

A Preliminary Study on Biosorption of Chromium by Rumen Consortia



Environment

KEYWORDS : Chromium, Diphenyl carbazide, Heavy metal, Atomic absorption spectrophotometer

Meignanalakshmi S

Department of Biotechnology, School of Bioengineering, SRM University, Kattankulathur-India, 603 203

Deepika J

Department of Biotechnology, School of Bioengineering, SRM University, Kattankulathur-India, 603 203

Vinoth Kumar S

Department of Biotechnology, School of Bioengineering, SRM University, Kattankulathur-India, 603 203

ABSTRACT

The present study has focused on the ability of rumen consortium to absorb the heavy metals. It is well known that the chronic inhalation of hexavalent chromium compounds increases risk of lung cancer. There has been an increase in chromium pollution in surface waters because of Electroplating, leather tanning, Coal and oil combustion and textile industries. Hence, a new method of using rumen fluid collected from slaughter house to treat chromium polluted area has been tried out in this study. The rumen consortium was inoculated in nutrient broth containing metal solutions in the concentration of 50-200ppm. The atomic absorption studies showed that the rumen consortium was able to tolerate a maximum of 150ppm chromium solution and the chromium removal was found to be 12.5% after 48 hrs. The percentage of chromium removal decreased with increasing metal concentration. The absorption was also confirmed by diphenyl carbazide method which was analysed in spectrophotometer at 540nm. From the studies mentioned above, it has been confirmed that the Cr(VI) in the maximum concentration of 150ppm has been absorbed by rumen consortia.

Summary of the paper: Heavy metal pollution has become a big environmental threat which slowly destroys the ecosystem in many ways either in water bodies or land. The removal of heavy metals using rumen consortia, slaughter house waste has been tried out in this study. The standard chromium solution which was prepared in the concentration of 100ppm was used for the experiments. To summarise, rumen fluid has the capacity to remove chromium solution in a maximum concentration of 150ppm. Hence this study will surely provide us a path to treat the effluents containing heavy metals in a much more effective manner.

1. Introduction:

The developing industrialization and the new agricultural practices carried over throughout the world have affected the environment. These practices leave toxic heavy metals like chromium, lead, zinc, cadmium and copper which tend to accumulate and destroy the ecosystem (Abbas et al, 2010). Chromium is the second most common inorganic contaminant of groundwater at hazardous sites (Horton et al, 2006). The conventional methods for heavy metal removal from effluents are precipitation, electrochemical, Ion exchange, reverse osmosis, coagulation and membrane separation (Agarwal et al, 2006). These processes are expensive and time-consuming so there has been a thirst for an alternative biological method which would be an effective replacement for the current methods.

Biosorption is a process where certain types of biomass bind to heavy metals from aqueous solution (Naja and Volesky, 2006). Bioremediation uses biological agents mainly microbes like yeast, fungi or bacteria to cleanup contaminated soil and water (Strong and Burgess, 2008). Since numerous types of pollutants are to be encountered in a contaminated site, diverse types of microorganisms are likely to be used for bioremediation (Watanabe et al, 2001). The permissible limits of chromium according to the Indian Standard (Baral, 2006) is 0.05 and 0.1 mg/l for potable and industrial discharge water respectively.

Biosorption studies have been carried out by several authors in various aspects. Kang et al (2006) applied a biofilm of *Pseudomonas aeruginosa* to remove Cr(III), Ni(II) and Co(II). Leonhauser et al studied the behavior of a biofilm of *Aeromonas hydrophila* and *Pseudomonas putida* in mercury removal. Ashok Kumar et al (2010) has used four cultures namely *Bacillus* sp, *Pseudomonas* sp, *Staphylococcus* sp and *Aspergillus niger* in a batch culture for the reduction of heavy metals. Among that, *Pseudomonas* sp reduced heavy metals more than other microbes.

In India, slaughterhouse waste has been increased due to the meat consumption and other commercial purposes. Apart from blood, skin, hair the digestive contents has become a large pollutant. In this study, we have focused on treating the heavy metal solution (Chromium) by using rumen fluid collected from

slaughter house waste. The future study aims in treating the industrial effluents, chromium contaminated soil using the rumen fluid. By this treatment, we can reduce the heavy metal and also use the slaughter house waste efficiently.

