

Chapter 15

Biosorption of heavy metals using fungal biosorbents – A review

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Introduction

In recent years, the soil ecosystem is always being contaminated with heavy metals due to rapid industrial development, mining, and other technologies. As a result, heavy metal contamination has become a serious issue and a matter of high attention throughout the planet (Iram et al., 2013; Ayangbenro and Babalola, 2017). Heavy metals are frequently discharged by industrial processes like steel manufacturing, chemical processing and electroplating (Chaturvedi et al., 2015; Zhou et al., 2016). The Comprehensive Environmental Response Compensation and Liability Act (CERCLA), USA reported that the maximum accepted concentration of certain heavy metals in water is 0.002, 0.01, 0.01, 0.015, 0.05 and 0.05 mg/L for Hg, As, Cr, Pb, Cd and Ag respectively (Chaturvedi et al., 2015). The Indian standards for heavy metals accepted limits in the soil is 3–6, 75–150, 135–270, 250–500, and 300–600 mg/kg for Cd, Ni, Cu, Pb and Zn respectively (Nagajyoti et al., 2010). Some of the heavy metals such as cobalt (Co), copper (Cu) and zinc (Zn) are required for normal body growth and functions of living organisms, whereas the higher concentrations of other metals such as cadmium (Cd), chromium (Cr), manganese (Mn), and lead (Pb) are studied to be very toxic for human and marine life (Ouyang et al., 2002).

Heavy metal contamination is presently a main conservational problem because metal ions remain in the environment due to their non-degradable nature. The toxicity and bioaccumulation tendency of heavy metals in the atmosphere is a severe threat to the health of all organisms. Unlike organic pollutants, heavy metals cannot be broken down using chemical or biological

procedures. Therefore, they can only be converted into fewer toxic species (Ayangbenro and Babalola, 2017). However, several conventional methods like filtration, reverse osmosis, chemical precipitation, oxidation and reduction, membrane technology, ion exchange and electrochemical treatment, are available for the elimination of heavy metals from a polluted atmosphere. Though these methods have some severe drawbacks related with them, the most significant one is their incapability to remove heavy metals observed at lower concentration (≤ 100 mg/L) (Dixit et al., 2015).

Lately, microorganisms have been recognized as biological adsorbents to remove heavy metals from effluent at affordable and in an eco-friendly way (Bai and Abraham, 2003; Elizabeth and Anuradha, 2000; Gadd, 1990; Veglio and Beolcni, 1997). Bioremediation is a method of cleaning up heavy metals by biomass (or microorganisms) through the methods of biodegradation, biosorption, bioaccumulation and bioconversion working in diverse techniques (Kulshreshtha et al., 2014; Mosa et al., 2016). Biosorption is a passive method and heavy metals become adsorbed on the surface of the biosorbent (Velásquez and Dussan, 2009) showing the resistance of biosorbent to heavy metals. The mechanisms such as intracellular (binding to compounds like proteins) and extracellular (chelation and cell wall binding) sequestration of heavy metals have been offered as mechanisms for heavy metals resistance in fungi (Fawzy et al., 2017). Numerous species of fungi use their spores and mycelium can absorb specific heavy metals like Cu, Cd, Pb, Hg, and Zn (Rajkumar et al., 2012). Amongst all microbes, fungal biomass, predominantly species of *Aspergillus niger*, *Penicillium chrysogenum*, *Rhizopus nigricans*, *Saccharomyces*, etc., have a great percentage of cell wall content, which exposes metal-binding features through biosorption mechanism (Verma et al., 2017).

Bioremediation

Bioremediation is a method that uses naturally happening or genetically engineered microbes like fungi, yeast and bacteria to alter dangerous substances into fewer toxic or harmless compounds. Microbes break down the diversity of organic compounds in nature to attain nutrients, carbon, and energy for growth and survival. Bioremediation helps the growth of microorganisms to degrade pollutants by using those pollutants as carbon and energy sources. Bioremediation has been used since the late 1970s to degrade petroleum product and hydrocarbons (Black, 2005). Bioremediation is an attractive substitute for physical and chemical methods, and the use of microbes play an important role in heavy metal remediation. Likewise, the use of microbes to remediate contaminated atmospheres is maintainable and helps to restore the natural state of the contaminated atmosphere with long term environmental benefits and cost-efficient (Dixit et al., 2015).

Though, the effectiveness of microbes depends on different factors i.e. concentration, chemical nature of contaminants, obtainability and physiological

structures of the atmosphere. So the mechanisms that affect the degradation possible of microbes are either disturbed with nutritional necessities or environmental factors that promote, based on the removal of toxic compounds and their shipping techniques, bioremediation is divided into two types: in situ and ex situ (Pande et al., 2020).

