BIOREDUCTION OF HEXAVALENT CHROMIUM USING MICROBES IN WATER AND SEDIMENTS OF COOVAM RIVER

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ABSTRACT

The present study has been attempt to analyze the status of heavy metal concentration on the bank of Coovum River at Chennai city. This study indicates that both urbanization and Industrialization have contributed to the large scale of pollutions currently observed. Hexavalent chromium pollution in this area has caused a major health hazard affecting people in this area due to chromite dust inhalation. Therefore it is necessary to remove the hexavalent chromium from the environment. The study is based on that reduction of Hexavalent Chromium (VI) by using microbes. The maximum tolerance of the identified strain to Cr VI was 100mg/100ml. These cultures were used for further investigation and biodegradation of the samples containing hexavalent chromium. The results indicated that isolated culture can be utilized to improve efficiency of biological treatment processes for effluents containing higher levels of Cr (VI).carried out to determine the concentration of heavy metal ions in and around this area.

Key words: Heavy metals, Pollution, Hexavalent, Chromium, Biodegradation.

INTRODUCTION

In the process of development, mining is one of the sector which contribute significantly, towards the deterioration of the environment in terms of air, water and land pollution. To achieve sustainable development, environmental protection measures should be introduced at the planning stage of a mining project. India is endowed with a wide range of mineral reserves and mineral production in the country maintained growing trend. India produces as many as 90 minerals including fuel minerals. Today water pollution is the biggest problem for human beings characterization by deterioration of the water quality as a result of various human activities which makes water unfit for drinking and domestic purposes. Many toxic heavy metals have been discharged into the environment as industrial waste, causing serious soil and water pollution. Water scarcity is increasing worldwide and pressure on the existing water resources is increasing due to growing demand of different sectors such as domestic, agriculture and industrial, hydropower etc. (Ayres.R.U1992) Water is an essential constituent of all animals, plants and human beings. The main sources of water pollution are chemical fertilizers and pesticides getting in an untreated sewage, dumping of waste and industrial effluents into rivers and streams running close in to the cities and to the low lands (Eneji.I.S,Sha'Ato.R and Annune.P.A,2011). Precipitation is accompanied by flocculation or coagulation and. one major problem is the formation of large amounts of sediments containing heavy metal ions(Gibbs,1995). It is caused by excavation of pits, solid waste disposal and other allied operations. Disposal of solid waste like overburden / waste rock, sub-grade ore and mineralized reject may result in environmental pollution causing disturbance of water level and water quality

(Dhar, 1993). This problem associated with heavy metal contamination is caused by natural oxidation when minerals are exposed to air and water. Sulfide oxidizing bacteria play an important role in the formation of AMD (Kleinmann *et al*., 1985).

The chromium is an important industrial metal used in diverse products and processes (Nriagu,1988). At many locations it is released to the environment due to leakage as a result of poor storage or improper disposal practice (Palmer and Wittbrodt, 1991; Calder, 1988). Aqueous chromium (VI) species are generally soluble and relatively weakly adsorbing, hence they can be mobile in groundwater (Kent *et al*.,1995). Cr (III), if uncomplexed, should be highly retarded in groundwater because its aqueous species are extensively adsorbed or insoluble (Kent *et al*.,1994; Anderson *et al*., 1994).

Hexavalent chromium are found in several ground water samples of Kanpur city. Generally contaminant flow was in the direction of ground water flow and much more depend upon the hydrological features of the area. It also inferred that shallow depth hand pumps were largely contaminated (Panday, 1997).

The surrounding soil and plants are also reported to be enriched in chromium content around the chromium rich ultramafic terrain in Pakistan (Kafay yatullah). Previous studies have shown that a high degree of heavy metal contamination in soil and plants have occurred in many places in the world, which could be related to the occurrence of ore deposits (Gough *et al.*,1989). Previous study shows that ground water and surface water chromite are contaminated with hexavalent chromium (R.K.Tiwary *et al.*, 2005). Chromium enters the environment, and persists in its most stable oxidation states, Cr (III) and Cr (VI). Trivalent form of chromium is relatively innocuous and immobile, while Cr (VI) moves readily through soils and Aquatic environment. Hexavalent compounds are acute toxic, mutagenic (Bianci *et al*., 1983; Beyersmann *et al*., 1984), teratogenic (Abbasi and Soni, 1984), and carcinogenic (Mancuso and Hueper., 1951; Waterhouse 1975; Yassi and Nieboer, 1988).

