REMOVAL OF COPPER AND CADMIUM BY METAL RESISTANT ORGANISMS ISOLATED FROM EFFLUENTS

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Abstract: Excess of heavy metals in the effluent are hazardous to the environment and a process like biosorption can be used to reduce the metal concentration to permissible level. The metal resistant bacterial, fungal, algal, and a consortium were isolated from effluents. Some of the isolates were able to tolerate metal concentration upto 200 ppm of $CuSO_4$ and $CdCl_2$. The batch experiment for biosorption of Cu and Cd salts was performed and metal concentrations were determined by ICP-AES. The ability of biosorption of heavy metals was determined for consortium and *Streptococcus spp* isolated from effluent sample. Bioremoval efficiency of bacterial culture was found to be 29.99% and of consortium was 8.464%.

Keywords: Biosorption, Heavy metals, Bioremoval efficiency, ICP-AES, Effluent, Metal concentrations.

Introduction: Activities like mineral excavation, smelting, disposal of tailing industrial effluent around mines, sludge dumping and water runoff are the main reasons for environmental metal pollution [1]. Accumulation of heavy metals has adverse effects on the flora and fauna, microorganisms and human beings. Biogeochemical cycles are affected and bioaccumulation of metals is observed.

Environmental cleanup is essential for human health and well-being as excess of heavy metals in the effluent are hazardous and poisonous to us. Prolonged exposure to heavy metals can cause deleterious health effects in human beings. Exposure to excess of cadmium can cause renal dysfunction, lung disease, kidney damage, bronchitis and excess of copper can cause anemia, liver and kidney damage, stomach and intestinal irritation [2].

The conventional methods for removing metal ions from aqueous streams include chemical precipitation, lime coagulation, ion exchange, reverse osmosis, solvent extraction and phytoremediation .These approaches prove to be less cost effective, inconvenient for practical use and present many limitations. Hence alternative methods of metal removal and recovery based on biological materials have been considered [3]. Biosorption may be used as an environment friendly technique. The major advantages of biosorption are low cost high efficiency, minimization of chemical and /or biological sludge no additional nutrient requirement regeneration of biosorbent and possibility of metal recovery.

Cu (II), Cd (II), Ni (II), Cr (IV) are toxic to the environment and can be removed by the use of microorganisms that are resistant to heavy metals. Tolerance and removal of toxic metal ions have been studied in bacteria, cyanobacteria , algae and fungi [4].

Sea weeds like Sargassum, Yeast and Fungi like *Saccharomyces cerevisiae*, *Penicillium chrysogenum* and bacteria like *Bacillus subtilis,* Escherichia coli K-12 and *Pseudomonas* *aeruginosa* and *Micrococcus lutea* have been reported to be used for biosorption.

Materials And Methods:

Sample Collection:

Samples were collected from effluents, Ulhasnagar river water and soil and water near mangrove, Borivali. Lab cultures were also used to check the metal adsorption capacity.

Enrichment:

The enrichment of Cu and Cd tolerant microorganisms was done in sterile Nutrient Broth, Saboraud's broth and Chu10 medium with 100 to 200 ppm $CuSO_4$ or $CdCl_2$. These were then kept on rotary shaker for 24-48 hrs at RT. The growth of the isolates in the enriched media was monitored colorimetrically at 530 nm.

Cultural Study:

The enriched cultures obtained by the enrichment procedure were isolated on Sterile Nutrient Agar, Saboraud's Agar and Chu10 medium with varying $CuSO_4$ or $CdCl_2$ concentrations. Isolates were studied and identified .For bacterial culture which could tolerate high concentration of $CdCl_2$ was identified to Genus level based on morphological, cultural and biochemical characteristics by referring to Bergey's manual of determinative bacteriology.

