



# Biosorption as Technique for Remediation of Heavy Metals from Wastewater using Microbial Biosorbent

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### ABSTRACT

Biosorption, a sustainable technology, utilizes living or non-living organism and their derivative as sorbents for the removal of heavy metals from wastewater. This review explores the mechanisms and applications of biosorption in addressing environmental challenges posed by heavy metal contaminants. Pretreatment methods enhance biosorbent performance by modifying cell wall structures through physical and chemical alterations, increasing metal binding capacity. Immobilization techniques like cell entrapment and cross-linking improve biosorbent stability and reusability in continuous systems, offering controlled particle size and ease of biomass separation. Differential Scanning Calorimetry assesses biosorbent thermal stability, providing insights into performance under varying conditions. Non-living microorganisms present advantages for biosorption, including resilience to toxic wastes and extended storage capabilities. Economic considerations are crucial when evaluating biosorbent modifications for enhanced performance. The review shows that biosorption using microbial biosorbents is a versatile and efficient method for heavy metal removal from wastewater, with applications in environmental remediation and sustainable water treatment practices. Future research should focus on novel biosorption strategies and optimization of existing techniques to effectively combat heavy metal pollution.


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### Introduction

Among all the biological processes available, biosorption is an alternative procedure that uses either live or dead natural materials such as plants, animals, and microorganisms. This is because it is simpler than other standard technology [1]. Microbial biosorbents have metal-sequestering

properties that help in the remediation of heavy metal ions in solution from 'ppm' to 'ppb' levels, as well as the ability to rapidly and efficiently sequester dissolved metal ions from dilute complicated solutions. Without a doubt, biosorption is an excellent technology for treating bigger and lower volumes of wastewater with complicated heavy metal concentrations [2]. Its use is significant in the removal of heavy metals from wastewater [1].

Biosorption is a metabolic energy process in which active or dormant microorganisms perform passively, quickly, reversibly, and independently [3, 4]. The term 'sorption' refers to a physicochemical process in which one material attaches to another or is absorbed and held by another substance. Sorption includes both the absorption and adsorption processes. Adsorption is the most prevalent kind of sorption employed in 'conventional' cleanup methods. Unless the mechanism (absorption or adsorption) is defined, sorption is the preferable approach, and it may be used in any system in which a sorbate interacts with a sorbent, resulting in an accumulation at the sorbate-sorbent interface. Thus, the prefix 'bio' denotes the presence of a biological element. In other words, biosorption is a physicochemical process that involves the removal of chemicals from a solution using biological components [1, 5-8].

The aim of this review is to draw the attention of researchers to the biosorption technique for the removal of heavy metals from wastewater using microbial biosorbent. Objectives consist of the understanding of the biosorption process and mechanisms, factors affecting biosorption, microbial biosorbent for biosorption, pretreatment, immobilization, and techniques for characterization of biosorbent.

### **Biosorption process and mechanism**

The biosorption process is an appealing approach that comprises a solid phase (sorbent or biosorbent) and a liquid phase (solvent) containing the dissolved species to be sorbed (heavy metal ion) [9, 10]. This process is characterized by the disequilibrium of surface forces caused by the contact of a solid surface with a liquid phase (sorbate), which forms a surface layer of solutes on the adsorbent and results in the accumulation of metals via physicochemical interactions of metal ions with cell components of biological species [4]. Microorganisms' production of extracellular polymeric substances (EPS) is inextricably linked to adherence processes that facilitate cellular adhesion to surfaces, as well as the formation of cell-to-cell aggregation in the form of flocs, biofilm, sludges, and biogranules, which are useful in the biosorption and biosequestration of metal or metalloid ions. EPS are complex

biopolymers made up mostly of polysaccharides, proteins, humic substances, uronic acid, nucleic acid, and lipids [11]. The use of non-living microorganisms may provide some advantages over living organisms, such as lower sensitivity concentrations of toxic wastes, no need for continuous nutrient supply, easy desorption and recovery, and storage for extended periods at room temperature without putrefaction occurring [12, 13].

