Australian Journal of Basic and Applied Sciences, 3(2): 1363-1368, 2009
ISSN 1991-8178

Chromate Resistance and Reduction by Bacterial Isolates

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Abstract: Extensive use of hexavalent chromium in various industrial applications has caused substantial environmental contamination. Utilization of Cr (VI) reducing microbial consortium has enhanced the efficiency of the process of detoxification of Cr (VI) to Cr (III). Chromium resistant bacteria such as *Pseudomonas fluorescens* and *Bacillus* sp isolated from heavy metal contaminated soil were examined for their tolerance to hexavalent chromium and their ability to reduce Cr (VI) to Cr (III). The detoxification process in cell suspensions and cell extracts was also studied. The influence of various factors such as pH, time interval and initial metal concentrations on the reduction of chromium by the bacterial isolates were studied. Both the bacterial isolates tolerated Cr (VI) at 100 ppm in minimal salt broth. At 25 ppm *Bacillus* sp and *Pseudomonas fluorescens* recorded maximum accumulation rates of 87.8 % and 93 % respectively. *Bacillus* sp. reduced 40 %, 68%, 81 %, 75 % and 60 % of Cr (VI) to Cr (III) and *Pseudomonas fluorescens* reduced 52%, 58%, 72%, 75% and 61% of Cr (VI) to Cr (III) at different levels of pH such as 5.0, 6.0, 7.0, 8.0 and 9.0 respectively. Maximal Cr (VI) reduction was observed at pH 7.0 to 8.0. The results indicate that the microbial consortia and the mono cultures of the above isolates can be useful for Cr (VI) detoxification of chromium contaminated environment.

Key words: Chromium, *Bacillus* sp., *Pseudomonas fluorescens*, Reduction.

INTRODUCTION

Hexavalent chromium is an important heavy metal widely used in the metallurgies refractory, chemical and tannery industries. Chrome plating, the deposition of metallic Cr, imparts a refractory nature to materials rendering the resistant to microbial attack and flexible over extended periods of time. More than 1,70,000 tons of Cr wastes are discharged to the environment annually as a consequence of industrial and manufacturing activities (Kamaludeen, S.P.B., K.R. Arunkumar, 2003) Several physical and chemical methods exist to remove heavy metals from the environments. However, these methods are reported to be impractical due to the operational high cost and subsequent generation of solid waste which is difficult to treat. Research in recent years indicated that many microorganisms can accumulate large concentration of metals (Ramteke, P.W., 2000). Microbial reduction of hexavalent chromium has practical importance because biological strategies are part of green technology that is cost effective and eco friendly. (Ganguli, A. and A.K. Tripathi, 2002April 4, 2009).

Bioreduction of Cr (VI) can occur directly as a result of microbial metabolism (enzymatic) or indirectly, mediated by bacterial metabolic products (such as H_2S) (Losi, M.E., C. Amrhein, 1994). A number of chromium resistant microorganisms have been reported including *Pseudomonas* spp (Mondaca, M.A., C.L. Gonzalez, 2005), *Microbacterium* (Pattanapipipaisal, P., N.L. Brown, 2001), *Desulfo vibrio* (Michel, C., M. Brugma, 2001), *Enterobacter* sp (Wang, P., T. Mori, 1990), *Escherichia coli* (Shen, H and Y. Wang, 1993), *Bacillus* spp (Campos, J., M. Maartinez-pacheco, 1995) and several other bacterial isolates (Holman, H.Y.N., D.L. Perry, 1999). However most of them have been isolated from tannery sludge, industrial sewage, evaporation ponds or discharge water or were purchased from culture collections. This paper deals with the isolation of Cr (VI) resistant bacteria from sewage sludge and sewage effluent and the influence of physiochemical and cultural factors in hexavalent chromium reduction by the bacterial isolates under monoculture.

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MATERIALS AND METHODS

Chromate resistant bacteria were isolated from sewage effluent and sewage sludge samples. For the isolation and enumeration of bacteria, samples were serially diluted and plated using the standard serial dilution and plate count technique (Jenson, V., 1968) in the nutrient agar for *Bacillus* sp and kings'B for *Pseudomonas fluorescens.* The influence of pH on bacterial growth and chromate reduction was assessed with the nutrient agar medium for *Bacillus* sp and Kings'B medium for *Pseudomonas fluorescens.* For the effect of pH, autoclaved culture medium was adjusted to pH 5.0, 6.0, 7.0, 8.0, and 9.0 with pre determined amounts of filter sterilized (0.22 µm) 1 M HCl or 1 M NaOH and incubated at room temperature. The inoculum used was 5% of the logarithmic phase cultures of bacterial isolates. Growth and Cr (VI) reduction were analysed for 18 hours interval (0, 18, 36, 54 and 72 hours).

