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Biosorption of Heavy Metals

Saba Shamim

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Abstract

Industrialization has led to introduction of heavy metals in the environment. Heavy metals are known to persist in the environment and become a risk for organisms. Microorganisms are present in industrial effluents. They have adopted different strategies to cope up with the harmful effects of these metals. These strategies can be metabolism dependent or independent. One such strategy is biosorption which is binding of metal ions with metal binding proteins present on the cell wall. Biosorption is exhibited by bacteria, algae, fungi and yeasts. Not only living organisms, but also residuals of dead bodies of microorganisms shows biosorbent properties like agricultural wastes including husk, seeds, peels and stalks of different crops. Different factors affect the rate of biosorption which includes temperature, pH, nature of biosorbents, surface area to volume ratio, concentration of biomass, initial metal ion concentration and metal affinity to biosorbent. Various models including Freundlich model and Langmuir model can be used to describe biosorption. Recovery of biosorbed metals can be done using agents like thiosulfate, mineral acids and organic acids. Choice of desorption agent should be carefully selected to prevent alteration of physical properties of a biosorbent.

Keywords: biosorption, heavy metals, bacteria, algae, fungi, yeasts

1. Introduction

Nature has gifted our earth with four spheres; biosphere, lithosphere, hydrosphere, and atmosphere. Together these spheres are important for maintaining a balanced ecosystem [1]. The industrial revolution in the past five decades is remarkable. Due to anthropogenic activities, increasing population, industrialization and urbanization, all spheres have become polluted [2–7]. There are two main sources of introduction of heavy metals in the environment (1) natural sources which includes volcanic emissions, forest fires, deep-sea vents, and geysers [8] and (2) anthropogenic sources which includes mining and smelting sites, metal-manufacturing plants, painting- and coating-industries and tanneries. These heavy metals are

released directly into the environment. Metals exhibit health issues [9] if their concentrations exceed allowable limits. Even when the concentration of metals does not exceed these limits, there is still a potential for bioaccumulation and associated chronic toxicity as heavy metals are known to be accumulative within biological systems [10]. These metals include arsenic, cadmium, chromium, copper, lead, mercury, nickel, and zinc [4, 11]. Industrial effluents are known to contain heavy metals which originate from metal plating, mining activities, smelting, battery manufacture, tanneries, petroleum refining, paint manufacture, pesticides, pigment manufacture, and printing and photographic industries [1, 11–14].

2. Heavy metals

Heavy metals are usually defined as metals having density more than 5 g/cm^3 [15]. They are classified as essential and non-essential metals. The metals which are need for normal cellular growth are essential metals e.g. zinc, nickel, copper, etc. Such metals are required in low concentrations (nM), but at higher concentrations (μM to mM) all heavy metals have detrimental effects to organisms [16, 17]. If the metals have no known biological function, they are called as non-essential metals e.g. e.g., lead, cadmium, mercury [18]. Such metals are toxic at any concentration [8]. The list of essential and non-essential heavy metals is given (**Table 1**). There are 90 naturally occurring elements in periodic table, 21 are non-metals, 16 are light metals and the remaining 53 (with As included) are heavy metals [19]. In periodic table, transition elements are mostly heavy metals. They have incompletely filled 'd' orbitals which allow heavy-metal cations to form complex compounds that may or may not be redox-active. In this way, heavy metals play an important role as 'trace elements' (cobalt, copper, nickel, and zinc) in sophisticated biochemical reactions and are important cofactors for metallo-proteins and enzymes [8]. The toxicity of heavy metal ions starts when their concentration becomes higher

Category of heavy metal	Example of heavy metals
Essential	Copper (Cu)
	Nickel (Ni)
	Iron (Fe)
	Zinc (Zn)
	Magnesium (Mg)
Non-essential	Lead (Pb)
	Mercury (Hg)
	Cadmium (Cd)
	Tin (Sn)
	Arsenic (As)

Table 1. Essential and non-essential heavy metals.

in the cells, due to which they form complex compounds [15, 18]. Microorganisms acquire resistance to these toxic metals by lateral gene transfer [20]. The interaction of microorganism with metal ions depends on factors like oxidation state of the metal ion, chemical/physical nature of metals, growth phase of microorganism etc. [21].

3. Methods for removal of heavy metals

Since last many decades, various physical and chemical methods were employed to remove metals from environment. The list is given below [5, 13, 14, 22–24].

Chemicals methods: Chemical precipitation, electrochemical treatment, oxidation/reduction.

Physical methods: Ion exchange, membrane technology, reverse osmosis, and evaporation recovery, filtration.

Biological methods: Microorganisms including bacteria, fungi or algae.