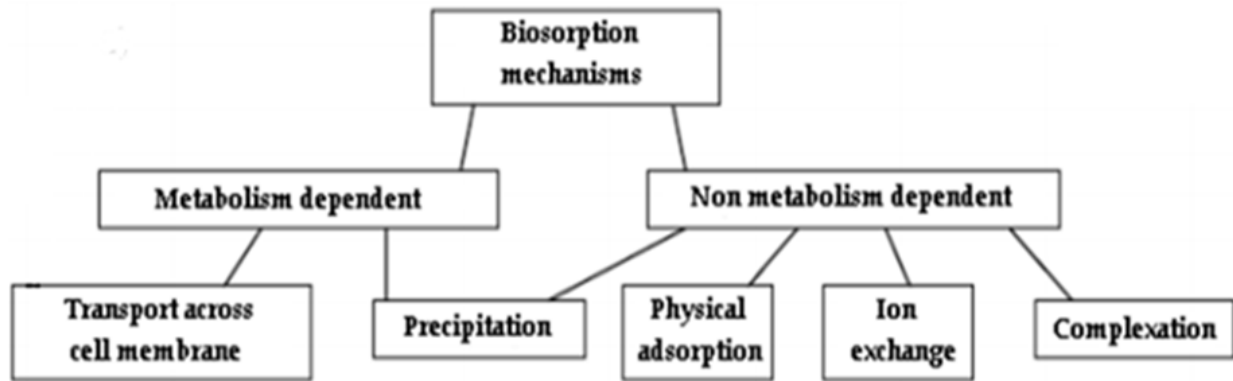
The processes involved in metal bonding must be thoroughly understood, and metal speciation in aqueous solutions must be considered since it plays an important role. A thorough understanding of microbe structure, which is highly complicated, clarifies many pathways for heavy metal entrapment. Metals are removed by a variety of methods, including extracellular immobilization, precipitation, intracellular detoxification, solubilization, and mobilization. Figure 1 illustrates the key processes involved in heavy metal biosorption.

Biosorption mechanisms have been classified into two types based on their dependency on the cell's metabolism: metabolism-dependent and non-metabolism-dependent systems. Biosorption may be classified into three types based on where the metal taken from the solution is found: extracellular accumulation/precipitation, cell surface sorption/precipitation, and intracellular accumulation [12, 14, 16-17].

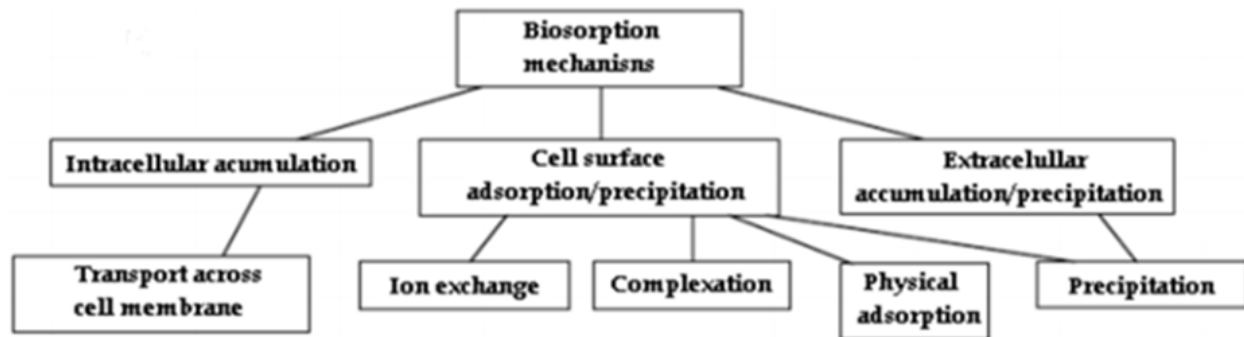
The transit of the metal across the cell membrane resulted in intracellular accumulation, which is determined by the cell's metabolism. This suggests that living cells may be all that is required for this type of biosorption to occur. It is frequently related to the microorganism's active defense mechanism, which responds in the presence of a hazardous metal. Metal absorption in non-metabolism-dependent biosorption occurs through a physicochemical interaction between the metal and the functional groups on the microbial cell surface. This can be accomplished via any of the following methods: physical adsorption, ion exchange, or chemical sorption, all of which are independent of the cells' metabolism. Microbial biomass cell walls are mostly composed of polysaccharides, proteins, and lipids, all of which include rich metal binding groups such as carboxyl, sulfate, phosphate, and amino. Non-metabolism dependent biosorption is rather fast and can be reversed [8, 14, 17].

