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Bioreduction of Hexavalent Chromium in a Non-nutritive Medium by Chromium Resistant Bacterial Isolates

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Reduction of toxic hexavalent chromium was studied by using four chromium resistant lyophilized strains of bacterial isolates of Sukinda mining area viz., *Micrococcus luteus, Pseudomonas putida, Serratia marcescens* and *Acinetobacter calcoaceticus.* The isolates tolerated hexavalent chromium beyond 500ppm and were selected for reduction. Amongst the four isolates a gram negative strain of *Acinetobacter calcoaceticus* showed highest amount of hexavalent chromium reduction of 38.1% when inoculated in a non-nutritive medium of Phosphate Buffer Saline(PBS) solution with 100 ppm Cr(VI) at 30°C for 24 hours at pH 7.0. *Acinetobacter calcoaceticus* was selected for parametric studies and was observed to exhibit highest reduction potential of 47.1% at pH 8.0, temperature 30°C/ 24 hours. Therefore, it is concluded that *Acinetobacter calcoaceticus* may be used in the bioremediation of hexavalent chromium toxicity.

Key words: Bioremediation, Hexavalent chromium, Non-nutritive medium, Chromium toxicity.

The rapid industrial explosion has resulted in introduction of new toxic chemicals into the environment resulting in deterioration of the environmental quality. The Sukinda mining area of Orissa's Jajpur district accounting for 97% of India's chromite ore deposits is among the ten most polluted places in the world [1]. Several decades of intensive mining has resulted in extensive hexavalent chromium pollution in soil and water in and around the area. The environment is sensitive to metals due to their longevity and toxicity [2]. Trivalent chromium, Cr (III) is an essential micronutrient for humans and is relatively less soluble [3] than the hexavalent chromium whereas the hexavalent chromium is toxic [4] , mutagenic [5] and carcinogenic [6] . A common water pollutant, the Cr(VI) form is toxic to most organisms because of its strong oxidizing properties. It is the water soluble, bioleachable form that can intracellularly reduced to Cr(V) and reacts with nucleic acids and other cell components to produce mutagenic and carcinogenic effects on biological systems [7] .Environmental clean-up strategies for Cr (VI) removal involve physicochemical or biological detoxification. Major limitations of physicochemical processes are the high-energy

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inputs, different chemical treatments and generation of unnecessary sludge, reactive chemical species as secondary wastes. These problems can be overcome by biological Cr(VI) detoxification, which is ecofriendly and an economically feasible technology [8,9,10] . In the present experiment, attempt has been made to reduce by four chromium resistant strains in a non-nutritive medium followed by optimization of conditions for maximum reduction of hexavalent chromium in order to evaluate the strains potential as an effective tool for bioremediation of hexavalent chromium pollution.

MATERIAL AND METHODS

Culture collection

The lyophilized cultures of four bacterial isolates viz. *Micrococcus luteus, Pseudomonas putida, Serratia marcescens* and *Acinetobacter calcoaceticus* were selected for hexavalent chromium reduction study. These bacteria were isolated from Sukinda mining area of Jajpur district, Orissa. The cultures were revived by inoculation into Luria Bertani (LB) broth followed by incubation at temperature 30°C for 24 hours .The cultures were streaked onto Luria Bertani agar (LA) plates, which were further incubated for 24 hours. Luria Bertani broth and agar used for bacterial culture revival and chromium reduction studies were procured from HiMedia Laboratories, Mumbai.

Estimation of heavy metal and pH tolerance

For estimation of chromium tolerance molten LA medium was supplemented with Cr(VI) of final concentration ranging from 100-1100mg/l by using filter (0.22 µm) sterilized $K_2Cr_2O_7$ solution. The isolates were later streaked onto the LA plates and incubated at 30°C for 24 hours and the resistance pattern or minimum inhibitory concentration (MIC) was noted down. The pH tolerance test was conducted to study the cardinal pH of the chromium resistant bacterial isolates. Five milliliter of the medium was taken in different test tubes and the pH was adjusted from 1-14 with help of 1N HCL, 1N NaOH. 100µl of the overnight culture (LB) was dispensed in to the test tubes and incubated at temperature 30°C for 24hours. A loopful of overnight culture was subcultured on to LA plates and all the plates were incubated at temperature 30°C for 24 hours. Then the cardinal pH of all the four isolates was determined from the observation.