However, these strategies were not the first choice as they are expensive, inefficient, labor-intensive, or the treatment process lacks selectivity [25, 26]. The research on bioremediation or biosorption-based remediation techniques in the past decades has concluded that bioremediation is a natural process and cost effective [4, 27–31].

4. Biosorption

Biosorption is defined as "ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated (by the use of ATP) or spontaneous physico-chemical pathways of uptake (not at the cost of ATP), or as a property of certain types of inactive, non-living microbial biomass which bind and concentrate heavy metals from even very dilute aqueous solutions" [1, 5, 32]. It is a complex process that depends on different-factors like cell physiology, physicochemical factors such as pH, temperature, contact time, ionic strength, and metal concentration, chemistry of the metal ions, cell wall composition of microorganisms [5, 33, 34]. Biosorption of different heavy metals e.g. cadmium, silver, lead, nickel etc. by using microorganisms like fungi, algae or bacteria was studied by different groups [34–42].

4.1. Significance

Bioremediation offer different advantages such as low operating cost, minimum ratio of disposable sludge volume, high efficiency in detoxifying very dilute effluents and even *in situ* remediation [30, 43, 44]. Bacteria detoxify heavy metals in a variety of different ways [45]. Although various types of tolerance mechanisms have been reported in bacteria for heavy metal stress, Cd detoxification has only been restricted to efflux pumps. The plasmid encoded *cad* systems in (*Staphylococcus aureus*) and the *czc* system (*Alcaligenes eutrophus*) are

best characterized. These systems actively maintain Cd ions outside the intracellular environment, hence avoiding its toxic effects. Consistently, many researchers reported that sensitive bacteria can accumulate 3–15 times more Cd than resistant strains [46]. The most important aspect of Cd ions is that they covalently bind to sulfhydryl groups. Although this is partially the cause for its high toxicity, this feature is also used by several organisms to render the metal harmless to the cell, through sequestration with metal-detoxifying ligands, the metal becomes less bioavailable.

4.2. Advantages of biosorption

Following are given the advantages of biosorption over conventional metal removal methods [47, 48].

1. Cheaper production of biomass (bacteria or fungi)

2. Use of biomass for removal of heavy metals

3. Multiple heavy metals uptake at a time

4. Treatment of large volumes of wastewater

5. No need for chemical additions as highly selective for uptake and removal of specific metals

6. Functional over wide range of conditions including temperature, pH, presence of other metal ions, etc.

7. Easy and cheaper desorption of metals attached to biomass

8. Reduced volume of waste or toxic materials production

4.3. Disadvantages of biosorption

The disadvantages of biosorption are stated below [49].

1. Saturation of active sites of metal binding ligands

2. Reversible sorption of metals on biomass

5. Biosorption mechanisms

The process of heavy metal ion binding to bacterial cell wall (peptidoglycan) can be metabolism dependent or independent [1].

5.1. Metabolism dependent biosorption

Metabolism dependent biosorption is exhibited by living biological material. It involves various mechanisms like chelation; a specific way in which ions and molecules bind to metal ions

and it involves the formation or presence of two or more separate coordinate bonds between a polydentate ligand and a single central atom, physical adsorption; adhesion of atoms, ions, or molecules from a gas, liquid, or dissolved solid to a surface. This process creates a film of the adsorbate on the surface of the adsorbent. It is a surface phenomenon. Generally the adsorption process is classified as physisorption, characteristic of weak van der Waals forces, or chemisorption, characteristic of covalent bonding. It may also occur due to electrostatic attraction, precipitation; it is the creation of a solid in a solution or inside another solid during a chemical reaction or by diffusion in a solid. When this reaction occurs in a liquid solution, the solid formed is called the "precipitate" and the chemical that causes the solid to form is called the "precipitant") or complexation (it consists of a central atom or ion, which is usually metallic and is called the coordination centre, and a surrounding array of bound molecules or ions, that are known as ligands or complexing agents. Many metal-containing compounds, especially those of transition metals, are coordination complexes). There may involve a single process or combination of these processes [50, 51]. If the metal binding to cell wall is metabolism dependent then it involves energy from ATP. The ligands present on the cell wall of biological material such as phosphoryl, carboxyl, carbonyl, sulfhydryl and hydroxyl groups immobilizes the metal ion [32] and then uptake occurs [5]. Other factors that effect the metal uptake by living biomass includes nature of heavy metals ions, conditions of the medium, cell wall composition, etc. [5]. The uptake process by living biomass involves adsorption to cell wall and entering into the cytoplasm [29, 31, 52, 53].

