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## Bioremediation of toxic metal ions-A Review

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### ABSTRACT

*The awareness of toxicological and ecological effects of toxic metals has attracted serious attention for decontaminating industrial waste waters prior to discharge into water bodies of sewage ponds, streams and rivers. Unlike organic pollutants metal ions are indestructible and have cumulative effect on ecosystem. Conventional methods of metal removal from industrial effluents are: chemical, precipitation, oxidation and reduction, membrane filtration technology, electrochemical precipitation, evaporation recovery and ion exchange resins. However these strategies suffer from various limitations. Though ion exchangers are efficient in removing toxic metal ions, they do not distinguish between essential ions ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ), which are present in excess over toxic metal ions. Bioremediation, the technology of using of living organisms or their principles in isolation for decontaminating toxic chemicals from the environment. Both the principles of biosorption and accumulation in microbes have been exploited for removing toxic metal ions from polluted effluents.*

**Keywords:** Biosorption, Bioaccumulation, fungi, biosorbent, toxic metals.

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### INTRODUCTION

The awareness of toxicological and ecological effects of toxic metals has attracted serious attention for decontaminating industrial waste waters prior to discharge into water bodies of sewage ponds, streams and rivers. Unlike organic pollutants metal ions are indestructible and have cumulative effect on ecosystem. Conventional methods of metal removal from industrial effluents are: chemical, precipitation, oxidation and reduction, membrane filtration technology, electrochemical precipitation, evaporation recovery and ion exchange resins. However these strategies suffer from various limitations. Though ion exchangers are efficient in removing toxic metal ions, they do not distinguish between essential ions ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ), which are present in excess over toxic metal ions. Bioremediation can be defined as the use of living organisms or their principles in isolation for decontaminating unwanted and toxic chemicals from the environment. Amongst living systems microorganisms are known to be versatile and live in extremes of hostile environment and hence have excellent capabilities for developing the process of bioremediation [1-7]. Microorganisms such as bacteria and fungi are known to coexist in the earth along with metals. They take part in global cycling of mineral elements leading to formation of deposits as well as their solubilization from ores. The novel mechanisms employed by microbes in interacting with metal ions had

been discussed in the preceding section in detail. These principles can be employed in developing decontaminating strategies for toxic elements.

### Bioaccumulation

Viable microbes accumulate toxic metal ions by transporting them across cell membrane into intracellular locations using both specific and nonspecific transporter proteins. Since the normal wild type organism suffers the above effects of metals it would be advantageous to have resistant organisms. The potential of the Ni-resistant hyper accumulator strain of *N.crassa* in removing nickel ions from highly toxic concentrations was demonstrated [8]. While the wild type *N.crassa* was efficient in removing Ni from low concentrations ( $4\text{mg L}^{-1}$ ), the Ni-R strain was capable of removing more than 90% of from high concentrations ( $120\text{ mg L}^{-1}$ ). Similarly cobalt-resistant strain of *N.crassa* capable of removing 90% of  $\text{Co}^{2+}$  ions from very high concentrations was also demonstrated ( $500\text{ mg L}^{-1}$ ) [9]. In all the above cases removal of toxic metal ions was not affected by the presence of excess of physiologically essential metal ions such as Mg, Ca, Na or K, which is advantageous over ion exchange resins. It should be noted that metal ion uptake in the above studies was shown to involve both biosorption and bioaccumulation. The amount of metal ion removed by any one of the processes varied with time of exposure. At initial time up to 1 hr biosorption predominated accounting for 90% of the metal ion taken up, while at later time points (6 h –24 h), bioaccumulation increased up to 60%. *Neurospora crassa* Cu metallothionein gene was expressed in *E.coli*, which showed superior ability in sequestering the metal ions [10]. Since the use of living organisms has practical limitations for treatment of industrial effluents under field conditions, the use of fungal biopolymers extracted from biomass was investigated as an alternative.

