Think India Journal
ISSN: 0971-1260 Vol-22, Special Issue-31
National Conference ETDAB-2019
Held on 23th & 24th December 2019
Organized by: Deptt. of Botany, Deogiri College, Aurangabad, M.S

Screening, Isolation and Detoxification of Cr (VI) by *Bacillus*

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Abstract:

Industrial pollution is generally referred to undesirable outcome when factories emits harmful by-product like metals and waste into environment such as emissions to air or water bodies, deposition on landfills etc or emission of toxic chemicals into atmosphere. The high amount of industrial waste in water cause serious impact on the ecosystem and humans. Chronic health issues are high due to soil contaminated with industrial waste. Harmful gases released in the air has increased in various illness and continue to affect humans on daily basis. So microorganisms can be used to detoxify such metals. In this paper species of *Bacillus* is used to detoxify the hexavalent chromium from the metal containing solution. The 10 bacterial isolates were screened by enriching the soil in the nutrient broth. Later the isolates were exposed to different concentration of Chromium stock from which one isolate showed maximum growth at 100 ppm of Cr (VI). The isolate was identified as *Bacilllus* species and then used for the detoxification of chromium.

Keywords: Hexavalent Chromium, *Bacillus*, bioremediation

Introduction

As the industrialization is growing day by day contamination of metals has become a serious problem. Virtually all metals whether essential or non-essential can exhibit toxicity above a certain threshold concentration. Industries like paint, tannery, electroplating, metal processing industries and mining areasconsist of chromium as its effluent. Discharges from the industry include chromium as one of their effluent can cause a serious damage to the human health and environment. The effluent containing chromium gets deposited in the soil which can make the soil and water contaminated and cause a chronic health issues for the living system. According to the Environment Protection Agency (EPA) concentration of chromium for drinking water which is permissible on basis of health consideration is < 0.1 ppm. As the chromium toxicity depend on its valence state the Cr (VI) is highly toxic then the Cr (III). As the chromium is toxic and no essential to plant so the plant do not posses a specific mechanism for the uptake of this metal for its metabolism. So there is a need for the removal of this heavy metal from the soil and water. Exposure to hexavalent chromium may cause several problems like ulceration of skin, transformation of mammalian cells, respiratory infections, allergic dermatitis(Bock, M., Schmidt, A., Bruckner, T., & Diepgen, T. L.2003).

There are various methods like chemical and physical for the remediation of the chromium. The method includes reverse osmosis, ion exchange and precipitation. As the chemical and physical methods are cost expensive it is not possible to use it on daily basis. The other conventional methods are also used like lime coagulation, solvent extraction, chemical precipitation etc. Bioremediation is a process which includes use of microorganism for the degradation of enivironmental contaminants to its less toxic form. As the biological method is very much cheap and easily available it can be used for the degradation and detoxification of chromium.Microorganisms utilizes the metal for its metabolism resulting it in the reduction of that metal. The accumulation of metal can be done by microbes by various process like intracellular accumulation, extracellular processes, methylation of metalloids etc. Various microorganism have the capacity for the uptake of chromium like *Candida utilis*, *Streptomyces nouresei*, *R.arrhizus (*Bagdwal *et.al*.,2004). In the current study we investigated the use of the *Bacillus*culture for the detoxification of hexavalent chromium from the metal containing solution.

Material and Methods:

A. Materials:

Garden soil, Nutrient broth, Nutrient agar,Sulphuric Acid: Water (1:1),Orthophospheric acid,1,5-Diphenyl Carbazide reagent (DPC), Distilled water, Potassium Dichromate stock solution (100 ppm), *Bacillus* Suspension.

B. Methods:

I. Enrichment and Isolation

For isolation 1 gm of soil sample was inoculated in Nutrient brothand flask was kept at 30˚C in incubator for 24 h. The enriched culture was then streaked on sterile Nutrient agar plates and the plates were incubated at 30˚C for 24 h and the colonies were selected and screened for morphological, cultural and biochemical characterization.