2. Materials and Methods:

2.1 Chemicals and solvents:

$K_2Cr_2O_7$, 3M sulphuric acid, Phosphoric acid and Diphenyl carbazide solution (DPCI) 0.5% in acetone.

2.2 Chromium solution preparation and inoculation of rumen fluid:

The Chromium (standard potassium dichromate) were taken in different concentration (50 – 200ppm), added to 100ml of nutrient broth and treated with rumen fluid and the readings were taken in Atomic absorption spectrometer for two successive days. The standard for chromium has been prepared from the stock solution (100ppm) in the range of 1-5ppm for atomic absorption reading.

2.3 Diphenyl carbazide method:

The presence of chromium in the inoculated sample was analysed by the diphenyl carbazide modified method suggested by Chakrabarty and Mishra (1992). 100 μ l of the culture filtrate was taken after centrifuging the sample at 8000rpm for 15 minutes. It was then diluted to 2.5 ml of distilled water. 0.1 ml of the diluted sample was taken in a separate test tube and to this 0.5 ml of phosphoric acid and 0.6 ml of sulphuric acid was added. This solution was made upto 10 ml with distilled water. Finally, 0.2 ml diphenyl carbazide solution was added and incubated for 10 minutes in room temperature. The absorption values were measured at 540nm.

3. Results:

3.1 Atomic absorption analysis of Chromium biosorption by rumen consortia

The percentage of chromium removal observed in different concentrations has been presented in the following Table 1 and Fig 1 & 2. The results indicated that upto 150ppm, the chromium removal was observed. At 150ppm level chromium removal was found to be 12.5%.

Table 1: Percentage of chromium removal after treating it with rumen fluid

S.No	Concentration of Chromium (in ppm)	Chromium removal (in %)	
		24 hrs	48 hrs
1	50	5.5	17.25
2	100	11.5	10.5
3	150	12.5	12.5
4	200	1.5	5.5

**Fig 1: Growth of rumen bacteria in different chromium concentration in nutrient broth**

- 1 a) Control (Chromium - 100ppm)
 b) Nutrient Broth with Chromium (50ppm)
 c) Nutrient Broth with Chromium (100ppm)
 d) Nutrient Broth with Chromium (150ppm)
 e) Nutrient Broth with chromium (200ppm)

**Fig 2: Growth of rumen bacteria in different chromium metal concentration**

- 2 a) Chromium (50ppm)
 2 b) Chromium (100ppm)
 2 c) Chromium (150ppm)
 2 d) Chromium (200ppm)

3.2 Detection of free chromium by Diphenyl carbazide method

The absorbance values taken for the different concentration of chromium treated with rumen fluid has been provided in the table 2.

Table 2: Presence of free chromium level after treatment with rumen fluid by Diphenyl carbazide method

S.No	Absorption of Cr (24hrs)	Absorption of Cr (48hrs)
RF1	0.003	0.001
RF2	0.023	0.002
RF3	0.017	0.005
RF4	0.036	0.015
M1	0.016	0.014
M2	0.031	0.027
M3	0.045	0.026
M4	0.053	0.047

RF1 - Rumen fluid in nutrient broth (Cr - 50ppm) RF2 - Rumen fluid in nutrient broth (Cr - 100ppm) RF3- Rumen fluid in nutrient broth (Cr - 150ppm)

RF4 - Rumen fluid in nutrient broth (Cr - 200ppm)
 M1 - Rumen fluid in Cr solution (50ppm)
 M2 - Rumen fluid in Cr solution (100ppm)
 M3 - Rumen fluid in Cr solution (150ppm)
 M4 - Rumen fluid in Cr solution (200ppm)

Discussion:

The significance of the study is that the absorption of hexavalent chromium has been tried with rumen fluid, an animal waste from slaughter house waste. The reason behind the study is that the grazing animals like goat, sheep (ruminants) consume the plants grown in heavy metal contaminated soil. Hence the digestive enzymes produced by the microbes in rumen environment will help in removing the heavy metals in standard solutions.

