



A Review

Brewer's Yeast: an Alternative for Heavy Metal Biosorption from Waste Waters

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Abstract

Heavy metal pollution is one of the most important environmental problems today. Thus, metal brings serious environmental pollution, threatening human health and ecosystem. In recent years, applying biotechnologies removing metal pollution became hot topic because of its potential application. Alternative process is biosorption, which utilizes various natural materials, including, yeast. The yeast biomass has been successfully used as biosorbent for removal of Ag, Au, Cd, Co, Cr, Cu, Ni, Pb, U, Th and Zn from aqueous solution. Yeasts of genera *Saccharomyces* are efficient biosorbents for heavy metal ions and it can be procured in large quantity at low cost. *Saccharomyces cerevisiae* can remove toxic metals, recover precious metals and clean radio-nuclides from aqueous solutions to various extents. *Saccharomyces* has the ability to differentiate between different metals such as selenium, antimony and mercury based on their toxicity. This low-cost biosorbent will make the process highly economical and competitive particularly for environmental applications in detoxifying effluents.

Keywords: brewer yeast, biosorption, heavy metals.

1. Introduction

Biosorption is defined as the removal of metal from solution by biological material [10]. Heavy metal pollution is one of the most important environmental problems today. A lot of industries produce and discharge wastes containing different heavy metals into the environment, such as mining and smelting of metalliferous, surface finishing industry, energy and fuel production, fertilizer and pesticide industry, metallurgy, electroplating, electric appliance manufacturing, metal surface treating etc. Thus, metal brings serious environmental pollution, threatening human health and ecosystem.

Three kinds of metals are of concern: toxic metals such Hg, Cr, Pb, Cu, Ni, Cd, As, precious metals such as Pt, Au, Ag, Pd, radionuclides such as U, Th, Ra, Am [29].

In Romania most heavy metal emissions are produced by industrial activities, but also mining activities, transport, as well as the spreading of fertilizer and sewage sludge discharge heavy metals into the environment.

In 2004, the yearly medium value of 0.075 mg/m³ for heavy metals was exceeded in 23 localities (Reia, Caransebeş, Moldova, Oţelu Roşu, Braşov, Cluj, Ploieşti, Floreşti, Azuga, Miercurea Ciuc, Gheorghieni, Odorheiu Secuiesc, Arad, Rm. Vâlcea, Zalău, Suceava, Copşa Mică, Mediaş, Alba Iulia, Zlatna, Baia Mare, Petroşani, Brad). Highest values were recorded in Zlatna - 0.186 mg/m³, Arad - 0.188 mg/m³ and Braşov - 0.156 mg/m³.

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The contamination of soil and water with heavy metals (mainly lead, zinc, cadmium) exceeds the alert limit in the zones Copșa Mică, Zlatna and Baia Mare (Agenția de Protecția Mediului a Județului Sibiu. Anuare privind starea mediului 1996 - 2005). Heavy metals like Cd, Zn or Ni exceeded in 2000 the admitted limits of the Romanian legislation in several lakes of the Danube Delta. It is alerting because heavy metals, like cadmium, have reached high limits (three times the allowed limits) in some fish species in the areas Caraorman and Matita.

Methods for removing metal ions from aqueous solutions consist of physical, chemical and biological technologies. Conventional methods for removing metals are reverse osmosis, electrodialysis, ion exchange, chemical precipitation, ultrafiltration, adsorption on activated carbon, evaporation, etc.

Hence the disadvantages like incomplete metal removing, high reagent and energy requirements, generation of sludge or other waste products that require careful disposal has made it imperative for a cost-effective treatment method that it is capable of removing heavy metals. Volesky [1] summarized the advantages and disadvantages of those conventional metal removal technologies.

In recent years, applying biotechnologies in controlling and removing metal pollution became hot topic in this field because of its potential application. Alternative process is biosorption, which utilizes various natural materials, including bacteria, fungi, yeast, algae. These biosorbents have metal-sequestering property and can be used to decrease the concentration of heavy metal ions in solution from ppm to ppb level. These natural compounds can sequester dissolved metal ions out of dilute complex solutions with high efficiency [32]. The major advantages of biosorption over conventional treatment methods include low-cost, high efficiency, minimisation of chemical or biological sludge, regeneration of biosorbent and possibility of metal recovery [28].