In situ methods of bioremediation

In situ methods involve the treatment of contaminants at their place with minimal conflicts. It not only protects the cost of transport but also makes use of risk-free microorganisms to eliminate chemical pollutants. Similarly, it avoids conflicts as it causes treatment at the contaminated site and does not contain any excavation. This is also a harmless mode for the bioremediation of dangerous compounds. For the treatment of chlorinated solvents, hydrocarbon, dyes and heavy metal contaminated sites, different in situ bioremediation techniques have been efficiently applied (Pande et al., 2019; Roy et al., 2015). Occasionally the in situ bioremediation methods are improved i.e. engineered in situ bioremediation; while others are applied as such without any improvement and are known as intrinsic bioremediation or natural attenuation. Most frequently used in situ methods are bioventing, biosparging and bioaugmentation.

Ex situ methods of bioremediation

Ex situ treatment is not provided at the requisite sites of pollution but treated elsewhere. It needs the contaminants to be excavated from the polluted sites and shipping of the contaminated soil or driving the groundwater to the treatment site. This procedure is independent of the ecological factors as agreed out in the lack of a natural environment, and so, the treatment technique of target contaminants can be changed by physicochemical approaches before and/or during the degradation. Depending upon the physical condition of the contaminant during bioremediation, ex-situ bioremediation of soil can be categorized as solid and slurry phase treatment (Pande et al., 2019).

Moreover, the bioremediation may be due to extracellular accumulation, intracellular accumulation, cell surface sorption, and enzymatic reduction. Lately, genetic engineering methods resulted in a change of morphological and physiological structures of microbial cells that improved their bioremediation ability (Abbas et al., 2014; Garg et al., 2012). Meanwhile, ex-situ bioaugmentation of heavy metal contaminated landfill soils by single strain fungal cultures or fungal consortia is efficient in the removal of a wide range of metals such as nickel (Ni), mercury (Hg), lead (Pb), zinc (Zn), copper (Cu), chromium (Cr), manganese (Mn), and iron (Fe) (Hassan et al., 2020; Hassan et al., 2019; Khan et al., 2019).

Biosorption

Biosorption is a process that associations the use of biomaterials for sorbing, sequestering and immobilizing organic or inorganic materials from aqueous solutions. Biosorption is defined as the comprehended and examined is based on the passive sequestration using non-metabolizing, non-living biomass. Such biomass is a complex chemical material whose numerous types of diverse chemically active groups may show some tendencies to act in adhering other chemical materials or ions, attracting them from solution and attaching them to the biomass solid material. In doing so, the solid mass or molecules of biomass *sorbent* becomes augmented in those substances of *sorbate(s)* that were attracted and sequestered. This is the very scientific foundation of a very beneficial and potent technique of sorption that is accomplished of removing and extracting specific chemical species from solution (Kotrba, 2011).

Bioaccumulation

The difference between the terms passive' biosorption from what may be termed bioaccumulation which is active, metabolically mediated transport and deposition of chemical species. Biosorption is a not metabolically checked procedure that defines the passive binding of metals to non-living organisms from aqueous solutions. On the other hand, the term bioaccumulation states to active procedures that need the metabolic activity of living organisms (Davis et al., 2003; Naja and Volesky, 2011). It is very inspiring to assess bioaccumulation quantitatively since the chemical transport may work both ways inside and outside the cell, through the cell wall and cell membranes, with some organelles (e.g. vacuole) helping as deposition or storage sites into the cell. In addition to it, some cells tend to yield extracellular chemicals. The occurrence and quantity of these possibly sorbent-binding substances may vary greatly with the level and type of cellular metabolic activities. The process is therefore complex and, correspondingly, bioaccumulation is thus difficult to study quantitatively (Kotrba, 2011; Fomina and Gadd, 2014). In the common sorption, two basic terms such as "absorption" and "adsorption" is used often. Adsorption is realized to involve the interphase aggregation or concentration of substances at a surface or interface. Such a procedure can take place on the interface of any two phases, like gas-liquid, gas-solid, liquid-liquid, or liquid-solid interfaces. Absorption, contrariwise, is a procedure in which the atoms or molecules of one phase interpenetrate near evenly between those of another phase to form a "solution" with it (Kotrba, 2011).

Mechanism of the biosorption process

Primarily, biosorption can be defined as the passive sequestering of metal ions by metabolically inactive biomass. This kind of metal uptake may take

place using any one or a mixture of several mechanisms like complexation, coordination, chelation, ion exchange or microprecipitation and entrapment (Robalds et al., 2016). The above mechanisms are associated with both living or nonliving cells of microbes but the previous two mechanisms such as entrapment and Micro-precipitation denote to immobilization of metal species earlier solidified found frequently external or even inside the cells like the extracellular polymeric capsule or cytoplasmic components (Kotrba, 2011). Physical adsorption occurs because of weak Van der Waals' attraction forces, while the so-termed chemisorption is a result of moderately strong chemical bonding among adsorbates and adsorbent surface functional groups (Bhatnagar and Sillanpää, 2010).