II. Estimation of chromium

The standard solution of Potassium dichromate was prepared with different concentration from 10-100 ppm by DPC method (1ml of supernatant,0.5ml sulphuric acid:water, 0.1 ml Orthophospheric acid, 8.5 ml Distilled water, 0.5 ml DPC reagent) and standard graph was plotted at 540 nm

Hexavalent chromium reacts with 1,5-Diphenyl carbazide in acidic conditions to produce a red violet colour which is spectrophotometrically determined at 540 nm.

III. Cr (VI) assay by 1,5-diphenyl Carbazide:

1) Effect of initial biomass concentration on percent sorption of Cr (VI):

To study the initial biomass of *Bacillus*concentration on percent sorption of Cr (VI). Test tube containing 10 ml of 20 ppm Cr (VI) solution with pH 7.0 was inoculated with 0.1 to 0.5 ml of cell suspension of *Bacillus*(OD =1.0). The tubes were then incubated at 30˚C on shaker at 100 rpm and after 1h retension time the content was removed and centrifuged at 8000 rpm and then the supernatant was collected and residual Cr (VI) and present sorption was estimated by DPC method.

2) Effect of Initial Cr (VI) concentration on percent sorption:

To study the effect of Cr (VI) concentration on percent sorption, the flask containing 20 ml of different Cr (VI) concentration ranging from 20-100 ppm with pH 7.0 were prepared which were inoculated with fixed inoculum of *Bacillus* (1%) (OD =1.0) and then incubated on the shaker at 100 rpm for 1h at 100 rpm at 30˚C then centrifuged and analyzed for residual Cr (VI).

3) Effect of pH on percent sorption of Cr (VI):

To study the percent sorption of pH, Flask containing 30 ml of 20 ppm Cr (VI) containing solution with different pH (3, 5, 7, 9,11) were inoculated with fix inoculum of *Bacillus* suspension (1%) (OD =1.0) and the flask were incubated at 30˚C for 1h on shaker at 100 rpm and then centrifuged and analyzed for residual Cr (VI).

4) Effect of Holding time on Cr (VI) sorption:

To study the effect of holding time, Flask containing 50 ml of 20 ppm Cr (VI) solution with pH 7.0 was inoculated with 1% *Bacillus* suspension (OD =1.0) and flask were kept on shaker at 100 rpm. After every 30 mins the sample was removed, centrifuged and further analyzed for residual Cr (VI).

5) **Effect of Holding temperature on Cr (VI) sorption:**

To study the effect of holding temperature, Tubes containing 10 ml of 20 ppm solution with pH 7.0 were inoculated with 1% *Bacillus* suspension (OD =1.0). The tubes were incubated at various temperature (10-50˚C) after 1h retention time the sample was centrifugedand the further analyzed for residual Cr (VI).

Results and Discussions

After 24h of incubation of soil sample in Nutrient broth the uniform turbidity was observed. After the enrichment the sample was streaked on the nutrient agar plates after 24h colonies were developed and further the colonies were studied for morphological characterization and standard biochemical tests were carried out for the identification(Bergey's Mannual of determinative bacteriology) and species of *Bacillus* was confirmed.

- **Cr Assay by 2-Diphenly carbazide:**

  Results of growth independent percent sorption obtained by calculating arithmetic mean by triplicates of results of Cr (VI) by *Bacillus* are recorded below:

1) **Effect of initial biomass concentration on percent sorption of Cr (VI) by *Bacillus*:**

