

Fungus — An alternative for bioremediation of heavy metal containing wastewater: A review

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The common filamentous fungi can sorb heavy metals (Zn, Cd, Pb, Fe, Ni, Ag, Th, Ra & U) from aqueous solutions to varying extents. Fungal biosorption largely depends on parameters such as pH, metal ion and biomass concentration, physical or chemical pretreatment of biomass, and presence of various legends in solution. When compared with commercial ion exchange resins, carbons and metal oxides, fungal derivatives generally perform well. Biomass can be regenerated by using various elutants. The availability of variety of biomass and their metal binding potential makes it a economical and sustainable option for developing effluent treatment process for removal and recovery of heavy metals.

Keywords: Bioremediation, Fungus, Wastewater

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Introduction

Rapid urbanization and industrialization has led to increased disposal of heavy metals and radionuclides into the environment. Physicochemical processes usually achieve removal of heavy metals from metal-bearing wastewater before discharging the effluents into natural water-body systems. But conventional treatment technologies¹ (precipitation & coagulation) become less effective and more expensive when metal concentrations are in the range of 1–100 mg/l. Adsorption on activated carbon is a recognized method for the removal of heavy metals from wastewater. The high cost of activated carbon limits its use in adsorption. The variety of materials tested as metal adsorbents includes gridish coal, crushed coconut shell, peat, bark, straw, waste tyre rubber and human hair. Recent developments in the field of environmental biotechnology include the search for microorganisms as sorbents for heavy metals.

Bacteria, fungi, yeast and algae can remove heavy metals and radionuclides from aqueous solution in substantial quantities²⁻⁴. Shumate and Strandberg⁵ defined biosorption as “a non directed physico – chemical interaction that may occur between metal/radio nuclide species and the cellular compounds of biological species”.

Fungi and yeasts accumulate micronutrients such as Cu, Zn, Mn and non – nutrient metals, like U, Ni, Cd, Sn and Hg in amounts higher than the nutritional requirement⁶. The potential of fungal biomass as biosorbent has been recognized for the removal of heavy metals and radionuclides from polluted waters^{7,8}.

Modes of Metal Ion Uptake

The metal ion uptake by living and dead cells can consist of two modes. The first uptake mode involves the surface binding of metal ions to cell wall and extra cellular material. The second mode of metal uptake into the cell across the cell membrane is dependent on the cell metabolism, and is referred to as intracellular uptake, active uptake or bioaccumulation⁹. The first mode is common to metal adsorption by both living and dead cells; the second mode, which is metabolism dependent, occurs in living cells. For living cells metal uptake is also facilitated by the production of metal-binding proteins. Therefore, metal uptake may take place by different modes, depending on whether the cells are dead or living.

Both living and dead cells are capable of metal adsorption. The use of dead biomass seems to be a preferred alternative for the majority of metal removal studies reported. The wider acceptability of dead cells is due to the absence of toxicity limitations, absence of requirements of growth media and nutrients in the feed solution, and the fact that biosorbed metals can

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Table 1 — Biosorption of metal ions by various fungal species

Biomass type	Metal studied	Biosorptive capacity mg/g	Isotherm applicability	References No.
<i>A. niger</i>	Cu	7.22	Freundlich & Langmuir	44
<i>A. oryzae</i>	Cd	2.2	Langmuir	53
<i>P. chrysogenum</i>	Cr ³⁺	27.2	Langmuir & Freundlich	22
	Ni ²⁺	19.2		
	Zn ²⁺	24.5		
	Zn	21.6		
	Pb	116	–	62
<i>P. spinulosum</i>	Cu	6.6	–	48
<i>S. cerevisiae</i>	U	94.7	–	30
<i>R. nigricans</i>	Cr	47	Langmuir & Freundlich	13
	Pb	13-105	–	3
<i>R. arrhizus</i>	Cr ⁶⁺	9.02	Freundlich	8
	Mn	12	–	57
	Cu	16	–	
	Zn	20	–	
	Hg	58	–	
<i>M. rouxii</i>	Pb	4.06	Thomas	63
	Cd	3.76		
	Ni	0.36		
	Zn	1.36		

be easily adsorbed and recovered, the regenerated biomass can be reused, and the metal uptake reactors can be easily modeled mathematically. The dead fungal cells have been shown to bind metals at level greater than live cells depending on the methods used to kill (pretreated) the live cells^{8,10-12}.