5.2. Metabolism independent biosorption

The metabolism independent process mostly occurs in biomass consisting of dead cells [54]. The adsorption process is the main key point behind such physicochemical biosorption mechanism. The adsorption process can be ionic interactions or physiochemical adsorption. Presence of anionic ligands on bacterial cell wall (carboxyl, amine, hydroxyl, phosphate, and sulfhydryl groups) also plays an important role in metal biosorption. Living biological mass is preferred over dead mass, because living cells have ability for continuous metal uptake, and self-replenishment [27, 29, 31]. Previously it is reported that adsorption is a rapid process while accumulation is slow and energy dependent [29, 31 52–53]. The fate of metal inside cell can be accumulation, detoxification and/or efflux depending on the nature of bacteria [31, 55, 56]. In past few decades, many groups worked on heavy metal resistant bacteria that can be used for bioremediation [27, 29, 31, 56–58]. Many workers reported that cells of bacteria of genera *Alcaligenes* and *Pseudomonas* can be used for bioremediation purpose [45].

5.3. Metal accumulation

In order to have the physiological effect on the growth of cells, heavy metals must enter the cell [19, 59, 60]. Metal uptake system in bacteria is grouped in two types; one is fast and unspecific, constitutively expressed and does not require ATP. They are usually driven only by the chemiosmotic gradient across the cytoplasmic membrane of bacteria. The second type of uptake system is highly specific, slow, inducible and dependent on ATP, in addition to the chemiosmotic gradient. They are only induced in times of need, starvation or a special metabolic situation [61].

As cell surface encounter metal ion, formation of a complex takes place, which is a pre-requisite for uptake of metals by the organism [59, 60]. Once surface sorption takes place, the metal is transported into the periplasmic space of Gram-negative cells and transported further into the cytoplasm [60]. When cell encounters high concentration of any heavy metal, the heavy metal ion is transported into the cytoplasm, accumulated inside the cell due to one type of metal uptake which is fast, unspecific, constitutively expressed and does not require ATP [61]. The cations of heavy metals interact with physiological ions Cd^{2+} with Zn^{2+} or Ca^{2+}, Ni^{2+} and Co^{2+} with Fe^{2+}, Zn^{2+} with Mg^{2+} thus inhibit the function of respective physiological cations. This result in oxidative stress in the cell [1].

6. Types of biosorbents

Biosorbents can be classified as living or non- living organic materials. They are discussed below in detail.

6.1. Living organic materials

6.1.1. Bacteria

Among microorganisms, bacteria constitute of being the most abundant, versatile, most diverse creature on this planet earth [48, 62]. They are basically classified on the basis of their morphology as rod, cocci or spirillum [48, 63]. A bacterium has relatively simple morphology consisting of cell wall, cell membrane, capsule, slime layer and internal structures mitochondria, Golgi apparatus, ribosomes, endoplasmic reticulum. Slime layer contains functional groups like carboxyl, amino, phosphate or sulfate for metals chelation [48, 62]. Cell wall in general, is responsible for surface binding sites and binding strength for different metal ions depending on different binding mechanisms. Various bacterial species e.g. *Bacillus*, *Pseudomonas*, *Escherichia* [48] exhibit biosorption property because of their small size and ability to grow in different environmental conditions [64–66].

Gram classification divides bacteria in two broad categories; Gram positive and Gram negative. Gram negative mostly constitute pathogens although pathogens are also reported in Gram positive. Gram positive bacteria are comprised of thick peptidoglycan layer connected by amino acid bridges, also known to contain polyalcohols and teichoic acids. Overall, Gram positive bacterial cell wall comprised of 90% peptidoglycan. Some teichoic acids are linked to lipids of lipid bilayer forming lipoteichoic acid. These lipoteichoic acids are linked to lipids of cytoplasmic membrane. They constitute linkage of peptidoglycan to cytoplasmic membrane. This results in cross linking of peptidoglycan forming a grid like structure. These teichoic acids are responsible for negative charge on cell wall due to presence of phosphodiester bonds between teichoic acid monomers [48]. On the other hand, Gram negative bacterial cell wall contains an additional outer membrane composed of phospholipids and lipopolysaccharides. Gram negative cell wall contains 10–20% peptidoglycan. The negative charge on the Gram negative bacteria is due to lipopolysaccharides, teichoic acids, teichuronic acids. Extracellular polysaccharides also exhibit the property of metal binding. They are not present in all Gram negative bacteria. Moreover, those species that contain them, they can be easily removed by chemical washing or mechanical disruption [49, 67].

6.1.1.1. Bacterial biosorption

Bacterial cell wall encountering the metal ion is the first component of biosorption. The metal ions get attached to the functional groups (amine, carboxyl, hydroxyl, phosphate, sulfate, amine) present on the cell wall [49, 67]. The general metal uptake process involves binding of metal ions to reactive groups present on bacterial cell wall followed by internalization of metal ions inside cell [48]. More metal is uptaken by Gram positive bacteria due to presence of glycoproteins. Less metal uptake by Gram negative bacteria is observed due to phospholipids and LPS [68, 69]. Biosorption of various metals by different bacteria is given in **Table 2**.

