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Removal of Hexavalent Chromium by a Consortium of Bacteria Isolated from Domestic Sewage

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Abstract: A consortium of sulphate reducing bacteria (CSRB) isolated from domestic sewage which can tolerate hexavalent chromium concentrations upto 2000 ppm. This consortium consists of a range of gram positive (spore and non spore forming) and gram negative bacteria. The reduction of Cr^{+6} to Cr^{+3} was observed by change in color from orange yellow to grayish black and settling of amorphous precipitate associated with the bacterial cell surfaces on the bottom of the culture vessels. The growth of 'CSRB' was affected by the concentration of Cr^{+6} in the medium. The reduction in growth of 'CSRB' was increased with the increase in the Cr^{+6}. At 2000 ppm Cr^{+6} the reduction in growth was 86.78% after 48 hours. The rate of reduction was dependent on the initial concentration of Cr^{+6} in the medium. 99.68 to 81.89% Cr^{+6} can be removed from concentrations ranging from 50 to 2000 ppm. Above 1000 ppm Cr^{+6} concentration there was a sharp fall in the reduction percent. The rate of Cr^{+6} reduction also depends upon the bacterial cell density. Zn^{+2} supported while Ag^{+} inhibited the reduction of Cr^{+6} when added to the culture medium.

Key words: Sulphate reducing bacteria, consortium, reduction, hexavalent chromium

Introduction

The industrialization is one of the major cause of water pollution. The industries like paper, textile, insecticide, electroplating and tanneries throwing their toxic wastes to the main water channels which is creating problems for aquatic as well as terrestrial life. Waste water containing hexavalent chromium (Cr^{+6}) is generated in many industrial processes including chrome leather tanning, electroplating, wood preservation, alloy preparation, printing and dying mills. The solubility, environmental mobility and toxicity of chromium depends upon its oxidation state. At physiological environmental pH levels, Cr^{+6} compounds are more soluble, more environmentally mobile and more toxic than trivalent chromium (Cr^{+3}). Cr^{+6} form divalent anions, chromate (CrO_4^{-2}) and dichromate ($Cr_2O_7^{-2}$) in waters.

Cr^{+6} is a strong oxidizing agent. As a result of chemical redox reactions, Cr^{+6} is reduced to Cr^{+3}, which readily forms insoluble chromium hydroxide at a neutral pH. In biological system Cr^{+6} passes easily through the cellular membrane and then is reduced to Cr^{+3} in the mitochondria and nuclei, as well as in the cytoplasm. Though Cr^{+3} is impermeable to biological membrane but the Cr^{+3} generated inside the cell bind stably to protein and interacts with nucleic acid (Ohtake *et al.*, 1990).

The conventional methods for removing dissolved chromium include chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, evaporative, absorption on coal and activated carbon, alum etc. Most of them are not practical, economic and efficient (Beveridge, 1989). The use of microbial bio-mass, as chromium absorbent, has not been as frequently used but offer an inexpensive alternative (Fude and Shigui, 1993). Some microbial species like *Pseudomonas, Aeromonas* and *Enterobacter spp.* (Ishibashi *et al.*, 1990; Wang *et al.*, 1990) have been shown to reduce chromium(VI) enzymatically, the same process can be achieved indirectly by sulphate reducers (Ehrlich, 1990). In the present studies, the ability of a consortium of sulphate reducing bacteria isolated from municipal sewage to reduce Cr^{+6}, were carried out.

Materials and Methods

Isolation of Chromium Resistent Bacterial Consortium: A chromium resistent bacterial consortium was isolated from domestic sewage. It was grown in a modified Fude *et al.* (1994) medium (gm/l) tryptone 15.0, $MgSO_4.7H_2O$ 2.0, Na_2SO_3 0.5, Iron citrate 0.5, Sodium lactate 0.4, $FeSO_4$ and sodium thioglycollate 0.5. The pH was adjusted to 7.5 by 0.1 N NaOH.

Inoculum preparation: For experimental cultures, sterilized 40 mL medium in screw capped culture tubes (50mL volume) was inoculated with 1 mL of the starter culture (optical density at 600 nm [OD_{600}] = 0.42) and incubated at 37°C anaerobically, for 20 hrs. 20% inoculum was used through out the studies, unless otherwise stated.

Reduction of Cr^{+6}: Potassium chromate (K_2CrO_4) was used as a source of Cr^{+6}. The removal of Cr^{+6} was investigated for 100, 200, 500, 1000, 1500 and 2000 ppm Cr^{+6} concentration. These concentrations were prepared by adding calculated amounts of K_2CrO_4 to the growth medium.