Metal absorption during precipitation can occur both in solution and on the cell surface. Furthermore, it may be determined by the cell's metabolism if, in the presence of harmful metals, the microorganism creates chemicals that aid in the precipitation process. Precipitation may not be

### (a) Cell Dependence on Metabolism



### (b) Metal Location on the Biosorbent



**Figure 1.** Biosorption Mechanisms Classification According to (a) and (b) [14, 15].

dependent on cell metabolism if it occurs following a chemical interaction between the metal and the cell surface [14, [17-18]. Complexation can develop on the cell surface as a result of metal-active group interaction. Microorganisms may also produce organic acids such as citric, oxalic, gluonic, fumaric, lactic, and malic acids and chelate harmful metals, resulting in the production of metallo-organic compounds. These organic acids generated by microorganisms' aid in the solubilization and leaching of metal compounds from their surfaces. Heavy metals can be biosorbed or complexed with carboxyl groups present in microbial polysaccharides and polymers. Ion exchange is the mechanism involved in biosorption; microorganism cell walls include polysaccharides, and bivalent metal ions exchange with the polysaccharide counterions [8, 17-18].

#### Factors affecting biosorption

Some variables that can influence metal absorption via biosorption include metal ion selectivity, biosorbent alterations, and the biosorbent's ability to [3, 19]. Furthermore, several physicochemical parameters influence biosorption processes [1, 5, 7, 15, 19]. However, the most important variables are solution pH, biosorbent quantity, biosorbent

amount, contact duration, temperature, agitation speed, and co-ions.

#### Solution pH

Solution The pH is the most important component determining the biosorption process. The solubility of biosorbate and the action of functional groups in biosorbents are pH-dependent. For example, hydrogen and hydroxide ions are heavily adsorbed, but the adsorption of other ions is influenced by the pH of the solution [1, 7]. A rise in pH improves the removal of cationic metals but inhibits the removal of anions [15]. Heavy metals are entirely liberated under highly acidic environments. At lower pH, more protons are predicted to be accessible, reducing the electrostatic attraction of cationic biosorbate to positively charged biosorbent sites. This situation may be due to a decline in biosorption below pH 7.5. When the pH of the solution increases, the surfaces of the biosorbent become negatively charged, and no exchangeable anions on the biosorbent's outer surface remain at a higher pH [7].

#### Biosorbate

Hossain (2013) reported that the physicochemical natures of biosorbates have a significant effect on

both capacity and rate of biosorption. Physical biosorption is non-specific in nature, hence any biosorbent is biosorbed to varying degrees on the surface of every biosorbent. However, readily ionized compounds are more biosorbed, whereas low-ionized chemicals are less so. It is worthy of note that pH controls ionization.

The initial metal concentration of the solution has a considerable impact on the biosorption process. If and only if the binding sites are not saturated, increasing the initial biosorbate concentration reduces removal efficiency because it increases the amount of biosorbed biosorbate per unit weight of biosorbent. Biosorption isotherms are often used to show the relationship between varying starting metal concentrations and metal absorption at a constant temperature and biomass content [1, 7, 20].

### *Biosorbent*

The nature of both biosorbent and biosorbate-regulated biosorption processes in which chemisorption occurs is due to the mechanism involved. The process of physisorption is independent of the type of biosorbent used. Each biosorbent has unique features and functional groups that are the primary metal binding factors [7]. According to Park et al. (2010) and Hossain (2013), raising the biosorbent concentration reduces the amount of biosorbed biosorbate per unit weight of biosorbent while increasing its removal rate. It was discovered that for bacterial cells with biosorbate biosorption at a proper pH, increasing the biosorbent dose reduces the maximal specific biosorbate uptake, most likely due to cell aggregation processes. At acidic pH levels, the preceding tendency is reversed, possibly due to partial breakdown of the bacterial cell wall contents. Electrostatic interactions between cells, interference between binding sites, and decreased mixing have all been postulated as contributing reasons to biosorbent concentration dependency [7].