### Biosorption

Inactivated microbial biomass is both economical and useful source for biosorption of toxic metals. Sufficient quantities of biomass can be obtained as waste product of fermentation industries employing bacteria, yeast and fungi. Rajendran *et al.*, [11] reported *A.niger* mutant M3 capable of absorbing 50% more nickel at 1.7 mM concentration than its parent strain. *Mucor nicheri*, *Penicillium chrysogenum* waste biomass obtained from industrial fermenters proved to be cost effective metal biosorptive agents on large scale [12]. Biosorb, an alkali extracted mycelial biomass of *Aspergillus niger* has been used as a model system to explore the basic mechanisms of biosorption of toxic metal ions. Biosorb was found to sequester  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$  both from dilute and concentrated solutions up to 10% of its dry weight. The binding of these ions to biosorb resulted in release of stoichiometric quantities of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  into the medium. This stoichiometry was related to the charge of the ions bound as binding of divalent ions released equimolar concentrations of Ca and Mg put together, while binding by univalent  $\text{Ag}^{+}$  caused the release of Ca/Mg in the ratio of 2:1 [13]. Efficient concentration of silver ions by *A.niger* biosorb was also demonstrated [14]. Biosorbent prepared from a cadmium-resistant species of *Curvularia* sp. isolated from industrial effluent was also shown to have excellent binding capacity for cadmium ions [15]. *Aspergillus niger* biosorbent was also used to determine its potential in sequestering toxic metal ions ( $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cr}^{3+}$ ) from polluted lake waters and industrial effluents. Especially  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  were sequestered with high efficiency from lake waters containing low concentrations ( $5\text{ to }50\ \mu\text{g L}^{-1}$ ). Up to 100 liter equivalent of these ions were sequestered into the biosorb under the experimental conditions. In comparison to Dowex-50, Biosorb was found to be an order of magnitude superior and also chromium ions from effluent of electroplating industry ( $434\text{ mg L}^{-1}$ ) could also be removed effectively by fungal biosorbent [14]. Biosorbent from *A.niger* was used to remove  $\text{Hg}^{2+}$  and  $\text{CH}_3\text{Hg}^{+}$  effectively [16].

Inactivated microbial biomass presents a very useful source for the biosorption of toxic metals. Due to low mechanical strength, low density and small particle size live biomass as such is not suitable for use in the detoxification of certain effluents. Susceptibility to biodegradation reduces the storage and shelf life of the biomass. In many cases biosorbents are ground to powder, sieved and mixed with granulating cross-linking agents and extruded to give the desired final size [17]. Immobilized *Rhizopus arrhizus* biomass has been extensively used to sequester uranium and other metal ions [17]. Fluidized beds of alginate and

polyacrylamide immobilized species of *Chlorella* and *Spirulina* have been used to remove a variety of metals like  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Au}^{3+}$  from mixtures [18]. Recently *A.niger* biosorbent in polysilicate matrix and used to concentrate  $\text{Hg}^{2+}$  and  $\text{CH}_3\text{Hg}^+$  from dilute solution. This material was used to evaluate the ground water levels of  $\text{Hg}^{2+}$  from industrial areas[19]. A number of commercial products with patents were developed for application of metal removal especially using microbial polymers. A potent commercial algal biosorbent AlgaSorb™ has been developed using a fresh water alga *Chlorella vulgaris* to treat wastewater. Another metal sorption agent AMT-BIOCLAIM™ has employed *Bacillus* biomass. Bio-Fix biosorbent uses a complex biomass preparation from a variety of sources including cyanobacterium (*Spirulina*), yeast, algae, plants and guar gums to give a consistent product and immobilized as beads using polysulfone [20]. Samuelson *et al* [21] reported recombinant *Staphylococcus xylosus* and *S.carnosus*, had gained  $\text{Ni}^{2+}$  and  $\text{Cd}^{2+}$  binding capacity and suggested that they could be used in bioremediation of heavy metals. They evaluated surface display systems for expression of two different polyhistidyl peptides His<sub>3</sub>-Glu-His<sub>3</sub>-His<sub>6</sub> and His<sub>6</sub>, which exhibited good metal binding activity.