Chromium (Cr^{+6}) is most toxic water pollutant. The value above 0.05 in drinking water may pose detrimental to human health. In higher concentration of Cr^{+6} may produce diseases like a corrosive action on the skin and mucous membrane. It is a strong oxidizing agent capable of being absorbed through the skin (Nriagu and Nieboer, 1988) it is also an irritant to the plant and animal. In contrast, trivalent compounds have relatively low toxicity (Van Weerelt *et al*., 1964) and are immobile under moderately alkaline to slightly acidic conditions.

In the present investigation we have isolated an indigenous bacterial strain capable of resisting chromium from Coovam effluent. The main aim is to establish a remediation route for detoxification of Cr (VI) using this indigenous microorganism with all optimized process parameters and conditions.

MATERIALS AND METHOD

To carry out the study water and soil samples collected from the Coovam River. Initial concentration of Hexavalent chromium in water samples and soil characteristic study has been carried out. Sample Characteristics of Chromium contaminated soil and water samples were collected in screw capped sterilized bottles from different area of Coovam River. The pH of the samples was determined with an ion-specific electrode (pH meter) and the pH of the soil and waste water samples were in the range of 7.3 - 7.8 and 8.3 – 8.5, respectively. This indicated that the chromite contaminated sites are slightly alkaline in nature. The range of Cr (VI) in the contaminated soil sample is in the range of 2-6 mg/l and in case of effluent it varied from 36-44 mg/l. Results of the XRF analysis of dump material show that the Cr_2O_3 content in the water body varies from 25.31 to 55.525% whereas dump material contains 5-9% Cr_2O_3. Iron percentage is also found high in the sample. The procedure of bioremediation of hexavalent chromium involves sample collection, isolation of microbes, minimum inhibitory concentration check, growth of microbes, strain identification, diphenyl carbazide assay, initial measurement of chromium (VI), bioreduction of chromium (VI), measurement of Cr (VI) after reduction. These processes are described below in detail.

ISOLATION OF MICROBES:

Microbes that are capable of residing in chromium contaminated soil must have developed the resistivity towards chromium. Therefore these microbes must be able to reduce Cr (VI) to Cr (III). Therefore, these soil and water samples used to grow chromium resistant microbes.Peptone and yeast extract (PYE) were used to prepare culture media. Microbes that have been grown on the Petri plate have to be checked for the highest concentration of chromium that they can resist and can have visible growth on the Petri plate. For this purpose, petri plates with increasing concentrations of $K_2Cr_2O_7$ were prepared. Plates were then streaked. After the incubation of 24 hours, the microbes grown on the plates were transferred to the plate with higher concentration of $K_2Cr_2O_7$. After the check for the MIC, a single strain capable of growing at higher concentration was selected for further investigation.

After the MIC check, the selected strain was used for the preparation of culture. The culture was prepared using the procedure mentioned earlier. Also, the plates were prepared. Incubation was done for 24 hours at 37°C. The selected strain was sent to 'Centre for Cellular and Molecular Biology' (CCMB) Hyderabad for the identification process.

Initial measurement of Chromium (VI):

Dissolve 250mg 1, 5-Diphenyl Carbazide in 50 ml acetone. Store it in brown bottle. Discard when solutions become discolored.1gm of the solid sample is mixed in 25 ml of distilled water and was filtered using filter paper. For the water sample we use 25 ml of it, the water and filterded soil samples is acidified by adding 5 drops of Phosphoric acid. Then 2ml of 0.2N H_2SO_4 and 2ml of Diphenyl carbazide is added. Pink coloration is developed. Then it is analyzed by a spectrophotometer.

Bioreduction of Chromium (VI):

A 250 ml flask with 50ml water sample or soil sample (filtered) and components of media are mixed. It is supplemented with 0.5g of lactose. After sterilization, 2ml of 24 hour grown culture was added to it. The setup was inoculated in the laminar air flow. After that, the incubation for a period of 24 hours and 72 hours was done in the incubator at a temperature of 37°C. After the approximate time, the evaluation of chromium (VI) was done following standard procedure. Controls were prepared with no culture being added to them.

Final measurement of Chromium (VI):

The bacterial strain, were precultured overnight in PYE broth and the cell pellets were collected by centrifugation (6000*g* for 10 min) followed by washing the cell pellets in phosphate buffer. After two washes of the cells in phosphate buffer, the cells were re-suspended in the similar buffer. Culture flasks containing minimal salts medium supplemented with Cr (VI) from the water and filtered soil sample (40 ml each) and 0.5g lactose were inoculated with the equal amounts of culture species. Media without Cr (VI) was inoculated with bacteria and uninoculated media containing Cr (VI) served as controls. All the cultures including controls (in duplicate) were incubated for 72 h at 37oC temperature.

