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Uptake of hexavalent chromium from aqueous solution employing live, dead and immobilized bacterial biomass

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ABSTRACT: Gram negative bacteria *Pseudomonas* sp. biosorbs chromium (VI) ion from its aqueous solution. The Biosorption study was carried out by live, dead and immobilized cells of *Pseudomonas* Sp at 100 ppm concentration of Cr (VI). The biomass and protein content of *Pseudomonas* Sp increased up to 48 hours. The bacterial isolate was found to utilize the Cr (VI) up to 100 ppm without affecting its metabolic activities. Hence in the present study the isolate was further investigated for its ability to biosorbs the Cr (VI) in live, dead and immobilized cells. The biosorption of Cr (VI) is 44%, 49.6% and 66.55% is maximum at 240 minutes by live, dead and immobilized cells of *Pseudomonas* Sp. @ JASEM

Aqueous effluent streams originating from production processes can contain heavy metals that due to their chemical and toxicological properties cannot be discharged directly into the environment. Heavy metals are of great concern when found in high concentrations in soils, surface water and ground water due to their nature (Murugesan and John ruby, 2005). Chromium is a toxic heavy metal introduced into water bodies by a wide range of large scale industrial uses. The largest users of chromium are the metallurgical, refractory and chemical industries. The pollution due to chromium mainly arises from chrome tanning activities. Only hexavalent chromium (Cr(VI)) and trivalent chromium (Cr(III)) are ecologically important because they are the more stable oxidation states. Being mutagenic, carcinogenic and teratogenic, Cr(VI) is approximately 100-fold more toxic than Cr(III) (Shen and Wang 1995). The unused solutions of Cr (III) salts discharged into tannery wastes have raised ecological concern, sometimes even more than the other waste streams. The concentration of chromium in the effluents from chrome – tanning yard is in the range of 2000 to 5000 ppm (Thyagarajan, 1992). Chromium (VI) being more soluble and consequently more biologically available, presents a real hazard while Cr (III) is considered relatively non – toxic. Of the two stable Cr valences, hexavalent (VI) Cr is about 100 fold more toxic than the (III). More over Cr (VI) is the predominant species involved in mutagenicity, carcinogenicity and teratogenicity (Babich *et al*., 1982). Chromium is hazardous to the environment, the waste water containing toxic chromium must be treated before discharged to natural environments. Since conventional method cannot completely remove toxic heavy metals and most of them require high energy or large quantities of chemical reagents and more practical economic methods have been explored. During the recent years, the study of microorganisms has contributed important insights into the basic problems. The biochemical versatility of microorganisms, a consequence of their genetic plasticity and their ability to modify their physiology as to ensure maximum competitiveness in an ever changing environment is one of the major driving forces to use them as biocatalysts for the control and remediation of chromium from the industrial effluent. The objective of this study was to isolate and characterize the culturable microbial community of a chromium-contaminated sites and to evaluate the Cr(VI) resistance and Cr(VI)-reducing ability using live, dead and immobilized bacterial biomass.

MATERIALS AND METHODS

COLLECTION OF SOIL SAMPLES

Soil samples are collected from garden soil contaminated with chromium containing synthetic effluent and also receiving wastes from common burning place and effluent treatment area. Samples are collected at different sites of the field by using sterile scalpel and they are transferred to sterile polythene bags and they are used for analysis.

ISOLATION OF CHROMIUM RESISTANT MICROORGANISMS

The soil samples was mixed well and 1g of soil was transferred into 100 ml of sterile distilled water and homogenized. From this 1 ml was taken and serially diluted up to 10^{-7} and from this dilution 1 ml of solution was transferred into sterile petriplates and incubated for two weeks. The colonies present on the petriplates ware selected and streaked on nutrient agar plates in pure form.

ESTIMATION OF SOLUBLE PROTEIN

Protein content of *Pseudomonas* Sp, treated in various concentrations of chromium was estimated (Lowry *et al*., 1951).