Aerobic hexavalent chromium reduction

In order to observe chromium reduction in a non-nutritive medium [11] like Phosphate buffer solution (pH 7.0), first the selected bacterial isolates were inoculated into LB with 100ppm of Cr (VI) and incubated at 30°C for 24 hours/ 100rpm in Incubator shaker (STM-225-IS). Cells were collected after centrifugation (Remi Compufuge) at 10,000rpm for 10 minutes. The collected cells were washed twice with PBS and resuspended in 50 ml PBS with 100ppm of Cr(VI). The reduction was observed at hourly intervals upto 6 hours and finally after 24 hours. Cr (VI) reduction was determined by measuring absorbance at 540nm using a spectrophotometer (Systronic 104). The isolate capable of highest reduction was selected for further studies.

Optimization of pH and temperature on chromium reduction

The influences of pH and temperature on highest hexavalent chromium reducing bacterial isolate was assessed with PBS medium and culture condition described for chromium reduction. For the effect of pH, autoclaved non-nutritive suspension medium was adjusted to pH 7 and 8 with 1N HCL or1N NaOH and incubated at 30°C. Later keeping the optimum pH for reduction constant temperature was varied viz., 20°C, 30°C and 37°C.

RESULTS AND DISCUSSION

Heavy metal and pH tolerance profile

It was observed that all the four isolates i.e *M. luteus*, *P. putida*, *S. marcescens* and *A. calcoaceticus* could tolerate chromium beyond 500ppm. Further *S. marcescens* and *A. calcoaceticus* could tolerate up to 1000mg/l of Cr(VI) . Most Cr(VI) resistant microorganisms are tolerant up to 10-1500 mg/l of Cr(VI) [7,12,13,14] . Chromium (VI) bacterial resistance above 2500 mg/l has been reported [15] in a Gram positive rod from a tannery effluent. Tolerance to such high levels of hexavalent chromium is due to microbial populations in metal polluted environment, contain microorganisms that have adapted to the toxic concentrations of heavy metals and become "metal resistant" [16] .

The pH tolerance profile reveals that the optimum pH of all the selected isolates ranges between 7-9. All isolates except *Pseudomonas putida* could tolerate a pH range of 4-12. Alkaline pH favours the growth of most of the isolates than acidic pH. It has also been observed [11] that isolates more tolerant to Cr(VI) grew better at pH 7-9.The reason may be attributed to adaptation of the isolates to their mostly alkaline natural habitat

Aerobic hexavalent chromium reduction

The results of the hourly hexavalent chromium reduction by the selected bacterial isolates in PBS (Fig. 1) indicates that rate of reduction is directly proportional to time. *A. calcoaceticus* reduced 20-28.5% of Cr (VI) within 3 hours, after that the rate was almost constant i.e ranged between 34.03% to 38%. This difference in trend of reduction in a non-nutritive medium may be due to decrease in physiological and metabolic activities of the isolates [17,11] and viability after some time and possible inhibition of biomass activity by prolonged chromate toxicity in a non-nutritive medium.

Table 1. Profile of hexavalent chromium resistant bacterial isolates

Isolate	Bacteria	Source	Gram's reaction	Optimum pH
VAB10	*Acinetobacter* calcoaceticus	COB plant (OMC Ltd.) waste water	Negative	7-10
VAB9	*Serratia marcescens*	COB plant (OMC Ltd.) Slurry	Negative	7-9
VAB8	*Pseudmonas putida*	*Dumsala canal sediment*	Negative	7-9
VAB6	*Micrococcus luteus*	COB plant (OMC Ltd.)soil	positive	7-8

Optimization of pH on chromium reduction

An hourly chromium reduction study in PBS presented in Fig. 2 indicates that, *A. calcoaceticus* reduced 47.1 % hexavalent chromium at pH 8. The trend increases with increase in time i.e up to twenty fourth hour. As Cr(VI) reduction is enzyme mediated changes in pH will affect the degree of ionization of the enzyme changing the protein conformation and affecting the enzyme activity[18]. Higher reduction by *A. calcoaceticus* was observed at pH 8. However percentage of reduction by *A. calcoaceticus* reduced after the fourth hour and was almost stable after that i.e 45.3% to 47.1%. This may be due to lack of nutrients in the medium leading to a decrease in cell viability with increase in time.

