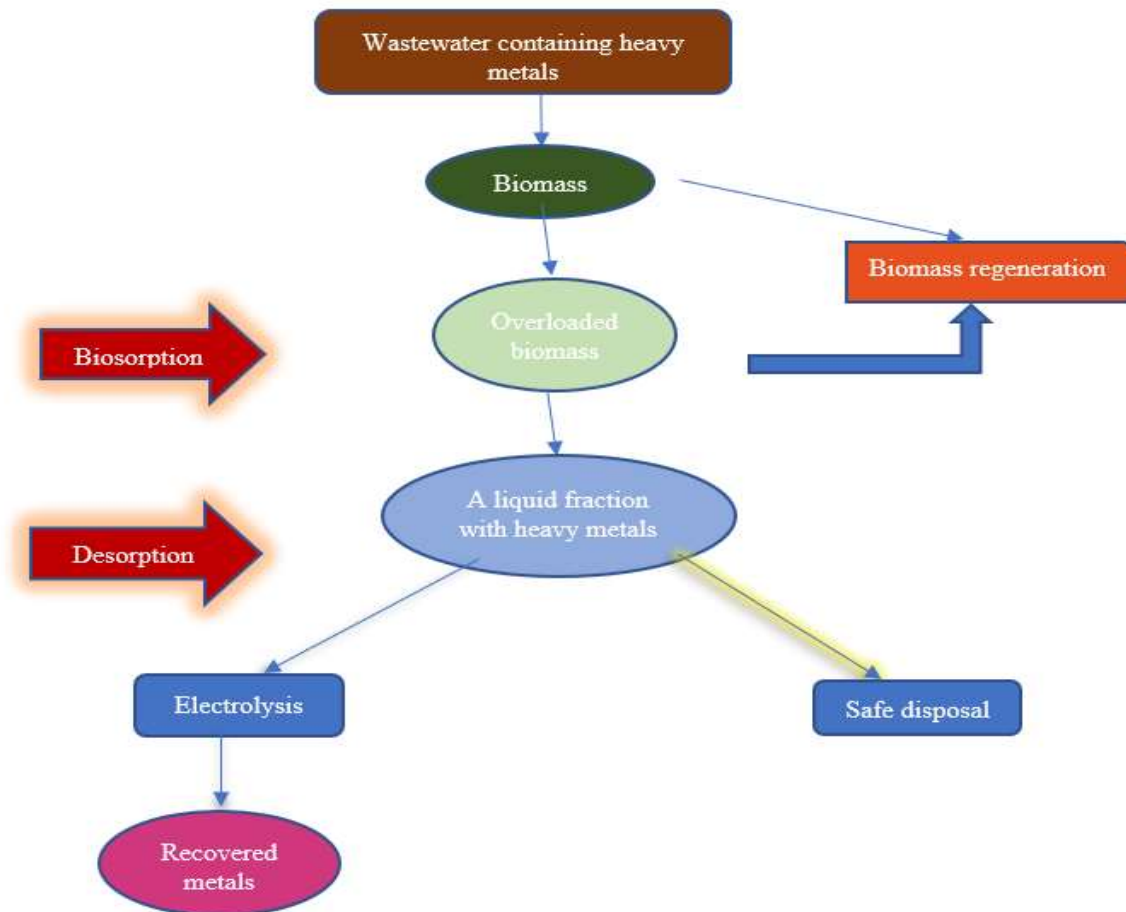


Figure 3. Heavy metals-containing wastewater treatment [9, 22]

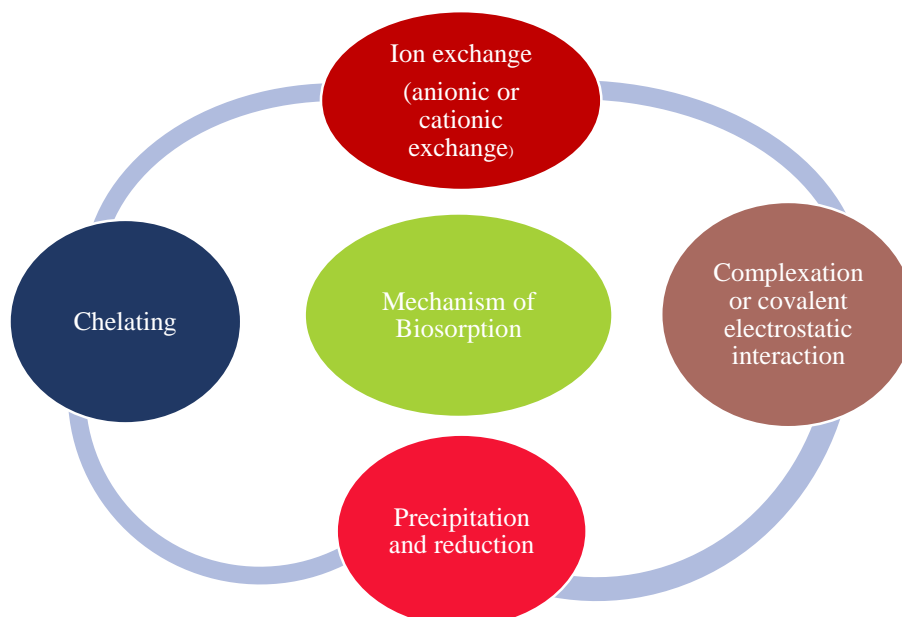


Figure 4. Shows biosorption mechanism [16]



Factors affecting biosorption

The efficiency of the mechanism of biosorption depends on the following environmental factors-

pH- The pH of a solution plays a significant role in heavy metal remediation because of being similar to the ion exchange process in some respect. It depends on the number of binding spots available on a cell’s surface. Fewer spots for metal cations at low pH are available to bind with them. Fewer metal cations can so be absorbed. However, as the pH rises, more active spots with a negative charge that attracts cations appear. [28, 43].