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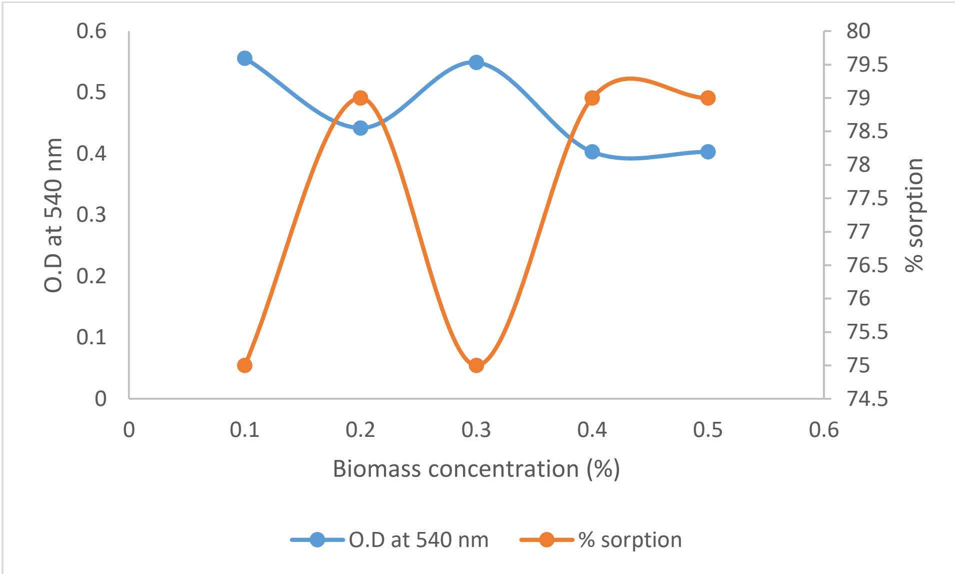
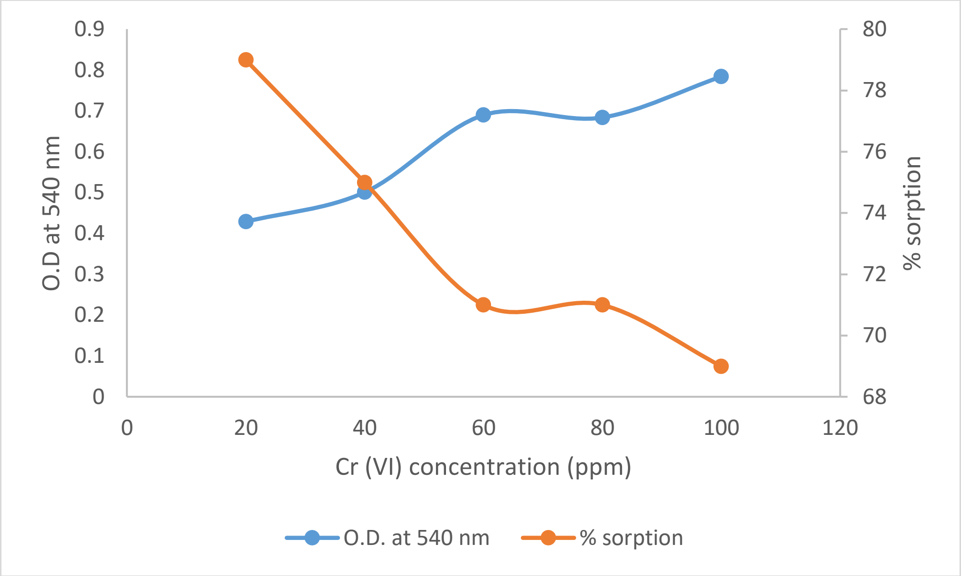