Growth of the bacteria was monitored at specific time intervals, by measuring optical density of the cultures at 540 nm. To compute the Cr (VI) reduction by growing cells, a 3 ml culture from each of the above flasks was centrifuged (6000 rpm for 15 min) and the supernatant analyzed for Cr (VI). In order to isolate the chromium resistant bacteria different soil samples and water samples were taken which is listed in the Table 1 below**.** Cr (VI) analysis by diphenyl carbazide assay and pH of the samples were measured. From these samples soil sample and water sample is selected for the isolation of chromium resistant bacteria. Soil sample and waste water sample is selected for further studies because of the high chromium content present in both of them.

RESULTS AND DISCUSSION

Isolation of Chromium Resistant bacterial Strains and Cr (VI) Tolerance study:

Soils contain a very large number of micro-organisms which can include a number of Cr (VI) utilizing bacteria. A single chromium resistance bacterial strain was isolated from the samples by culture methods. The isolated strains is tested for their chromate tolerance at different concentrations (25-200mg/100ml) in solid agar medium. Microbes grown on 125mg $K_2Cr_2O_7$/100ml distilled water are shown in Figure 1 & 2.

Figure 1: Showing growth of microbe at 125mg $K_2Cr_2O_7$/100ml distilled Water

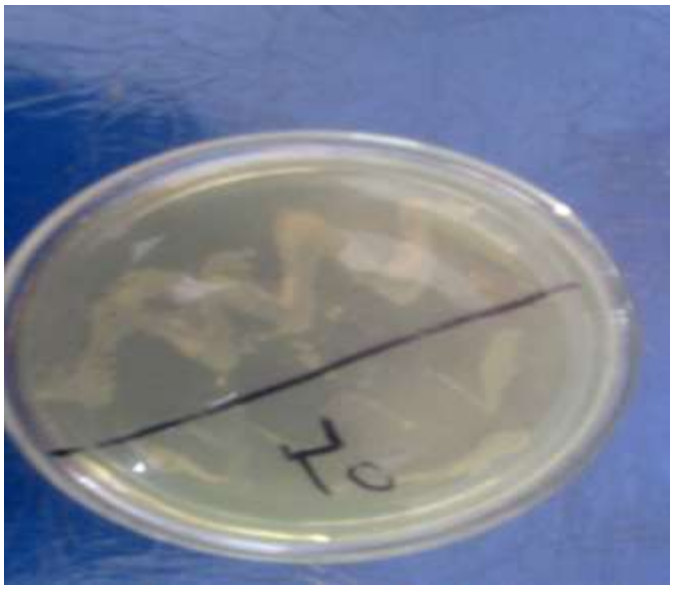
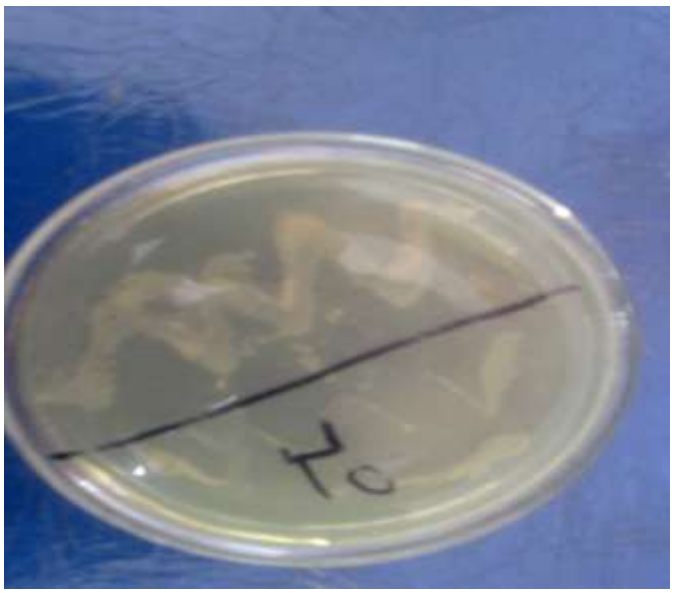


Figure 2: Showing growth of microbe at 125mg $K_2Cr_2O_7$/100ml distilled water



Bioreduction of chromium

The initial Chromium (VI) concentration of water sample taken was 0.755 ppm. The water sample was collected from Coovam River. After 24 hour, the samples were taken and 3 ml of it was centrifuged and filtered with whattman filter paper no 42. The supernatant after filtration was measured at 540 nm. The reduced concentration of the sample was 0.261ppm. After 72 hours, another set of samples were taken and 3 ml of it was centrifuged and filtered with whattman filter paper no 42. The supernatant after filtration was measured at 540 nm. The reduced concentration was 0.022ppm.