Determination Of Maximum Metal Resistance Of Isolates:

After enrichment and purification 18 isolates were selected based on their growth rate .Heavy metal resistance study was done by determining the maximum metal tolerance ability of the culture in nutrient broth with varying concentration of heavy metal. Analytical grade $CuSO_4$ and $CdCl_2$ were dissolved in sterilized deionised water to form desired stock solutions. Individual isolates were inoculated in 10 ml sterile nutrient broth containing 100, 200, 300, 400 till 1000 ppm of each metal salt individually. Isolates were inoculated in the tubes with varying concentrations of heavy metals .Tubes were incubated at 37º C for 24 h. After 24 h growth of bacteria was measured at 600 nm using nutrient broth containing equal amount of metal salt as a blank [5]. Two supplementary culture media were set up as negative and positive controls. The positive control flask contained the culture medium, but inoculated with the specific test isolate, while an uninoculated culture medium was used as the negative control. .In order to check if the action is bactericidal or bacteriostatic from the incubated broth tubes cultures were streaked on nutrient media. On the basis of MMT values the isolates which could tolerate higher Cu and Cd salts, concentration of the metal salts were selected for biosorption studies [6].

Batch Biosorption Experiments were conducted in Erlenmeyer flasks (500ml), 100ml solution of analytical grade $CuSO_4$ and $CdCl_2$ of pH 4.5 adjusted with dilute HNO_3 were taken in different flasks with1.0g of dried biomass (*i.e.*1.0% w/v), were agitated at 100 rpm at room temperature. Initial concentration of metals (Ci) was 200 mg/L. Sample was drawn after 24 hrs and analyzed as follows an aliquot of 30 ml of the medium was taken , centrifuged (4000 ×g, 4°C, 15 min). After the biosorption experiment samples were centrifuged and supernatant and sediment were acid-digested using concentrated Nitric Acid.

The heavy metal concentrations were determined using the Inductively Couple Plasma Optical Emission Spectrometer [ICP-OES] (Spectro Ciros CCD, Spectro Analytical Instruments, Kleve, Germany at IIT Mumbai. [6],[7].

The amount of metal adsorbed at equilibrium q max was calculated using following equation: q max (Adsorption capacity) = V/w (Ci - Cf) Where Ci and Cf are the liquid phase concentrations of the metal at initial and equilibrium respectively, V is the volume of the solution and w is the dry weight of biomass/adsorbent [8].

Results And Discussion:

The colony characteristics of the bacterial culture on Nutrient agar with metal salts were studied. The morphology observed of the consortium culture grown in the presence of 200ppm of Cu ++ was rods and cocci in singles and fungal filaments, and that of bacterial culture inoculated in 150ppm of Cd++ was Gram positive cocci in singles and chains .From the Table (1) it is evident that bacterial culture and consortium can tolerate high concentration of Copper Sulphate and Cadmium Chloride. *Aspergillus* spp was found to tolerate 40 ppm of Copper, whereas Streptococcus spp could tolerate upto 200 ppm of Cd. It has also been reported that Cr-resistant *Aspergillus* spp survived to a maximum level of 10,000 mg L–1 of Cr(VI) Chromium-resistant *Micrococcus* spp. also survived to a concentration of 8000 mg L–1 of Cr(VI). MMT values reported in this study confirmed the emergence of highly heavy metal resistant microorganisms in the polluted sites.

Cadmium tolerant isolate was found to be *Streptococcus* spp. as identified using Bergey's manual [9].

Table-1: Determination of Maximum Metal Tolerance by isolates grown in the presence of metals.

Conc. of Cu/Cd ppm	*Aspergillus* spp. $CuSO_4$	*Chlorella* spp. $CdCl_2$	Consortium Bac+Fungal $CuSO_4$	*Streptococcus* spp. $CdCl_2$
20	+	+	+	+
40	+	+	+	+
60	-	+	+	+
80	-	-	+	+
100	-	-	+	+
120	-	-	-	+
140	-	-	-	+
160	-	-	-	+
180	-	-	-	+
200	-	-	-	+

Key = + Growth – No growth

Table-2 shows the result of the batch biosorption as estimated by metal concentration present in solution before and after biosorption as determined by the technique ICP-AES (Ion Coupled Plasma Atomic Emission Spectroscopy). It was found that there was a decrease in the $CuSO_4$ and $CdCl_2$ concentration in solutions containing consortium and *Streptococcus* spp. respectively. Whereas fungal and algal culture did not show significant decrease in Cu and Cd concentration after the batch biosorption.