### *Contact time*

Metal sorption is often quick and complete in less than an hour. To identify an adequate contact duration between biosorbents and metallic ion solution, metal ion biosorption capabilities must be calculated as a function of time. Ideally, the removal is greater in the beginning and reduces progressively over time. The explanation for this might be because the biosorbents had a bigger surface area accessible for metal biosorption at the start. As the surface biosorption sites are depleted, the rate at which biosorbate is moved from the

outside to the interior sites of the biosorbent particles determines the absorption rate [5, 7].

### *Temperature*

Most biosorbate removal is endothermic, and raising the temperature often improves biosorbate removal by increasing surface activity and kinetic energy of the biosorbate, which may harm the biosorbent's physical structure [15]. According to Vijayaraghavan & Yun (2008), Park et al. (2010), and Kushwah et al. (2015), temperature appears to have a minor or no effect on biosorption processes between 20 and 35 °C. As a result, room temperature is typically ideal for biosorption processes [15].

### *Agitation speed*

Increased agitation speed improves biosorbate removal rate by reducing mass transfer resistance, but it may harm the biosorbent's physical structure [1].

### *Co-ions*

Co-ions denote the presence of other ions in a solution, which might influence the biosorption of metal ions (primary ions) on biomass, also known as competitive biosorption. To be honest, wastewater is a combination of many metals, and competition between co-ions is typical. This interaction can be synergistic, antagonistic, or non-interactive, and cannot be anticipated by single-metal research. During the biosorption of biosorbents, metal ions frequently compete for surface binding sites [7]. The existence of other ions in solution might significantly complicate the evaluation of the sorption system, depending on how the new solute species interacts with both the sorbent and the original one. Appropriate and meaningful assessment of a sorbent system including three or more metallic ions becomes significantly harder, if not impossible. Percentage removal is only useful for rudimentary orientation, such as a qualitative comparison, which is frequently used for rapid and extremely approximate screening of biosorbent materials [2].

## **Microbial biosorbent for biosorption**

Algae, bacteria, fungi, and yeasts are examples of biosorbents that are commonly employed in microbial biosorption processes [2, 4, 14, 16, 21-22]. Microbial cells are classified into two types: prokaryotic cells and eukaryotic cells. Prokaryotic cells have a considerably simpler and smaller structure than eukaryotic cells, and they lack a genuine membrane-bound nucleus. It often lacks vast, complicated internal membrane systems but

does have a plasma membrane. In contrast, eukaryotic cells have a membrane-enclosed nucleus and several membranous organelles. They have more complex morphology and are often bigger than prokaryotes. Eukaryotic organisms include algae, fungi, protozoans, higher plants, and mammals. Bacteria and archaea are prokaryotic organisms. The cell wall structure and reactivity to gram staining distinguish most bacteria as gram-positive or gram-negative. Most bacteria and yeast are unicellular. Bacterial cells typically have a diameter of 0.5-1.0  $\mu\text{m}$ , however, some can be larger than 50  $\mu\text{m}$ . Eukaryotic cells can range in diameter from 2  $\mu\text{m}$  to over 200  $\mu\text{m}$  [2]. The biosorption processes are influenced by both inherent and external variables [4].

### *Algae biosorbents*

Algae are eukaryotic creatures that possess chlorophyll, which facilitates oxygenic photosynthesis [14]. Algae are a wide and diversified category of basic plant-like creatures that range from unicellular to multicellular forms and may be found in both aquatic and terrestrial habitats. They have a high sorption capacity [2, 22]. Unlike bacteria and fungi, algae seldom create poisonous chemicals. Potential metal cation-binding sites in algal cell components include amine, carboxyl, hydroxyl, imidazole, phosphate, sulfate, and sulfhydryl, as well as chemical functional groups found in cell proteins and sugar. Algae cell walls may biosorb metals in a reversible manner, comparable to an ion-exchange resin. As a result, the biosorption mechanism may be viewed as being reliant on the composition of the algal cell wall. Algal cell walls can be composed of polysaccharides such as mannan, xylan, alginic acid, and chitin. These components, coupled with the proteins, can give acid-binding sites such as amino, amine, hydroxyl, imidazole, phosphate, and sulfate groups. The biosorption method does not include Van der Waals forces in the cellulose network of cell walls. Thus, the metal biosorption process involves both ionic charge and covalent bonding. It is assumed that proteins and polysaccharides are the primary components involved in biosorption. Covalent bonding might occur between amino and carboxyl groups, as well as ionic charge bonding between carboxyl and sulfate groups linked with these components [16].

