Removal Of Heavy Metal (Chromium) By Microbes And To Study The Impact Of TDS On Its Removal Efficiency

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Abstract- **Industrial operations such as electroplating, steel manufacturing, leather tanning, wood preservation, ceramics, glass manufacturing and chemical processing and fertilizer applications release alarmingly higher amounts of heavy metals into the natural environment, which are detrimental to human health. Of the important metals Chromium (VI) is regarded as toxic; whereas, others, such as copper, nickel, cobalt and zinc are not as toxic, but their extensive usage and increasing levels in the environment are of serious concerns .Various techniques have been employed for the treatment of metal bearing industrial effluents, which usually include precipitation, adsorption, ion exchange, membrane and electrochemical technologies but these techniques are expensive, not environment friendly and usually dependent on the concentration of the waste which are ineffective in very diluted solutions. Therefore, the search for efficient, eco-friendly and cost effective remedies for wastewater treatment has been initiated. Heavy metal resistant bacteria have significant role in bioremediation of heavy metals in wastewater. Two microbial species *Pseudomonas Putida* and *Bacillus subtillis* were selected for removal of chromium. Waste water also contains high TDS along with heavy metal. So, the objective of this work is to examine the capacity of bacteria in removing the heavy metal and also to study the impact of varying TDS concentration on removal efficiency for chromium by selected microbial species.**

Index Terms- Heavy Metals,Chromium (VI),TDS,Bioremediation

I. INTRODUCTION

Earth's surface comprises of 70% water is the most valuable natural resource existing on our planet. Without this invaluable compound, the life on the Earth would not exist. Although this fact is widely recognized, pollution of water resources is a common problem being faced today. Heavy metal pollution occurs directly by effluent outfalls from industries, refineries and waste treatment plants and indirectly by the contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rain water. Modern industry is, to a large degree, responsible for contamination of the environment. Lakes, rivers and oceans are being overwhelmed with many toxic contaminants. Among toxic substances reaching hazardous levels are heavy metals. Heavy metals are the group of contaminants of concern, which comes under the inorganic division. Some strong toxic metal ions such as Hg are very toxic even in lower concentration of 0.001-0.1 mg/ L. Metals are extensively used in several industries, including mining, metallurgical, electronic, electroplating and metal finishing. The presence of metal ions in final industrial effluents is extremely undesirable, as they are toxic to both lower and higher organisms. Under certain environmental conditions, metals may accumulate to toxic levels and cause ecological damage. Of the important metals Chromium (VI) is regarded as toxic; whereas, others, such as copper, nickel, cobalt and zinc are not as toxic, but their extensive usage and increasing

levels in the environment are of serious concerns .Various techniques have been employed for the treatment of metal bearing industrial effluents, which usually include precipitation, adsorption, ion exchange, membrane and electrochemical technologies but these techniques are expensive, not environment friendly and usually dependent on the concentration of the waste which are ineffective in very diluted solutions. Therefore, the search for efficient, eco-friendly and cost effective remedies for wastewater treatment has been initiated. It was only in the 1990s that a new scientific area developed that could help to recover heavy metals and it was bioremediation. The early reports described how abundant biological materials could be used to remove, at very low cost, even small amounts of toxic heavy metals from industrial effluents. The principle advantages of biological technologies for the removal of pollutants are they can be carried out in situ at the contaminated site, usually environmentally benign (no secondary pollution) and they are cost effective. Of the different biological methods, bioaccumulation and biosorption have been demonstrated to possess good potential to replace conventional methods for the removal of metals.

Some confusion has prevailed in the literature regarding the use of the terms "bioaccumulation" and "biosorption" based on the state of the biomass. Herein, therefore, bioaccumulation is defined as the phenomenon of living cells; whereas, biosorption mechanisms are based on the use of dead biomass. To be precise, bioaccumulation can be defined as the uptake of toxicants by living cells. The toxicant can transport into the cell, accumulate intracellularly, across the cell membrane and through the cell metabolic cycle Conversely, biosorption can be defined as the passive uptake of toxicants by dead/inactive biological materials or by materials. Metal-sequestering properties of non-viable biomass provide a basis for a new approach to remove heavy metals when they occur at low concentrations.That aspect of biosorption makes the eventual recovery of this waste metal easier and economical**[4]**.