Sr. No.	Metals	Bacteria	Temperature (°C)	pH	Agitation	Time	Wt (g/L)	q(mg/g) or % removal	References
1.	Arsenic	*Bacillus* sp. KM02	—	—	—	—	—	—	[108]
		Kocuria sp.	—	—	—	—	—	—	[109]
		Bacillus sp.	—	—	—	—	—	—	[110]
2.	Cadmium	*Pseudomonas putida* mt2	—	—	—	—	—	—	[111–114]
		Cupriavidus metallidurans CH34	—	—	—	—	—	—	[111–114]
		Enterobacter cloacae	25	5	240	2	0.1	58.9%	[115]
		Stenotrophomonas maltophilia	28	5	140	2	20	0.12	[116]
		Actinomycetes sp.	30	6	150	24	5	32.63	[116]
3.	Chromium	*Micrococcus* sp.	35	5	120	24	—	92%	[117]
		Bacillus licheniformis	28	3.5	120	48	—	95%	[116]
		Staphylococcus saprophyticus	27	2	150	3	0.2	24.1	[118]
		Enterobacter cloacae	25	4	240	2	0.1	55.8	[115]
		Pseudomonas aeruginosa	25	—	—	—	—	1.07	[119]
		Micrococcus sp.	35	5	120	24	—	92%	[117]
4.	Cobalt	*Rhodopseudomonas palustris*	—	—	—	—	—	—	[120]
5.	Copper	*Stenotrophomonas maltophilia*	25	5	140	2	20	0.57	[116]
		Bacillus licheniformis	28	2.5	120	48	—	32%	[121]
		Geobacillus thermodenitrificans	25	5	100	12	—	51	[122]
		Bacillus cereus	25	5.5	—	24	1.0	50.32	[119]
		Pseudomonas aeruginosa	25	—	—	—	—	0.67	[119]
		Thiobacillus thiooxidans	30	5	786	2	0.25	39.84	[123]
		Enterobacter cloacae	25	5	240	2	0.1	78.9	[115]
		Staphylococcus saprophyticus	27	3.5	150	2	0.2	14.5	[118]
6.	Gold	*Cupriavidus metallidurans* CH34	—	—	—	—	—	—	[125]

Sr. No.	Metals	Bacteria	Temperature (°C)	pH	Agitation	Time	Wt (g/L)	q(mg/g) or % removal	References
7.	Lead	*Enterobacter cloacae*	25	5	240	2	0.1	67.9	[115]
		Bacillus sp.	30	5–9	100	24	—	69.34	[124]
		Pseudomonas sp.	30	5–9	100	24	—	90.41	[124]
		Micrococcus sp.	30	5–9	100	24	—	84.27%	[124]
		Bacillus cereus	25	5.5	—	24	1.0	36.71	[119]
		Geobacillus thermodenitrificans	25	4	100	12	—	53	[122]
		Stenotrophomonas maltophilia	25	5.0	140	2	20	0.41	[116]
8.	Mercury	*Enterobacter cloacae*	25	4	240	2	0.1	43.23	[115]
9.	Nickel	*Actinomycetes* sp.	30	5	150	24	5	36.55	[116]
		Micrococcus sp.	35	5	120	24	—	90%	[117]
10.	Selenium	*Cupriavidus metallidurans* CH34	—	—	—	—	—	—	[111–114]
11.	Silver	*Cupriavidus metallidurans* CH34	—	—	—	—	—	—	[111–114]
12.	Zinc	*Pseudomonas aeruginosa*	25	—	—	—	—	1.33	[119]
		Geobacillus thermodenitrificans	25	5	100	12	—	18	[122]

Where, Wt = weight of used adsorbent; Q = uptake removal of pollutant (mg/g); Agitation = speed of shaker (rpm); T = Temperature of the experiment (°C).

Table 2. Bacteria and their biosorption features regarding different metals [48, 126, 127].

6.1.2. Algae

Algae are aquatic plants that lack true roots and stems. It can range from micro algae to macroalgae. They are autotrophic. They can grow in big biomass even when less nutrition is provided. They are considered good biosorbent material [48, 70–73] because of their big size, high sorption capacity and no production of toxic substances. Mostly they are classified as microalgae (fresh water or green algae), macroalgae (marine or brown algae) and red algae. Among these three classes, brown alga is reported to have higher metal uptake capacity. The following features are responsible for binding of heavy metal ions to algae surface; algae species, ionic charge of metal and chemical composition of metal ion solution. Metal ion binding sites on algal surface includes sulfhydryl, hydroxyl, phosphate, sulfate, imidazole, amine, carboxyl groups [74]. The metal uptake mechanism of algae is similar to that of bacteria that is bonding of metal ions with the surface followed by internalization. According to Abbas et al., [48], either of two mechanisms in algal biosorption is involved: (1) ion exchange method where ions present on algal surface Ca, Mg, Na, K they are displaced by metal ions, (2) complexation between functional groups and metal ions.

