# REMOVAL OF CHROMIUM (VI) FROM LEACHATE USING BACTERIAL BIOMASS

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**Abstract:** Contamination of fresh water bodies by chromium from industrial origin is a big problem. Biosorption is a potential method over chemical treatment to remove lethal metals from wastewater. The aim of the present study was to remove chromium from the leachate using non-living bacterial biomass of *Bacillus* spp isolated from soil. The leachate was sampled from Sisdol Landfill Site and biosorption process was performed by using dead biomass of environmental bacteria under optimized conditions obtained from biosorption of fortified solutions. Atomic absorption spectrophotometer was used to analyze the concentration of chromium in leachate after the completion of the biosorption process.

Maximum (99%) removal of chromium from the leachate was obtained at pH 2 by using 0.25 gm biomass of Bacillus spp for a contact period of 5 hours. Thus, chromium was substantially removed from the leachate using non-living biomass of Bacillus spp in acidic condition. Microbial biomass can be employed for removal of low amount of toxic metals from wastewater and the application of microbial biomass should be recommended for treatment of industrial wastewater before discharging into aquatic systems.

**Keywords:** Bacillus spp; Biosorption, Chromium; Leachate; Sisdol Landfill Site.

## INTRODUCTION

Environmental pollution due to toxic metals is one of the major concerns nowadays and chromium is one of them. Wastewater containing chromium is discharged into aquatic systems mainly by industrial activities and the level of contamination is increasing because of rapid industrialization (Pal and Vimala, 2011). Chromium is mainly derived from mining and refining ores, steel plants, leather tanning, aluminum productions, industrial and household sludge, iron sheet cleaning, chrome plating, fly ash from incinerators, radioactive materials, paints, alloys, inorganic chemical production, batteries, pesticides or preservatives and metal plating (Pal and Vimala, 2011). Chromium causes carcinogenic and mutagenic health hazard in living organisms when they get access to drinking water supply or food chain (Ahalya et al, 2005). It is also reported to cause epigastric pain, nausea, vomiting, severe diarrhea and hemorrhage in humans (Prasad and Abdullah, 2010). Therefore, removal of chromium to an environmentally safe level in a cost effective manner is of great importance and in this situation, biological treatment involving nonliving (dead) bacteria offers the reduction of toxic metal levels to an acceptable limit (Kanchana et al, 2011). Chromium occurs in the aqueous system as both trivalent and hexavalent forms. Hexavalent chromium is much more toxic than trivalent chromium so the reduction of Cr (VI) to Cr (III) serves as key processes for removal of Cr (VI) contaminated wastewater (Owlad et al, 2009).

The leachate from the landfill and industrial wastewater containing dissolved metals can be removed by different physico-chemical methods such as reverse osmosis, solvent extraction, lime coagulation, ion exchange, chemical precipitation, membrane separation process, oxidation-reduction, filtration, adsorption, incineration, recovery by evaporation, neutralization, electro dialysis and electrochemical treatment. However, these methods are expensive requiring high energy and reagents, incomplete metal removal as well as generate toxic sludge (Pal and Vimala, 2011). The use of microbial biomass as a biosorbent is a potential alternative over chemical methods for removal of metals from wastewater by a method known as biosorption. Biosorption is a passive removal of metals using non-living biomass as adsorbent material which is metabolism-independent, in contrast to bioaccumulation, a metabolism-dependent process (Hussein et al, 2003). The present study attempts to investigate the biosorptive potential of Bacillus spp for chromium (VI) from the leachate under the laboratory conditions.

## MATERIALS AND METHODS

The study was conducted at the Environment and Climate Study laboratory of Nepal Academy of Science and Technology (NAST). The leachate was collected in a sterile plastic bottle from leachate stream of Sisdol

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Landfill Site, Okharpauwa, Nuwakot. The leachate was filtered through whatmann filter paper and diluted to 100 folds. The diluted leachate was stored at 40C. Biosorption experiments were carried out by nonliving biomass of Bacillus spp isolated from soil. Firstly, biosorption was performed in synthetic solutions of chromium to optimize pH. Later, chromium was removed from leachate under obtained optimum pH with exposure time of 5 hours in laboratory conditions.