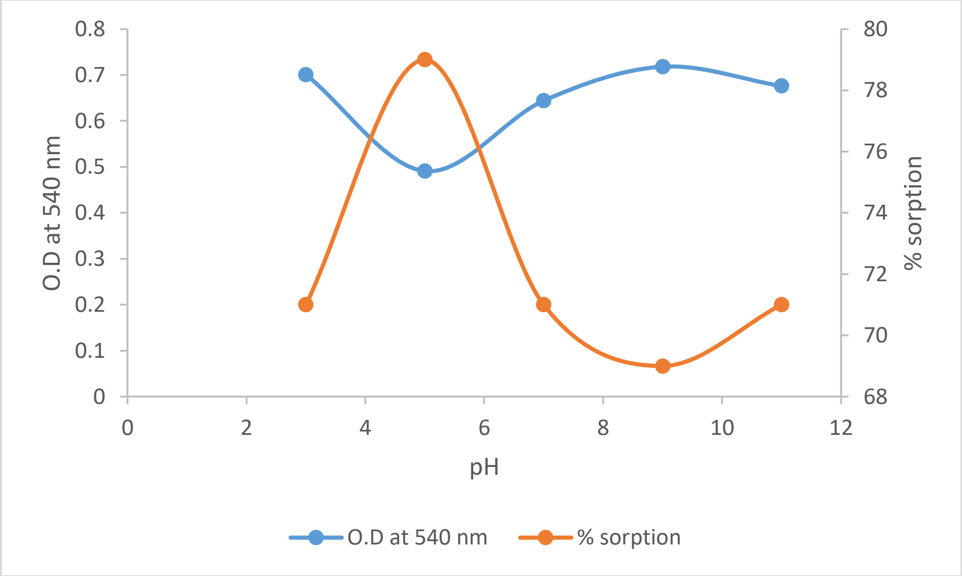
It is suggested that the increase in biomass concentration leads to interface between the binding sites Fourest and Roux (1992). Hence, this factor needs to be taken into consideration in any application of microbial biomass as biosorbent. From the above graph it was found that the highest sorption was at 0.2 concentration of biomass that is 79%. As there is constant absorption it may due to interference between binding sites at higher concentration (de Rome and Gadd, 1987). High biomass concentration may exhibit a "screen" effect of the dense layer of cells which restricts the access of metal ions to the binding sites (Pons and Fuste, 1993

2) Effect of Cr (VI) concentration on percent sorption by *Bacillus*:



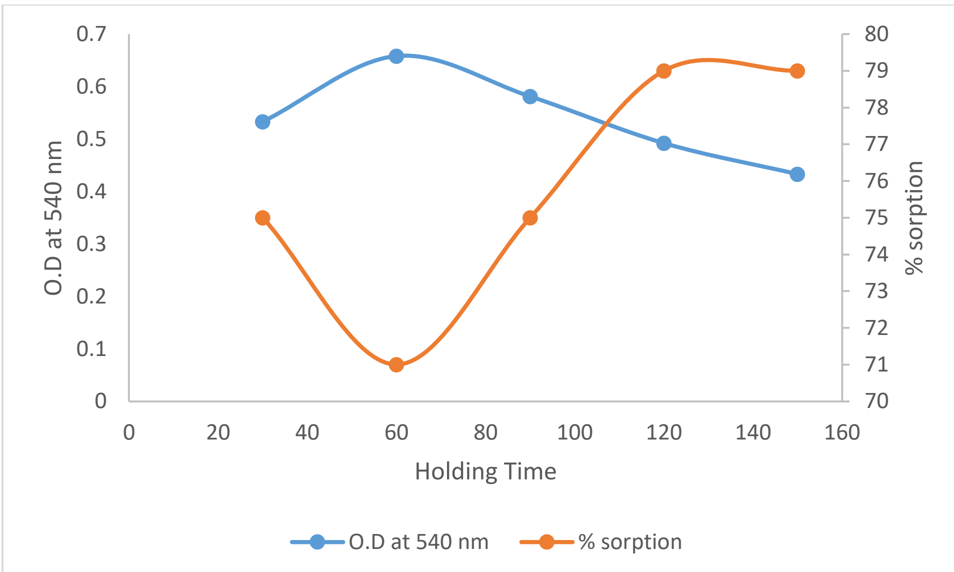
From the above graph it showed that Cr (VI) concentration increased from 20-100 ppm and the maximum Cr (VI) adsorption was achieved at 20 ppm which was found to be 79% subsequent increase in Cr (VI) concentration does not showed further increase in percent sorption. Zhou and Kiff (1991) reported that copper removal efficiency of *Rhizopusarrhizus* was higher at low initial concentration. Puranik *et.al*. (1995) also reported that there was no further increase in cadmium uptake by *Streptomyces pimprina* in response to increase in metal concentration.