6.1.2.1. Biosorption by algae

According to Abbas et al., [48], algal cell wall is made up of polysaccharides (alginic acid, chitin, xylan, mannan) which provides functional groups (sulfate, hydroxyl, phosphate, imidazole, amino, amine) known to act as metal binding sites [74]. As far as metal binding mechanism is concerned, ionic charge and covalent bonding are hypothesized. Carboxyl and sulfate groups are involved in ionic bonding whereas amino and carboxyl groups are involved in covalent bonding between metal ion and functional group. In response to metal ions, phytochelatins are produced inside the algal body [48]. Biosorption of various metals by different bacteria is given in **Table 3**.

Sr. No.	Metals	Algae	Temperature (°C)	pH	Agitation	Time	Wt (g/L)	q(mg/g) or % removal	References
1.	Arsenic	*Spirogyra hyalina*	25	—	180	2	1	9.8	[128]
2.	Cadmium	*Bifurcaria bifurcate*	-	4.5	175	3	2.5	95	[129]
		Oocystis	28	7.5	72	60–80	28–51	-	[130]
		Pithophora spp. (filamentous)	25	-	-	9 days	0.17–14	-	[131]
		Sargassum sp. (brown algae)	30	5	150	-	-	22.2	[132]
		Sargassum tenerrimum	25	6	150	24	4	0.4 mmol/g	[133]
		Fucus vesiculosus (brown algae)	25	6	-	2	0.25	1.12 mmol/g	[134]
		Ascophyllum nodosum	25	6	-	2	0.5–1	114.9	[135]
3.	Chromium	*Pithophora spp.* (filamentous)	25	-	-	9 days	-	-	[131]
		Sargassum sp.	30	4	-	6	2–5	68.9	[136]
		Spirogyra sp. (green algae)	30	4	180	3	1–3	265	[132]
		Sargassum sp. (brown algae)	30	3	150	-	-	20.2	[132]
4.	Cobalt	*Spirogyra hyalina*	25	—	180	2	2.5	7.856	[128]
5.	Copper	*Calotropis procera*	25	4	150	6	2	14.5	[137]
		Oocystis	28	5.5	60–80	72	4.4–6.0	-	[130]
		Sargassum filipendula	25	4.5	175	6	5	-	[138]
		Microalgae	30	-	150	-	5	0.66	[139]
		Sargassum sp. (brown algae)	30	4	150	-	-	18.6	[132]
		Fucus vesiculosus (brown algae)	25	5	-	2	0.25	0.97	[134]
		Ascophyllum nodosum	25	4	-	2	0.5–1	70.9	[135]
7.	Lead	*Calotropis procera*	25	4	150	6	2	22.8	[137]
		Oocystis	28	5.5	60–80	72	16–80	-	[130]
		Pithophora spp. (filamentous)	25	-	-	9 days	0.12–0.13	-	[131]
		Fucus vesiculosus (brown algae)	25	5	-	2	0.25	1.04	[134]

Sr. No.	Metals	Algae	Temperature (°C)	pH	Agitation	Time	Wt (g/L)	q(mg/g) or % removal	References
8.	Mercury	*Sargassum* sp. (brown algae)	30	4	100	-	-	14.8	[132]
			25	-	180	2	1	20	[128]
		Cladophora fascicularis *Spirogyra hyaline*	25	-	180	2	2.5	39.2	[128]
9.	Nickel	*Sargassum* sp. (brown algae)	30	5	150	-	-	26.1	[132]
		Fucus vesiculosus (brown algae) *Ascophyllum nodosum*	25	5	-	2	0.25	0.80	[134]
			25	6	-	2	0.5–1	50	[135]
12.	Zinc	Microalgae	30	-	150	-	5	0.72 mmol/g	[139]
		Sargassum sp. (brown algae) *Ascophyllum nodosum*	30	3	150	-	-	15.4	[132]
			25	6	-	2	0.5–1	53.2	[135]
13.	Iron	*Sargassum* sp. (brown algae)	30	3	150	—	—	14.6	[132]

Table 3. Algae and their biosorption features regarding different metals [48].