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BIOSORPTION OF CHROMIUM USING BACERIAL BIOMASS

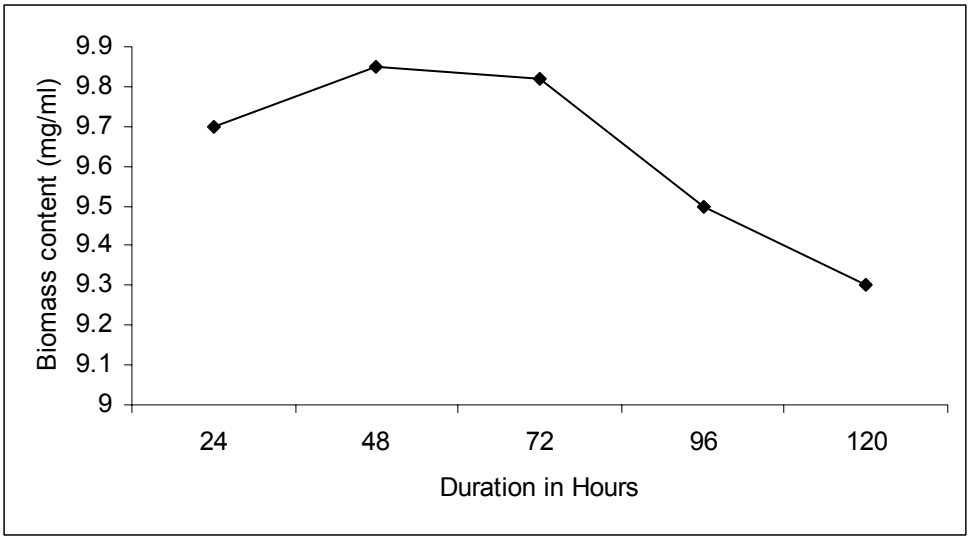

Fig. 1 Growth profile of *Pseudomonas* Sp, with 100 ppm Cr (VI) concentration at various time intervals

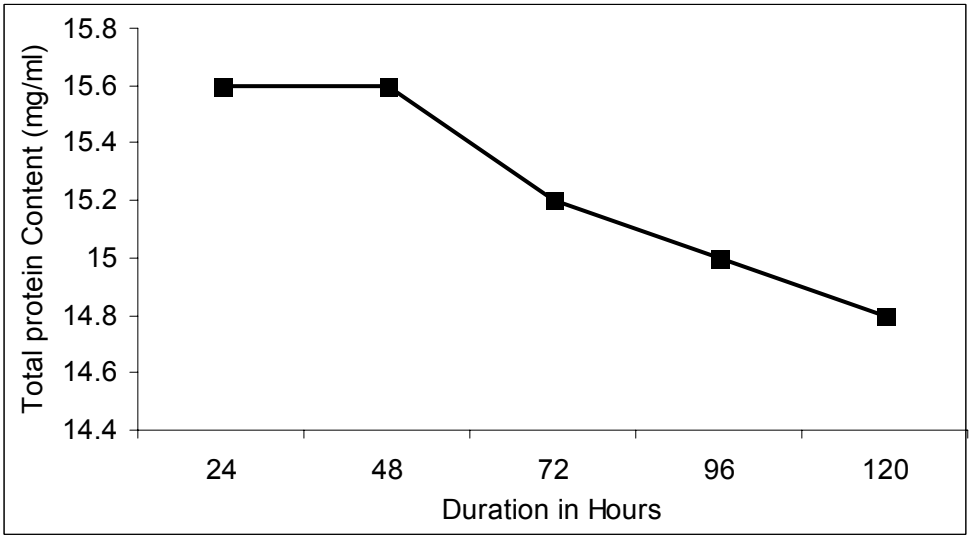

Fig.2 Soluble Protein Content (mg/ml) of *Pseudomonas* Sp, with 100 ppm Cr (VI) at various time intervals

