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# EFFECT OF METAL ON HEXAVALENT CHROMIUM REDUCTION BY *ACENETOBACTER CALCOACITICUS*

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**ABSTRACT**
Due to chromium mining, Sukinda in the district of Jajpur is the most polluted area in the world with hexavalent chromium, which is highly toxic to living organisms. The natural habitat of Sukinda is also characterized by presence of a large number of toxic and non-toxic metals affecting reduction of hexavalent chromium by the native novel microbial flora. This necessitates study on multiple metal effects on hexavalent chromium reduction by *Acenetobacter calcoaciticus* isolated from the mining area. It showed a broad range of tolerance to metals like $Fe^{2+}$, $Cu^{2+}$, $Ni^{2+}$, $Hg^{2+}$ and $Co^{2+}$ up to concentration of 1000ppm, 900ppm, 1000ppm, 100ppm and 300ppm respectively. The bacteria was selected for parametric studies and observed to exhibit highest reduction 89.39% in a nutritive culture medium supplemented with 1ppm of Cupper at pH 8.0, temperature 30ºC for 24h. Therefore, *A. calcoaceticus* may be used for bioremediation of hexavalent chromium toxicity in different chromium contaminated sites.

## INTRODUCTION

In view of increasing human need enhanced mining activities along with rapid industrialization has contaminated the environment with various pollutants. Sukinda valley of Jajpur district, Orissa is one of the most polluted places in the world (Black Smith Institute, 2007) accounts for 97% of India's chromites ore deposits and last 70 years of intensive open cast mining generated 30 million tones of hexavalent chromium wastes polluting soil, water, ruined agricultural fields. Chromium an essential micronutrient in animal physiology required for normal carbohydrate and lipid metabolism and a priority pollutant is well known for the mutagenicity (Petrilli and Flora, 1977) and carcinogenicity (Gruber and Jennette, 1978) of its hexavalent form in humans, experimental animals (IARC, 1990) and Plants (Flora *et al*., 1990). It is water-soluble; bioleachable form that can intracellularly reduced to $Cr^{5+}$ and reacts with nucleic acids and other cell components to produce mutagenic and carcinogenic effects on biological systems (McLean and Beveridge, 2001). Conventional chemical treatment of $Cr^{6+}$ waste generates large volume of sludge, gases and not being cost expensive, it is imperative to develop safe and cheaper alternatives, where the metal resistant microorganisms are of primary importance in bioremediation of metal contaminated sites. Bioreduction of $Cr^{6+}$ occurs directly due to microbial metabolism or indirectly by bacterial metabolites (Losi *et al*., 1994a). Many researchers (Aravindhan *et al*., 2007; Rahman *et al*., 2007) have investigated and demonstrated the feasibility for treatment of $Cr^{6+}$ contaminated sites and industrial effluents using either pure culture or consortium of bacteria. However, the natural habitat also contains large number of toxic and non-toxic metals affecting chromium reduction (Verma and Singh, 1995). Therefore, an attempt has been made in the present investigation to study effect of different metals effects on $Cr^{6+}$ reduction.

## MATERIALS AND METHODS

### Culture collection

A lyophilized Gram-negative bacteria *A. calcoaciticus* maintained in the laboratory was taken for effect of different metals on hexavalent chromium reduction study. The culture was revived by inoculated into the Luria Bertani broth, incubated at temperature 30ºC overnight were streaked in Luria Bertani agar plates and incubated for 24h. Luria Bertani broth and agar were used for bacterial culture revival and chromium reduction studies were procured from Hi-Media Laboratories, Mumbai.

### Estimation of metal tolerance

Metal tolerance of *A. calcoaciticus* was studied in molten Luria Bertani broth medium supplemented with different metal such as iron, copper, nickel, mercury and cobalt with final concentration ranging from 100-1000mg/L by using filter (0.45$\mu$m) sterilized solution of ferrous sulphate, cupper sulphate, nickel sulphate, mercuric chloride and cobalt chloride and medium without metal was kept as control. 0.1mL of fresh culture was added to conical flask containing respective metal solution and incubated at 30ºC for 24h. The isolate was streaked onto the Luria Bertani agar plates and further incubated and minimum inhibitory concentration (MIC)

was observed.