Temperature- Temperature affects the stability of metal ions and metal-cell complexes. The adsorption rate of biomass is increased by higher temperatures, but this can damage the sorption material [38].

Contact time- The duration of contact between the biomass and the metal-containing solution has an effect on biosorption. The lesser the contact time, the fastest the biosorption. Hence, most of the metals are adsorbed in the beginning.

Concentration and age of biomass- Since there is a high concentration of biomass present, there are relatively few bonded metals compared to the total volume of dry matter, but there is a significant degree of metal removal from the solution. Higher cell aggregates arise when there is a higher concentration of biomass, which might disturb the reactor’s balance. [39, 42].

Presence of other ions in solutions- The wastewater is contaminated with various contaminants, including different kinds of metals and the presence of other substances dissolved in a solution which can inhibit the biosorption of metals. This results from competition between other ions and removed metal ions for binding sites on cell surfaces.

Table 2. The difference between biosorption and bioaccumulation on basis of the following characteristics: [13][31]

Characteristics	Biosorption	Bioaccumulation
Affinity of metals	The affinity of metals is high under favorable conditions	Toxicity will affect metal uptake by living cells, but in some instances, there is high metal accumulation
Tolerance of temperature	Occurs within a modest range of temperature	It gets inhibited by low temperatures
Selectivity	Poor because varieties of ligands are involved	Better than biosorption
Rate of uptake of metal	Fast rate, a few seconds for outer cell wall accumulation	Usually more slowly than that biosorption. It takes time for intercellular accumulation to occur
Versatility	Metal uptake may be affected by anions or other molecules. The extent of metal uptake is usually pH dependent	Requires an energy source. It is dependent on plasma membrane ATPase activity. Frequently accompanied by the efflux of another metal
Cost	Usually, low. Biomass can be obtained from industrial waste. Only the production of biosorbent and transportation cost is there	Usually, high
pH	A wide range of pH is required	A living cell is affected heavily by a significant change in pH
Energy demand	Usually, low	It is required for the growth of a cell

ECONOMIC ASPECTS OF BIOSORPTION TECHNOLOGY

The important economic aspects of biosorption technology are:

- (i) The biomass used must be natural i.e., largely and cheaply available [7].
- (ii) The selectivity elimination of heavy metal must be under a wide range of pH, temperature, and rapid kinetics of adsorption and desorption [7].
- (iii) Microbes should have a high surface-to-volume ratio., and
- (iv) A superior capability to detoxify heavy metals [11].



The following crucial factors must be considered when evaluating how living biosorbents perform:

- (i) The physiological state of an organism,
- (ii) The cells' age and the micronutrients that were available for them to use as they grew, and
- (iii) The environmental conditions during the biosorption process such as pH, temperature, etc.

Table 3. It shows the advantages and disadvantages of the biosorption process [5]

Advantages	Disadvantages
It has high selectivity in terms of the removal and recovery of heavy metals	The potential for biological process improvement is limited because cells are not metabolizing
Low capital investment and operational costs	Early saturation can be a concern; regardless of the value, metal desorption is needed before further usage when metal interaction sites are occupied.
Reduced volume of hazardous waste	There is a saturation of active sites of metal-bound, ligands.
Ability to treat large volumes of wastewater due to rapid kinetics	There is a reversible sorption of metals on biomass.
Use of naturally abundant renewable biomaterial that can be cheaply produced	There are possibilities of increasing costs.
Ability to handle multiple heavy metals and mixed wastes	The disposal of biosorbent at the end of life.
Less need for additional expensive reagents	They require nutrients and metals cannot be separated directly with biosorbent

CONCLUSION

The conventional methods for removing heavy metal ions are widely known, but they have certain disadvantages: chemical precipitation generates activated carbon and sludge wastes, and ion exchange resins are produced from non-renewable, unsustainable resources. Hence, these traditional methods have certain disadvantages (Journal of Chemical Science and Technology Oct. 2014, Vol. 3 Iss. 4, PP. 74-102 - 75) like generation of toxic sludge or other waste products, incomplete metal removal, high energy requirements, and reagent. The use of cost-effective alternative technologies is mostly driven by rising environmental consciousness and the legislative constraints placed on effluent discharge. An alternative method for the removal of heavy metal ions uses microbial biomass. For instance, bioaccumulation—a natural biological phenomenon—occurs when microorganisms use proteins to take up and sequester metal ions in the intracellular space for use in cellular processes (e.g., enzyme catalysis, signaling, stabilizing charges on biomolecules). For many years, resource recovery was not given primary importance in wastewater treatment plants (WWTPs). But the Water Environment Federation (WEF) has recently adopted a Nutrient Energy Water (NEW) model for upgrading existing wastewater treatment facilities so that they can produce value-added products (VAPs) from sewage sludge. Therefore, in today's globalized world where natural resources are consumed excessively, sewage sludge is now regarded as a resource rather than a waste. Value-Added Products like energy, carboxylates, nutrients, and metals should be recovered from sewage sludge via a sustainable approach.

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