**Isolation of Bacillus spp from soil**

Ten gram of moist soil was weighed and added to 95 ml of distilled water in a conical flask resulting 10-1 dilution. From this dilution, 1 ml was taken and added to 9 ml of distilled water in a flask giving 10-2 dilution. Similarly dilutions up to 10-5 were made. For each dilution, 0.1 ml of soil dilutions was transferred to replicate agar plates. A sterilized glass rod was used to spread sample on the surface of the agar and the plates were incubated at 370C for 24 hours. The colony per plate was counted as CFUs per gram of soil. The isolated colony was picked and inoculated into nutrient broth and MacConkey agar. Biochemical reactions were done for identification of Bacillus spp.

**Preparation of bacterial biomass**

The bacteria of interest was inoculated into nutrient broth and incubated at $37^0$C for 24 hours. The broth was then centrifuged at 3,500 rpm for 25 min and the pellet was collected by pouring off supernatant gently. The pellet was oven dried at $55^0$C and crushed uniformly. The biomass was then kept in air tight test tubes to be used in the biosorption process.

**Preparation of metal solutions**

An aqueous solution of hexavalent chromium was prepared by dissolving potassium chromate ($K_2C_rO_4$) salt to obtain a stock solution of 1000 mg/l. This solution was used to prepare required experimental solutions. The pH of each solution was adjusted to desired value by using 0.25M HCl and 0.25M NaOH.

**Biosorption process**

Metal solutions of required concentration were taken in a glass vessel and pH was set at 2, 3, 5, 7, 8 and 9. Biomass dose was added to the solutions and agitated at a speed of 115 rpm at room temperature (RT). After five hours contact time, solutions were filtered through membrane filter and the filtrate was analyzed by atomic absorption spectrophotometer (AAS). The biosorption of leachate was then conducted under the optimized conditions. The percentage of chromium removal i.e. R (%) was calculated using the following equation:

$$R (\%) = (C_i - C_f)/C_i \times 100$$

where Ci and Cf represent initial and final Cr (VI) concentration respectively.

## RESULT AND DISCUSSION

*Bacillus* spp was isolated from soil by spread plate method and the organism was identified on the basis of biochemical reactions. The microbial biomass of Bacillus spp was prepared by centrifugation technique and harvested in a dry form by oven dry. The bacterial biomass was then used for biosorption of chromium in leachate. The bacterial adsorbents use the functional groups present in microbes to form complexes with metal ions during the sorption of metal (Sethuraman and Balasubramanian, 2010). Many microbial species such as bacteria, fungi, yeast and algae are known to be capable of adsorbing metals on their surface or accumulating within their structure (Vinita and Radhanath, 1992). However, the sorption of metals by microbial cells can be affected by pH, biomass dose, initial metal concentration, etc. Bacteria are usually used as biosorbents because of their small size, their ubiquity, their ability to grow under controlled conditions, and their resilience to a wide range of environmental situations (Wang and Chen, 2009).

Sisdol Landfill Site is a place for dumping of solid wastes generated by people of Kathmandu Valley. Although this Landfill Site has a facility of semi-aerobic system to reduce leachate contamination of soil and water, the system is not working properly; instead leachate makes the way directly to the Kolfu River alarming aquatic flora and fauna. Besides, the people downstream the River using metal contaminated water for many purposes (such as bathing, irrigation, water for cattles) would enter food chain and ultimately has health hazard. So, the removal of metals by the use of bacterial biomass in the laboratory could be an alternative approach for prospective insitu removal of toxic metals from the leachate of Sisdol Landfill Site.

Metal biosorption by bacterial biomass mainly depends on the components of the cell, especially through cell surface and the spatial structure of the cell wall. Peptidoglycan, teichoic acids and lipoteichoic acids are all important chemical components of bacterial surface structures. Various proteins are also involved in metal binding for certain kinds of biomasses. Some active sites and functional groups especially carboxyl group have been found to bind metal ions (Wang and Chen, 2009). Bacillus spp is a gram-positive, aerobic, rod-shaped bacterium and ubiquitous in soil and water. Its parietal structure is well known and is composed primarily of peptidoglycan and teichoic acid. Peptidoglycan is a polymer of acetylglucosamine and acetylmuramic acid, which carry mainly carboxylic and hydroxyl functional groups. On the other hand, teichoic acid is a polymer of copyranosyl glycerol phosphate, which carries mostly phosphate and hydroxyl groups. The use of dead cells in biosorption is most advantageous for wastewater treatment as the dead organisms are not affected by toxic wastes; they do not require a continuous supply of nutrients and they can be regenerated and reused for many cycles. Dead cells may also be stored or used for extended periods at room temperature without putrefaction (Sivaprakash et al, 2009).

