Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2014, 6 (2):118-124
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Removal of hexavalent chromium from aqueous solution using marine isolates from Vishakhapatnam beach

Prithwijit Sarkar, Shreya Bhagavatula* and Sangeetha Subramanian

Industrial Biotechnology Division, School of Bio-Sciences and technology, VIT University, Vellore

---

ABSTRACT

Study was aimed at removing hexavalent chromium from aqueous solution using a microorganism screened and isolated from a marine water sample. Chromium, a heavy metal which is toxic beyond a certain concentration, needs to be removed from polluted environmental samples. In this study, enrichment media was used to screen for the presence of bacteria that could survive in a chromium environment. They were plated, sub-cultured and streaked to obtain a pure culture. Two isolates were characterized primarily, by staining methods. They were used to for the removal of chromium [VI] from aqueous solution. One of the bacterial isolates showed to significantly decrease the chromium concentration by 61.1%. Hence there was an effective removal of chromium [VI] from the solution using the microorganism isolated from the marine water sample.

Keywords: Hexavalent chromium, marine microorganism, enrichment, removal efficiency.

INTRODUCTION

Increasing levels of pollution demand new and innovative strategies to combat and prevent the rise of contaminants in the environment. The environment faces immense threats due to indiscriminate human activities. Prevention and lowering of pollution levels is necessary in today's world so that the current and future generations can live in a safe environment with minimum health risks [1].

One of these major pollutants is the heavy metal Chromium (Cr [VI]). It is released into the environment from effluents of tannery industries, steel plants, textile and paint industries and galvanization units. Since it is a commercially important heavy metal, it is present in high concentrations in industrial waste water [2]. Inappropriate and improper waste water treatment steps lead to the pollution of soil and water with these harmful effluents. Relatively high concentrations of chromium are toxic to humans, plants and animals. Main diseases associated with chromium toxicity in humans are skin dermatitis, ulceration in the GI tract and can also be carcinogenic to some animals [3, 4].

It is a soil and water pollutant, and strategies to remove this from the waste waters include physical and chemical methods like precipitation, coagulation, ion exchange and absorption [5]. However, studies have shown that the toxic hexavalent chromium does not precipitate readily by the already existing precipitation methods [6]. Chromium removal processes involving activated carbon are efficient but then it is fairly expensive and leads to increase in costs. However, using a biological method would be beneficial as it would be more sustainable and reduce the cost in the removal operations [7]. That is the main objective of this research study. The reduction of chromium

concentrations in aqueous solution by using two different marine microorganisms isolated from the beaches of Vishakhapatnam was studied. Bacteria and fungi are present everywhere in abundance. The availability and ease to work with microorganisms is the key advantage in employing a biological method to remove chromium from polluted environments.

In this study, microorganisms that thrive in environments enriched in hexavalent chromium were isolated by an enrichment technique and batch reactor studies were carried out in shake flasks to check for chromium level reduction.

MATERIALS AND METHODS

Sample Collection and Materials required:-

The marine water sample was collected from the beaches of Visakhapatnam (Figure 1) during the month of September, 2013. It was collected in a sterile plastic bottle and was stored at room temperature. For the screening, isolation and chromium removal assay of the microorganism, nutrient agar, nutrient broth, agar powder, distilled water, potassium dichromate, 6M sulfuric acid, acetone and Di-phenylcarbazide (DPC) were used . Instruments used were autoclave, laminar air flow hood, orbital shaker (set at 120 rpm), UV-visible spectrophotometer and cooling centrifuge.

Figure 1: Marine sample collection

Enrichment technique

To screen for chromium removing bacteria from the marine water, an enrichment media was used. The microorganisms that can thrive on chromium can only grow in such media [8]. Nutrient broth was prepared by adding 1.3g of HiMedia Nutrient broth and 4g of sodium chloride, dissolved in 100ml of distilled water and was then divided into three conical flasks. The first flask was used as a control. To the other two flasks were made to have two different chromium concentrations; potassium dichromate was added such that the second flask had a chromium concentration of 100µg/ml and the third flask had 500µg/ml (Figure 2). They were subject to sterilization at 121°C for 20 minutes, and then they were cooled to room temperature. 5ml of the marine water sample was inoculated into the second and third flasks under sterile conditions in a laminar air flow. After inoculation, the three flasks were kept in an orbital shaker for 48 hours at 37°C. This was used as the inoculums for the screening and isolation of the microorganism.