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms are various and not fully understood. According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into a) metabolism dependent and b) non-metabolism dependent. According to the location where the metal is removed from solution, biosorption can be classified as a) extracellular accumulation, b) cell surface sorption/precipitation, c) intracellular accumulation [28]. Transport of the metal across the cell membrane yields intracellular

accumulation, which is dependent on the cell's metabolism. This kind of biosorption may take place only with viable cells. During non-metabolism biosorption, metal uptake is by physico-chemical interaction between the metal and the functional groups present on the microbial cell surface. This type of mechanism is relatively rapid and can be reversible [9].

There are several chemical groups that could attract and sequester the metals in biomass like acetamido groups of chitin, amino and phosphate groups in nucleic acids, amino, sulfhydryl and carboxyl groups in amino acids and proteins, hydroxyls in polyaccharides etc. The presence of some functional group does not guarantee their accessibility for sorption, perhaps due to the steric, conformational or other barriers [20].

Types of biomass

Some types of biosorbents would be broad range, binding and collecting the majority of heavy metals with no specific activity, while others are specific for certain metals. Recent biosorption experiments have focused attention on waste materials, which are by-products or the by-products from large scale industrial operations.

For example waste mycelia available from fermentation processes, olive mill solid residue [14], activated sludge from sewage treatment plants, biosolids, aquatic macrophytes, etc. [27].

Although many biological materials can bind heavy metals, only those with sufficiently high metal-binding capacity and selectivity for heavy metals are suitable for use in a full-scale biosorption process. A large number of biomass types have been investigated for their metal binding capability under various conditions. Another challenge is that the application of biosorption is facing up with great difficulty [13].

Great efforts have to be made to improve biosorption process, including immobilization of biomaterials, improvement of regeneration and re-use, optimization of biosorption process etc.

The importance of metallic ions to fungal and yeast metabolism has been known for a long time. The presence of heavy metals affects the metabolic activities of fungal and yeast cultures, and can affect commercial fermentation processes, which created interest in relating the behavior of fungi to the presence of heavy metals. The results from such studies led to a concept of using fungi and yeasts for the removal of toxic metals (such as lead and cadmium) from wastewater and recovery of precious metals (such as gold and silver) from process waters [4].

Both living and dead fungal cells possess a remarkable ability for taking up toxic and precious metals.

Biomass could be provided from activated sludge or fermentation waste from industries like those of food, dairy and starch. Also, organisms (e.g., bacteria, yeast, fungi and algae) coming from their natural habitats are good sources of biomass [8]. Apart from the microbial sources even agricultural products such as wool, rice, straw, coconut husks, peat moss, exhausted coffee [2], waste tea [7], walnut skin, coconut fibre, cork biomass [26], defatted rice bran, rice hulls, soybean hulls and cotton seed hulls [23], are depicted as good biomass sources. However, sea weeds, molds, yeasts, bacteria have been tested for metal biosorption with encouraging results [24].

Seaweeds are large group of marine benthic algae. They offer several advantages for biosorption because of their larger surface area. This feature offers a convenient basis for the production of biosorbent particles suitable for sorption process. They contain many polyfunctional metal-binding sites for both cationic and anionic metal complexes. Potential metal cation-binding sites of algal cell components include carboxyl, amine, imidazole, phosphate, sulphate, sulfhydryl, hydroxyl and chemical functional groups contained in cell proteins and sugars. Brown algae stand out as very good biosorbent of heavy metals [21]. Their cell walls contain fucoidin and alginic acid. The alginic acid offers anionic carboxylate and sulfate ions at neutral pH.

Bacteria: Different bacterial species present differences in relation to their number of surface binding sites, binding strength for different ions and the binding mechanisms. Gram positive cell walls and surfaces have a negative charge density owing to the peptidoglycan network, a macromolecule consisting of strands of alternating glucosamine and muramic acid residues, which are often N-acetylated. Carboxylate groups at the carboxyl terminus of individual strands provide bulk of anionic character to the cell wall [34].

Fungi and yeasts: The majority of fungi show filamentous or hyphal growth. Cell walls of fungi present a multi-laminate architecture where up to 90% of their dry mass consists of amino or non-amino polysaccharides. The fungal cell walls can be considered as a two phase system consisting of chitin framework embedded on an amorphous polysaccharide matrix. The cell walls are rich in polysaccharides and glycoprotein's such as glycans (-1-6 and -1-3 linked D-glucose residues), chitin (-1-4 linked N-acetyl-D-glucosamine), chitosan (-1-4 linked D-glucosamine), mannans (-1-4 linked

mannose) and phosphormannans (phosphorylated mannans). A lot of metal binding groups, like amine, imidazole, phosphate, sulphate, sulfhydryl and hydroxyl are present in the polymers [12].