Table-2: ICP-AES readings. Analysis done at IIT Mumbai		
Samples	**$CuSO_4$ in ppm**	**$CdCl_2$ in ppm**
Streptococcus Sp before biosorption	-	72.87
Streptococcus Sp after biosorption	-	51.06
Consortium before biosorption	76.8	-
Consortium after bio sorption	70.3	-
Algal culture before biosorption	-	53.4
Algal culture after biosorption	-	72.44
Aspergillus before biosorption	40.34	-
Aspergillus after biosorption	76.001	-

Key: - Sample not done.

Cadmium adsorbed by bacterial culture was 7.3 mg/g and Copper adsorbed by consortium was 1.95 mg/g during this study.

Adsorption capacity in mg/g of *Mucor rouxii* for Cd was 6.94 mg/g, *Penicillium chrysogenum* was 11mg/g whereas *Chlorella vulgaris* showed 111mg/g and Streptomyces showed 3.4 mg/g as has been reported by Ahluwalia [10]. Adsorption capacity observed in this study is comparable with the cultures reported in the literature.

Bio-removal Efficiency of Cu by consortium was 8.464% and of Cadmium by *Streptococcus* spp was 29.99% as found in this study. As reported by Basha, *Bacillus licheniformis* showed maximum heavy metal removal ability (98.34%), *Salmonella typhi* showed a maximum of 92.4%, *Pseudomonas fluorescence* showed maximum of 94.8% and *Escherichia coli* showed maximum of 92.06% of Cadmium removal ability from the textile dye effluents .These results were reported after 48 hours of time of contact with metal [11]. It is found that about 67 - 82% of Pb and 73-79% of Cd was removed within 30 days [7]. At equilibrium, adsorption was 99.8% of Ni, 98.0% of Pb, 99.2% of Cu and 99.8%of Cd from dried paper mill sludge with an initial conc. of about 5 mg /l of aqueous solution [8].

In the present study the time of contact was 24 hours in batch biosorption study. It is recommended that the time of contact should be increased to get more metal removal. Based on the present study, it can be concluded that it is possible to develop new bioremediation strategies with the inoculation of HMRB (High Metal Resistant Bacteria) in order to enhance biological-extraction of metals from metal-polluted environments and either consortia or single bacterial culture can be utilized.

Based on this study it may be concluded that microbes can tolerate high concentrations of heavy metals as they are armed with various resistance and catabolic potentials.

This catabolic potential of microbes is enormous and is advantageous to mankind to manage a cleaner and healthier environment through bioremediation. However it is essential to conduct more studies at gene level to know the biosorption potential and heavy metal resistance ability of the microorganism. This study may be helpful to develop affordable ecofriendly technology for the treatment of textile dye effluents before being released in to the environment [11].

Conclusion:

Pollutants find way in soil, water and air through industrial activities so environmental scavenging (bioremediation) need was felt. Microbial ecosystem can drastically alter the fate of the metal entering into aquatic or soil environments by adsorbing the metal. Microorganisms can be used for the reduction of environmental pollution and will be cost effective as compared to chemical methods. Metals from Microorganism can also be recovered.

Ideally, the bioremediation of industrial effluents should be linked with a selective recovery of the metals. Using this process, it is possible to recover different heavy metals with high yield and purity. The selective recovery of metals allows companies to sell or recycle metals for industrial processes without generation of hazardous wastes. This strategy can be easily implemented and constitutes a positive approach to the wastewater treatment because it combines the minimization of environmental liabilities with financial benefits from reselling or recycling the metal

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