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# EFFECT OF VARIOUS PARAMETERS ON REMOVAL OF CHROMIUM (VI) USING *PSEUDOMONAS AERUGINOSA 4442*

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**ABSTRACT**

Industrialization and human activities makes intense impact on the environment by disposing industrial effluent containing heavy metals. Remedial procedures using biomaterials, such as, bacteria, algae, fungi, yeast, and agricultural waste biomass are regarded as a cost-effective technique for the treatment of high volume and low concentration of complex waste water containing heavy metals. In current research a microbial remedy is suggested to treat the chromium bearing effluent which includes application of bacterial culture i.e. *Pseudomonas aeruginosa* 4442 and effect of different condition was studied on biosorption of chromium. The major findings include parameter which showed highest percent accumulation of chromium i.e. Nutrient broth (82%), Glucose (72%), Yeast extract (67%), incubation period (120h), shaking conditions, 20h active culture ( 65%), $Mn^{2+}$(88%) and $Cu^{2+}$(81%) and pretreatment of culture with triton (0.1%) given highest accumulation(72%). The recent study proposes the application of *Pseudomonas aeruginosa* 4442 for biosorption of chromium with obtained optimum parameters.



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## INTRODUCTION

Increased industrialization and human activities have large impact on the environment through disposal of waste containing heavy metals. Remedial procedures using biomaterials, such as, bacteria, algae, fungi, yeast, and agricultural waste biomass are regarded as a cost-effective technique for the treatment of high volume and low concentration complex waste water containing heavy metals. Heavy metals comprise an ill-defined group of approximately 65 metallic elements of density greater than 5with diverse physical, chemical and biological properties. Heavy metal pollution is a growing problem all over the world. Industrial zones are responsible to release pollutants through their effluents and these toxic substances contaminates soil, water bodies and sediments. When such toxic substances accumulate in the environment and in food chain, they can profoundly disrupt biological processes. Chromium compounds are extensively used in many industries which include tannery, textiles, metal electro-plating, paint, pigment industry etc. which are responsible to release hexavalent toxic form of chromium into effluents. Chromium metal in its two forms Cr(VI) and Cr(III) are commonly found in the industrial effluents discharged from various chromium related industries' (VI) due to its strong oxidizing capacity is toxic to the ecosystem whereas Cr(III) is less harmful (Wang *et al.*,1990). Hexavalent chromium at a concentration of 10 mg/kg of body weight causes liver necrosis, nephritis and even death in human beings (Dikshit *et al.*, 1989). The properties of heavy metals which warrant their reclamation from effluents are their toxicity and their commercial value (Kasam and Baecker, 1988). It is reported that, through the conventional methods such as precipitation, ion-exchange, evaporation, reverse osmosis effective treatment of chromium bearing effluents can be done (Chand *et al.*, 1994) which are expensive. Heavy metals accumulation process using biological system is grouped under "biosorption", which involves various mechanisms such as intracellular uptake and storage, surface binding or some undefined mechanisms (Gadd, 1990). In current research a microbial remedy is proposed to treat the chromium bearing effluent which includes application of bacterial culture for removal of specific metals from their dilute solutionsas suggested by Townsley *et al* in 1986.

## MATERIALS AND METHODS

**Effect of growth medium and various carbon and nitrogen sources:** To study the effect of various carbon and nitrogen

sources on growth and percent accumulation of Cr (VI) by *P. aeruginosa* 4442, Minimal broth medium with and without 100 ppm of Cr (VI) was used. To study the effect of carbohydrate peptone (1%v/v), different carbohydrates (2%w/v) were added and for nitrogen 1 % w/v different nitrogen sources were added to minimal broth. Flasks containing Minimal nutrient medium plus carbohydrate and nitrogen sources were inoculated with 1 % biomass of *P. aeruginosa* and incubated on rotary shaker at 110rpm for 48h at $30^0$C. After incubation the medium was harvested and growth was estimated in terms of turbidity, dry weight and percent Cr (VI) accumulation.

**Effect of incubation condition on sorption of Cr (VI):** Nutrient broth (pH 7.0) medium with and without 20-500ppm of Cr (VI) was inoculated with 1% (w/v) biomass and incubated on rotary shaker at $30^0$ C and one set of flasks were incubated at stationary condition. The flasks were incubated for 5 days and percent accumulation of Cr (VI) was studied at shaker and stationary conditions for various Cr concentrations.