Modeling of Biosorption

The adsorption isotherm models of Langmuir, Freundlich, Brunauer – Emmett – Teller (BET) and Scatchard have been used to describe the biosorption equilibria (Table 1) Langmuir model can be described as:

$$q = Q_o b C_f / 1 + b C_f$$

where q is the uptake of metal per unit weight of the adsorbent, Q_o is the maximum uptake, b is the constant related to energy of adsorption, and C_f is the equilibrium (residual) concentration of ions¹³. Langmuir model is based on the assumption that maximum adsorption occurs when a saturated monolayer of solute molecule is present on the adsorption surface, and the energy of adsorption is

constant and that there is no migration of adsorbate molecule in the surface plane.

Freundlich isotherm has the form

$$q = K_f C_f^{1/n}$$

where q is the uptake of metal per unit weight of biosorbent, C_f is the equilibrium concentration of metal ion in solution, K_f and n are the Freundlich constants denoting adsorption capacity and intensity of adsorption respectively¹³. Higher the values of K_f and n and lower the value of b , the higher the affinity of the biomass.¹⁴ Freundlich model is basically empirical and was developed for heterogeneous surfaces. The model is a useful means of data description.

BET isotherm represents the multi-layer adsorption at the adsorbent surface and assumes that a Langmuir isotherm applies to each layer¹⁰.

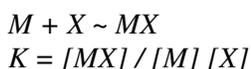
$$C_e / (C_s - C_e) = 1/BQ^o + (B - 1/BQ^o) (C_e / C_s)$$

where C_s is the saturation concentration of the metal ion, Q^o is the amount adsorbed per unit weight

of biomass for monolayer biosorption, B is constant relating to the energy of interaction with the surface, C_e is the equilibrium metal ion concentration.

These models are mathematical representations of biosorption equilibria and, by providing metal uptake capacities for various fungal strains, serve as a means of comparing different species in terms of metal removal.

Scatchard¹⁵ developed a model to describe the attraction of proteins for small molecules and ions, and also used to describe the biosorption equilibria¹⁶⁻¹⁸. The interaction of metal ions with the cell surface binding sites can be described by:



where K is the association constant, M is the metal ion under consideration, X is a binding site on the biomass surface and MX is the metal ion biosorbed on biomass. The plot of the amount of bound metal ion on biomass divided by equilibrium metal ion concentration vs the amount of bound metal ion gives a typical Scatchard plot. The equation can be represented as follows:

$$MX/M_e = K (X_o - MX)$$

where M_e and X_o are the equilibrium metal ion concentration of potential binding sites. The slope of the plot gives $-k$ and the abscissa intercept gives X_o . The total number of binding sites may be extrapolated from data in which the complete saturation of the biomass binding sites is not observed.

Metal Uptake by Living Cells

The living cells of *Penicillium*, *Aspergillus*, *Rizopus*, *Mucor*, *Saccharomyces* and *Fusarium* have been shown to biosorb metal ions^{8,11,19-21}. *Penicillium* biomass has been found to biosorb heavy metals (Cr, Ni, Zn, Pb & As)²². The living cells of *Penicillium*, *Rizopus* and *Saccharomyces* can biosorb radionuclides (U, Th & Sr)²³⁻²⁵. Metal ion uptake by living cells is a function of cell age, composition of growth media, contact time, pH of metal solution and temperature. Biosorption of metals (Cu, Zn, Cd, Pb & U) by non growing cells of *Penicillium*, *Aspergillus*, *Saccharomyces*, *Rhizopus* and *Mucor* reach equilibrium in 1 – 4 h^{16,26,27}. Biosorption kinetics of metals is usually biphasic in nature, consisting of an

initial rapid phase, contributing up to 90 per cent biosorption, and lasting for 10 min. Second phase is slower and lasts up to 4 h.

Cell age also affects the biosorption of metal ions. Increased biosorption has been observed during the lag period or early stages of growth and declines as cultures reached stationary phase. *A. niger*, *P. spinulosum* and *T. viride* showed a similar uptake pattern^{16,28}. Volesky & May⁴ observed that 12 h old cultures of baker's yeast were able to biosorb 2.6 times more uranium than 24 h cultures.