The soil sample was collected from the same area. Initial concentration of chromium (VI) was 0.998ppm. After 24 hours first set of the soil sample was measured and the concentration was 0.58ppm.Then the second set of soil sample after 72 hours was measured and the concentration was 0.43 ppm. Table 6 shows the result of reduction after different time intervals.

Table 1: Bioreduction of samples after different time intervals.

Samples	**Initial Concentration of Cr (VI) (**ppm)	**Concentration of Cr (VI) after 24 hours of bioreduction(**ppm)	**Concentration of Cr (VI) after 72 hours of bioreduction(**ppm)
Waste Water Sample	0.755	0.261	0.022
Soilsample (OB Dump)	0.998	0.58	0.43

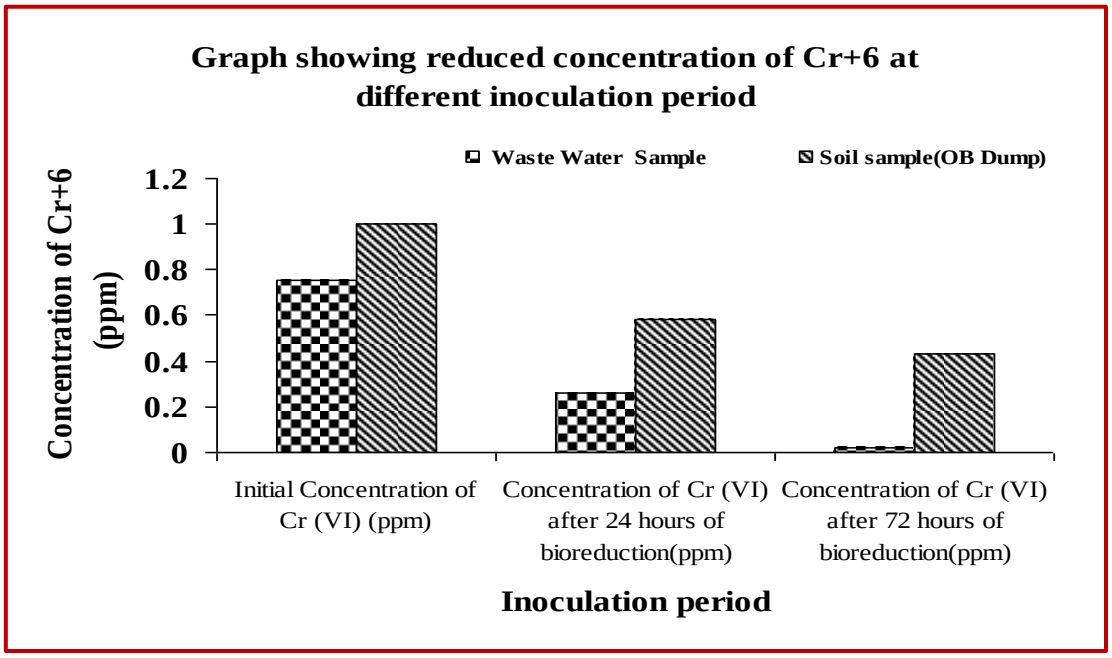


Figure 3: Showing reduced concentration of Cr^{+6} after different period of time

CONCLUSION

Hexavalent chromium is a highly toxic. Hence the present investigation has examined the presence of indigenous organisms from the Cr (VI) contaminated soil and water. The identified microorganism has high

resistance to Cr (VI) and has significant capability to completely degrade Cr (VI) in the waste water. The result shows that the bioreduction of Cr (VI) in the soil sample 47% and 97% in waste water sample within 72 hours while the degradation of soil and water sample was 42% and 66% in 24 hours respectively. Hence, it can be concluded that the identified chromium resistant microorganism is more effective in cleaning wastewater. Even though, the condition is very bad at present, but if the same continues in future groundwater source will be completely polluted and become unfit for drinking and other purposes. This observation indicates contamination of the environment. Thus dumping of waste polluted materials should be avoided and they should not be let into the river. Hence lot of precaution should be done to avoid consequence.

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