**Effect of culture age of *P. aeruginosa 4442* on % accumulation of Cr (VI):** To study the effect of culture age, on Cr (VI) accumulation, growth of *Pseudomonas aeruginosa* 4442 was harvested at the different phases of incubation of 2, 4, 6…28 hours. Growth in terms of absorbance and dry weight was taken. Dried biomass was taken and its absorbance was adjusted to1.0 using sterilized distilled water at 600nm and 1% (v/v) of this was added to 100ppm of Cr (VI) solution with pH 7.0.It was incubated at $30^0$C on a rotary shaker at 100rpm for 1hr. After incubation, sample was centrifuged and supernatant was analyzed for residual Cr (VI) content. From it percent sorption of Cr (VI) at various cell ages was studied.

**Effect of cations and anions on accumulation of few metal ions:** Effect of cations on percent sorption of metals on *P. aeruginosa* 4442 was studied by using single metal and multi-metal solution. Multi-metal solution was prepared from metal salts of $Cu^{2}$, $Mn^{2+}$, $Zn^{2+}$, $Ni^{2+}$ and $Cr^{6+}$ Metal solution of 100ppm each was mixed in equal volume so as to get multi metal solution. To it, 1% (w/v) of biomass of *P. aeruginosa 4442* was added and allowed to react for 1hr at $30^0$C on rotary shaker and after incubation the samples were withdrawn and analyzed for residual metal concentrations. To study the effect of different anions on percent sorption of different metals, metal solutions were prepared containing 100 ppm concentration of each 5 metals and to it 1% (w/v) biomass of *P. aeruginosa 4442* was mixed with equal volume of each 1mM anion and was allowed to react for1 hr at $30^0$C, samples were withdrawn and centrifuged. Supernatant was analyzed to estimate % Cr (VI) sorption of each metal in the presence of different anions.

**Effect of pre-treatment of *P. aeruginosa 4442* on Cr (VI) uptake:** To study the effect of various pre-treatment procedures on percent sorption by *P. aeruginosa* 4442, 1% (w/v) of biomass of *P. aeruginosa* 4442 was added to pre-treatment materials as shown above for boiling equal volume of sterilized distilled water was used. The pre-treatment material and biomass was allowed to react with each other for 30 minutes. After reaction time, the pre-treated material was removed by centrifugation. Pre-treated pellet was added to 100ml of 100 ppm of Cr (VI) and allowed to react for 1hr on shaker at 100rpm at $30^0$C. Sample were withdrawn, centrifuged and supernatant was analyzed on AAS for residual Cr (VI) and from it % adsorption of Cr (VI) by various pre-treated biomass were estimated.

## RESULTS AND DISCUSSION

**Effect of growth medium and various carbon and nitrogen sources:** From the results (Table 1), it was observed that growth in terms of turbidity and dry weight in minimal broth and nutrient broth and percent accumulation of Cr (VI) by *P. aeruginosa* 4442 cells showed more growth in nutrient broth as compared to minimal broth. The Cr (VI) accumulation in nutrient broth was maximum 82 % as compared to 56 % in minimal broth.

**Table 1. Growth of *P. aeruginosa* 4442 in minimal and nutrient broth with and without Cr (VI)**

| Medium | Growth in terms of Absorbance at 600nm | Dry wt. (g/100ml) | % accumulation of Cr (VI) |
|---|---|---|---|
| Nutrient broth ($M^-$) | 0.324 | 0.68 | - |
| Minimal broth ($M^-$) | 0.085 | 0.35 | - |
| Nutrient broth+ Cr (VI) | 0.256 | 0.42 | 82 |
| Minimal broth+ Cr (VI) | 0.036 | 0.12 | 56 |

Legend-($M^-$) = Without metal (Cr)