### Environmental pollution and bioremediation

The awareness of toxicological and ecological effects of toxic metals have attracted serious attention for decontaminating industrial waste waters prior to discharge into natural water bodies of sewage ponds, streams and rivers. Unlike organic pollutants metal ions are indestructible and have a cumulative effect on ecosystem. Conventional methods of metal removal from industrial effluents are: Chemical precipitation, Oxidation and reduction, Membrane filtration technology, Electrochemical precipitation, Evaporation recovery, Ion exchange resins. All these technologies are expensive and energy intensive. For example, ion exchangers are efficient in removing toxic metal ions, but cannot distinguish between essential ions via:  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$  etc and non essential metals like  $\text{Cd}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$ , etc. One of the most potential methods have been the use of waste biomass especially from microbial sources to sequester metal ions. Both the principles of biosorption and accumulation in microbes have been exploited for removing toxic metal ions from polluted effluents. Inactivated microbial biomass is both economical and useful for biosorption of toxic metal ions. *A.niger* biosorbent prepared by alkali-extraction of mycelia biomass has been used as a model system to explore the potential in biosorption of toxic metal ions like  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$  etc, both from dilute and concentrated solutions. The biosorbent was able to remove toxic metal ions from both the conditions up to 10% of its weight. In comparison to Dowex-50, biosorb was found to be an order of magnitude superior. Mercury and methyl mercury are particularly known for their highly toxic effects and *A.niger* biosorbent was used to remove the both mercury species inorganic ( $\text{Hg}^{2+}$ ) and methyl mercury ( $\text{CH}_3\text{Hg}^+$ ) from aqueous solutions[22]. The spent decontamination solutions of nuclear power reactors contain the dissolved corrosion deposits along with large amounts of radio activity removed from the system surfaces and are to be processed for disposal. Out of these radionuclides, the major concern is  $^{60}\text{Co}$  with its long half life (5.26 yrs) and high  $\gamma$ -energies. Removal of  $^{60}\text{Co}$  from low concentrations (parts per billion) with 10-fold excess of non radioactive Fe in chelated forms along with  $\text{Ni}^{2+}$  and  $\text{Cr}^{3+}$  compounds .

Microorganisms such as bacteria and fungi are known to exist in the earth along with metals. If the mechanisms employed by microbes in interacting with metal ions are clearly understood their principles can be employed in developing decontaminating strategies for toxic metals. Microorganisms take up metal ions by two broad mechanisms: Biosorption and Bioaccumulation.

## APPLICATIONS

Metal Biosorption and applications in Bioremediation: As already discussed the term biosorption refers to accumulation or binding of metal ions to the cell surface/cell wall by an energy independent process. This generally involves binding of metal ions by a number of anionic ligands viz. phosphoric, carbonyl, sulfhydryl and hydroxyl groups present on the cell wall polymers. Cell walls of bacteria, fungi and algae have been extensively studied. They contain complex polysaccharides with varying proportion of the above mentioned functional groups. The peptidoglycons of cell walls of gram positive bacterium *Bacillus subtilis* contain carboxylic acid groups contributed by glutamic acid residues which have been implicated as the major metal binding sites. This apart from the phosphoryl groups contributed by teichoic acids of the cell wall [26]. Teichoic acid and teichuronic have been shown to be the metal binding sites in *Bacillus licheniformis*. In *Pseudomonas fluorescens* cell wall binding was shown to have high affinity and low affinity binding sites. This dependent on the metal ion and the binding groups. In case of  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ , and modification of  $-\text{COOH}$  group resulted in loss of high affinity binding sites. Similar data also reported for  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$  binding to *E.coli* cell walls.

### CONCLUSIONS

In conclusion, the PPG has been employed as a novel, mild and highly efficient solvent system for the convenient preparation of benzimidazoles in excellent yields from o-phenyldiamine and a wide variety of aryl aldehydes using  $\text{ZnCl}_2$  as catalyst. In addition low cost, recyclable solvent system and ready availability of catalyst, an environmentally benign procedure makes this methodology a useful contribution to the existing procedures available for the synthesis of benzimidazole derivatives.

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