Optimization of temperature on chromium reduction

The hexavalent chromium reduction profile was monitored at different temperatures

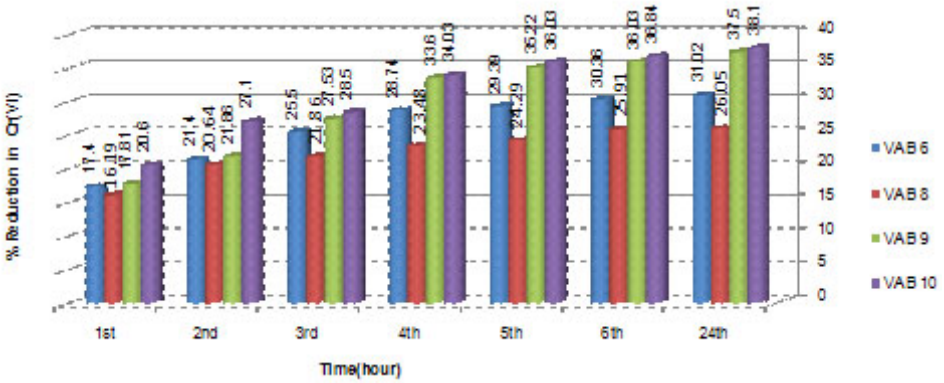

Fig. 1. Hexavalent chromium reduction (hourly) of bacterial isolates in PBS

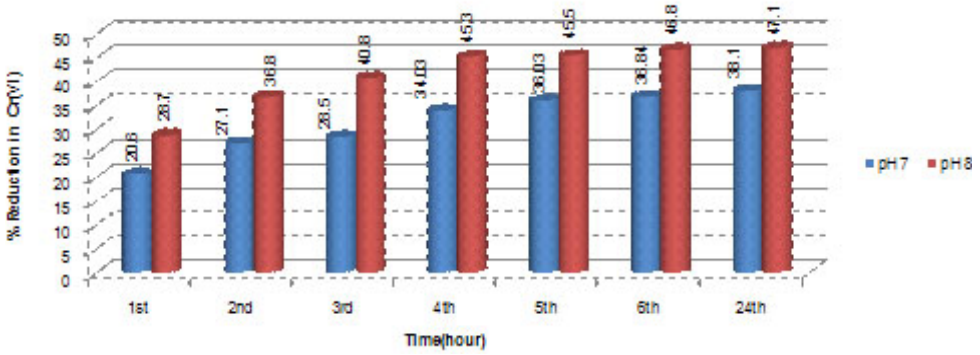

Fig. 2. Hexavalent chromium reduction by *A . calcoaceticus* in PBS at pH 7 and 8

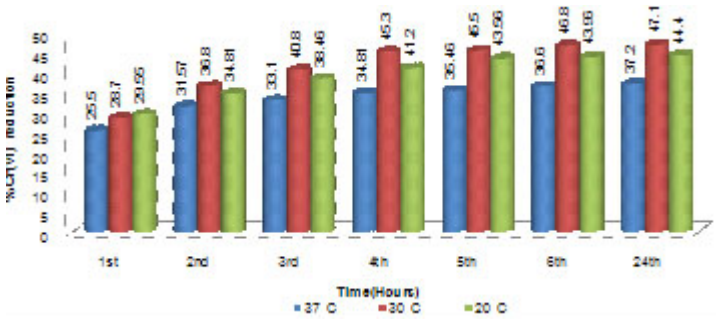

Fig. 3. Hexavalent chromium reduction by *A. calcoaceticus* in PBS at different temperatures

ranging from 20°C-37°C in PBS for 24 hours keeping constant the pH at 8.0. Highest reduction (47.1 %) was observed (Fig. 3) at optimum temperature of 30°C and percentage of reduction decreased with increase in temperature. This is possibly because of decreased enzyme activity with increase in temperature. This has been attributed [2] to loss of viability or metabolic activity of the cells on prolonged incubation at higher temperature .Similar results were also obtained earlier by researchers who reported [11,17] an optimum temperature of 30°C-37°C for chromate reduction .It has also been reported[19] that no chromate reduction was observed at 4°C and 60°C.Temperature is an important selection factor for bacterial growth and affects enzymatic reactions necessary for chromate reduction. The percentage of reduction by *A. calcoaceticus* was observed to decrease after the fourth hour(45.3%) and was almost stable till 24th hour (47.1%).This may be due to lack of nutrients in the medium leading to a decrease in cell viability with increase in time.

CONCLUSIONS

The results demonstrate that the locally isolated strains are tolerant to high Cr(VI) concentrations and has the ability for hexavalent chromium reduction. Especially the gram negative strain of *Acinetobacter calcoaceticus* showed high efficiency in reducing hexavalent chromium even in a non-nutritive medium. Selection of the native flora for bioremediation purposes in the chromium contaminated sites area be advantageous, because it will minimize inhibitory effects from other components that present along with Chromium, as the isolates will have developed some degree of resistance to these components. However, before large scale implementation of the strain for bioremediation its molecular characteristics, pathogenicity and chromate reduction ability in presence of various other metals generally found in the area needs to be assessed in a laboratory scale.