### *Bacterial biosorbents*

Bacteria are the most abundant and adaptable types of microbes. They account for around 1018g of total live terrestrial biomass. Some bacterial species, including *Bacillus*, *Pseudomonas*, *Streptomyces*, *Escherichia*, and *Micrococcus*, have been examined for metal and organic absorption [2, 16]. Gram-

positive bacteria were dyed purple using the gram staining technique, and gram-negative bacteria were colored pink or red. Gram-negative cells have far more complicated chemical and structural surfaces than gram-positive cells. Gram-positive cells have thicker peptidoglycan layers than gram-negative bacteria, which makes their walls tougher [2]. Bacterial cells have polysaccharide slime layers and can easily give amino, carboxyl, phosphate, and sulfate groups for metal biosorption. Bacterial biomass is often generated as a waste byproduct of industrial processes; that is, it is deliberately replicated on a massive scale. Bacterial uptake capabilities range from 0.23-0.90 mmol/g [16]. Bacteria are utilized as biosorbents due to their small size, widespread distribution, capacity to develop under-regulated settings, and resilience to a wide variety of environmental conditions [2]. Heavy metal binding to the surface of the bacterial cell wall occurs in two stages. The first step includes the interaction of metal ions with reactive groups on the cell surface, whereas the second stage involves the deposition of successive metal species in increasing concentrations [16]. Bacteria may have the ability to biosorb a wide range of elements or, depending on the species, be element-selective. Microorganisms may be adapted for a certain element or combination of elements in the future utilizing recombinant DNA technology, which is based on genetic manipulation with endo-restrictive nucleases [2].

### *Fungi and Yeasts Biosorbents*

Fungi and yeast are eukaryotic organisms that are easy to cultivate. It has a high biomass production and may be genetically and morphologically modified. Fungal organisms are commonly utilized in a wide range of large-scale industrial fermentation processes [2, 14]. For example, *Aspergillus* strains are used to produce gallic acid, citric acid, and enzymes such as amylases, glucose isomerase, pectinase, lipases, and glucanases, whereas *Saccharomyces cerevisiae* is employed in the food and beverage sectors. The biomass may be obtained in large numbers at a low cost and as a byproduct of well-established commercial fermentation processes for heavy metal and radionuclide biosorption. This makes fungus an attractive raw material for developing acceptable biosorbents [2]. Fungal cell walls are complex macromolecular structures made up mostly of chitins, glucans, mannans, and proteins, but they also contain other polysaccharides, lipids, and colors such as melanin. The cell wall has been identified as the primary site for heavy metal ion sequestration by fungal biomass. The fungal cell wall is composed of up to 90% various forms of polysaccharides [16]. Heavy metal ions may be efficiently absorbed by yeasts from the genera

*Saccharomyces*, *Candida*, and *Pichia*. Most yeasts may absorb a wide spectrum of metal ions or are highly selective to a single metal ion. *Saccharomyces cerevisiae* as biosorbents is of particular interest [2].

### Microbial Aggregate Biosorbents

Microbial aggregate biosorption techniques are particularly effective for treating wastewater with low levels of heavy metal. Because of the hierarchical and self-maintained micro-ecosystem produced in microbial aggregates, they have a high potential for eliminating heavy metals under a variety of situations. Furthermore, these microbial biomasses may be cultured and propagated in wastewater treatment systems such as bioreactors, which not only enhances heavy metal removal efficiency but also keeps the microbial aggregate micro-ecosystems stable [23]. Table 1 depicts the removal efficiency of living and dead biosorbents for the biosorption of heavy metals.