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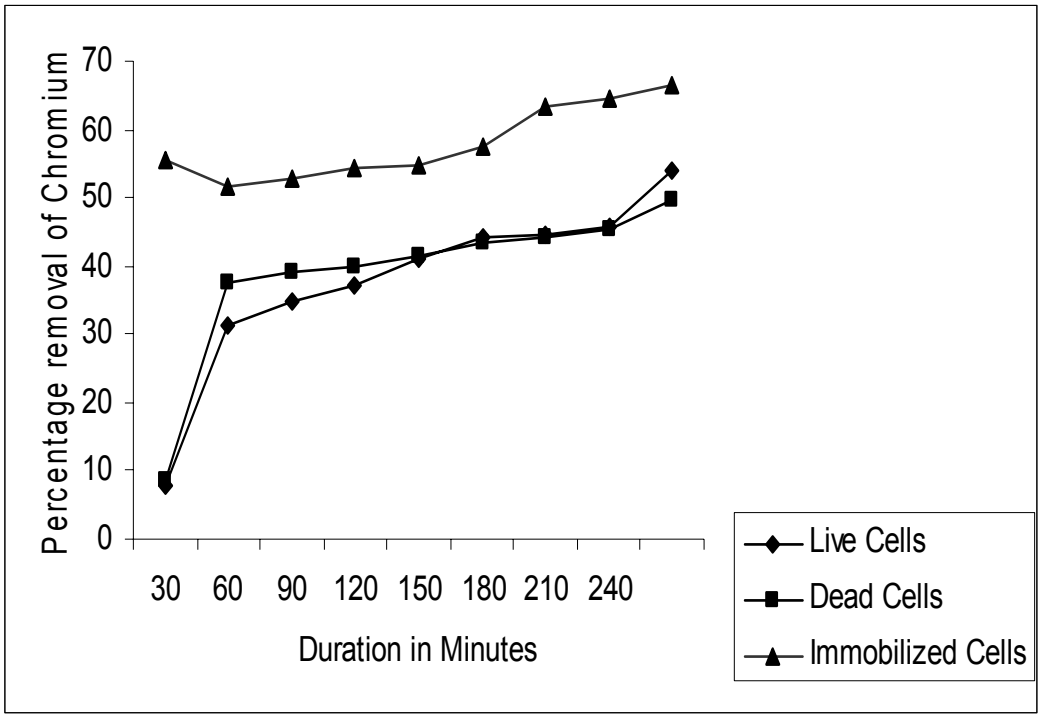

Fig.3 Biosorption of Cr (VI) on live, dead and immobilized *Pseudomonas* sp, grown with Chromium in the medium

Biosorption studies were conducted using live dead and immobilized cells *Pseudomonas* Sp. The growth media (Dextrose – 1.0g; Peptone – 0.5g; NaCl – 0.5g; Yeast extract – 1g; Beef extract – 0.15g; Agar – 2g) was prepared with 100 ppm of Chromium and inoculated with 18 hrs old culture in to the media. After inoculation, the flasks were kept for incubation on a rotary shaker at 120 rpm for 24 hrs. After 24 hrs, one set of flasks grown with Cr (VI) were autoclaved at 15 lb pressure at 121°C for 20 minutes to kill the cells. Again, one set of flask grown with Cr (VI) were harvested by centrifuging them in cold centrifuge to recover the cells. The cells were washed twice with distilled water. The biomass was estimated by dry weight method. A known weight of the biomass was taken and diluted to 100 ml and 100 ppm of Cr (VI) was added. The flasks were kept on a rotary shaker at 80 rpm and incubated for 48 hrs. Every half an hour, the sample was drawn out, centrifuged and the supernatant was analyzed for Cr (VI) concentration.

BIOSORPTION OF Cr (VI) USING IMMOBILIZED BIOMASS OF BACTERIA

The seed culture of *Pseudomonas* Sp, was grown and the cells were harvested by centrifuging at 1000 rpm for 10 min and the cells were washed and suspended in 0.1%(W/V) NaCl. Then, 2 % (W/V) of sodium alginate was added to the cells (2% W/V) biomass suspension and mixed thoroughly without forming any air bubbles in the slurry. The slurry containing the cells was extended as drops through a small tube into 4% $CaCl_2$ solution. The gel beads were kept in 4% $CaCl_2$ solution at 5^oC for about an hour for complete gelatin. The beads then were washed with sterile distilled water and used for metal biosorption study.

ESTIMATION OF HEXAVALENT CHROMIUM

Hexavalent chromium was estimated spectrophotometrically using diphenyl carbazide reagent (APHA, 1995). To 0.1 ml of the sample 0.43 ml of 3M $H_2 SO_4$ was added followed by 1 ml of 2, 5 diphenyl carbazide solution. It was mixed and makes up to 25 ml. After 10 minutes pink color was developed and it was read against reagent blank at 540 nm in a spectrophotometer

RESULTS AND DISSCUSION

In biosorption of Hexavalent chromium four bacterial strains were isolated and one is dominant which is gram negative rod, non-motile and oxidase positive. It is identified as *Pseudomonas* sp which shows more bioadsorption capability. Screening study of the above isolated organism was conducted by taking 100 ppm of Cr (VI) concentration in the growth media. The growth profile of *Pseudomonas* Sp., with 100 ppm Cr (VI) concentrations at various time