6.1.3. Fungi

Fungi are eukaryotic living organism which includes yeasts, mushrooms, molds, etc. The cell wall structure of fungi offers good metal binding properties. Fungi in living and dead both forms can be used as biosorbent material [48, 75]. Metal uptake by fungi involves two processes (i) active uptake or bioaccumulation or intracellular uptake, it is dependent on cell metabolism and (ii) biosorption or passive uptake which involves binding of metal ions to surface of cell wall and it is independent of cell metabolism. The energy independent metal uptake mechanism can be affected by temperature, metabolic inhibitors, etc. Metal uptake by fungi was reported both active and passive. Active uptake occurred only with living cells. In this case, the interaction of metal ions with cell surface functional groups may involves ion-exchange, complexation or just physical adsorption.

6.1.3.1. Biosorption by fungi

According to Das et al., [69] fungal cell wall exhibit excellent metal biding properties due to its components. The cell wall of fungus is composed mainly of chitins, mannans, glucans, in addition to lipids, polysaccharides, pigments e.g. melanin [48, 76–78]. Fungal cell wall is reported to be made up of 90% polysaccharides. The functional groups which are involved in metal binding includes carboxyl, phosphate, uranic acids, proteins, nitrogen containing ligands, chitin or chitosan [48, 79]. Biosorption ability of fungal cells can be manipulated by physical of chemical treatments including autoclaving, heat processes or dimethyl sulfoxide, laundry detergent, orthophosphoric acid, formaldehyde, gluteraldehyde, NaOH, respectively [69]. Macrofungi also called as mushrooms, grow wild in all types of environments

ranging from forests to polluted soils and water bodies. They uptake the metals in their fruiting bodies, mycelia and sporocarps [48]. Biosorption of various metals by different fungi and mushrooms is given in **Tables 4** and **5** respectively.

6.1.4. Yeasts

Yeasts are famous organisms while studying biosorption. *Saccharomyces cerevisiae* is well known yeast which is considered a model system to study biosorption. They are easy to grow, non-pathogenic and give high biomass yield using simple growth medium [80]. The availability of complete genome information makes its genetic engineering an easy job [75, 81]. They are also considered ideal experimental organism in molecular biology experimentation [75, 82–84]. The property of biosorption by yeast cells is affected by various factors including properties of metal ions (valency, radius), cell age of *S. cerevisiae* cells, conditions of culture (composition of growth medium, carbon source), biosorption conditions (initial concentration of metals and biomass, availability of metal ions, temperature, pH, other ions in growth medium) [75]. Moreover, the large size of yeast makes them promising candidates for metal bioremediation. *Saccharomyces cerevisiae* is a widely studied yeast strain. Its different forms are already studied for its biosorption properties including immobilized versus fess cell, living versus dead cells, engineered versus non engineered cells, cultural versus waste cells, etc. [69, 85–89].

Sr. No.	Metals	Bacteria	Temperature (°C)	pH	Agitation	Time	Wt (g/L)	q(mg/g) or % removal	References
1.	Arsenic	*Penicillium chrysogenum*	25	3–4	190	—	1	24.5	[140]
2.	Cadmium	*Aspergillus cristatus*	25	6	120	2	0.4	23.2	[142]
		Aspergillus niger	25	4.75	125	6	0.7	13	[143]
		Hydrilla verticillata	25	5	150	0.33	3–9	15	[141]
3.	Chromium	*Aspergillus niger*	28	4.5	150	1	10	16.39	[144, 145]
		Pleurotus ostreatus	25	4.5	150	3	2	1.97	[146]
		Trichoderma viride	-	6	150	0.75	3.75	4.66	[147]
		Mucor	35	5.5	-	-	-	-	[148]
		Penicillium canescens	20	6	100	4	2	34.8	[149]
4.	Copper	*Pleurotus ostreatus*	25	4.5	150	3	2	4.0	[150]
		Fomes fasciatus	25	5.5	200	1	1	32.2	[151]
		Aspergillus lentulus	35	6	180	0.41	4	-	[152]
5.	Lead	*Rhizopus nigricans*	25	5.5	225	-	25	80.8	[153]
		Trichoderma longibrachiatum	25	7	-	0.33	-	71	[154]
		Pleurotus ostreatus	25	5.5	-	3	2	4.84	[155]
6.	Mercury	*Aspergillus flavus*	30	5.5	100	8	10	95.3%	[156]
		Aspergillus fumigatus	30	5.5	100	8	10	95.3%	[140]
7.	Nickel	*Aspergillus niger*	25	4.5	150	3	1	7.69	[157]

Table 4. Fungi and their biosorption features regarding different metals [48].