### Pretreatment of biosorbent

Pretreatment of biosorbent helps to achieve optimal performance in a biosorption process [2]. Cell wall alteration can have a significant impact on metal ion binding. Several strategies have been used to modify microbial cell walls in order to increase the metal binding capacity of biomass and better understand the biosorption mechanism. Physical treatments include heating and boiling, freezing and thawing, drying, and lyophilization. The different chemical treatments used for biomass modification include washing the biomass with detergents, cross-linking with organic solvents, and alkaline or acid treatment. Pretreatments may change the surface characteristics/groups by eliminating or concealing the groups, or by exposing more metal binding sites. Some approaches have been shown to improve metal biosorption to some extent. Alkali treatment of fungal cultures has been demonstrated to greatly improve metal absorption capacity, but acid treatment of biomass has essentially little effect on metal biosorption [2, 24]. According to Park et al. (2010), pretreatment or alteration of biomass raises the cost of producing biosorbents. Nonetheless, improved sorption performance may offset the expense of modification processes. Thus, economic considerations should be considered while evaluating the influence of alteration on performance enhancement.

Because the cell wall plays an essential role in metal biosorption by nonliving cells, heat or chemical sterilizing, as well as crushing, can improve metal biosorption. As a result of cell membrane

disintegration, damaged cells have a higher accessible surface area and disclose internal components as well as a significant number of surface binding sites. Some alterations can be applied during microbe development or in pre-grown biomass. As a result, the environment in which bacteria develop influences their cell components or surface phenol type, which impacts their biosorption capability [2, 24].

### Immobilization of biosorbent

Many strategies have been explored to immobilize biomass, which may be classified into three categories: cell immobilization on inert supports, entrapment inside a polymeric matrix, and cross-linking [15]. Entrapment and cross-linking, two of the many recognized immobilization strategies available for making biosorbents usable, have been shown to be useful for biosorption [5, 6]. Immobilization of microorganisms inside a polymeric matrix has demonstrated better promise in fixed or fluidized bed reactors. The advantages include particle size management, biomass regeneration and reuse, simple separation of biomass and effluent, high biomass loading, and little clogging under continuous-flow circumstances [19, 21]. The immobilization method is a critical component for the practical use of biosorption, particularly with dead biomass. Several matrices have been used to immobilize cells. Important immobilization matrices utilized in biosorbent immobilization include sodium or calcium alginate, polysulfone, polyacrylamide, polyurethane, and silica, to name a few. The polymeric matrix controls the mechanical strength and chemical resistance of the final biosorbent particle that will be used in subsequent sorption-desorption cycles. So, it is critical to select the immobilization matrix [2].

Nonetheless, care must be taken to prevent the practical issues that arise during the immobilization process, namely mass transfer limits and increased process costs. After immobilization, the biomass is generally kept inside the interior of the matrix; consequently, mass transfer resistance is critical in determining the rate of biosorption. The existence of mass transfer resistance often slows the achievement of equilibrium; nonetheless, a proper immobilization matrix should allow all active binding sites to access the solute, although at a slower rate [19].

For industrial biosorption applications, it is critical to adopt an appropriate immobilization approach to create commercial biosorbents that preserve the capacity of microbial biomass to adsorb metal(s) during continuous treatment operations. Free microbial cells are small particles with low density, poor mechanical strength, and little rigidity, which