### Effect of metal on $Cr^{6+}$ reduction

To five sets of 100mL of Luria Bertani broth (pH 8.0) containing 100ppm of $Cr^{6+}$ with 5% inoculums of *A. calcoaciticus* and 1ppm each of filter sterilized ferrous sulphate, cupper sulphate, nickel sulphate, mercuric chloride and cobalt chloride was incubated at 30ºC for 24h at 100rpm in an incubator shaker. A control set was run without metal. Cells were collected after centrifugation at 10,000rpm for 10 minutes. Supernatant was analyzed for residual chromium by 1, 5-Diphenyl carbazide method (APHA, 1992). $Cr^{6+}$ reduction was determined by measuring absorbance at 540nm using a spectrophotometer.

## RESULTS AND DISCUSSION

### Culture profile

A lyophilized Gram-negative bacteria *A. calcoaciticus* isolated from Sukinda mining area could tolerate $Cr^{6+}$ 1000ppm and reduced 85% of $Cr^{6+}$ in form of $K_2Cr_2O_7$ supplemented in Luria Bertani broth (pH8.0) at 30ºC for 24h. (Mishra *et al*., 2010)

### Metal tolerance profile of *A. calcoaceticus*

*A. calcoaceticus* showed a broad range of tolerance (Table 1) to heavy metals such as iron, cupper, nickel, mercury and cobalt up to concentration of 1000ppm, 900ppm, 1000ppm, 100ppm and 300ppm respectively. Apart from all the metals, the highest tolerance was observed towards iron and nickel. These observations assume great significance (Srinath *et al*., 2001; Luli *et al*., 1983) because effluents from any metal related to industry or mines have several metal ions or contaminants. Moreover, natural habitats are generally characterized by the co-existence of a large number of toxic and non-toxic cations. Tolerance to other metals has an added advantage of withstanding the presence of other metal ions while performing the desired activity.

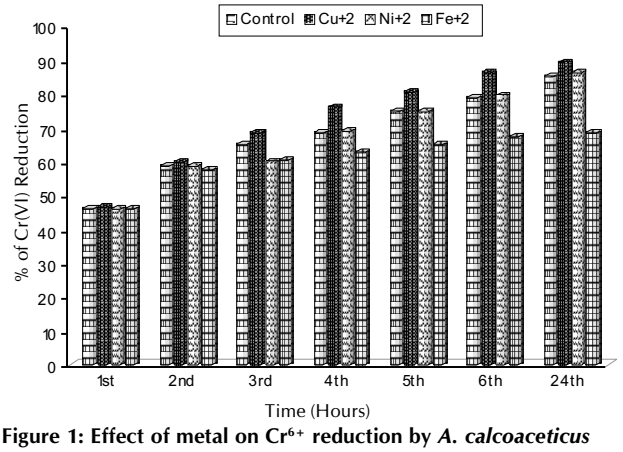


**Figure 1: Effect of metal on $Cr^{6+}$ reduction by *A. calcoaceticus***

### Effect of metal on aerobic $Cr^{6+}$ reduction

Fig. 1 showed hexavalent chromium reduction by *A. calcoaceticus* in the presence of different metals. In 1ppm of cupper, 89.39% $Cr^{6+}$ reduction was observed at 30ºC/24h/pH 8. In the presence of iron, 68.44% hexavalent chromium was reduced and the rate of reduction decreased in presence of nickel as compared to control. Although the organism showed more tolerance to iron, no change in chromium reduction was observed. The increase in reduction in presence of cupper may be due to enhanced enzyme activity of chromate reductase (Elongovan *et al*., 2006; Pal and Paul, 2004; Faisal and Hasnain, 2004) as it also acts as micronutrient for optimal growth of the bacteria.

**Table 1: Metal tolerance profile of *A. calcoaceticus***

| Metal | Concentration of different metal (ppm) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | 100 | 200 | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 |
| $Fe^{2+}$ | + + + | + + + | + + + | + + + | + + | + + | + + | + + | + | + | + |
| $Cu^{2+}$ | + + + | + + + | + + + | + + | + + | + + | + + | + | + | + | - |
| $Ni^{2+}$ | + + + | + + + | + + + | + + | + + | + | + | + | + | + | + |
| $Hg^{2+}$ | + + + | + | - | - | - | - | - | - | - | - | - |
| $Co^{2+}$ | + + + | + + | + | + | - | - | - | - | - | - | - |

+ + +: Luxuriant growth, + +: Moderate growth, +: Mild growth, -: No growth

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