Sr. No.	Mushrooms	Metals	References
1.	Volvariella volvacea (edible Mushroom) – mycelia, sporocarps	Cadmium, lead, Copper, Chromium	[158]
2.	Ganoderma lucidum	Chromium	[69, 159]
3.	Coriolopsis strumosa	Copper	[160]
4.	Daedalea tenuis	Copper	[160]
5.	Lentinus strigosus	Copper	[160]
6.	Lenzites malaccensis	Copper	[160]
7.	Phellinus xeranticus	Copper	[160]
8.	Rigidoporus lineatus	Copper	[160]
9.	Rigidoporus microporus	Copper	[160]
10.	Trametes lactinea	Copper	[160]
11.	Ganoderma lucidum	Copper	[159, 160]
12.	Agaricus macrospores	Cadmium, mercury, copper	[161]

Table 5. Mushrooms and biosorption of different metals [48].

6.1.4.1. Biosorption by yeast

The free form of yeast cells is not considered good candidates for biosorption [86]. Free cells face the problem of separation of solid liquid phase. This problem seems to be less effective in flocculating cell [90]. Pretreatment of yeast cells can result in increased surface to volume ration for binding of metal with the metal binding sites. It is reported that pH above 5 optimizes the metal biosorption in yeast cells [91]. According to Abbas et al., [48] in yeasts, higher concentration of heavy metals can be accumulated by bioaccumulation process than biosorption. However, general biosorption is responsible for the major uptake of heavy metals for many filamentous fungi. Biosorption of various metals by different yeasts is given in **Table 6**.

Sr. No.	Metals	Yeasts	Temperature (°C)	pH	Agitation	Time	Wt (g/L)	q(mg/g) or % removal	References
1.	Cadmium	Saccharomyces cerevisiae	25	7	100	2	2	12.3	[69]
2.	Chromium	Saccharomyces cerevisiae	25	5.2	150	1	80	55.3%	[162]
		Candida utilis	25	5.5	160	1	1.0	28	[162]
3.	Cobalt	Saccharomyces cerevisiae	25	7	100	2	2	8.2	[162, 163]
4.	Copper	Saccharomyces cerevisiae	25	7	100	2	2	29.9	[162]
		Candida pelliculosa	30	6	120	120	13.3	95.04%	[164]
		Schizosaccharomyces pombe	25	4	-	96	-	74.85	[165]
5.	Lead	Mucor rouxii	25	5.0	125	15	—	17.13	[166]
6.	Mercury	Saccharomyces cerevisiae	25	7	100	2	2	76.2	[162]
7.	Nickel	Saccharomyces cerevisiae	25	7	100	2	2	14.1	[162]
8.	Zinc	Saccharomyces cerevisiae	25	7	100	2	2	11.8	[162]

Table 6. Yeasts and their biosorption features regarding different metals [48].

6.2. Non-living organic materials

6.2.1. Wastes of agricultural or food industry

The wastes of agriculture or food industry includes agricultural byproducts as corn cobs, soya bean hulls, cotton seeds hulls [92] or fruit peels. They contain cellulosic material in their cell wall which is known to contain functional groups like phenolics or carboxylic. On the basis of cation exchange between functional groups and metal ions, the binding of metal ion with functional group results in biosorption and thus removal of metal ion from medium [49].

7. Factors affecting biosorption

Biosorption process is affected by following factors.

Temperature: For efficient removal of metal ions from environment, the optimum temperature needed to be investigated. It is generally assumed that biosorption is carried out between 20 and 35°C. High temperatures above 45°C may results in damage to proteins which in turn affects metal uptake process [48, 93–95].

pH: It is a very important parameter. It affects solubility of metal ions and binding sites of biomass. At lower pH, the biosorption of metals is affected [96, 97]. General range of pH for metal uptake is between 2.5–6. Above this limit, metal uptake ability of biosorbent gets compromised [48].

Nature of biosorbents: Metal uptake is reported in different forms like biofilms, freely suspended microbial cells or immobilization of microbial cells. It can be altered by physical or chemical treatments. Physical treatments include autoclaving, drying, boiling, sonication, etc. Chemical treatment as the name indicates involves chemicals like acid or alkali to improve biosorption capacity. According to Wang and Chen, [75], the fungal cells are deacetylated which affects the structure of chitin resulting in the formation of chitosan-glycan complexes which have results high metal affinities. Abbas et al., [48] also report about effect of age, growth medium components on biosorption as they might result in cell wall composition, cell size and EPS formation.

Surface area to volume ratio: This property plays an important role in efficient removal of heavy metal from medium. The surface area property plays a significant role in case of biofilms [48]. The binding of metal ions with microbial cell wall is previously reported [98]. Although intracellular metal adsorption is energy-consuming process but still microorganisms prefer it over wall adsorption.