Some types of biosorbents would be broad range, binding and collecting the majority of heavy metals with no specific activity, while others are specific for certain metals. Recent biosorption experiments have focused attention on waste materials, which are by-products or the by-products from large scale industrial operations. For example waste mycelia available from fermentation processes, olive mill solid residue [14], activated sludge from sewage treatment plants [19], biosolids [22], aquatic macrophytes [27], etc.

Brewer's yeast as biosorbent for heavy metal ions

Brewer's yeast is made from a one-celled fungus called *Saccharomyces cerevisiae* and is used to make beer. It also can be grown to make nutritional supplements. Brewer's yeast is a rich source of minerals, especially chromium, an essential trace mineral that helps the body maintain normal blood sugar levels, selenium, amino acids, protein and the B-complex vitamins.

Saccharomices cerevisiae is a species of budding yeast. "Saccharomyces" derives from Greek, and means "sugar mold", "cerevisiae" comes from Latin, and means "of beer". It is perhaps the most useful yeast owing to its use since ancient times in baking and brewing. It is believed that it was originally isolated from the skins of grapes (one can see the yeast as a component of the thin white film on the skins of some dark-colored fruits such as plums, it exists among the waxes of the cuticle). It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *E. coli* as the model prokaryote. It is the microorganism behind the most common type of fermentation. *Saccharomices cerevisiae* cells are round to ovoid, 5 – 10 µm in diameter. It reproduces by a division process known as budding [29,32]. In general, yeast cells have a cell wall, cytoplasmic membrane, cytoplasm and inclusions, a single nucleus, mitochondria, Golgi apparatus, vacuoles, cytoskeleton.

The yeast biomass has been successfully used as biosorbent for removal of Ag, Au, Cd, Co, Cr, Cu, Ni, Pb, U, Th and Zn from aqueous solution. Yeasts of genera *Saccharomyces*, *Candida*, *Pichia* are efficient biosorbents for heavy metal ions. Most of yeasts can bond a wide range of metal ions or be strictly specific in respect of only one metal ion. *Saccharomices cerevisiae* as biosorbents is of

special interest [23]. A number of literatures have proved that *S. cerevisiae* can remove toxic metals, recover precious metals and clean radionuclides from aqueous solutions to various extents.

The advantages of *S. cerevisiae* as biosorbents in metal biosorption, biosorptive capacity of *S. cerevisiae*, the selectivity and competitive biosorption by *S. cerevisiae* were depicted in detail by Wang and Chen [32].

There are three types of *Saccharomyces cerevisiae*: lab culture, living and non-living brewer's yeast which can be used as biosorbent [36]. Based on literature data, the magnitude order of metal uptake capacity by *S. cerevisiae* can be estimated as the followings: for lead, biosorptive capacity by *S. cerevisiae* is above tenth and less than 300 mg Pb/g dry weight biomass, for copper, less than 20 mg Cu/g dry weight yeast, for zinc, usually less than 30 mg Zn/g dry weight, for cadmium, usually above 10 but less than 100 mg Cd/g dry mass, for chromium and nickel, seldom more than 40 mg/g dry mass, for precious metals, such as Ag, Pt, Pd, around 50 mg/g dry weight yeast. Biosorptive capacity of radionuclide uranium by *S. cerevisiae* is usually between 150 and 300 mg U/g dry weight biomass [32].

The biosorption of heavy metals using microorganisms like *S. cerevisiae* is affected by several factors. These factors include the specific surface properties of the biosorbent and the physico-chemical parameters of the solution such as temperature, pH, initial metal ion concentration and biomass concentration [31].

Non-living biomass appears to have advantages in comparison to the use of living cells. Killed cells may be stored or used for extended periods at room temperature, they are not subject to metal toxicity and nutrient supply is not necessary. The pretreatment and killing of biomass either by physical or chemical treatments or crosslinking are known to improve the biosorption capacity of biomass [3].

Biosorption application is facing to great challenge, some investigators proposed several suggestion. For the future of biosorption, there are two trends of biosorption development for metal removal. One trend is to use hybrid technology for pollutants removal [13], especially using living cells. Another trend is to develop good commercial biosorbents just like a kind of ion exchange resin, and to exploit the market with great endeavor [16].

The difficulties existing for biosorption application urge people to consider applying the hybrid technology which comprise of various processes to treat real effluents.