II. LITERATURE REVIEW

A batch equilibrium method was used to determine the sorption of chromium, copper, manganese and zinc by P. aeruginosa AT18. The heavy metal adsorbates used in this study were chromium (Cr2O3), copper (Cu(NO3)2), manganese (MnSO4) and zinc (ZnSO4). Single stock solutions of chromium (60 mg L_1), copper (50 mg L_1), manganese (50 mg L_1) and zinc (80 mg L_1) were prepared by dissolving appropriate quantities of pure metal powders in 1% nitric acid A set of 250 mL Erlenmeyer flasks containing 100 mL of metal solution was used in the experiments. Cell suspensions (10 mL) were exposed to metal solutions for 72 h on a rotary shaker at 150 rpm. Biomass was separated by centrifugation at 10 000 rpm for 15 min, and the supernatants were analysed for residual metal concentration. Metal adsorbed by P. aeruginosa AT18 biomass. effect of pH on biosorption, the biomass of P. aeruginosa AT18 strain was conditioned to different pH environments (ranging between 3 and 8). Suspensions of pH conditioned biomass (10 mL) were then contacted with metal solutions of the corresponding pH.effect of contact time was also cheked by taking samples at various time interval from 0 to 72 hours. The results obtained in the experimental assays show that P. aeruginosa AT18 has the capacity for biosorption of the metallic ions Cr3+, Cu2+ and Zn2+ in solutions, although its capacity for the sorption of manganese is low (22.39 mg Mn2+/g of biomass) in comparison to the Cr3+, Cu2+ and Zn2+ ions, as shown by the individual analyses. removal efficiency was found to be 99.6% and 96% for chromium and copper, respectively**[6].** The tolerance to As, Hg, Co, Fe and Cr was determined in different Colombian *Bacillus sphaericus* native strains, as well as the biosorption and bioaccumulation in living biomass. In addition, biosorption of Cr in dead cellswas also determined. Living cells of the twomost tolerant strains had the capacity to accumulate between 6 and 47% of Co, Hg, Fe and As. Living and dead cells of *B. sphaericus* OT4b31 showed a biosorption of 25 and 44.5% of Cr respectively, while *B. sphaericus* IV(4)10 showed a biosorption of 32 and 45%. These results are due to the absence of an active metabolism in dead cells and to the pH adjustment. S-layer proteins may possibly have the ability to entrap metallic ions, either on living or dead cells. This can be an interesting alternative for bioremediation processes of heavy metals**[5].**Experimental metals used in the study were chromium (VI), copper (II) and iron (III) in the form of their respective metal

solutions. A synthetic multi-element standard solution of liquid media containing 150 mg L-1 of each Cr, Cu and Fe ions was prepared from their respective stock solutions (1000 mg L-1). Chemicals used for preparing these stock solutions where potassium dichromate, copper sulphate and ferric chloride. Stock solutions of these metals were prepared using deionized water and autoclaved separately. Prior to addition of stock solution of metals the liquid media were autoclaved at 121°C for 20 min. All the additions were performed aseptically. *B. licheniformis* (NCIM 2471) was obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India. A set of three samples was prepared as described above and pH was adjusted to 3.5 for all three samples. After inoculation, samples were incubated at temperatures i.e. 20°C, 28°C, 37°C respectively and incubated at 120 rpm for 48 hrs. Maximum % removal of metal ion was observed at 28°C. Removal efficiencies were 95%, 52% and 38% for Fe (III), Cr (VI) and Cu (II) respectively at 28°C**[3].** The study describes the sorption of Cr, Cu, Mn and Zn by *Pseudomonas aeruginosa* AT18 isolated from a site contaminated with petroleum and heavy metals. The concentrations studied were 50, 49, 60 and 70 (mg L_1) for Cr, Cu, Mn and Zn, respectively. The solution pH and ionic strength were very important factors in the metal biosorption performance and the biosorption capacity of *P. aeruginosa* AT18 for Cr3+, Cu2+, Mn2+ and Zn2+. In aqueous solution, the biosorption increased with increasing pH in the range 5.46– 7.72. The results obtained in the experimental assays show that *P. aeruginosa* AT18 has the capacity for biosorption of the metallic ions Cr3+, Cu2+ and Zn2+ in solutions, although its capacity for the sorption of manganese is low (22.39 mg Mn2+/g of biomass) in comparison to the Cr3+, Cu2+ and Zn2+ ions, as shown by the individual analyses. However, 20% of the manganese was removed from an initial concentration of 49.0 mg L_1, with a Qm value similar to that obtained in solutions containing mixtures of Cr3+, Cu2+, Mn2+ and Zn2+. The chromium level sorbed by *P. aeruginosa* AT18 biomass was higher than that for Cu, Mn and Zn, with 100% removal in the pH range 7.00–7.72 and a Qm of 121.90–200.00 mg of Cr3+/g of biomass. The removal of Cr, Cu and Zn is also a result of precipitation processes**[7].** The tannery effluent and effluent contaminated soil were collected from a common effluent treatment plant (CETP) at Ranipet, Vellore dist. Tamil Nadu, India. The tannery effluent was collected in sterile bottles. The effluent contaminated soil was also collected in a sterile polythene bags from nearby canals. The samples were transported to the laboratory on the same day itself. The microbiological analysis (fungi, bacteria and actinomycetes) of the samples were carried out on reaching the laboratory, as per the American public health association (APHA) (Clesceri et al., 2005). The tannery effluent and effluent contaminated soil were serially diluted and then spread plate technique was performed on a mineral salt medium (MSM) media containing lead acetate at the concentration of 500 mg/L. The MSM media consists of (in g/L) Na2HPO4, 4.0; KH2PO4, 1.5; NH4Cl 1.0; MgSO4•7H2O, 0.2; C6H8O7FeNH3, 0.05; along with modified Hoagland trace element solution (in g/3.6 L) BH3, 11.0; MnCl2•4H2O, 7.0; AlCl3, 1.0; CoCl2, 1.0; CuCl2, 1.0; KI, 1.0; NiCl2, 1.0; ZnCl2, 1.0; BaCl2, 0.5; KBr, 0.5; LiCl, 0.5; Na2MoO4, 0.5; SeCl4, 0.5; SnCl2•2H2O, 0.5; NaVO3•H2O, 0.1; pH 7.0). After incubation, morphologically different colonies were isolated and purified by repeated streaking on agar plates. The isolates were then stored in agar slants for further studies. The strain with higher lead bioremediation was selected for further biochemical and molecular characterization. The bacterium was tested for the activity of bioremediation of lead at pH range of 3 to 8. The cells were inoculated into six 100 mL MSM broth containing lead acetate at the concentration of 1250 mg/L with the pH adjusted to 3 to 8 at progression of pH 1 in each of the six broths, respectively. The lead acetate concentration was chosen to be 1250 mg/L as this was the highest concentration at which VITKAS-2 showed growth. The pH was adjusted using sodium hydroxide and acetic acid. The setup was stored for 24 h at 25°C and shaken at 150 rpm. To confirm that the Pb had been taken up by the bacteria, the MSM culture supernatant was subjected to atomic absorption spectrophotometer.lead were found to be removed was 40%**[1].** The liquid and solid waste samples were collected from landfills and industries of Doon Valley Uttarakhand viz. New Tehri, Chamba, University campus Badshahithaul, Srinagar, Pauri, Rishikesh, Haridwar, Dehradun, Mussorie, Kotdwar,