Concentration of biomass: The concentration of biomass is directly proportional to the metal uptake [48, 98, 99]. It is reported that electrostatic interaction between the cells plays an important role in metal uptake. At a given equilibrium, the biomass adsorbs more metal ions at low cell densities than at high densities [100]. Metal uptake depends on biding sites. More biomass concentration or more metal ions restricts the access of metal ions to binding sites [48, 101].

Initial metal ion concentration: The initial concentration provides an important driving force to overcome all mass transfer resistance of metal between the aqueous and solid phases [102]. Increasing amount of metal adsorbed by the biomass will be increased with initial concentration of metals. Optimum percentage of metal removal can be taken at low initial metal concentration. Thus, at a given concentration of biomass, the metal uptake increases with increase in initial concentration [48].

Metal affinity to biosorbent: Physical/chemical pretreatment affects permeability and surface charges of the biomass and makes metal binding groups accessible for binding. It can be manipulated by pretreating the biomass with alkalis, acids detergents and heat, which may increase the amount of metal uptake [48, 94].

8. Kinetics of biosorption

Before going in the details of studying kinetics of biosorption, one should understand the quality of a biosorbent. For observing the quality of a biosorbent, two factors should be considered (i) how much metal ion is attracted by the biosorbent, (ii) to which extent metal ions are retained on biosorbent in an immobilized form. The metal uptake by the biosorbent can be calculated by checking the difference in initial quantities of metal ions in medium to that remained in the medium after biosorption takes place. This is studied by the following Eq. 1 [48, 49, 94]:

$$q = \frac{V(Ci - Ce)}{M} \tag{1}$$

q = amount of metal biosorbed by biomass (mg/g); V = Volume of metal solution (L); Ci = Initial concentration of metal (mg/L); Ce = Concentration of metal (mg/L) at equilibrium; M = Mass of adsorbent.

Units = milligrams of solute sorbed per gram of dry biosorbent material (when engineering process – mass balance calculations are to be considered) or mmol/g (when the mechanism or stoichiometry are to be considered).

According to Abdi and Kazemi [49], in order to observe biosorption kinetics of any heavy metal, sorption performance of a biosorbent must be taken into consideration. For it, a biosorption isotherm should be studied. A biosorption isotherm is the plot of uptake of metal (q) versus equilibrium solute concentration in the solution (Cf). For studying the isotherm plots, parameters including temperature, pH and ionic strength are kept constant whereas metal concentration is varied. Literature showed that confusion prevails regarding pH because it is common believe that pH of a medium changes during whole process of biosorption. Biosorption isotherms are typically described by two models (i) Freundlich and (ii) Langmuir. These models are two - parameters models which are vastly used to describe the equilibrium state for adsorption of metal ions experimental work [48].

Freundlich model: Freundlich and Kuster in (1907) published first mathematical equation to describe the isotherm. It is a non-liner sorption model. It involves monolayer sorption of

metal with active sites and is described by continuous interactions between adsorbed molecules [49, 103]. It is given by Eq. 2:

$$qe = K Ce_n^1 \qquad (2)$$

K = mg/g or l/mg; 1/n or n = Freundlich constant related to adsorption capacity; n = Freundlich constant related to adsorption intensity.

Langmuir model: Langmuir in 1918 published a model for describing gas or liquid adsorbed on solid material. It describes the monolayer sorption of metal with active sites and do not involve interactions between adsorbed molecules [48, 49]. It is given by Eq. 3:

$$qe = \frac{qmaxbCe}{1 + bCe} \qquad (3)$$

qe = Amount of metal ion removed (mg/g); Ce = Equilibrium concentration (mg/L); b = Langmuir constant related to affinity; qmax = maximum metal uptake (mg/g) under the given conditions.

k, n = Freundlich and Langmuir constants (n value greater than 1.0 shows that sorption is favorable physical process) [49, 104].

9. Desorption and recovery of metals

After biosorption of heavy metal from environment, its recovery is another crucial step which involves desorption of metal from biosorbent. According to previous literatures [105–107], various agents were used for this purpose which includes complexing agents (thiosulfate, EDTA), mineral acids (HNO_3, H_2SO_4, HCl), organic acids (acetic acid, citric acid). Before choosing the recovery agents, it should be kept in mind that chosen recovery agent should given least harm to physical properties of a biosorbent so that its efficiency of metal binding must remain in its original state to ensure its maximum efficiency for metal binding [94, 106, 107].

10. Conclusions

Biosorption is eco-friendly and cheap method of removing metals from the environment. Previous researches conducted during last five decades provided vast amount of information about different types of biosorbents and their mechanism of metal uptake. More research is needed to explore new biosorbents from environment. A deep insight is required not only on method of metal removal, but also its efficient recovery so that it can be obtained in usable form.

Author details

Saba Shamim

Address all correspondence to: sabashamimgenetics@gmail.com

Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore (UOL), Lahore, Pakistan

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