Figure 2: Enrichment technique

Figure 3: Sub-culture by streaking

Screening and isolation:-

After 48 hours of incubation, the flasks were checked for growth and turbidity. The next step was to isolate the microorganism from the enrichment media. 2.8g of HiMedia Nutrient Agar, 2g of Agar powder and 4g of salt concentration was taken in two conical flasks each, and the volumes were made up to 100ml using distilled water. The chromium concentration was made to 100µg/ml and 500µg/ml in the two conical flasks containing nutrient agar, similar to the broth used in the enrichment step.

12 test tubes were taken and they were filled with 9ml of 4% saline solution. They were used for the serial dilution of the enriched culture that was obtained after 48 hours of incubation. These test tubes, along with the conical flasks were sterilized in the autoclave and after cooling down to room temperature, serial dilution was performed under

sterile conditions. 1ml of the inoculum was added to the first test tube, and then subsequently the serial dilution was done upto 10^{-4} and 10^{-5} dilutions. The nutrient agar plates were allowed to cool and solidify in the laminar air flow. Spread plate technique was done in four petri dishes, one for each dilution of each of the two chromium concentrations. The plates were incubated for 48 hours at 37°C.

Sub-culturing:-
After 48 hours of incubation, colonies were obtained in all four plates. Two distinct colonies were taken from the 10^{-4} and 10^{-5} plates which had 500µg/ml of chromium concentration. Two nutrient agar plates were prepared and these two colonies were subsequently sub-cultured on them. Sub-culturing was done by the method of streaking (Figure 3), so that a pure culture could be obtained. The plates were incubated for 24 hours at 37°C.

Morphological studies and staining:-
The streaks obtained in the two plates after 24 hours of incubation were observed. The texture and morphology of the colonies were observed and noted. For further characterization, Gram's staining and spore staining was done for both the isolated microorganisms. In Gram staining, a smear was prepared on a clean glass slide by air drying and heat fixing it. To the smear, a drop of Crystal violet solution was added and allowed to stand for 60 seconds, and washed with distilled water. A few drops of Gram's Iodine was added and left for 30 seconds, and decoloriser was added and the slide was tilted. Finally, Safranine was added and the slide was allowed to stand for 60 seconds. Similarly, spore staining was done by adding Malachite green on a smear, followed by exposure to steam for 5 to 7 minutes and then finally adding Safranine as the counter stain for 60 seconds. Then the slides were washed, dried and viewed under the microscope under 10X and 40X magnification [9].

Standard graph preparation:-
DPC (Diphenyl Carbazide) method was used to check the removal efficiency of chromium [VI]. For this, the standard DPC method was followed [10, 11]. 250mg of DPC was added in 100 ml of acetone to give a 0.25% DPC solution. Test tubes containing different chromium concentrations ranging from 0.5 to 2.5 mg/l were prepared from a stock solution of 100mg/l chromium concentration. One test tube was used as the blank. Their volumes were made upto 20 ml. In each test tube 330µl of concentrated 6M sulfuric acid and 400µl of 0.25% DPC solution were added. These test tubes were incubated for 10 minutes and the color development was observed. The OD values were taken at 540nm using UV-visible spectrophotometer. A graph for chromium concentration versus the OD values was plotted.

Removal of Chromium [VI] - analysis:-
Nutrient broth was prepared with the addition of potassium chromium and it was sterilized. It was cooled to room temperature and the initial chromium concentration was found out using the DPC method. In two conical flasks, the two different isolates were inoculated. Four colonies of each microorganism were inoculated. These conical flasks were incubated for 24 hours at 37°C. Then the culture was centrifuged at 10,000 rpm for 10 minutes. The pellets of cells were discarded and the supernatants were transferred to separate test tubes. The final concentration of chromium was found out by using the DPC method by reading the absorbance value at 540nm in a UV-visible spectrophotometer. The removal efficiency of chromium [VI] was finally calculated.