Biomass also depends on the culture conditions and growth media composition. Avery & Tobin²⁹ observed that live cells of *S. cerevisiae* incubated in presence of glucose (2%, w/v), with 100 μ M Sr^{2+} resulted in stimulation of Sr^{2+} uptake. Volesky³⁰ indicated that *R. nigricans* cultures grown on glucose pentone, sucrose and potato-dextrose medium exhibited a uranium uptake capacity of 0.47, 0.45 and 0.34 m mole U/g of biomass respectively. The growth medium controls the composition and structure of cell wall, which in turn affects biosorption.³¹

Biosorption of metal ions strongly depends on pH. The biosorption of Cr, Ni, Zn and Pb by *P. chrysogenum* was inhibited below pH 3.0. It was found to increase with pH from acidic (2.0) to basic range (7.0)^{21,22}. Barros *et al*¹¹ observed that Cd biosorption on various fungal strains was pH sensitive. *A. oryzae*, *A. niger*, *F. solani* and *Candida utilis* were found to perform better in the acidic range. The change in the sorption capacity with pH can be explained on the basis of proton-competitive adsorption reaction³².

Biosorption is also affected by biomass concentrations⁸. Low Cd uptake was observed at high concentrations of *A. niger*¹¹. The amount of Cr biosorbed per unit weight of biomass decreased with an increase in concentration of *R. arrhizus*, *R. nigricans*, *A. oryzae* and *A. niger*^{1,7,13}. The lower uptake at higher biomass concentrations can be attributed to the electrostatic interactions of the functional groups at the cell surfaces. The cells at higher concentration in suspension attached to each other, thus lowering the cell surface area in contact with the solution.

Metal uptake discussed so far was mainly due to the passive mode. The biosorption of heavy metal ions on the cell surface occurs by ion exchange and complexation reaction with functional groups like carboxyl, amine, amides, hydroxyl, phosphate and

sulphydryl groups^{33,34}. Metals (Ag, Au & U) has also been removed as a result of precipitation/crystallization on fungal and algal surfaces^{23,24,35}.

Metal uptake can also take place by an active mode, which is dependent on the cell metabolic cycle, and metal ions are transported into the cell material across the cell wall. Metal uptake by active mode has been observed for Cu, Cd, Ni, Zn, Co, Mn, Sr, Mg and Ca^{30,36-38}. Gadd³⁹ suggested that at high metal concentrations, active mode might not contribute significantly to metal uptake, especially for filamentous fungi.

Metal Uptake by Dead Cells

Dead biomass in industrial applications offers certain advantages over living cells. Systems using living cells are likely to be more sensitive to metal ion concentration (toxicity effects) and adverse operating conditions (pH & temperature). Constant nutrient supply is required for systems using living cells and recovery of metals and regeneration of biosorbent is more complicated for living cells. Dead biomass can be procured from industrial sources as a waste product from fermentation processes. Food and beverage industry can serve as a source of yeast (*S. cerevisiae*) and chemical and pharmaceutical industries as sources of *A. niger*, *T. reessi*, *R. arrhizus* and *R. nigricans*. Waste biomass of *M. meihie*, *R. arrhizus*, *A. niger*, and *P. chrysogenum* procured from fermentation industries has been found effective in biosorption⁴⁰⁻⁴⁵.

Cells can be killed by physical treatment methods using heat treatment⁴⁶⁻⁴⁸, autoclaving and vacuum drying^{8,49} or chemicals like acids, alkalies and detergents^{11,22,50-52} or other chemicals like formaldehyde⁵³ or by mechanical disruption⁵⁴. Pretreatment methods have usually shown an increase in the metal sorption capacity for a variety of fungal species. Alkali treatment (usually with NaOH) of fungal biomass for 4 – 6 h at 95 – 100°C deacetylates chitin present in the cell wall to form chitosan – glucan complexes with higher affinity for metal ions^{55,56}.