These results confirmed that nutrient broth being a complete medium supported for maximum growth and subsequently Cr (VI) absorption. On the contrary less growth was observed in minimal broth and hence less Cr (VI) accumulation. Cells of *Ralstoniametallidurans* CH34 (Anders *et. al.,* 1999) exhibited higher specific metal uptake when grown in complex medium instead of simple medium. This could be ascribed to anabolic metabolism where the readily available source of nutrients like peptone, yeast extract, tryptone in media provides essential amino acids and growth factors that are incorporated into the cell polymers binding to metals. The biomass growth study and percent accumulation of Cr (VI) were carried out using various organic and inorganic nitrogen sources (peptone, yeast extract, beef extract, ammonium sulphate) as well as various carbon sources (dextrose, starch, lactose, glycerol etc.) The growth showed variation in response to various nitrogen sources. Growth was more in organic sources of nitrogen compared to inorganic one, maximum growth was obtained in presence of yeast extract (67%) followed by beef extract (61%) peptone (56%) and tryptone (54%). The effect of carbon source on growth of *P. aeruginosa* 4442 in presence of peptone and dextrose as carbon source, gave highest 72% of Cr (VI) accumulation. It can be concluded that dextrose being monosaccharide was easily degraded by the *P. aeruginosa 4442* (Table 2).

**Table 2. Effect of various C sources and percent accumulation of Cr (VI)**

| Carbon and N sources (C-2%,N-1%) | Growth in terms of Absorbance at 600nm | Dry wt. (g/100mL) | Percent accumulation of Cr(VI) |
|---|---|---|---|
| Glucose | 1.149 | 0.89 | 72 |
| Lactose | 0.003 | 0.06 | 5 |
| Mannitol | 0.06 | 0.08 | 5 |
| Sucrose | 0.986 | 0.68 | 52 |
| Starch | 0.116 | 0.18 | 22 |
| Glycerol | 0.097 | 0.03 | 37 |
| Peptone | 0.88 | 0.76 | 56 |
| Yeast extract | 0.924 | 0.78 | 67 |
| Tryptone | 0.899 | 0.45 | 54 |
| Beef extract | 0.90 | 0.57 | 61 |
| Urea | 0.669 | 0.47 | 46 |
| $NH_4Cl$ | 0.002 | 0.028 | 14 |
| $(NH_4)2SO_4$ | 0.002 | 0.09 | 26 |

Stoll and Duncan (1996) carried out experiments on the removal of $Cu^{2+}$, Cr (VI), $Cd^{2+}$, $Ni^{2+}$ and $Zn^{2+}$ showed that uptake of metals increased in yeast cells when grown with glucose as a source of carbon and energy. Similarly, Rama Krishna *et al.,* (2005) suggested that the effect of various electron donors on Cr (VI) reduction using *Ganoderma lucidum* (a wood rotting fungus) in a bioreactor reduction system. Among 5 electron donors (peptone, acetate, dextrose, molasses and sewage) peptone showed maximum Cr (VI) reduction, followed by molasses.

**Effect of incubation condition on sorption of Cr (VI):** From the results (Table 3 and 4), it was observed that as the concentration of Cr (VI) increased there was decrease in its accumulation. This increase was substantial for 72 hrs after which it was slow.

**Table 3. Effect of incubation period on percent accumulation of Cr (VI) by *P. aeruginosa 4442* (shaking conditions)**

| Conc. of Cr(VI) (ppm) | Incubation period (h) | | | | |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | 120 |
| | Percent Cr (VI) accumulation | | | | |
| 50 | 43 | 48 | 54 | 68 | 72 |
| 100 | 35 | 37 | 40 | 48 | 62 |
| 150 | 20 | 25 | 28 | 40 | 41 |
| 200 | 18 | 24 | 30 | 35 | 40 |
| 250 | 14 | 18 | 21 | 31 | 32 |

**Table 4 Effect of incubation period on percent accumulation of Cr (VI) by *P. aeruginosa 4442* (stationary conditions)**

| Conc. of Cr (VI) (ppm) | Incubation period (h) | | | | |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | 120 |
| | Percent Cr (VI) accumulation | | | | |
| 50 | 25 | 32 | 48 | 59 | 62 |
| 100 | 18 | 20 | 26 | 40 | 41 |
| 150 | 14 | 18 | 25 | 40 | 40 |
| 200 | 12 | 17 | 19 | 28 | 30 |
| 250 | 10 | 13 | 16 | 22 | 25 |

Further, it was observed that the process of agitation and stationary when applied incubation at shaker condition showed increase in percent Cr accumulation, it was due to proper mixing of the sorbate and sorbent system that facilitates proper contact between the Cr (VI) ions in solution and the *P. aeruginosa 4442* biomass binding sites and thereby promotes effective transfer of sorbate ions to the sorbent sites. This observation is in agreement with the biosorption Cr (VI) by *Rhizopus arrhizus* by Niyogi *et al*., (1998).