Metal biosorption and bioaccumulation

Not like with other biomass types, the capability of microbes to interact with and to accumulate a variety of metal ions from their aqueous environs has been examined widely in the last two decades due to the hazard of heavy metal toxicity (Kotrba, 2011). Microorganisms can accumulate metals both by a metabolism-independent, passive, or a metabolism dependent, active method. Therefore, complete accumulation is influenced by two features of the cell: sorptivity of the cell envelope and ability for taking up metals into the cytosol. Passive adsorption and is dependent on component-specific transport systems but active uptake into the cytosol is typically slower (Gadd, 1988a,b). Many biomaterials like dead cells of bacteria, moulds, yeasts, and algae have been used as active metal-removing agents (Ahluwalia and Goyal, 2007; Rahim et al., 2012). Among these, fungal biomass has developed as an outstanding biosorbent since of large cell size, a high percentage of cell wall material, simple medium/nutrient necessities, high biomass harvest, and easy genetic and morphological manipulations (Verma et al., 2017).

Living cells of *Lentinus edodes* were used for biosorption of Zn, Cd, and Hg (Bayramoğlu and Arica, 2008). Açıkel and Alp (2009) showed that the biomass of *Rhizopus delemar* was used for Ni and Cu removal in single metal systems. Tsekova et al. (2010) found that the immobilized and free cells of *Aspergillus niger* strain B 77 were observed to remove Mn, Fe, Ni, Cu, Zn, Cd, and Pb. The Ca-alginate cells were observed to be a more effective biosorbent than the other cells. Sen and Ghosh (2011) observed that the biosorption of Cr (VI) by *Fusarium solani*. Damodaran et al., (2013) reported that the bioaccumulation of Cr(VI), Cu(II), Zn(II), Cd(II), and Pb(II) by *Galerina vittiformis* having light brown fruiting body was isolated from a municipality landfill. Li et al. (2013) observed that the bioremediation possible of the fungi can be improved by the adding of chemical and biological chelators. The *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* were involved in bioremoval of Zn(II), Fe(III), Cu(II) and Cd(II) in a great

salty atmosphere. Kurniati et al. (2014) stated that the bioremoval of Hg by *Aspergillus flavus* strain KRP1 from agitation as well as static systems.

Factors influencing the heavy metal sorption

The amount of biosorption efficacy of bacterial cells for heavy metal ions be determined by upon many external operating factors like solution pH, temperature, contact time, initial concentration of metal ions, biosorbent dosage and agitation rate (Liang et al., 2014; Chaturanga et al., 2014; Gupta et al., 2014).

Solution pH

The solution pH is one of the most significant ecological factors. The pH value of solution powerfully impacts not only the site detachment of the biomass' cell surface but also the solution chemistry of the heavy metals like hydrolysis, complexation by organic and/or inorganic ligands, precipitation, redox reactions, the speciation and the biosorption obtainability of the heavy metals (Esposito et al., 2002; Fiol et al., 2006). pH also disturbs the solubility of the metal ions in the solution, where H^+ ions exchange some of the positive ions from the biomass surface. The biosorption ability of metal cations raises with an increase in pH values, and this may be due to the extra negative binding sites showing on biomass surface (Aksu and Gülen, 2002). At low pH values, the attaching sites of the cell wall are blocked and related with hydrogen ions that delay the entree of metal cations due to repulsive forces to the functional groups on the surface of biomass. On the conflicting, biosorption efficacy of metal anions increases with reduction in pH ranges due to the increase in positively charged biomass surface, while at increase pH, the repulsive forces among the negatively charged surface of biomass and metal anions decrease the metal uptake ability (Aryal et al., 2010; Ziagova et al., 2007). For diverse biosorption system of metal ions, the optimal pH is changed. Volesky (1990) stated that the optimal pH range is 5–9 for biosorption of copper by *S. cerevisiae*, and 4–5 for uranium. Biomass of Cu(II) and Pb(II) by *Aspergillus flavus* and *Aspergillus niger* respectively. The pH value is 8–9 for biosorption of Cu(II) by *A. flavus* and *A. niger* showed maximum sorption capability for Pb(II) at pH value is 4–5.4 (Iram et al., 2015).

Temperature

The temperature of the solution disturbs the sorption of metal ions on biosorbents. Biosorption of heavy metals is frequently altered with an increase in temperature due to the rise in surface action and kinetic energy of the solute, but the damage of some binding sites obtainable for metal ions can take place at higher temperatures (Aryal and Liakopoulou-Kyriakides, 2014).