Tobin *et al*⁵⁷ investigated the uptake of 17 metal species by heat killed *R. arrhizus* at a pH 4.0. A linear relationship was observed between maximum biosorptive capacity of a metal ion by biomass and ionic radii of the various cations. Higher uptake capacity was observed for larger ions, the exception being Cr³⁺ and the alkali metal ions. Alkali metal ions

were not prone to biosorption, due to inability of metal ions to form complexes with the legend groups present on the fungal cell surface. The fungal biomass possesses exceptionally high biosorptive capacity for radionuclides, such as U and Th (in excess of 180 mg/g)^{40,58}. Similarly for Ag, the uptake by alkali treatment *A. niger* has been observed to be as high as 100 mg/g⁵⁹.

The biosorption of metal ions has been observed to be pH dependent. Uranium removal by *Penicillium* was more or less the same in the pH range of 2.5 – 9.5⁶⁰. This showed that there were differences in the behavior of fungal cell wall surfaces. The biosorption of Pb, Cd, Ni and Zn was severely inhibited at pH below 4.0^{10,43,61}. Fourest *et al.*,⁶² observed that Zn biosorption on *M. miehei* and *P. chrysogenum* was at pH less than 4.0 and for *R. arrhizus*, which exhibited a higher Zn uptake, it was 5.8. The metal uptake for *R. arrhizus*, *M. miehei* and *P. chrysogenum* increased from 16 – 35, 3 – 32 and 4.5 – 22 mg/g respectively, when pH of the reaction mixture was controlled at 7.0. Under uncontrolled conditions of pH, the drop in pH may create an undesired competition for metal ions from protons, thus lowering the metal uptake capacity.

Yan and Viraraghavan,⁶³ observed that biosorption column of *Mucor rouxii* biomass was able to remove metal ions (Pb, Cd, Ni & Zn) not only from single-component metal solutions but also from multi-component metal solutions. The metal removal capacities of the biomass for Pb, Cd, Ni and Zn were 4.06, 3.76, 0.36 and 1.36 mg/g respectively. For a multi-component metal solution containing Cd, Ni and Zn were 0.36, 0.31 and 0.40 mg/g respectively.

The uranium uptake by *R. arrhizus* was reduced in the presence of Fe and Zn²⁴ and for *Penicillium* Fe alone-inhibited uranium biosorption and Zn had no significant effect^{20,47}. This shows the variability in the metal biosorption, which occurs in fungal species. The nature of biosorption on fungi is complex and extreme caution is warranted in drawing any general conclusion on fungal biosorption. *P. chrysogenum* exhibits selectivity for Pb over metals such as Cd, Cu, Zn and As. The biosorptive uptake for metals decreased in the following order: Pb > Cd > Cu > Zn > As. Yakubu and Dudeney⁵⁴ showed that biosorption of uranium on *A. niger* was substantially reduced in the presence of Cu, Zn & Fe and the preferential order for biosorption was : Fe > U > Cu > Zn. Zhou and Kiff⁶⁴ indicated that Mn, Zn, Cd, Mg and Ca inhibited

Cu biosorption by *R. arrhizus*. Brady *et al.*,⁶¹ observed that biosorption of metals on aerobically grown yeast treated with hot alkali was selective. The metal uptake in two three – metal systems followed the order: Cu > Cr > Cd and Cu > Pb > Ni.

The presence of anions also affects the biosorption of metal ions. Biosorption is reduced in the presence of ethylenediamine tetra acetate (EDTA), sulphate, chloride, phosphate, carbonate, glutamate, citrate and pyrophosphate^{44,64,65}. The presence of EDTA has been found to severely affect the biosorption of Cu, La, U, Ag, Cd and Pb. The presence of carbonates severely inhibits the uranium biosorption⁶⁶.

Regeneration and Elution of Metals from Loaded Fungal Biomass

The application of fungi and yeast as biosorbents depends not only on the biosorptive capacity, but also on the ease with which biomass can be regenerated and reused. The cell surface-bound metal might be easily removed by the use of elutants, but the intracellular metal can only be released by destructive treatment.

Various elutants have been screened for recovery of U from *Rhizopus* and *Penicillium* biomass^{23,67}. Mineral acids (H₂SO₄, HCl) are effective in recovery of sorbed U, but can cause damage to the biomass and reduce U biosorptive capacity in subsequent uses. Sodium bicarbonate was found to be an effective elutant. Galun *et al.*,⁶⁰ observed that U was desorbed by ammonium carbonate and sodium bicarbonate. EDTA was less effective as an elutant, but it did increase U biosorption two fold for subsequent use.