**Effect of culture age of *P. aeruginosa 4442* on % accumulation of Cr (VI):** It was evident that distinct morphology of cell of bacteria exhibited during the growth cycle. Thus, culture age can also influence the surface character and there by absorption of Cr (VI) significantly (Table 5). The isolated *P. aeruginosa* 4442 when allowed to grow in sterile nutrient broth containing 50ppm of Cr (VI) and pH7.0 at $30^0$ C on shaker condition and growth was monitored in terms of turbidity, dry weight and percent Cr (VI) accumulation. It was observed that initially upto 6-8 hrs growth was very slow and then it showed significant growth in its log phase till 18 hrs. From 16-20 hrs Cr (VI) accumulation was between 60-65%. The growth rate remained constant for a very short span of time and then there was decrease in Cr (VI) uptake. Chang and coworkers (1997) reported an increase in biosorption of lead and cadmium by *P. aeruginosa* with increasing culture age. Uranium removal by *Saccharomyces cerevisiae* in logarithmic phase was 4.6 times more than the older phase cells (Volesky and May-Philips, 1995).

**Table 5. Effect of aging of culture on % accumulation of Cr (VI)**

| Incubation time (h) | Absorbance at 600nm | Dry wt. (g/ 100ml) | % accumulation of Cr (VI) 50ppm |
|---|---|---|---|
| 2 | 0.054 | 0.71 | 5 |
| 4 | 0.069 | 0.75 | 7 |
| 6 | 0.092 | 0.81 | 9 |
| 8 | 0.108 | 0.83 | 22 |
| 10 | 0.156 | 0.91 | 30 |
| 12 | 0.19 | 0.98 | 35 |
| 14 | 0.234 | 1.07 | 55 |
| 16 | 0.382 | 1.26 | 60 |
| 18 | 0.64 | 1.94 | 62 |
| 20 | 0.89 | 2.34 | 65 |
| 22 | 0.987 | 2.59 | 59 |
| 24 | 1.326 | 2.87 | 59 |
| 26 | 1.533 | 3.15 | 57 |
| 28 | 1.705 | 4.21 | 57 |

**Effect of cations and anions on accumulation of few metal ions:** Industrial effluents with presence of single metal ion species can never occur. It is usually a mixture of numerous metals and non-metals as cations and anions. The percent sorption of 5 metals under study was carried out as single metal system and multi-metal system containing 100 ppm of each metal. From the (Table 6) it was observed that the percent sorption of each metal as a single species was more compared to that of multi-metal systems and the preferential sorption of metals in single metal system by *P. aeruginosa* 4442 was found to be manganese, $Cu^{2+}$, $zn^{2+}$, $Cr^{6+}$ and $Ni^{2+}$.

**Table 6. Effect of cations on percent sorption of metals Of metals by *P. aeruginosa 4442***

| Metal | % accumulation of metal ions from | |
|---|---|---|
| | Single metal solution | Multi-metal solution |
| $Zn^{2+}$ | 78 | 62 |
| $Cu^{2+}$ | 87 | 81 |
| $Mn^{2+}$ | 88 | 52 |
| $Cr^{6+}$ | 71 | 58 |
| $Ni^{2+}$ | 69 | 54 |

Percentage of $Cu^{2+}$ removal was not significantly affected by the presence of other metal ions on the contrary the uptake of $Mn^{2+}$ and $Cr^{6+}$ was greatly influenced in presence of other metal ions. Gokusungur*et al*. (2005) observed that the competitive biosorption capacities of the ethanol treated yeast for all metal ions of lead; $Cu^{2+}$ and $Cd^{2+}$ were lower than that under the non-competitive conditions. The decrease of metal uptake under competitive condition was thought to be a response to increased competition between same charged species for binding site.