The effect of varying concentrations (0, 25, 50, 75 and 100 mg l^{-1}) of Cr (VI) as $K_2Cr_2O_7$ on the tolerance of bacteria was examined and the initial pH was adjusted to 7.0. in duplicate. Growth and Cr (VI) reduction analysis were carried out by similar procedure employed in effect of pH on chromium reduction experiment. The time course of Cr (VI) reduction was evaluated at 2.0 mg l^{-1} concentration and incubated at room temperature with the initial pH of 7.0. The Cr (VI) reduction and growth of the isolates were evaluated at 0, 3, 6, 9, 12 and 24 hours. The bacterial growth rate was determined by measuring the optical density of the cell suspension at 620 nm at 18 hours time interval (0, 18, 36, 54 and 72 hours) using a spectrophotometer (ELICO SL 150 UV visible).

Hexavalent chromium in the culture was analysed by centrifuging the contents at 8000 rpm after the incubation period. The supernatant was used for hexavlent chromium estimation. It was estimated by reacting 1 ml of 1N H_2SO_4 and 0.4 ml of the reagent (4.0 g of pthallic acid and 0.25 g of 1, 5 diphenyl carbazide (DPC) in 100 ml of 95 % ethanol (Barlett, R. and B.R. James, 1979). Percent Cr (VI) removal was calculated as follows,

$$\text{Percent metal removal} = \frac{(X-Y)}{X} \times 100$$

Where,
X = OD before treatment
Y = OD after treatment

RESULTS AND DISCUSSION

Results:

The effect of pH on the chromium reduction by the bacterial isolates (*Bacillus* sp and *Pseudomonas fluorescens)* is presented in fig.1. The growth and Cr (VI) reduction by bacterial systems were maximum at pH 7.0. *Bacillus* sp and *Pseudomonas fluorescens* exhibited varying levels of Cr (VI) reduction as the concentration of Cr (VI) increased from 25 to 100 mg L^{-1}. The bacterial cells accumulated more of Cr (VI) up to 36 hours after that the accumulation became marginal.

Initial pH of the culture medium was considered as a factor for growth and Cr (VI) reduction by the isolates. Both the isolates grew better at pH 7.0. The pH levels of 5.0 and 9.0 inhibited the bacterial growth and Cr (VI) reduction. *Bacillus* sp. removed 40 %, 68%, 81 %, 25 % and 60 % of chromium (VI) and *Pseudomonas fluorescens* removed 52%, 58%, 72%, 75% and 61% chromium (VI) at different levels of pH 5.0, 6.0, 7.0, 8.0 and 9.0, respectively. Both the isolates recorded maximum reduction of chromium (VI) at pH 6.5 to 8.0.

The effect of chromium on the growth of the bacterial isolates is presented in fig.2. The growth of the isolates was slightly inhibited by 100 mg/L of Cr (VI) when compared to lower concentrations (0, 25, 50 and 75 ppm of Cr (VI)). The biosorption potential by *Bacillus* sp. and *Pseudomonas fluorescens* decreased with increasing levels of Chromium. At 25 ppm concentration *Bacillus* sp. and *Pseudomonas fluorescens* recorded maximum accumulation rates of 87.8 and 93 % respectively. The time course of Cr (VI) reduction by the isolates (at 2.0 mg/L of Cr (VI)), *Pseudomonas fluorescens* showed the highest reduction rate, reducing100% within 18 hours of incubation (fig.3). In the case of *Bacillus* sp, it takes 24 hours time for complete reduction.