Temperature appears to disturb biosorption only to a smaller extent within the range from 20 to 35 °C (Vegliò and Beolchini, 1997). Advanced temperatures typically improve sorption due to the raised surface action and kinetic energy of the solute (Sağ and Kutsal, 2000; Vijayaraghavan and Yun, 2007); though, physical destruction to the biosorbent can be predictable at higher temperatures. Owing to the exothermic nature of some adsorption methods, an enhance in temperature has been observed to decrease the biosorption ability of the biomass (Mameri et al., 1999; Suhagini et al., 1999).

Brady and Duncan (1994) reported that temperature (5–40 °C) had a slight effect on the accumulation level of Cu^{2+} , Co^{2+} or Cd^{2+} using free cells of *S. cerevisiae* in suspension. Adsorption reactions are usually exothermic, so biosorption ability raises with reduction of temperature (Kapoor and Viraraghavan, 1997a,b). In the value of 15–40 °C, the highest equilibrium biosorption capability for Pb(II), Ni(II) and Cr(VI) ions using the inactive *S. cerevisiae* reached a temperature of 25 °C. The reduction in ability at higher temperature among 25 and 40 °C discovered that the methods of biosorption for these metal ions by *S. cerevisiae* are exothermic. The reduction of biosorption ability at a higher temperature may be owing to the destruction of active binding sites in the biomass (Özer and Özer, 2003).

Contact time

The contact time is also one of the most significant factors for the management of wastewater by biosorbents. At the very opening, biosorption continues fast, and most metals are biosorbed. However, for attaining equilibrium, significant the interaction time among biosorbent and metal is significant to determine the biosorption behavior. It is very significant for economical profitable exploitation. If equilibrium is optimally attained within a fewer period, biosorption takes place fast. Various scientists assessed the result of contact time on biosorption of heavy metals (Merrin et al., 1998; Prakasham et al., 1999; Srinath et al., 2003).

The biosorption method of heavy metal using *S. cerevisiae* typically completes quickly. The biosorption of metals like copper, zinc, lead and uranium by *S. cerevisiae* is a rapid method and often reaches equilibrium within a few hours (Kapoor and Viraraghavan, 1997a,b). The adsorption of Pb(II) and Cu(II) by *A. flavus* and *A. niger* exposed that nearly 82% and 83% removal of heavy metal ions and saturation level of entire biomass were documented for 10 min (Iram et al., 2015).

Initial metal ion concentration

The initial solute concentration appears to affect biosorption, with a greater concentration resulting in a high solute uptake (Ho and McKay, 1999; Ho and McKay, 2000; Binupriya et al., 2007). This is because, at lesser initial solute concentrations, the ratio of the initial moles of solute

to the obtainable surface area is low; later, the fractional sorption becomes independent of the initial concentration. Since the initial metal ion concentration offers the essential driving force to overwhelm the resistance to the mass transfer of metal ions among aqueous and solid phases, but reductions the sorption percentage (Aryal et al., 2010). The biosorption of Cu(II) and Pb(II) by the biomass of *A. flavus* and *A. niger* was 20.75–93.65 mg/g and 3.25 to 172.25 mg/g respectively, with initial metal concentration 200–1400 ppm (Iram et al., 2015).

Biosorbent dosage

The biosorbent dosage powerfully impacts the extent of biosorption. In various examples, lower biosorbent dosages produce advanced uptakes and lower percentage removal effectiveness (Aksu and Çağatay, 2006). A raise in the biomass concentration usually increases the quantity of solute biosorbed, due to the augmented surface area of the biosorbent, which in turn increases the number of binding sites (Esposito et al., 2001). Equally, the amount of biosorbed solute per unit weight of biosorbent reduction with increasing biosorbent dosage, which may be due to the complex interaction of numerous parameters. The significant factor such as an increase in biosorbent doses is that the available solute is inadequate to cover the entire available sites on the biosorbent, normally resulting in low solute uptake (Tangaromsuk et al., 2002). Gadd et al. (1988) reported that the interference among binding sites due to augmented biosorbent dosages cannot be overruled, as this will result in low specific uptake.

Agitation rate

The degree of biosorption method can be influenced by using external film diffusion. With suitable agitation, this bulk transfer resistance can be reduced. While raising the agitation speed, the diffusion rate of a solute from the majority of liquid to the liquid border layer nearby atoms becomes great due to better turbulence and the reduction in thickness of the liquid border layer (Evans et al., 2002). Beneath these circumstances, the range of the external diffusion coefficient becomes higher (Shen and Duvnjak, 2005). Lastly, at advanced agitation speeds, the boundary layer becomes thin, which typically improves the rate at which a solute diffuse through a boundary layer.