RESULTS AND DISCUSSION

Chromium degrading microorganisms were successfully screened and isolated from sea water. Enrichment was done with chromium [VI] concentration in the broth so that only the microorganisms that can survive in an environment rich in chromium can survive and thrive in it. The turbidity seen in the enriched broth indicated that there was a growth of microorganisms that can survive and possibly degrade chromium [VI].

The spread plates also contained the same chromium [VI] concentration as that of the enriched broth, and there were several distinct colonies present in all four spread plates which had different chromium concentrations and different inoculums dilutions. Two of the colonies that were selected and sub-cultured were suspected to aid in the removal of chromium from the aqueous solution.

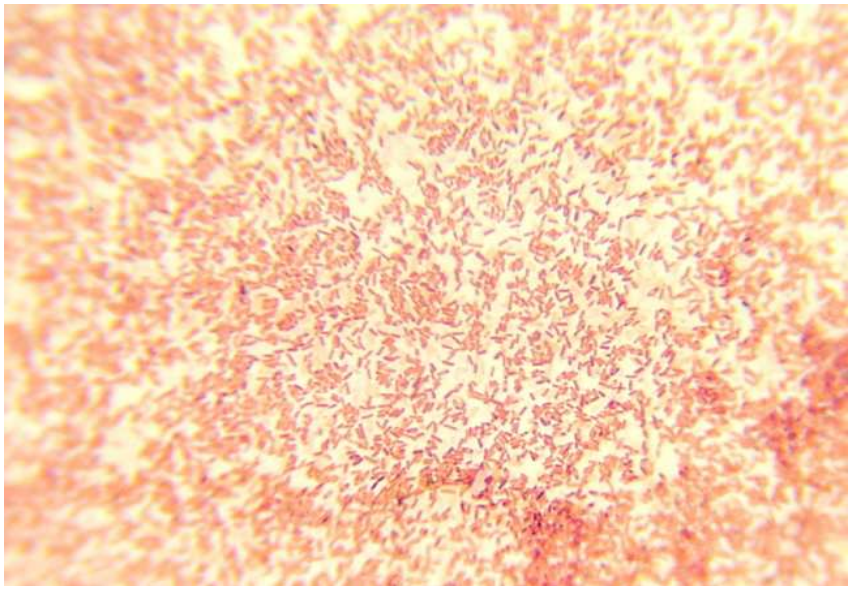

Figure 4a: Isolated Bacterium 1

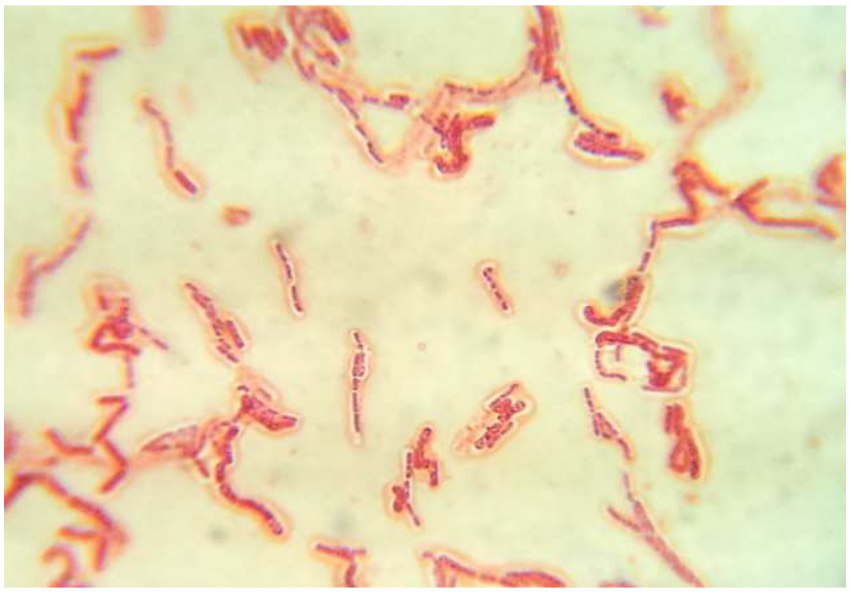

Figure 4b: Isolated Bacterium 2

Staining techniques were performed and it was observed that both the bacteria were Gram negative and non-sporulating. Study of the morphological characteristics was done and it was observed that (Figure 4a and 4b) the first isolate were short rods (Bacterium 1) and the second isolate were long rods arranged in chains (Bacterium 2).