Rudraprayag, Devprayag, Nainital, Haldwani, Lakshar and Uttarkashi. *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *Aspergillus niger*. All the four microbes were isolated from the soil and sludge by serial dilution and pour plating method. Cells were cultured in nutrient broth with the following composition: beef extract (3.0 g), peptone (10.0 g), dissodium phosphate (1.0 g), sodium chloride (5.0 g), dissolved in one liter of distilled water. Final pH was around 7.4-7.6. The medium was autoclaved at 121ºC for 20 minutes. Cultures were maintained in agar slants (nutrient broth plus 30 g/L agar). They were allowed to grow in the synthetic media having different heavy metal solutions to make capable of heavy metal resistant. The concentration of metals Zn, Mn, Mg, Cu, Cr, Co, Cd Ni and Pb were 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml. Cells were inoculated in nutrient broth (100 mL/flask) and kept under agitation in a rotary shaker, at 80 rpm, for 48 hours at 35 ± 2ºC. Cells to be used in biosorption experiments were separated by centrifugation. Experiments of heavy metals biosorption were done in Erlenmeyer flasks containing 150 mL of each samples and 15.0 ± 1.0 mg of cells. To ensure equilibrium, cells and waste were maintained in contact for 48 hours, under constant agitation, at 30-350C ± 2ºC. In all experiments, cells were obtained from only one cultivation and collected from the same flask at the same growth stage. After 48 hours, cells were separated from the medium and residual metal concentrations were monitored by ICP-MS. The average Ni reduction was 48% and Cu reduction was recorded as 65% by *Bacillus*. The average Cu reduction was 42%, Cr reduction 45% and most reduction was recorded in case of Pb and it was 93% by *Staphylococcus*. *Aspergillus niger* reduced the zinc and cadmium only. The average Zn reduction was 58%, and Cd reduction was recorded as 50%. The average Ni reduction was 56% and Cu reduction was recorded as 68% by *Pseuomonas*.**[2].**

III. CONCLUSION

From literature review, it has been observed that many microbial species are capable for biological removal of heavy metals such as Chromium,Cadmium,Cobalt,Lead,Nickel.it can work effectively at dilute concentration of heavy metal. Major advantage of biological removal of heavy metal are cost effective,environmentally friendly,can reuse the heavy metal after its removal as compared to other conventional techniques available.