Mycoremediation

Fungi, otherwise known as *Eumycota*, are a nonphotosynthetic, filamentous, saprobic group of microorganisms that rely on organic substrates as their sole source of carbon and energy for growth and metabolism. Yeasts are one of the subclasses of fungi, which are unicellular, while fungi are multi-celled

(Willey et al., 2016). Due to their innate ability to degrade a wide range of materials from natural recalcitrant polymers like lignin to synthetic xenobiotics like plastic, fungi are used in bioremediation. This is termed as *mycoremediation*. Fungi possess higher biomass because of their filamentous nature and are potential biosorbents for bioremediation of heavy metal-laden effluents and landfills (Goltapeh et al., 2013). The interaction between the fungal hyphae and metal species is dependent on the physiology and chemical structure of the former.

Features of fungal and yeast biomass

Metals, metallic species and metal ions are inherently involved in the metabolic activities of the fungal cell. Metal ions of iron, zinc, nickel, are required as co-factors or ligands for the activation of enzymes that catalyze numerous metabolic reactions. These uptake and transport of these metal ions are both passive and active. Similarly, the same metal ions at higher concentrations have proved to be toxic to the cellular process such as the synthesis of protein, nucleic acids, and corrosion of the cell wall. The inhibitory role of metals at higher concentration in biochemical reactions stimulate the fungal cell to develop several metal tolerance/resistance mechanisms (Volesky and Holan, 1995).

These mechanisms include both sequestration and bioaccumulation of metal ions by the organism based on the aspect of the toxicity. The passive metal uptake and binding to the cell wall due to its chemical makeup and constituents is a common “suicidal” strategy of the cell when faced with uncommon concentrations of heavy metals in the external cellular environment. Nevertheless, this natural tendency of adsorption by the fungal biomass is a blessing in disguise, when it comes to bioremediation of a heavy-metal contaminated soil or water resource.

Though sequestration is not localized solely on the cell wall, it is the primary site of biosorption. The fungal cell walls contain glucans, mannans, glycoproteins, chitosan and chitin, along with other biomolecules such as proteins, and lipids. Ultrastructural studies of the cell wall revealed two phases which help us determine how cell wall acts a biosorbent layer to metal ions. While the outer layer is composed of glucans, mannans, or galactans and an interwoven microfibrillar layer consist of parallel arrangement of chitin chains which confers a crystalline nature to it. Pigments, polyphosphates, and inorganic ions are also part of the two phases of the fungal cell wall. Yeast cell walls which are made of non-cellulosic glucans and an outer layer of mannoprotein, also contribute to the adsorption property of the organism. While fungal cell wall is dominated by chitin-chitosan complex, the yeast cell walls possess a mannan-glucan arrangement (Goltapeh et al., 2013).

The positively charged chitin-chitosan complex in cell walls is often interspersed by negatively charged phosphates and glucuronic residues. This contributes to the observation of varied mechanisms of sequestration. Meanwhile, metal ions can also accumulate inside the cell in its cytoplasm or specialized organelles (Sağ, 2001).

Mechanisms of fungal biosorption

Metabolism dependent and independent biosorption

As discussed earlier, the uptake of metal ions is both passive and active and is dependent on the metabolic state of the cell. Active metal uptake is a metabolism dependent transport of metal ion across the membrane and is energy-driven. In metabolism-dependent mechanisms, metal ions are entrapped in inter- and intrafibrillar capillaries, spaces of the polysaccharide material, and diffusion through the cell wall and membranes of fungi. These mechanisms are primarily employed by live fungal cultures. Whereas the passive sequestration of metal ions is independent of the metabolic reactions of the cell and can be carried out by both live and dead biomass of fungal cells. Mechanisms of metabolism-independent biosorption of metal ions are physical adsorption, ion-exchange, complexation, and chelation (Danesh et al., 2013). Precipitation and crystallization are processes which are often coupled to metabolism if necessary, to the physiology of the cell.

Extracellular precipitation of metals

Precipitation of metals as mycogenic crystals i.e., by the secretion of extracellular metabolites is often combined with an internal metabolic process. This process is a metal tolerance mechanism exhibited by fungal cells in the presence of toxicity. Certain wood-rotting fungi were found to be capable of transforming zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) into zinc oxalate dihydrate ($\text{C}_2\text{O}_2\text{Zn} \cdot 2\text{H}_2\text{O}$), copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) into copper oxalate hydrate ($\text{C}_2\text{CuO}_4 \cdot x\text{H}_2\text{O}$), cadmium sulfate ($3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$) into cadmium oxalate trihydrate ($\text{C}_2\text{CdO}_4 \cdot 3\text{H}_2\text{O}$) and lead nitrate ($\text{Pb}(\text{NO}_3)_2$) into lead oxalate (PbC_2O_4). They can remove heavy metals from a metal-amended substrate by precipitation as metal oxalates (Kaewdoug et al., 2016). Biomineralization is a precipitation mechanism through which metals can be extracted out of a source or an ore. The fungal strains *Aspergillus* sp. UF3 and *Fusarium oxysporum* UF8 produced calcite and co-precipitated heavy metals Lead and radionuclide Strontium as carbonates. The production of the enzymes, carbonic anhydrase in synergism with urease by the calcifying fungi helps the biomineralization process (Dhami et al., 2017). A strain of *Aspergillus niger* isolated from a metal-contaminated soil precipitated nickel in the environment internally as well as externally into small rectangular crystals of nickel-oxalate dihydrates (Magyarosy et al., 2002). One of the