The effects of cation on the removal of Cr^{+6} were investigated by adding various amounts of Zn^{+2} as $ZnCl_2$ and Ag^{+} as AgCl.

The mechanism of chromium reduction was tested by adding sodium molybdate (Na_2MoO_4) 20mM final concentration to inoculated and un-inoculated cultures. Molybdate act as an inhibitor of sulphate reduction in microorganisms (Tugel *et al.*, 1986). In this experiment the molybdate stops the active reduction and will discriminate between an active bacterial process and an abiotic process caused by the sulphide in the medium.

Analytical methods: Chromates were determined spectrophotometrically by diphenylcarbazide method (Urone, 1955). Total chromium was assayed by using A.A. spectrophotometer Perkin Elmer 2380 USA.

Results and Discussion

Isolation of sulphate reducing bacterial consortium: The sulphate reducing bacterial consortium was isolated from domestic sewage and designated as 'CSRB'. This 'CSRB' consists of gram negative and gram positive both spore forming, non spore forming, rod shaped bacteria in a ratio of 2:3(2:1). The cell size of gram negative bacteria varies from 1.5 to 3 μm in length to 0.4 to 0.5 μm in width whereas the gram positive bacteria was in the range of 4 to 8 um in length to 1 to 1.5 μm in width. The spore size was in the range of 0.6 to 0.9 μm. The 'CSRB' can grow at pH 6-8 (optimum pH 7.0) and at 30 to 40°C (optimum, 37°C). Fude and Shigui (1993) have isolated a consortium of sulphate reducing bacteria from electroplating sludge and Fude *et al.* (1994) reported the possibility of the utilization of the same for the removal of Cr^{+6} from liquid wastes.

Effect of Cr^{+6} on growth of 'CSRB': The effect of hexavalent chromium on the growth of 'CSRB' was tested under anaerobic conditions. Fig. 1 show that the increase in the concentration of Cr^{+6} retard the growth of 'CSRB'. The reduction in growth at 100, 500, 1000, 1500 and 2000 ppm Cr^{+6} level was 32.5, 65.14, 68.85, 84.57 and 86.78%, respectively as compared to control (medium without Cr^{+6}). The 'CSRB' can tolerate Cr^{+6} upto 2000 ppm. The sulphate reducing bacterial consortium isolated by Fude and Shigui (1993) can tolerate Cr^{+6} upto 2500 ppm. Shimada and Matsushima (1988) have isolated a strain of *Pseudomonas sp.* which can grow in presence of 1000 ppm K_2CrO_4 whereas, the strain of Wang *et al.* (1989) can tolerate 10mM chromate. Cr^{+6} resistance in different strains have also been reported by various worker such as Horitsu *et al.* (1983) in *Pseudomonas ambigua*, Serpokrylov *et al.* (1985) in *Aeromonas dechromaticans*, Efftathiou *et al.* (1977) in *Streptococcus lactis*, Wang *et al.* (1989) in *Enterobacter cloaceae* and Fude *et al.* (1991) in *Desulfovibrio sp.* etc.

Reduction of Cr^{+6}: The reduction of Cr^{+6} by the medium presented in Fig. 2 reveal that media had reduced the Cr^{+6} upto 400 ppm concentration. The reduction was high in lower concentrations which was upto 35.69% at 50 ppm. Above 400 ppm Cr^{+6} concentration no reduction by the medium was observed. Fig. 3 show that the reduction of Cr^{+6} in presence of bacteria was dependent on the initial concentration of Cr^{+6} in the medium, especially above 200 ppm. The time required for maximum reduction increase in proportion to the amount of Cr^{+6} added. When the concentration of Cr^{+6} exceeds from 1000 ppm, there was a sharp increase in reduction. The 'CSRB' reduced 99% Cr^{+6} at 50 ppm in 120 hrs whereas the same reduction percentage was achieved in 240 hrs at 500 ppm concentration. At 1000 ppm concentration 98.5, at 1500 ppm 90.17 and at 2000 ppm 81.89% reduction was achieved in 312 hrs. The change in the color of the medium from orange yellow to grayish black and white precipitation was also observed in the experiments. It is also observed that when the culture inoculated in a medium containing sodium molybdate (Na_2MoO_4) no production of H_2S and reduction of Cr^{+6} taken place.