The optimum biosorption of chromium (1 ppm) was obtained at pH 2 using biomass of *Bacillus* spp (0.25 gm)

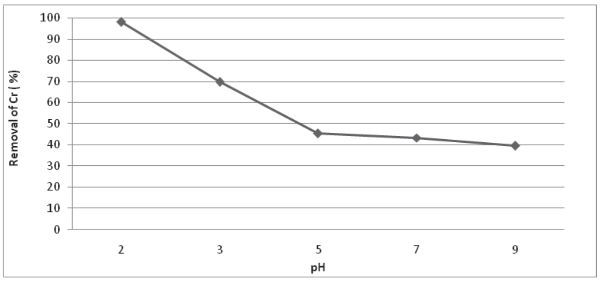


**Figure 1: Effect of pH in chromium biosorption**

in fortified solution (Figure 1). Maximum adsorption of chromium at pH 2 is consistent with the other studies conducted elsewhere (Ahalya et al, 2005; Ilhan et al, 2004). Hexavalent chromium exists as $CrO_4^{2-}$, $HCrO_4^-$, $H_2CrO_4$ and $Cr_2O_7^{2-}$ in solution. In low pH solutions, $HCrO_4^-$ is the prevalent form which shifts to other forms such as $H_2CrO_4$ and $Cr_2O_7^{2-}$ as the pH increases. At lower pH the carboxyl and amino groups on the surface of biomass are protonated which results in a strong electrostatic attraction between chromium anions and positively charged biosorbents (Prasad and Abdullah, 2010). Thus, adsorption of Cr (VI) at pH 2 suggests that the negatively charged chromium species (chromate in the medium) bind through electrostatic attraction to positively charged functional groups on the surface of biosorbents. As the pH is increased, the overall surface charge on the cells become negative and biosorption decreases. In alkali conditions, carboxylate group exists in deprotonated form and has net negative charge. As a result, the surface charge of the biosorbents become negative and biosorption of Cr (VI) decreases (Sivaprakash et al, 2009).

On the contrary, rate of adsorption is decreased with increasing concentration of chromium (Figure 2) and this finding is similar with the study conducted by Ahalya et al (2005). This is due to the increase in the number of ions competing for the available binding sites in the biosorbents and also due to the lack of binding sites for the complexation of chromium ions at higher concentration levels. At lower concentrations, all metal

ions present in the solution would interact with the binding sites and thus facilitates maximum adsorption.

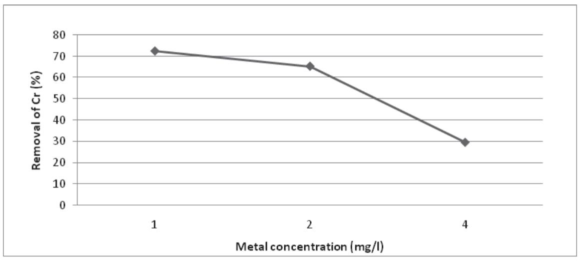


**Figure 2: Effect of metal concentration in chromium biosorption**

At higher concentrations, more chromium ions are left unabsorbed in solution due to the saturation of binding sites (Ahalya et al, 2005).

The maximum removal of chromium in aqueous solutions was obtained at pH 2 with 0.25 gm *of Bacillus* spp as biosorbents. Under this optimized condition, 99% of chromium from the leachate was removed during the biosorption process (Table 1).

**Table 1: Biosorption of chromium in leachate using Bacillus spp.**

| Sample | Concentration of Cr (mg/l) | | Cr removal (mg/l) | Cr removal R (%) |
|---|---|---|---|---|
| | Before bio-sorption | After biosorp-tion | | |
| Leachate | 1 | 0.01 | 0.99 | 99 |

## CONCLUSION

Chromium was removed from the leachate using non-living biomass of *Bacillus* spp at acidic condition. Microbial biomass can be employed for removal of low amount of toxic metals such as chromium from wastewater and bioremediation of metals can be achieved in a relatively cost-effective manner. The application of microbial biomass should be encouraged for treatment of wastewater as well as groundwater contaminated with toxic metals.


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