**Table 7 Effect of anions on percent sorption of metals study by *P. aeruginosa4442***

| Anion | Percent sorption of metals | | | | |
|---|---|---|---|---|---|
| | $Cr^{6+}$ | $Zn^{2+}$ | $Ni^{2+}$ | $Mn^{2+}$ | $Cu^{2+}$ |
| Control | 68 | 60 | 75 | 59 | 76 |
| $K_2HPO_4$ | 49 | 38 | 78 | 63 | 60 |
| Acetate | 66 | 65 | 53 | 51 | 62 |
| KCl | 38 | 79 | 58 | 59 | 64 |
| EDTA | 68 | 65 | 68 | 53 | 53 |
| Carbonate | 54 | 57 | 66 | 54 | 78 |
| Sulphate | 54 | 30 | 74 | 40 | 66 |
| Citrate | 54 | 44 | 40 | 52 | 76 |
| Nitrate | 49 | 40 | 66 | 36 | 40 |
| Borate | 49 | 66 | 23 | 33 | 25 |

Table 7 showed the effect of various anions on percent sorption of various metals. Chromium sorption was significantly reduced in presence chloride and EDTA, presence of nitrate has reduced % sorption in case of $Zn^{2+}$ and $Cu^{2+}$ and presence of borate has reduced $Zn^{2+}$ and $Ni^{2+}$ sorption by *P. aeruginosa 4442*. All the anions studied showed a different degree of reaction with respect to % removal of metal ions under study. In case of Cr (VI) removal EDTA showed near about equal efficiency as that of control, followed by acetate (66%). Presence of carbonate and sulphate showed reduction and KCl showed near about 50% reduction. Presence of KCl, borate, acetate showed to increase % removal of $Zn^{2+}$ while sulphate, citrate, $K_2$ $HPO_4$ showed reduction. In case of $Ni^{2+}$ sorption, presence of anions showed % sorption in the decreasing order of $K_2$ $HPO_4$ > sulphate>EDTA> carbonate>KCl> Acetate>citrate and borate. For $Mn^{2+}$ maximum % sorption was in the presence of $K_2HPO_4$, which was more compared to control, rest of the anions resulted in interfering the % removal and in case of $Cu^{2+}$, carbonate showed increase in % sorption (78%) followed by citrate, which was just equal to that of control. Rest of the anions showed decrease in % sorption and presence of borate was found to have negative effect on % sorption (25%) of copper. The presence of anions also affects the biosorption of metal ions. Kapoor and Viraraghavan (1997) reported that the biosorption capacity decreased in the presence of sulphate, chloride, phosphate, carbonate, glutamate, citrate and pyrophosphate.

**Table 8. Effect of pre-treatment on percent sorption of Cr (VI)**

| Pre-treatment with | Percent Cr (VI) sorption |
|---|---|
| Control | 61 |
| Acetone (1%) | 67 |
| Triton (0.1%) | 72 |
| HCl (1M) | 69 |
| NaOH (1M) | 65 |
| Boiling | 43 |

**Effect of pre-treatment of *P. aeruginosa 4442* on Cr (VI) uptake:** The loss of specific groups of molecules in the cell wall can affect the metal binding ability of the cell wall. For example, the loss of specific molecules from the core region of the lipopolysaccharide of a rough mutant strain (D21f2) of *E. coli* resulted in a more open structure that allowed the binding of both larger cations and more monovalent cations (Coughlin *et. al.*1983). From the Table (8), it was evident that various pre-treatments showed increase in % sorption of Cr (VI). McHale and McHale (1994) documented that any pre-treatment which affects the cell wall character will in turn affect the nature of metal uptake. The interior of the cells also contains many metal binding components so the treatments which permeabilizing the cells such as grinding (Gadd and white, 1989) detergents and chemicals (Strandberg *et al*., 1981) increase the metal uptake. *P. aeruginosa* 4442 showed increased % sorption of Cr (VI), when it was treated with 1M HCl, 1M NaOH, triton and acetone treatment while there was decrease in % sorption of Cr(VI) by boiling. Similar results were observed by Mahadevan and Tatum (1965). They stated that alkali treatment of the bacterial cell removes most of the cell wall materials containing $COO^-$ and $PO_4$ groups further Muzzarelli *et. al*., (1980) interpreted that the alkali treatment of the cells improved their capacity to chelate various metal ions.

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