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REFERENCES

1. Blacksmith Institute Report.The world's worst polluted places. A project of Blacksmith Institute. 2006; 16-17.
2. Aravindhan,R.,Sreeram, K.J.,Rao,J.R. and Nair,B.U. Biological removal of carcinogenic chromium(VI) using mixed *Pseudomonas* strains. *J.Gen. Appl. Microbiol.,* 2007; **53**(2): 71-79.
3. Bahijri, S. M. A. and Mufti., A. M. B. Beneficial effects of chromium in people with type 2 diabetes and urinary chromium response to glucose load as a possible indicator of Status. *Biological Trace Element Res.,* 2002; **85:** 97-110.
4. Lock, K., and Janssen., C. R. Ecotoxicity of chromium (III) to *Eisenia fetida, Enchytraeus albidus* and *Folsomia candida*. *Ecotoxical. Environ. Safety.,* 2002; **51**: 203-205.
5. Feng, Z. W. H., Rom, W. N. , Costa, M. and Tang, M. S. Chromium (VI) exposure enhances polycyclic aromatic hydrocarbon-DNA binding at the p53 gene in human lung cells. *Carcinogenesis,* 2004; **24**: 771-778.
6. Park, R.M., Bena, J.M., Stayner, L.T. et al. Hexavalent chromium and lung cancer in the chromate industry: A quantitative risk assessment. *Risk Analysis,* 2004; **24**: 1099-1108.
7. McLean, J. and Beveridge, T. J. Chromate Reduction by a Pseudomonad Isolated from a Site Contaminated with Chromated Copper Arsenate, *Appl. Environ. Microbiol.,* 2001; **67**(3): 1076-1084.
8. Kamaludeen, S. P., Arunkumar, K. R., Avudainayagam, S., Ramasamy, K. Bioremediation of chromium contaminated environments. *Indian J. Exp. Biol*., 2003; **41**: 972-985.
9. Camargo, F. A. O., Okeke, B. C., Bento, F. M., and Frankenberger, W. T. Hexavalent chromium reduction by immobilized cells and the cell-free extract of *Bacillus* sp. ES 29. *Bioremed. J*., 2004; **8**(1-2): 23-30.
10. Pal, A., Dutta, S. and Paul, A.K. Reduction of hexavalent chromium by cell-free extract of *Bacillus sphaericus* and 303 isolated from serpentine soil. *Curr. Microbiol*., 2005; **51**: 327-330.
11. Camargo,F.A.,Bento,F.M.,Okeke,B.C and Frankenberger.,W.T.Chromate reduction by chromium-resistant bacteria isolated from soils contaminated with dichromate. *J. Environ. Qual.,* 2003; **32**: 1228-1233.
12. Basu, M., Bhattacharya, S. and Paul,A.K. Isolation and characterization of chromium-resistant bacteria from tannery effluents.*Bull. Environ. Contam. Toxicol*.,1997; **58**: 535-542.
13. Losi, M.E. and Frankenberger, W.T. Chromium-resistant microorganisms isolated from evaporation ponds of a metal processing plant.*Water Air Soil Pollut*., 1994; **74**: 405-413.
14. Bopp, L.H, Chakrabarty, A.M. and Ehrlich, H.L.Chromate resistance plasmid in *Pseudomonas fluorescens. J Bacteriol*, 1983; **155**(3): 1105-1109.
15. Shakoori, A.R., Makhdoom, M. and Haq, R.U. Hexavalent chromium reduction by a dichromate-resistant gram-positive bacte-rium isolate from effluents of tanneries. *Appl. Microbiol. Biotechnol.,* 2000; **53**: 348-351.
16. Kasan,H.C. and Baecker ,A.A.W.,Activated sludge treatment of coal gasification effluent in a petro-chemical plant-II.Metal accumulation by heterotrophic bacteria.*Wat. Sci. Tech*.,1989; **21**(4/5): 297-303.
17. Losi, M.E., Amrhein, C. and Frankenberger,W.T. Environmental biochemistry of chromium. *Rev. Environ. Contam. Toxicol*.,1994a; **36**: 91-121.
18. Farrell, S.O., and Ranallo,R.T. Experiments in biochemistry.A hands-on approach. Saunders College Publ., Orlando, FL.2000
19. Wang, P., Mori, T., Toda, K. and Ohtake ,H. Membrane-associated chromate reductase activity from *Enterobacter cloacae*. *J. Bacteriol*., 1990; **172**:1670–1672.