The elution of Ni, Cu, Zn and Cd biosorbed on *Penicillium* biomass can be achieved by using dilute hydrochloric acid or dilute sodium hydroxide solution. Elution with 0.1 N NaOH and washing to pH 5.5 – 6.0 resulted in a 2 – 6 fold increase in mycelial uptake in subsequent use. No significant changes in biosorption of Ni, Cu, and Zn were observed when using HCl alone. The use of mineral acids as an elutant has been widely studied^{10,41,50,68}. Zhou and Kiff⁶⁴ found no decrease in biosorptive capacity in subsequent uses when 0.1 N HCl was used as an elutant. Concerns over the damage to biosorbent structure from using mineral acids as an elutants prompted us to investigate less aggressive elutants. 0.1 M CdCl₂ and MgSO₄ solutions were able to elute Cd and Ni biosorbed by *A. niger*¹⁰.

Huang *et al.*⁴⁹ observed that Cd (II)-loaded *A. oryzae* can be effectively regenerated using a strong acid. For

Table 2 — Cost comparison for various adsorbents^{32, 66}

Source	Price of adsorbents*
	\$/kg
Biomass	1-5
Activated carbon	2.0-5.5
Ion-exchange resins	13-30

* Includes transportation and drying costs

eight adsorption-desorption runs, 35 mg of Cd (II) per gram of dry weight of *A. oryzae* was removed. There is another significant factor which determines the efficiency of desorption is S/L ratio i.e ratio of metal laden biosorbent (mg) to the elutant volume (ml). From among four different S/L ratios (8, 10, 20 and 40), optimum⁶⁹ was found to be 10 and 8 (elutant volume 20 & 25 ml) giving 90-95 percent recovery of Cr (VI) ions. However, a higher S/L (40) i.e an elutant volume of 5 ml gave a maximum of 48 percent recovery. Kuyucak and Volesky⁷⁰ also reported a S/L ratio of 10-12 percent as favorable for successful elution of cobalt from *Ascophyllum nodosum*.

The Cr uptake by polysulfone entrapped *R. nigricans* indicate that the loss of adsorption efficiency after the 25th cycle amount to 4-5 mg Cr/g biomass⁶⁹. The study suggested that the biosorption of Cr (VI) ions to *R. nigricans* biomass was almost completely reversible and the regeneration capacity was nearly 75-78 percent. Adsorption –desorption operations illustrating stabilized performance of the biosorbent in repeated cycles has been reported for removal of Cu, Zn and Cd by *Saccharomyces cerevisiae*^{71, 72}.

Comparison with Other Adsorbents

Metal removal capacity of fungal biomass is comparable with other conventional solids such as activated carbon and other solids. Huang *et al.*,⁴⁹ observed that Cd (II)-loaded *A. oryzae* can be effectively regenerated using a strong acid. For eight adsorption-desorption runs, 35 mg of Cd (II) per gram of dry weight of *A. oryzae* was removed, while activated carbon (Nuchar SA) removed only 14 mg of Cd (II) per gram of dry weight in five cycles.

The cost of culturing biomass for biosorption has been shown to be competitive (Table 2) with the cost of conventional adsorbent (ion-exchange resins and activated carbon)^{34,43,49,53,54,73,74}.

Applications to Practice

Important factors that govern the application of an adsorbent to be used in practice include: (a)

Effectiveness in removing pollutants; (b) Availability of the adsorbent; (c) Cost of the adsorbent; (d) Ease of regeneration and subsequent use of the biosorbent; and (e) Ease with which the biosorbent can be used in various reactor configurations. Source of raw biomass and cost of immobilization (if required) are important factors in determining overall cost of biosorbent material. Thus biosorption offers an economical and technically feasible option for treatment of wastewater. Fungal biosorption is also of natural geochemical importance in the concentration of metals in soils rich in fungi.⁶⁶

Conclusions

Heavy metal removal capacity of fungal biomass is determined by several factors with pH being the most important variable. Pretreatment methods have usually shown an increase in the metal sorption capacity for the variety of fungal species. Fungal biomass can be regenerated relatively easily and used again. Most significantly, the metal removal capacity of fungal biomass is as good or better than other conventional adsorbents. The biosorption-based process can be considered as a replacement of an existing metal-removal technology or can also be used as a polishing unit for an existing treatment facility.

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