The microbiological removal of Cr^{+6} from the liquid waste involves the production of H_2S in the medium. The H_2S produced by the bacteria, act as a reducing agent for the Cr^{+6}. This phenomenon has been observed by Smillie *et al.* (1981) in marine environment. Wang *et al.* (1989; 1990) have also reported the reduction of Cr^{+6} to Cr^{+3} by bacterial metabolic product 'H_2S'. They observed that H_2S reduced Cr^{+6} to Cr^{+3} and form insoluble chromium hydroxides which subsequently precipitates in the medium as reported earlier. In the present studies, in higher concentrations 'CSRB' didn't grow well and started its metabolic activity upto 48 hrs of incubation at 37°C and the amount of Cr^{+6} in the supernatant was not very much different from the control. Because as long as there is no production of H_2S no reduction of Cr^{+6} takes place.

The reduction of Cr^{+6} via H_2S mechanism was confirmed when the cells were grown in presence of molybdate, which act as an inhibitor of sulphate reduction by microorganisms. Because gram +ive and Gram -ive rods predominated during the precipitation of Cr, it is possible that of all the bacteria in 'CSRB' consortium were H_2S producers.

Effects of bacterial cell density on the removal of Cr^{+6}: The rate of reduction of Cr^{+6} also dependent upon the cell density. Higher the cell density greater will be the

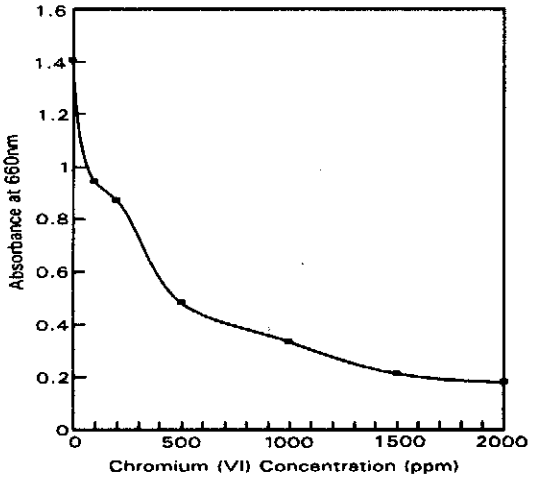

Fig. 1: Growth of 'CSRB' after 48 hrs. in different concentrations of chromium

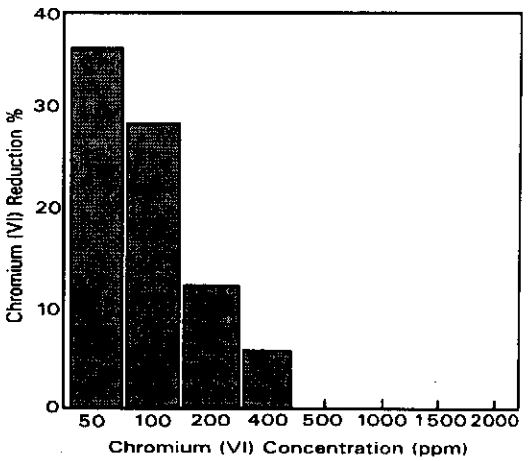

Fig. 2: Reduction of μ chromium (VI) by the medium after 48 hrs.

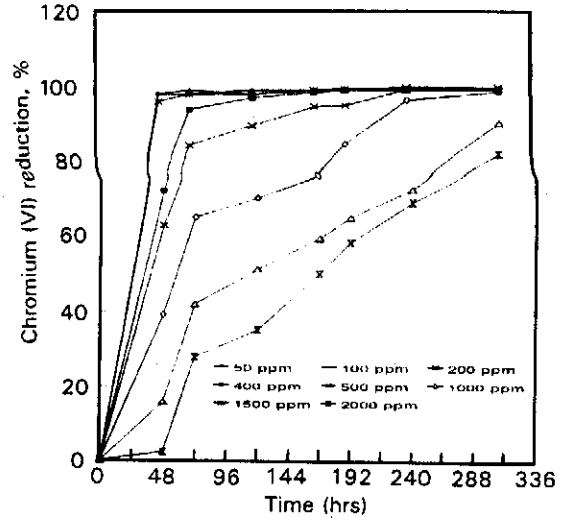

Fig. 3: Course of removal of chromium (VI) by 'CSRB' at different initial concentrations