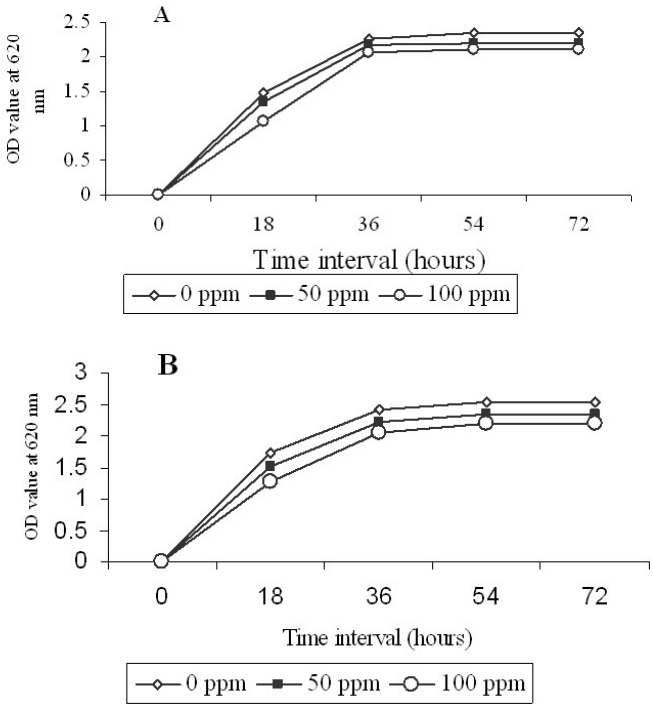

Fig. 1: Effect of Cr (VI) concentration on the growth of *Bacillus* sp (A) and *Pseudomonas fluorescens* (B)

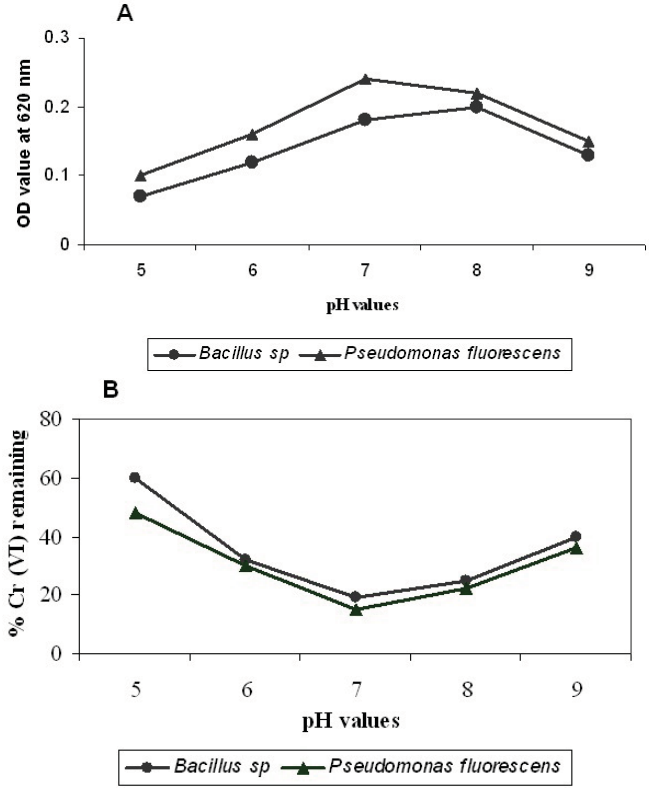

Fig. 2: Effect of pH on growth (A) and Cr (VI) reduction (B) in *Bacillus* sp and *Pseudomonas fluorescens*

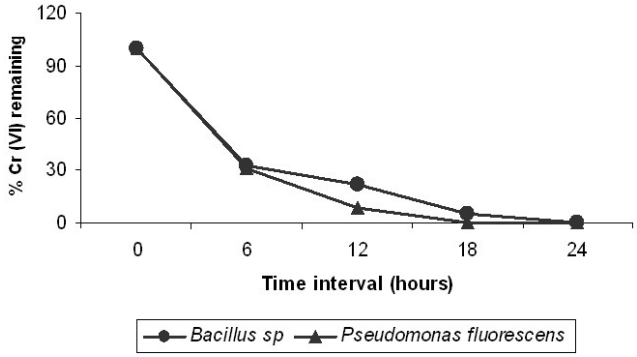

Fig. 3: Effect of incubation time on Cr (VI) reduction by the *Bacillus* sp and *Pseudomonas fluorescens*

Discussion:

Microorganisms with the ability to tolerate and reduce Cr (VI) can be used for detoxification of environments contaminated with Cr (VI). In this study, the isolation and screening of Cr (VI) resistant bacterial isolates and characterization of Cr (VI) reduction by the selected isolates have been attempted (Table 1& 2). Most of the isolates were able to reduce chromium at different rates.