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intervals are presented in fig.1. In this case the growth was retarded and the lag phase was extended after specific time intervals. The biomass content was found to be 9.7 mg/ml, 9.48 mg/ml, 9.82 mg / ml, 9.5 mg/ ml and 9.3 mg/ml at 24, 48, 72, 96, 120 hrs respectively (fig.1). It was found to be 9.85 mg/ml occurs at 48 hours. It was reduced to 9.3 mg/ml at 120 hours Rocio Ramirez *et al*, (2004) reported that *C . maltosa* strain was found to tolerate chromate concentrations as high as 100 µg/ ml, it also showed ability to reduce Cr (VI). Chromate reduction occurred both in intact cells as well as in cell-free extracts. The sensitive mutant stains of *Schizosaccharomyces pombe* exhibited a high bioaccumulation ability, with a dry biomass of 810 µg g^{-1} after 30 min, while the tolerant mutant had a significantly lower ability than the wild-type strain. It was found that the tolerant mutant strains utilized the total organic chromium of 35% among them 50% are organically bound chromium (Klara Czako-Ver *et al*., 1999). The protein content of *Pseudomonas* was found to be maximum 15.9 mg/ml at 48 hours. The protein content is 15.6 mg/ml, 15.9 mg/ml, 15.2 mg/ml, 15.0 mg/ml and 14.8 mg/ml at 24, 48, 72, 96 and 120 hours respectively. The protein content was increased to 15.9 mg/ml at 48 hrs and it was decreased to 14.8 mg/ml at 120 hrs (fig.2). The increase in the protein content is due to the synthesis of metal binding protein and the decrease in the protein content after 48 hrs may be due to stress conditions and low L-Cysteine content of the test organisms. Andreoni *et al*., (1997) showed an increase in the protein content from 20g/ml to 357 g/ml at 1500 mg/l concentraion of lead, this can be due to increase of the synthesis of metal binding protein.

The bacterial isolates *Pseudomonas* Sp, was found to utilize the Cr (VI) up to 100 ppm with out affecting its other metabolic activities. In the present study *Pseudomonas* Sp, was further investigated for its ability to biosorbs the Cr (VI) in live, dead and immobilized forms to verify the Cr (VI) utilization of this culture is passive or active mode. The biosorption of chromium by live bacterial isolate is 7.7% in initial stage and it was increased to the maximum of 54% at 240 minutes (fig.3). Yi-Tin wang *et al*, (1995) reported that cell – free extracts of *Bacillus* Sp, contain soluble type of enzyme are responsible for Cr (VI) reduction. The potency of *Aspergillus niger* was evaluated in shake flask culture by absorption of chromium was 75% at pH 6 (Shaili Srivastava and Indu Shekhar Thakur, 2006). The biosorption of chromium by dead cells of *Pseudomonas* Sp is 4.8% in initial stage and the absorption is increased to 49.6% at 240 minutes (fig.3). Sudha bai *et al*., (1998) have showed that the dead fungal biomass behaved like inorganic adsorbents, the pH of the medium, concentration of the metal ion, contact time and agitation influence the metal bioadsorption. Bagirarh *et al*., (1999) reported that the metal uptake by *Spirulina platensis* was increased with an increasing concentration up to 40 ppm. The maximum uptake of 44 mg/g dry weight biomass was obtained.

In the present study the experiments were carried out to determine the metal binding efficiency of alginate immobilized biosorbent. In the case of alginate entrapped biosorbent higher metal uptake was recorded, this is due to the better porosity of the beads which would allow metal ions to be freely transported through the matrix. The adsorption capacity of alginate immobilized biosorbent is 55.5% in initial and it was increased to 66.55% at 240 minutes (fig.3). Flavio Camargo *et a*l, (2004) revealed that the *Bacillus* sp. ES 29 cells immobilized to Celite displayed the highest rate (k = 0.443 at 3 mL h^{-1}) of Cr(VI) reduction. Using initial Cr (VI) concentrations of 2 to 8 mg L^{-1} at flow rates of 3 to 6 mL h^{-1} the immobilized intact extracts reduced 98% of the influent Cr (VI).

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