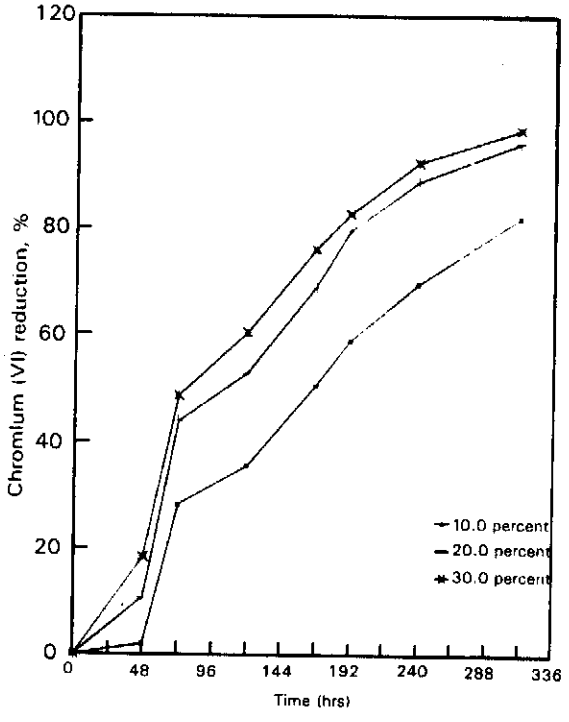

Fig. 4: Effect of inoculum size on the reduction of Chromium (VI)

reduction. Fig. 4 show that an increase in the inoculum size increased the rate of Cr^{+6} reduction. By 30% inoculum size about 20% greater reduction of Cr^{+6} was observed with respect to 10% inoculum. The increase in inoculum size also increased the initial rate of reduction.

The dependance of Cr^{+6} reduction on the cell density has also been reported by Wang *et al.* (1989). They reported that higher the cell density the greater will be the rate of reduction. This may be because of the increase in the

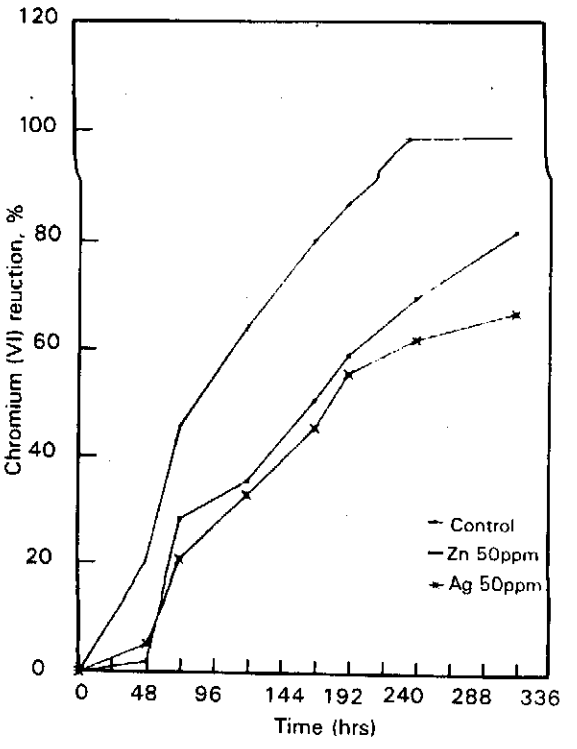

Fig. 5: Effects of cations on the reduction of chromium (VI) by 'CSRB'

bacterial cells in inoculum which in turn increase the rate of H_2S production, so the fast reduction of Cr^{+6} was observed.

Effects of cations on the removal of Cr^{+6}: Fig. 5 show the effect of the addition of Silver and Zinc on the removal of Cr^{+6} by 'CSRB'. The presence of Silver (Ag^{+}) inhibited while zinc (Zn^{+2}) supported the removal of Cr^{+6} from the medium. Zn^{+2} had increased the Cr^{+6} reduction to 98.25 as compared to control 81.89% in 312 hrs. The decline in the reduction by Ag^{+} was 18.50 with respect to control. Our results are in line with Fude and Shigui (1994), who reported that Ag^{+} inhibit the removal of Cr^{+6} while Zn^{+2} enhance the removal of Cr^{+6}. Ishibishi *et al.* (1990) identified Hg^{+} and Ag^{+}as strong inhibitors of chromate reduction.

The efficiency of removal of Cr^{+6} depends upon the characteristics of waste and microbes (Fude *et al.*, 1991; Ohtake *et al.*, 1990). The removal of Cr^{+6} in presence of heavy metals like Zn^{+} show that there is a strong interdependence among the members of 'CSRB' consortium. Because of its heavy metals tolerance and immobilization qualities of 'CSRB' it may be used for the bio-remidiation of heavy metal waters from variety of industries.

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