Mechanism of Removal[4]:

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. There are various mechanisms and are not fully understood. They may be classified according to following:

- Metabolism dependent and
- Non -metabolism dependent

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This means that this kind of accumulation may take place only with viable cells. It is often associated with an active defense system of the microorganism, which reacts in the presence of toxic metal. During non-metabolism dependent process metal uptake is by physico-chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This process i.e., non-metabolism dependent is relatively rapid and can be reversible. In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface .Further, it may be dependent on the cell's' metabolism if, in the presence of toxic metals, the microorganism produces compounds that favor the precipitation process. Precipitation may not be dependent on the cells' metabolism, if it occurs after a chemical interaction between the metal and cell surface.

1) Transport across cell membrane

Heavy metal transport across microbial cell membranes may be mediated by the same mechanism used to convey metabolically important ions such as potassium, magnesium and sodium. The metal transport systems may become confused by the presence of heavy metal ions of the same charge and ionic radius associated with essential ions. This kind of mechanism is not associated with metabolic activity. Basically bioaccumulation by living organisms comprises of two steps. First, a

metabolism independent binding takes place where the metals are bound to the cell walls followed by metabolism dependent intracellular uptake, whereby metal ions are transported across the cell membrane.

2) Physical adsorption

In this category, physical adsorption takes place with the help of van der Waals' forces. hypothesized that uranium, cadmium, zinc, copper and cobalt biosorption by dead biomasses of algae, fungi and yeasts takes place through electrostatic interactions between the metal ions in solutions and cell walls of microbial cells. Electrostatic interactions have been demonstrated to be responsible for copper biosorption by bacterium *Zoogloea ramigera* and alga *Chiarella vulgaris* for Chromium biosorption by fungi *Ganoderma lucidum* and *Aspergillus niger*.

3) Ion Exchange

Cell walls of microorganisms contain polysaccharides and bivalent metal ions exchange with the counter ions of the polysaccharides. For example, the alginates of marine algae occur as salts of K+, Na+, Ca^2+, and Mg^2+. These ions can exchange with counter ions such as CO^2+, Cu^2+, Cd^2+ and Zn^2+ resulting in the uptake of heavy metals. The copper uptake by fungi *Ganoderma lucidium* and *Aspergillus niger* was also up taken by ion exchange mechanism.

4) Precipitation

Precipitation may be either dependent on the cellular metabolism or independent of it. In the former case, the metal removal from solution is often associated with active defense system of the microorganisms. They react in the presence of toxic metal producing compounds, which favor the precipitation process. In the case of precipitation not dependent on the cellular metabolism, it may be a consequence of the chemical interaction between the metal and the cell surface. The various biosorption mechanisms mentioned above can take place simultaneously.

REFERENCES

1] Anish Saini1, Rohini Kumar M., Karthik Senan, Shakti Sagar, Vivekanandan K. E. And Jabez Osborne; Remediation Of Lead (Pb) By A Novel *Klebsiella Sp*. Isolated From Tannery Effluent Of Ranipet, Vellore District; Aafrican Journal Of Biotechnology; Vol.12(32),pp.5069-5074,7August,2013

2] Ashok Kumar, Balwant Singh Bisht, Vishnu Datt Joshi ; "Biosorption Of Heavy Metals By Four Acclimated Microbial Species, *Bacillus Spp., Pseudomonas Spp., Staphylococcus Spp.* And *Aspergillus Niger";* BIOL. ENVIRON. SCI. ; 2010, 4(12), 97-108

3] Deepti Pranay Samarth, Chandrashekhar Jangluji Chandekar And Rama Kaustubh Bhadekar; "Biosorption of heavy metals from aqueous solution using *Bacillus Licheniformis";* International Journal Of Pure And Applied Science & Technology; 10(2) (2012), pp. 12-19

4] K. Tarangini ; thesis on "Biosorption of Heavy Metals using Individual and Mixed cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis"* ; January – 2009

5] Lina Velasquez, Jenny Dussan ; "Biosorption And Bioaccumulation Of Heavymetals On Dead And Living Biomass Of *Bacillus Sphaericus" ;* Journal Of Hazardous Material ; 167 (2009) 713–716

6] Manisha Nanda,Dinesh Sharma,Arun Kumar; "Removal of heavy metals from industrial effluent using bacteria"; International Journal Of Environmental Science;Volume 2,Nov 2,2011

7] Rosa Maria Perez Silva, Arelis Abalos Rodriguez,Jose Mannuel Gomez Montez De Oca,Domingo Cantergo Moreno; "Biosorption Of Chromium,Copper,Manganese And Zinc By *Pseudomonas Aeurogens* AT18 Isolated From A Site Contaminated With Petroleum Science Direct"; Science Direct ; 100 (2009) 1533–1538