**Table 1.** Removal Efficiency of some living and dead biomasses for different heavy metals [18].

| Type of biomass                         | Heavy metal | Initial concentration (ppm) | PH  | Removal efficiency (% or mg/g unless) |               |
|---|-------------|-----------------------------|-----|---------------------------------------|---------------|
|   |             |                             |     | Live biomass                          | Dead biomass  |
| <i>Bacillus sphaericus</i>              | Cu(II)      | 5                           | 7   | 63.25%                                | 82.2%         |
|   | Ni(II)      | 5                           | 7   | 52.7%                                 | 59.46%        |
|   | Cr(VI)      | 5                           | 7   | 66.6%                                 | 76.5%         |
| <i>Chlamydomonas reinhardtii</i> (Alga) | Pb(II)      | 200                         | 6   | 8%                                    | 40%           |
|   | Cu(II)      | 200                         | 6   | 28%                                   | 55%           |
| <i>Tetraselmis suecica</i> (Alga)       | Cd(II)      | 6                           | 7.8 | 23.08% (24 h)                         | 76.92% (24 h) |
|   |             |                             |     | 56.04% (72 h)                         | 56.04% (72 h) |
| <i>Streptomyces ciscaucasicus</i>       | Zn(II)      | 150                         |     | 57%                                   | 73%           |
| <i>Fusarium</i> spp.                    | Zn(II)      | 80                          | 6   | 18%                                   | 12%           |
| <i>Bacillus cereus</i>                  | Cr(VI)      | 100                         | 2   | 86.79%                                | 89.87%        |
| <i>Bacillus pumilis</i>                 | Cr(VI)      | 100                         | 2   | 87.79%                                | 89.23%        |
| <i>Pantoea agglomerans</i>              | Cr(VI)      | 100                         | 3   | 83.64%                                | 85.5%         |
| <i>Mucor rouxii</i> (treated with NaOH) | Pb(II)      | 10                          | 5   | 35.69                                 | 25.22         |
|   | Zn(II)      | 10                          | 5   | 11.09                                 | 16.62         |
|   | Cd(II)      | 10                          | 5   | 8.46                                  | 8.36          |
|   | Ni(II)      | 10                          | 5   | 7.75                                  | 6.34          |
| <i>Aspergillus niger</i>                | Pb(II)      | 100                         | 7   | 79.4%                                 | 28.9%         |
| <i>Penicillium austurianum</i>          | Pb(II)      | 100                         | 7   | 75.57%                                | 98.85%        |
| Ureolytic mixed culture                 | Ni(II)      | 100                         | 6   | 17%                                   | 13%           |
| <i>Saccharomyces cerevisiae</i>         | Pb(II)      | 100                         | 7   | 61.2%                                 | 88.68%        |
| <i>Mucor arcindloides</i>               | Pb(II)      | 100                         | 7   | 62.91%                                | 93.13%        |
| Anaerobic mixed culture biomass         | Pb(II)      |                             |     | 35                                    | 51.56         |
|   |             | 50                          | 4   |                                       |               |
|   |             | 50                          | 4   | 13.6                                  | 29            |
|   |             | 50                          | 4   |                                       |               |
|   | Cd(II)      |                             |     | 11.8                                  | 28            |
| <i>Trichoderma reesi</i>                | Pb(II)      | 100                         | 7   | 35.31%                                | 80.7%         |

can lead to solid-liquid separation issues, biomass swelling, inability to regenerate/reuse, and the development of a high-pressure drop in the column mode in real applications. To achieve adequate flow rates in a fixed or extended bed reactor, high hydrostatic pressures are necessary. High pressures can disintegrate free biomass. The use of immobilized cell systems can help to prevent these issues. The immobilization of biomass in solid structures would result in a biosorbent material with the appropriate size, mechanical strength, stiffness, and porosity for usage in practical operations. Immobilized materials can be employed in the same way as ion exchange resins and activated carbons are, such as in adsorption-desorption cycles.

Furthermore, the immobilization procedure converts the biomass to a spherical form, allowing it to be employed as traditional adsorbents [5, 6]. Sizes range from 0.5 to 1.5 mm, with high exterior porosity, chemical and physical resistance, which are typical of commercial adsorbent particles [15].

### Techniques for characterization of biosorbents

Characterization of biosorbent is critical for determining the efficiency of any biosorbent used for biosorption. The approaches are often applied before and after each biosorption experiment [5]. The degree of biosorption at a biosorbent's active