widely studied yeast, *Saccharomyces cerevisiae* is identified to produce H₂S resulting in the precipitation of cadmium as insoluble sulfides (Minney and Quirk, 1985).

Chelation and complexation of metal ions

In addition to precipitation, complexation of the metal ions by the production of an efficient metal-ion chelator such as organic acids, and other metal-binding complexes such as siderophores, is often observed in fungi and yeast. Siderophores are high-affinity iron-binding molecules that chelate the Fe³⁺ ions in the extracellular space. Organic acids such as citric acid and oxalic is also an excellent metal-ion chelator, and are highly involved in the metal speciation (Gadd, 1999). Several brown-rot fungi are known to exhibit metal tolerance to copper, by the production of extracellular oxalate to chelate copper to copper oxalate precipitate (Akgul and Akgul, 2018). Various white-rot fungi have also displayed the same strategy to resist the toxicity of zinc, cobalt and calcium (Jarosz-Wilkolazka and Gadd, 2003).

Passive uptake of metal ions

The fungal cell surface is a mosaic of a large variety of functional groups such as carboxyl (-COOH), amide (-NH₂), thiol (-SH), phosphate (PO₄³⁻), and hydroxide (-OH), sulfate, imino, sulfonate, imidazole, sulfydryl, carbonyl, thioether, and other moieties. A certain pseudo-ion-exchange process in which metal ion is exchanged for a counterion in the biomass or resin. Moreover, dead fungal biomass shows a strong affinity for metal ions due to the lack of protons produced during metabolism. *Rhizopus arrhizus*, a fungal strain capable of uranium biosorption was identified to exchange hydrogen ions from biomass for uranyl ions (Treen Sears et al., 1984). Similarly, *Aspergillus niger*, a versatile ascomycete, released potassium ions, in addition to calcium and magnesium ions, during the biosorption of lead, cadmium and copper, indicating an ion-exchange process (Kapoor and Viraraghavan, 1997a,b). These plethora of positively and negatively charged functional groups on the fungal cell wall, especially amino groups of chitin (R₂-NH) and chitosan (R-NH₂), that contain carboxylate groups and fungal phenolic polymers such as melanins that contain phenolic units, are responsible for the physical adsorption, electrostatic interactions, providing them with metal-binding sites (Sağ, 2001; Gadd, 1994, Oyewole et al., 2019).

However, it is interesting to note that none of the mechanisms occurs singularly. The fungal cells do not engage in one single metabolism-dependent or -independent process, rather they take part in a step-wise metal sequestration strategy. These strategies often involve both metabolism dependent and independent mechanisms such as the production of extracellular metabolites to chelate the metal ions as well as passive adhesion and entrapment of metal ions to the fungal cell wall, occurring simultaneously (Pepper et al., 2011).

Immobilization of fungi and biosorption

Cell immobilization is a technique enhances cell density and metal exposure, all the while protecting it from the external stress indicators. The fungal cells are often bound to a support molecule such as agarose, chitin by entrapment, encapsulation, or retention by permeable membranes (Tampion and Tampion, 1987). An *Aspergillus niger* strain was immobilized with alginate into a microsphere biosorbent and used for Th (IV) removal. It was capable of removing up to 303.95 mg/g when compared to its' non-immobilized counterpart (Ding et al., 2019a,b). Mycelial pellets that are either self-immobilized or stabilized with composite materials such as alginate, chitosan, pectin, biochar colloids show higher metal removal efficiency when compared to either biosorbent alone (Wang et al., 2019; Chen et al., 2019; Legorreta-Castañeda et al., 2020).

Fungal metal resistance genes (MRG)

We have discussed earlier that the intracellular uptake of non-essential metal ions is often coupled with the expenditure of metabolic energy. They are also often accompanied by the excretion and production of complexation or chelator molecules such as glomalins, siderophores and metallothioneins.