Various biotechnology-based processes, such as biosorption, bioreduction and bioprecipitation were suggested. Consequently, application of living cells rather than dead cells for biosorption has gained attention again [15].

Another trend requires the improvement of biomaterials immobilization, as well as the optimization of the parameters of biosorption process and physicochemical conditions, including reuse and recycling [3].

The sources and type of biosorbent play a major role in determining the overall cost of the biosorbent material. If the biomass needs to be specifically cultured for this purpose, manufacturers will incorporate maintenance and production expenses in the total cost, as well as a commercial fee.

These low-cost biosorbents will make the process highly economical and competitive particularly for environmental applications in detoxifying effluents of e.g.: metal-plating and metal-finishing operations, mining and ore processing operations, metal processing, battery and accumulator manufacturing operations, thermal power generation (coal-fired plants in particular), nuclear power generation.

Saccharomyces cerevisiae can remove toxic metals, recover precious metals and clean radionuclides from aqueous solutions to various extents. *S. cerevisiae* is a product of many single cell and alcohol fermentations, it can be procured in large quantity at low cost. *Saccharomyces* has the ability to differentiate between different metals such as selenium, antimony and mercury based on their toxicity. This property makes *S. cerevisiae* useful in analytical measurements [19].

Table 1 presents some data on the biosorptive capacities of the yeast (in various forms) for different metal ions reported in literatures.

Based on data presented in this table and other literature data the magnitude order of metal uptake capacity by *S. cerevisiae* can be estimated as the followings: for lead, biosorptive capacity by *S. cerevisiae* is above tenth and less than 300 mg Pb/g dry weight biomass, for copper, is less than 20 mg Cu/g dry weight yeast, for zinc, usually less than 30 mg Zn/g dry weight, for cadmium, usually above 10 but less than 100 mg Cd/g dry mass, for chromium and nickel, seldom more than 40 mg/g dry mass, for precious metals, such as Ag, Pt, Pd, around 50 mg/g dry weight yeast.

Biosorptive capacity of radionuclide uranium by *S. cerevisiae* is usually between 150 and 300 mg U/g dry weight biomass.

Table 1. Metals biosorption by *Saccharomices cerevisiae* (mg/g)

Metal	Source or form of biosorbents	Biosorption capacity	References
Pb	Free cell	79.2	[19]
Pb	Lab cultivated, then dried at 100 °C	270.3	[11]
Pb	Ethanol treated waste baker's yeast	17.5	[5]
Cu	Waste yeast fermentation autoclaved	4.93	[35]
Cu	Free cells	6.4	[19]
Cu	Waste yeast from brewery,	8.1	[6]
Zn	Waste yeast from fermentation industry	3.45	[5]
Zn	Free cells	23.4	[33]
Hg	Free cells	64.2	[19]
Co	Free cells	9.9	[19]
Ni	Waste yeast from fermentation	1.47	[35]
Fe	Whiskey distillery spent, lyophilized	16.8	[19]
Pd	Immobilized cells of waste yeast	40.6	[19]
Pt	Immobilized cells of waste yeast	44	[18]

Biosorption Mechanism

The elucidation of the mechanism of biosorption is necessary to enable the technology to be developed.

The key factors controlling and characterizing these mechanisms are: the type of biological ligands available for metal sequestering, the status of the biomass, i.e. living/non-living, the chemical, stereochemical and coordination characteristics of the targeted metals and metal species, the characteristics of the metal solution such as pH and the presence of competing co-ions [32]. Microorganisms possess an abundance of functional groups that can passively adsorb metal ions. The term adsorption can be used as a general term and includes several passive, i.e. non-metabolic, mechanisms such as: complexation, chelation, co-ordination, ion exchange, precipitation, reduction.

1. Complexation

Complex formation of metal ions with organic molecules involves ligand centres in the organic species i.e. the presence of an atom or atoms having lone pair electrons to donate.

Complexation may be electrostatic or covalent and the simplest case is complexation by a mono-dentate ligand such as RNH_2 .

To approach and elucidate biosorption mechanisms, a significant part of the recent advances in biosorption are based on the classification of elements according to the hard-soft acid-base classification Pearson's. Hard acids", metals such as Na, K, Ca, Mg, often essential nutrients for microbial growth, bind preferentially to oxygen ligands. Soft acids, metals such as the precious metals Ag, Au, Pt, Pd are

bound covalently to the cell wall by "soft bases", ligands containing nitrogen or sulfur.