**Table 2.** Method of Characterizing Biosorbent and their Function

| No. | Characterization Technique                     | Major Purpose  |
|-----|--|--|
| 1.  | Atomic Absorption Spectroscopy (AAS)           | Biosorbate (heavy metals) concentration in aqueous phase   |
| 2.  | Inductively Coupled Plasma (ICP)               | Metal concentration in aqueous phase   |
| 3.  | UV-Vis spectrophotometer                       | Biosorbate (metal or dye) concentration in aqueous phase by measuring its colour intensity       |
| 4.  | Fourier Transform Infrared Spectroscopy (FTIR) | Active sites of the biosorbent   |
| 5.  | Scanning Electron Microscope (SEM)             | Visual confirmation of surface morphology of the biosorbent                                      |
| 6.  | Transmission Electron Microscope (TEM)         | Visual confirmation of inner morphology of biomass (especially cell)                             |
| 7.  | X-ray diffraction (XRD)                        | Analysis of crystallographic structure and chemical composition of metal bound on the biosorbent |
| 8.  | Electrons Spin Resonance Spectroscopy (ESR)    | Active sites of the biosorbent   |
| 9.  | Nuclear Magnetic Resonance (NMR)               | Active sites of the biosorbent   |
| 10. | Potentiometric titration                       | Active sites of the biosorbent and its amounts   |
| 11. | Energy Dispersive X-ray Spectroscopy (EDS)     | Element analysis and chemical characterization of metal bound on the biosorbent                  |
| 12. | X-ray Photoelectron Spectroscopy (XPS)         | Oxidation state of metal bound on the biosorbent and its ligand effects                          |
| 13. | X-ray Absorption Spectroscopy (XAS)            | Oxidation state of metal bound on biosorbent and its coordination environment                    |
| 14. | Thermo-gravimetric Analysis (TGA)              | Thermal stability of the biosorbent  |
| 15. | Differential Scanning Calorimetry (DSC)        | Thermal stability of the biosorbent  |

site may be determined using advanced analytical methods [15]. Park et al. (2010) and Fomina & Gadd (2014) further explained the analytical techniques

available in biosorption research characterization and their uses, as shown in Table 2.

Most of these analytical approaches need expensive instruments and are prohibitively expensive to perform as routine measurements. Above all, the information they provide may not always be relevant in understanding and assessing biosorption mechanisms [15]. Nonetheless, diverse approaches can always give unique but complementary information on the biosorption of a specific pollutant. As a result, combining several approaches is critical for investigating biosorption processes [15, 24].

### Conclusion and recommendation

Inferences drawn from the review as well as the recommendation made for future studies are as follows.

- Biosorption can be achieved by active or inactive microorganisms. The utilization of non-living micro-organisms may give some advantages compared to living organisms,

such as lower sensitivity concentration of toxic wastes, lack of requirements for a continuous supply of nutrients, easy desorption and recovery, and storage for extended periods at room temperature without putrefaction occurring.

- The pH of the Solution is the most influencing factor affecting the biosorption process. Temperature seems to affect biosorption processes to a lesser extent or does not influence within the range from 20-35°C. Therefore, room temperature is usually desirable for the biosorption processes.
- Microbial cells used as biosorbents are fundamentally classified into prokaryotic and eukaryotic. Prokaryotes are represented by bacteria and archaea whereas eukaryotic cells are more complex morphologically and are usually larger than prokaryotes which include algae, fungi, protozoa, higher plants, and animals.
- The most frequently used biosorption processes of microbial origin include algae, bacteria, fungi, and yeasts. Unlike other biosorbents such as bacteria and fungi, algae generally do not produce toxic substances. Yeasts of species *Saccharomyces*, *Candida*, and *Pichia* are efficient biosorbents for heavy metal ions. Yeasts of *Saccharomyces cerevisiae* as biosorbents are of special interest. Bacteria



species such as *Bacillus*, *Pseudomonas*, *Streptomyces*, *Escherichia*, and *Micrococcus* have been tested for uptake metals or organics. Microbial aggregates have vast potential in removing heavy metals under various conditions because of the hierarchical and self-maintained micro-ecosystem that is established in microbial aggregates.

- Entrapment and cross-linking techniques of immobilization of biomass have been found to be practical for biosorption. Immobilization of microorganisms within a polymeric matrix has proved greater potential, with regard to continuous systems which include fixed and fluidized bed reactors.
- Concerning the characterization of biosorbents, different methods can always provide distinctive, but complementary, information on biosorption of a target contaminant. Hence, it is important to combine different techniques to explore the mechanisms of biosorption.

### Contribution of authors

Conceptualization (Yusufu Luka, Abdu Zubairu & Bitrus Kwaji Highina), methodology and visualization (Adebare Johnson Adeleke, Abdulhalim Musa Abubakar & Mujahid Umar Yunus), data curation, formal analysis, investigation and visualization (Yusufu Luka, Abdulhalim Musa Abubakar, Yagana Abubakar Musti & Mamoudou Hamadou).

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### Conflict of Interest

The authors declare no conflict of interest.

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