Table 1: Identification of the isolated bacterial strains

Sl.No	Shape	Gram staining	Spore	Motility	Oxidase	Methyl red	Name of the organism
1.	Rods	+	-	+	+	+	*Bacillus* sp
2.	Rods	-	-	-	+	+	*Pseudomonas*

Table 2: Physico-chemical characteristics of the soil

Sl.no	pH	EC(dSm^{-1})	Organic carbon (%)	Pore space (%)	Bulk density g/cc	Particle density g/cc	Total Cr ($Mg\ kg^{-1}$)
1.	6.67	0.89	4.62	16.33	0.75	1.96	90.07

The bacterial isolates tolerated over a wide range (0 to 100 mg L^{-1}) of Cr (VI) concentration. The Cr (VI) resistance above 2500 mg L^{-1} has only been reported by Shakoori *et al.*(2000) and they isolated a gram positive bacterial strain (probably a *Bacillus* species) from a tannery effluent that grew in media containing potassium dichromate up to 80 mg L^{-1}. Most Cr (VI) resistant microorganisms are able to tolerate up to 1500 mg L^{-1} of Cr (VI) (McLean, J. and T.J. Beveridge, 2001).

The growth of the isolates and Cr (VI) reduction were dependent on pH and the initial Cr concentration. The optimum pH for the growth of the isolates was 7.0 to 8.0 and extreme pH (5.0 and 9.0) restricted the bacterial growth and Cr (VI) reduction. Irrespective of the pH, while increasing the incubation period and Cr (VI) concentration the mean Cr (VI) reduction also found to be increased. These results suggest that the extent of Cr (VI) reduction is dependent on the availability of electron donors and not on the loss of Cr (VI) reduction capacity of the organism due to mutagenic effects (Wang and Shen, 1997; Branco *et al*., 2004).

The relationship between pH and Cr (VI) concentration was not surprising because chromate (CrO_4^{2-}) is the dominant Cr (VI) species in an aqueous environment at pH 6.5 to 9.0 (15). Optimal pH for growth of Cr (VI) resistant bacteria was reported at 7.0 to 7.8, but Cr (VI) forms are soluble over a wide pH range and generally mobile in soil water systems (4). However since Cr (VI) reduction is enzyme mediated, changes in pH will affect the degree of ionization of the enzyme, changing the protein's conformation and affecting the enzyme activity (Camargo, F.A.O., F.M. Bento, 2003).

The Cr (VI) concentration affected the growth and Cr (VI) reduction *Pseudomonas fluorescens* showed the highest rate of Cr (VI) resistance and reduction when compared to *Bacillus* sp. The time for total reduction of Cr (VI) increased with increasing concentration of Cr (VI). The Cr (VI) reduction ability of the bacteria was growth dependent and *Pseudomonas fluorescens* was a more efficient Cr reducer than *Bacillus* sp.

Reduction of the transition metal chromium as chromate by bacteria under aerobic conditions is a common phenomenon. Trimble and Ehrlich (1968) observed aerobic reduction of the transition metal manganese as MnO_2 with glucose by *Bacillus* sp and by Coccus strain. Troshanpv (1969) reported that some of this bacterial isolates could reduce the trasition metal iron as Fe (III) aerobically, although he found that bacterial iron reduction was frequently more sensitive to oxygen than Mn (IV) reduction.

Evidently high chromate concentrations prevent multiplication of the strains but do not prevent them from reducing the chromate. The chromate resistances in these isolates are not related to its ability to reduce chromate, more over since earlier work (Bopp, L.H., A.M. Chajrabarty, 1983; Bopp, L.H. and H.L. Ehrlich, 1988) showed that the determinant for chromate resistance resides on a plasmid, the chromate reduction traits must reside on the chromosome in *Pseudomonas fluorescens*. The reduction by these strains appears to be enzymatic, since it is catalyzed by cell extracts.

Enzymatic chromate reduction by these organisms appears to be a form of respiration in intact cells and may have beneficial environmental effects. The product of chromate reduction is Cr (III), which is several orders of magnitude less toxic than Cr (VI) (Mearns, A.J., P.S. Oshida, 1976). Further more, Cr (III) forms insoluble hydroxides at neutral pH and precipitates, thus making it less available to biological systems. Although the results described above have began to clarify the interactions of some bacteria with chromate, more research is required before all mechanisms of chromate resistance and chromate reduction are fully understood.

Conclusion:

The hydrogen ion and Cr (VI) concentration are important environmental factors regulating remediation strategies for ecosystems polluted with natural or anthropogenic Cr (VI). The native isolates will be highly effective in Cr (VI) bioremediation, which is potentially more cost effective than traditional physical or chemical methods in the treatment of environment contaminated with Cr (VI). The present study reveals the capacity of bacterial cells to bind metallic ions in an ecofriendly manner and it is clear that the bacterial cell may act as nucleation sites for the removal of metals from the environment.

ACKNOWLEDGEMENT

The authors are thankful to the Ministry of science and technology, Department of biotechnology, New Delhi for the financial support to carry out this research work.

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