3) Effect of pH on percent sorption of Cr (VI) by *Bacillus*:



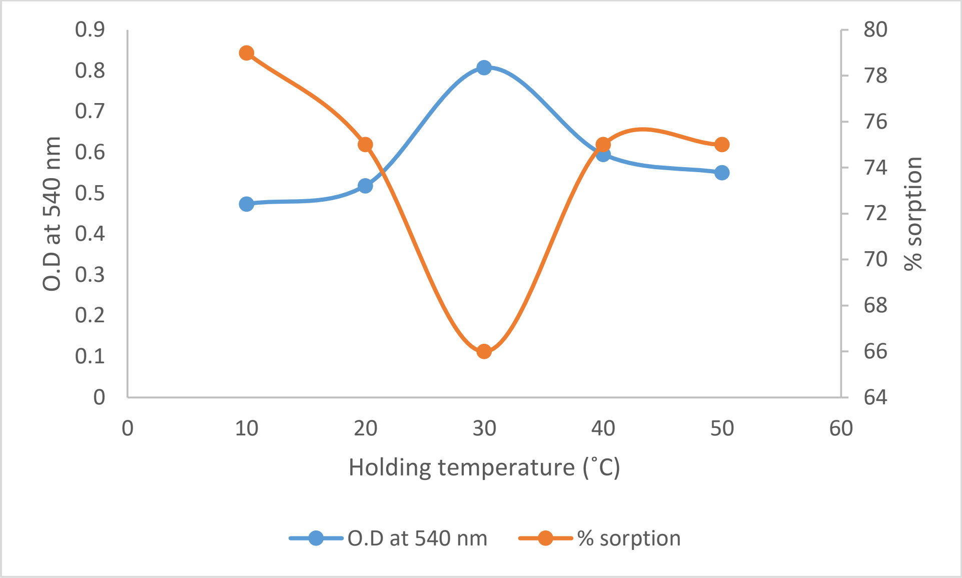
From above graph it showed that the percent sorption of Cr (VI) was found to be 79% at pH 5.0. From the result it is evident that the percent sorption of Cr (VI) was higher at acidic pH range, since the extreme acidic condition might have made opening and hence the availability of more binding sites on cell surfaces, thus facilitating biosorption of Cr (VI). Zhou and Kiff (1991) suggested that at low pH value, cell wall ligand are closely associated with H^3O^+ that restrain access of metal ions to ligands as a result of repulsive forces.

4) Effect of holding time on percent sorption of Cr (VI) by *Bacillus:*



From above graph it showed that the percent removal of Cr (VI) increased with time and reached to a maximum at a particular time termed "equilibrium time" after which percentage adsorption remained constant. The sorption of Cr (VI) was observed to be 79% after 120 mins. So there was no further increase in Cr (VI) adsorption.

5) Effect of Holding temperature on percent sorption of Cr (VI) by *Bacillus:*



From the above graph it showed that the maximum absorption of Cr (VI) was found to be 79% at 10˚C. There was decrease in percent sorption with increase in temperature. The metabolism uptake of zinc, copper and uranium by *Candida utilisi* (Failla *et.al*., 1976), *Saccharomyces cerevisiae* (de Rome and Gadd, 1987) and *Pseudomonas Sp*. (Pons and Fuste, 1993), respectively was not affected significantly over temperature ranging from 4 to 45˚C.

Conclusion

In the past decades research in metal sorption by certain chemical physical and biological methods has provided a wide understanding. The chepeast method by using microorganism in bioremediation has a wide impact than other methods. The metal absorption mechanism is quite complicated and fully un-understood. The mechanism of the metal sorption can be influence by biomass, pH, temperature, time, and concentration.

In this study reports isolation of *Bacillus* organism from soil was isolated that has the ability to reduce the Chromium (VI). It was found that Strain can reduce the Cr (VI) concentration under low biomass (0.2%), low concentration (20 ppm), low pH (5.0), for high time (120 min) and at low temperature (10˚C). It has an ability to reduce hexavalent chromium (VI) without any nutrient, which suggest that it can be applicable for bioremediation of Cr (VI). As the isolated strain has the potential to detoxify the Cr (VI) it can be suggested that it can be used as a potent organism for the process of bioremediation.

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