Heavy metal tolerance by Metallothioneins

Metallothioneins (MTs) are small, cysteine-rich proteins whose production is induced by the stress created by a higher concentration of metal ions such as cadmium and lead. They form complexes with toxic metals by binding to them and preventing them from interfering with cellular functions (Pepper et al., 2011). They detoxify metals by chelating metal ions such Cd, Hg, Zn, Pb, Ag via thiolate bonds in their cysteine residues, and is found in animals as well as plants (Nedkovska and Atanassov, 1998). The molecular mechanism behind the chelation of metals by metallothioneins is well-studied in the yeast model organism *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. CUP1, MTI and MTII genes encode metallothioneins that are specific for copper resistance in *Saccharomyces cerevisiae* and *Candida glabrata* respectively, even though it's promoter can ensure metallothionein production if homologous ORFs (Open Reading Frames) from *Drosophila* or other MT genes present. It is important to note that the metallothionein (MT) gene is upregulated in response to heavy metal stress (Macreadie et al., 1994). A study by Shoaib et al. (2019) found a necrotrophic fungus *Macrophomina phaseolina* that could tolerate up to 3000 ppm of chromium stress by the expression of MT genes. A metal-tolerant ectomycorrhizal *Hebeloma mesophaeum*, isolated from a lead smelter site, was found to sequester substantial amounts of Cadmium inside its vacuoles in complexation with metallothioneins.

An increase in basal levels of metallothionein HmMT3 transcripts also correlated with the hike in cadmium tolerance of the isolate (Sácký et al., 2019).

Fungal production of zinc transporters, siderophores, and others

Siderophores are low molecular weight ferric iron-binding organic molecules, that have a hydroxamate functional groups in fungi, to chelate iron ions and supply it to the cell. Whereas, in bacteria, the metal-binding receptor is catecholate functional group (Willey, 2016). A report by Brandenburger et al. (2017), identified a putative nonribosomal peptide synthetase CsNPS2 gene, which encodes for a fungal type VI siderophore synthetase. Moreover, the production of extracellular metabolites is also a stress response to toxic metal concentrations. Cadmium (Cd) was found to induce bioaccumulation of glutathione (GSH) mediated by c-glutamylcysteine synthetase (c-GCS) in the ectomycorrhizal fungus *Hebeloma cylindrosporium* (Khullar and Reddy, 2019). The genes such as metallothionein, Zn transporter, glutathione S-transferase, and the stress protein genes in *G. intraradices* was found to be activated under the presence of Cd, Zn, and Cu (Miransari, 2017). The genes coding for GSH biosynthesis enzymes, γ -glutamylcysteine synthetase (*Hc γ -GCS*) and glutathione synthetase (*HcGS*) were highly regulated when *Hebeloma cylindrosporium* by arsenic stress (Khullar and Reddy, 2020).

Metal resistance genes and mycorrhizal fungi

Interestingly, the metal resistance genes (MRGs) are observed considerable higher in symbiotic fungi associated with plant rhizosphere, wherein the arbuscular mycorrhizae (AM fungi) aid the plant in hyperaccumulation and phytoextraction of metals from the contaminated environment (Ow., 1996). The arbuscular mycorrhizal fungal protein glomalin which is a putative homolog of heat shock protein 60 (hsp60), is an alkaline-soluble glycoprotein produced by several AM fungi such as *Gigaspora rosea*, *Rhizophagus irregularis* and *Glomus intraradices* (Gadkar and Rillig, 2006; Javaid, 2011). This putative homolog gene is considered as a functional molecular marker to selectively identify AM fungi (Magurno et al., 2019). The transcription of this gene is regulated in response to heavy metal stress, and the protein is secreted in copious amounts. They bind irreversibly to metal ions such as Cu, Cd, Pb, Mn, Al, thereby decreasing their bioavailability to the plant root system (Gonzalez-Chavez et al., 2004; Aguilera et al., 2011).

Batch studies of fungal biosorption

Batch studies are the most effective technique employed by several researchers to determine the metal removal efficiency of different fungal and yeast strains, and their optimal conditions necessary to implement them in situ or

ex situ (Lu et al., 2020; Kumar et al., 2019; Godlewska-Żyłkiewicz, 2019; Noormohamadi, 2019).

Equilibrium modeling

Despite various means of biosorption by live, dead or immobilized fungal biomass, the interactions of biosorbent and the biosorbate has to be evaluated for their equilibria. Owing to the complex two-phased structure of the fungal cell wall, the equilibrium of the biosorption is often difficult to be identified. On the other hand, numerous studies report the use of adsorption-type isotherm to study the mechanism of fungal cell-metal interaction. Meanwhile, like the prior discussion on the factors affecting the biosorption between the metal ion and the functional groups of the biosorbate, the adsorption is also dependent on the same factors (Kotrba, 2011). There are innumerable adsorption isotherm models proposed for determining the interaction between the sorbent and sorbate. Few of them repeatedly used are Langmuir, Freundlich and Brunauer-Emmett-Teller models.