Alavi Moghadam et al. 2008 reported a higher chromium adsorption using wheat bran as the absorbent. It was observed at lower pHs, and a maximum chromium removal (87.8 %) was obtained at pH of 2. Adsorption of chromium by wheat bran reached to equilibrium after 60 minutes.

The tolerance data with a high range of heavy metal concentrations revealed the Cr-resistant isolates, especially the fungal one, could tolerate chromium toxicity at up to 10,000 mg L⁻¹ chromium (Shankar Congeevaram et al, 2007).

Biosorption of chromium by *Pseudomonas aeruginosa* (0.07-45.9%) was reported by Hassen et al 1998.

Bacillus spp., *Pseudomonas* spp., *Staphylococcus* spp., and *Aspergillus niger*, isolated from soil when treated with waste sample collected from industry reduced various heavy metals. Among them, *Staphylococcus* sp. reduced Cr 4.108 mg l⁻¹ (45%) and Pb 0.813 mg l⁻¹ (93%) (Ashok kumar et al, 2010).

From the present study, rumen consortia was found to be effective in removing the chromium and it has efficiently (12.5% of 150mg l⁻¹ Cr) proved in the chromium removal from the solution

REFERENCE

- A. Hassen, N. Saidi, M. Cherifh & A. Boudabous (1998). Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. *Bioresource Technology* 65 73-92. | | Abbas, M., Parveen, Z., Iqbal, M., Riazuddin, Iqbal, S., Ahmed, M., Bhutto, R. (2010). Monitoring of toxic metals (cadmium, lead, arsenic and mercury) in vegetables of Sindh, Pakistan. *Kathmandu University Journal of Science, Engineering and Technology*, 6, 60-65. | | Agarwal A, Kumar V, Pandey B.D, (2006) Remediation options for the treatment of electroplating and leather tanning effluent containing chromium - a review, *Mineral Processing and Extractive Metallurgy Review*.27 99-130. | | Alavi Moghadam; Nameni M; Arami M, (2008). Adsorption of hexavalent chromium from aqueous solutions by wheat bran. *International Journal of Environmental Science and Technology*, 5 (2), 161-168. | | Ashok Kumar, Balwant Singh Bisht, Vishnu Datt Joshi. (2010). Biosorption of Heavy Metals by four acclimated microbial species, *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *Aspergillus niger*. *Journal of Biogical and Environmental Sciences*, 4(12), 97-108. | | Chakrabarty AR, Mishra RK. (1992) Speciation and determination of chromium in waters. *Chemical Speciation Bioavailability* 4: 131±134. | | Horton R, Apel W A, Thompson V S, Sheridan P P, (2006). Low temperature reduction of hexavalent chromium by a microbial enrichment consortium and a novel strain of *Arthobacter aureescens*, *BMC Microbiology*.6 1-8. | | Kang S Y, Bremer P J, Kim K W, Mcquillan A J, (2006). Monitoring metal ion binding in single-layer *Pseudomonas aeruginosa* biofilms using ATR-IR spectroscopy, *Langmuir* 22: 286-291. | | Naja G, Volesky B, (2006). Behaviour of the mass transfer zone in a biosorption column, *Environmental Science Technology*. 40 161-173. | | Saroj S. Baral, Surendra N. Das, Pradip Rath (2006). Hexavalent chromium removal from aqueous solution by adsorption on treated sawdust. *Biochemical Engineering Journal*. | | Shankar Congeevaram, Sridevi Dhanarani, Joonhong Park, Michael Dexilin, Kaliannan Thamaraiselvi. (2007). Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *Journal of Hazardous Materials* 146: 270-277. | | Strong PJ and Burgess JE, (2008) Treatment methods for wine-related and distillery wastewaters: a review. *Bioremediation Journal* 12: 70-87. | | Watanabe K, Kodoma Y, Stutsubo K, Harayama S. (2001) Molecular characterization of bacterial populations in petroleum-contaminated ground water discharge from undergoing crude oil storage cavities. *Applied and Environmental Microbiology* 66: 4803-4809.