Several mechanisms might be involved in the immobilisation of metals and it is now evident and confirmed by several researchers, that the biosorption of precious metals is a two step mechanism comprising first covalent bonding and then in-situ reduction [13].

2. Chelation

Organic molecules containing more than one functional group with donor electron pairs can simultaneously donate these to a metal atom. This can result in the formation of a ring structure involving the metal atom a process termed 'chelation'.

In general, since a chelating agent may bond to a metal ion in more than one place simultaneously, chelated compounds are more stable than complexes involving mono-dentate ligands. Stability tends to increase with the number of chelating sites available on the ligand.

Thus chelation of metals by donor ligands of biopolymers leads to the formation of stable species.

The next figure present the structure of a metal ion chelated with ethylenediamine tetracarboxylic acid (EDTA), a hexa-donating compound with two nitrogen and four oxygen donor atoms.

3. Co-ordination

Metal atoms have preferences for specific donor atoms ("hard/hard"/"soft/soft") and the stereochemical arrangements that play an important role in the binding with the available ligands on the microbial cell. Limited information of surface

complexation models, based on the theory of surface co-ordination chemistry, is available to describe metal biosorption, however the preferences of the metal species should be considered to explain observed metal biosorption capacities and to elucidate biosorption mechanisms [9].

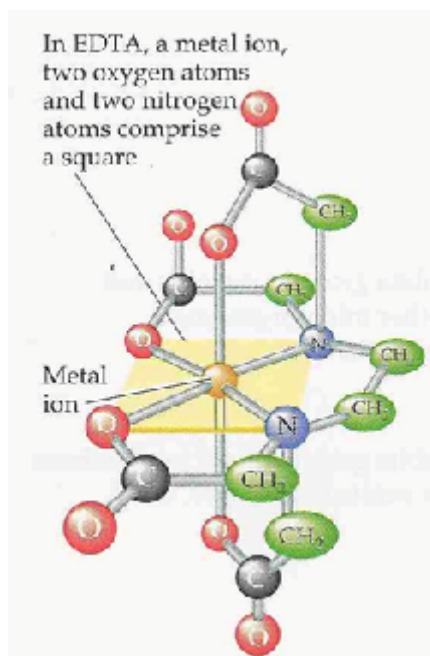


Figure 1. Structure of a metal ion chelated with EDTA

4. Ion exchange

The gram-positive bacteria, principally members of the genus *Bacillus*, have enhanced capacity for metal binding because of a significant negative charge density.

This is due to the structure of the cell wall with teichoic and teichuronic acids attached to the peptidoglycan network. The phosphodiester groups of teichoic acid and the carboxylate groups of the teichuronic acid thus contribute ion-exchange capacity to the cell wall.

5. Precipitation

Metal precipitation is also involved in biosorption. The precipitates may be formed and remain in contact with or inside the microbial cells or may be independent of the solid phase of the microbial cell. In the later case, the presence of the solid phase-microbial cell or biofilm also plays a favourable role in the phenomenon of precipitation.

The term precipitation in most cases refers to the formation of insoluble inorganic metal precipitates. However, in the case of metal biosorption by microbial cells, organic metal precipitates may also be formed.

6. Reduction

The removal of toxic hexavalent chromium from aqueous solution by biosorption by different biomass types has been extensively reported. This removal is often associated with the simultaneous reduction of Cr(VI) to Cr(III), thus inactivated fungal biomass e.g. *Aspergillus niger*, *Rhizopus oryzae*, *Saccharomyces cerevisiae* and *Penicillium chrysogenum* remove Cr(VI) from aqueous solutions by reduction to Cr(III) when contacted with the biomass [11, 30].

Also soft metals like gold and palladium are first bound on sites on and within the cell wall and these sites act as nucleation points for the reduction of metals and growth of crystals and elemental gold and palladium have been obtained. The biosorption mechanism is a two-step process: initiation of the uptake at discrete points by chemical bonding, then reduction of the metal ions [18].

Conclusions

Biosorption is being demonstrated as a useful alternative to conventional systems for the removal of heavy and precious metals from industrial effluents. Brewer yeast remove toxic metals, recover precious metals and clean radionuclides from aqueous solutions to various extents. This low-cost biosorbent will make the process highly economical and competitive particularly for environmental applications in detoxifying effluents.

The development of the biosorption processes requires further investigations concerning the regeneration of biosorbent material, finding the best physical and chemical conditions to improve the rate of biosorption.

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