After the standard graph was plotted the micro organism was checked for its efficiency in removal of chromium [VI].

Initial and final OD values were noted. There was a notable decrease in the concentration of chromium with Bacteria 2 (long rods in chains). The Bacterium 1 (short rods) did not show any notable decrease in the level of chromium concentration.

The corresponding chromium concentration observed from the standard graph was seen to reduce from 0.131mg/l to 0.08mg/l after 24 hours of incubation with Bacterium 2. The percentage reduce in chromium concentration can be calculated using the formula.
% reduce in chromium concentration = (initial chromium concentration - final chromium concentration) x100/initial chromium concentration

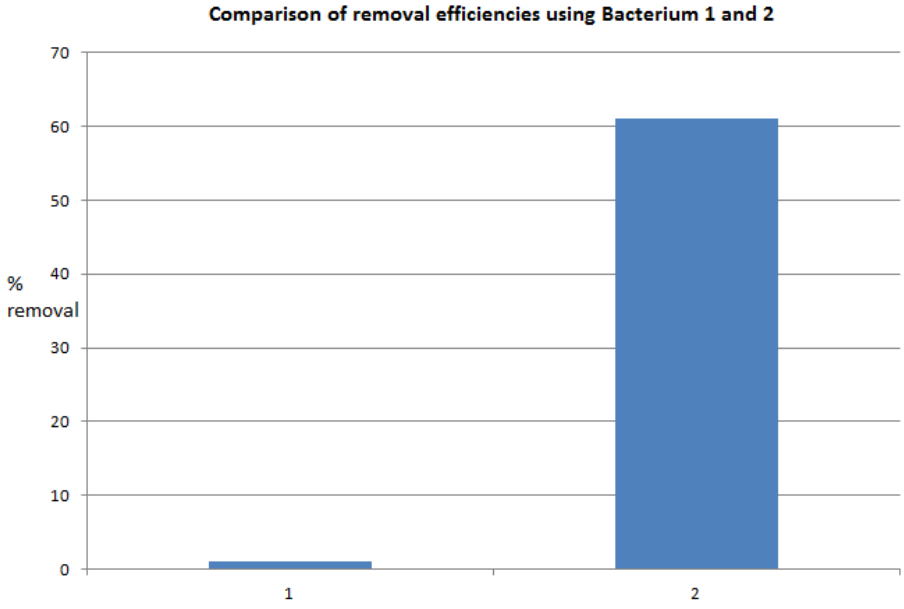

Figure 5: Comparison of removal efficiencies of Bacteria 1 and 2.

There was around **61.1% removal observed using Bacterium 2** and 1.03% removal using Bacterium 1. This shows that the particular marine microorganism Bacterium 2 was efficient in removal of Cr [VI]. (Figure 5)

Cell walls of the marine bacteria are responsible in the adsorption of the toxic heavy metal ions. There are certain bacteria which adsorb the heavy metal ions like *Bacillus megaterium*, *Bacillus licheniformis*, *Nostoc muscorum*, *Pseudomonas sp.* etc [12, 13]. There is a specific electron donor group in the cell wall which reduces the Cr[VI] state to Cr[III] state after the heavy metal ions get adsorbed on the surface of the bacteria [14]. The chemical component responsible for the adsorptions can be peptidoglycan, glycerol, ribitol, lipoprotein and porins [15]. Instances have been reported where microorganisms like *Enterobacter cloaceae* show accumulation of hexavalent chromium along with simultaneous exopolysaccharide production [16]. Apart from bacterial strains, there have been recent studies showing that fungal biomass of *Aspergillus niger* [17] and even the brown seaweed *Sargassum filipendula* [18] have been observed to remove chromium from aqueous solutions.