Langmuir model is a non-linear isotherm which reveals monolayer coverage where all sites are alike, and each site can bind only one adsorbed species and the adsorption of a single species is not influenced by neighboring occupied sites. Its linearized form is expressed as the following:

$$Q = \frac{Q_{\max} b_L C_f}{1 + b_L C_f}$$

Where, Q_{\max} , which corresponds to the maximum achievable uptake by a system; and b_L , which is related to the affinity between the sorbate and sorbent.

Modeling of the biosorption between the fungal strain *Rhizopus arrhizus* showed that one gram of dry fungal biomass could bind up to 312 μmolg of Zn^{2+} ions (Zhou, 1999). When a strain of *Aspergillus* sp. was used to remove platinum (PtIV) and palladium (PdII) ions, the adsorption saturation was reached at 5.49 mg and 4.28 mg per gram of live fungal cultures. Similarly, another *Aspergillus* sp. isolate was able to bind to up to 50.65 mg and 83.40 mg of Uranium (UVI) ions when treated with active and inactive fungi microspheres (Ding et al., 2019a,b). One gram of dried biomass *Trichoderma* fungus was able to adsorb 25.05 mg of lead(II) from aqueous solutions (Zarei et al., 2019). Many more studies have been reported where isotherm modeling has been used to determine the maximum adsorption capacity of fungal biomass, live, dead, immobilized or otherwise. The adsorption data was found to fit well to Langmuir isotherm model, in all these cases. The interpretation behind this modeling could be attributed to the above-discussed nature of the biosorbent (fungal cell wall), as well as the mechanism of biosorption (Kotrba, 2011).

This isotherm model is a purely empirical model wherein physical meaning to parameters are avoided. The linearized form of the model is as given below:

$$Q = K_F C_f^{1/n_F}$$

Where, K_F , which corresponds to the binding capacity; and nF , which characterize the affinity between the sorbent and sorbate

The biosorption data that is well suited to this model can be asserted as heterogeneous surfaces with a logarithmic diminution of the affinity during surface coverage. Most studies of fungal biosorption fit well to Langmuir isotherms rather than Freundlich. While the data is not completely unfit, it is better suited to the former.

Adsorption kinetics

Most biosorption reactions reach their equilibria depending on primary factors such as available binding sites and continue forward to desorption depending on the mechanism of biosorption. The adsorption system that is implemented on ground zero in a treatment plant necessarily requires the understanding of the sorption rate as well as the sorption type, external film adsorption (physisorption) or intraparticle diffusion (chemisorption). These can be identified with the help of empirical kinetics models such as pseudo-first-order and pseudo-second-order. The pseudo-first-order rate equation, otherwise known as Lagergren's model, Lagergren's first-order is described by the following linear equation

$$\ln(q_e - q) = \ln q_e - k_1 t$$

Where q is the metal concentration in the solid phase at time t , and q_e is the equilibrium metal concentration in the solid phase.

Whereas, pseudo-second-order parameters are derived from plotting t/q against t , and the model is linearized into the following form

$$t/q = t/q_e + 1/k_2 q_e^2$$

Both kinetics models describe the adsorption rate based on the adsorption capacity determined by the above-described equilibrium modeling, but the latter assumes second-order dependence on different sites of sorption on the biosorbent. This assumption of the pseudo-second-order model makes it more suitable for complex cell wall structures as that of fungal hyphae. Several batch tests conducted with various fungal biosorbents such as *Phanerochaete chrysosporium* with intracellular mineral scaffold (Lu et al., 2020), *Simplicillium chinense* QD10 (Jin et al., 2019), and *Aspergillus terreus* (Shokoohi et al., 2019) for the biosorption of metals Cd, Pb, and Cr respectively, affirm the assumption.

Desorption and recovery of metals from fungal biosorbents

The main objective of desorption from the fungal biomass is to regenerate the biosorbent for repeated use in a batch reactor system, as well as the recovery of metal ions (leachate) for economically feasible uses. The metals are recovered from the biosorbent as chlorides, carbonates, bicarbonates,

hydroxides of sodium, potassium or calcium (Shen and Chirwa, 2020; Saad et al., 2019; Salar, 2018).

Conclusion

While fungal biosorbents have been extensively studied for their metal removal capacities, not much is known about their equilibria and adsorption kinetics. Similarly, more research is required in the field of batch reactor studies to determine the desorption and recovery rate of metals from the fungal biomass. Whereas, the potential of applying arbuscular fungi with correlation to phytoremediation in metal-contaminated landfills are high, compared to ex-situ batch reactor designs. Since in situ bioremediation is considered more suitable for heavy metal contamination, research focus could be shifted towards the role of mycorrhizal fungi in metal remediation (Kotrba, 2011).

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