The presence of hexavalent chromium in wastewater is a potential hazard to aquatic animals and humans. Heavy metal contamination is one of the most noteworthy environmental problems of this century [19 ,20 ,21].There are various mechanisms proposed, kinetic models used and adsorption isotherms employed for the efficient removal of hexavalent chromium from industrial and municipal wastewaters using biosorbents. Metal removal treatment systems using microorganisms are cheap because of the low cost of sorbent materials used and may represent a practical replacement to conventional processes.

CONCLUSION

Chromium as quoted is a very toxic substance to the environment and contributes towards the environmental pollution [22]. Therefore novel and cost effective methods should be employed for its removal. This study involves a marine microorganism to reduce Chromium. The efficiency was found out to be 61.1%. This efficiency proves the

study on removal of chromium was successful. Further study in this area needs to be done to employ these marine microorganisms in large scale bioremediation and environment clean-up process.

Acknowledgements
The authors thank VIT University for the facilities provided to carry out the project.

REFERENCES

[1] P. Sarkar; S. Bhagavatula; K. Ramanathan; V. Shanthi; *J. Chem. Pharm. Res*., **2013**, 5, *8*, 48-53
[2] E. Vaiopoulou; P. Gikas, *Water Research*, **2012**, 46, *3*, 549-570.
[3] A.K. Shanker; C. Cervantes; H. Loza-Tavera; S. Avudainayagam. *Environ. Int.*, **2005**, 31, 739–753.
[4] S. Park; W.Y. Jung. *Carbon Sci*., **2001**, 2, 15–21.
[5] F. Pagnanelli; M. Trifoni; F. Beolchini; A. Esposito; L. Toro; F. Vegliò. *Process Biochem*, **2001**, 37, 115–124.
[6] S Singha; U. Sarkar; S. Mondal; S. Saha. *Science of The Total Environment*, **2013**, 447, 472-487
[7] Y Hou; H. Liu; X. Zhao; J. Qu; J.P. Chen. *Journal of Colloid and Interface Science*, **2012**, 385, *1*, 147-153
[8] D.H. Tambekar; S.S. Gayakwad. *Science Research Reporter*, **2013**, 3, *1*, 87-90
[9] P. Sarkar; S. Bhagavatula; V. Suneetha, *Journal of Applied Pharmaceutical Science*, **2014**, 4, *1*, 61-65
[10] R.T. Pflaum; L.C. Howick. *J. Am. Chem. Soc*., **1956**, 78, *19*, 4862–4866
[11] M. Pettine; S. Capri. *Analytica Chimica Acta*, **2005**, 540, *2*, 239-246
[12] B. Preetha; T. Viruthagiri; *Sep. Purif. Technol*., **2007**, 57, 126.
[13] Z. Aksu; T. Kutsal; S. Gun; N. Haciosmanoglu; M. Gholaminejal. *Environ. Technol*., **1991**, 12, 915.
[14] M.X. Loukidou, T.D. Karapantsios, A.I. Zouboulis, K.A. Matis; *Ind. Eng. Chem. Res*., 2004, 43, 1748.
[15] D. Park, Y. S. Yun, J.M. Park; *Chemosphere*, **2005**, 60, 1356.
[16] A. Iyer; K. Mody, B. Jha. *Marine Pollution Bulletin*, 49, 11–12, December **2004**, 974-977
[17] Y. Khambhaty, K. Mody, S. Basha, B. Jha. *Chemical Engineering Journal*, **2009**, 145, *3*, 489–495
[18] C. Bertagnolli, M.G.C Silva, E. Guibal, *Chemical Engineering Journal*, **2014**, 237, 362-371
[19] B. Volesky; Z.R. Holan; *Biotechnol. Prog*, **1995**,11, 235.
[20] F. Veglio; F. Beolchini; *Hydrometallurgy*, **1997**, 44, 301.
[21] D. Park; Y.S. Yun; K.H. Yim; J.M. Park. *Bioresour. Technol*, **2006**, 97, 1592.
[22] M. Gueye; Y. Richardson; F.T. Kafack; J. Blin. *Journal of Environmental Chemical Engineering*, **2014**, 2, *1*, 273-281