



Removal of Chromium from contaminated effluent and simultaneously Green Nanoparticle synthesis using *Bacillus subtilis*

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ABSTRACT

The wide spread industrial application of chromium leads to the dangerous effects to the environment. In recent years the removal of heavy metals from the industrial effluents using microorganisms is becoming a novel approach in recent years. *Bacillus* species exhibited the property of biosorption of Cr (VI) to Cr (III) and it simultaneously converted the Cr into nanoparticle. The Cr removal studies were carried out using different concentration of potassium dichromate solution (50, 100, 150, 200 and 250 ppm) in the growth media, using various pH ranges (2, 3, 5, 7 and 9) and biomass at different dosage (1-5 ml) under 37°C. The batch experiment was carried out with a initial Cr concentration (100 mg/l), pH 7 and 1ml of biomass dosage. The maximum percentage of removal was found to be 99% and along with it nanoparticle was also synthesized. The nanoparticles synthesised were characterised using UV-Visible Spectrophotometer, Fourier Transmission Infra-Red Spectrophotometer (FTIR), X-ray Diffraction (XRD) and Scanning electron microscope (SEM). Thus the study proves that the strain would completely remove chromium from the aqueous solution and it provides a facile green technology for the synthesis of nanoparticle.

Keywords: Chromium, *Bacillus subtilis*, Nanoparticle, Green Technology, FTIR, XRD.

1. INTRODUCTION

The heavy metal recovery from dilute solution remains a subject of great interest from both fundamental and applied research view point. Among the heavy metals chromium holds a prominent place as it has a wide variety of industrial coatings for wear resistance and thermal protection [1]. But in view of toxicity concentration chromium in the effluent must be brought down to permissible limit [2]. Otherwise

it be brought down to permissible limit [2]. Otherwise it will cause serious threat to the humans and animals.

Chromium, one of the metals exists in two stable oxidation states, Cr (III) and Cr (VI). Almost all regulatory agency in worldwide has listed Cr(VI) as a priority toxic chemical that should be controlled, and the maximum acceptable concentration in drinking water should be 0.05 mg L⁻¹ [3]. The presence of strong oxidants can change Cr (III) to Cr (VI).

Several techniques for Cr (VI) removal such as ion exchange, filtration, electrochemical precipitation, activated carbon adsorption, bio sorption, etc., have been reported in literature [4]. Biosorption is an emerging technology and has received increasing attention for the removal and recovery of heavy metals from effluent in recent years [5]. The chemicals or enzyme present in these biomaterial act as a reducing agents and crystallize the metal in ionic form to zero valent metal form. The resultant crystals usually occur as nanosized particles [6]. Thus the ideal process for chromium detoxification should be able to trap the Cr (VI) and convert them into a less toxic Cr (III). Thus the present study explored the use of *Bacillus subtilis* strain in bioremediation of chromium from the aqueous solution and develops a green technique for the synthesis of chromium nanoparticle. Therefore the process addresses two issues (i) bioremediation of heavy metal pollutants (ii) nanobiosynthesis by greener process.

2. MATERIALS AND METHODS

2.1. Bacterial strain

The bacterial strain *Bacillus subtilis* a heavy metal tolerant microorganism obtained from the department Environmental science, PSG College of Arts and science in Coimbatore was used in the present study. The culture was maintained in 4⁰C and sub cultured every month. A loopful of culture was taken from the slant and it was inoculated into the Luria-Bertani (LB) medium containing (g L⁻¹): yeast extracts 0.5; tyryptone 1.0; Nacl 1.0; adjusted to pH 7.0 followed by incubation at 30⁰C and kept in an orbital shaker. The 24h grown culture was used as a seed culture.

2.2. Inoculum and culture conditions

50 ml of the culture medium containing (gL⁻¹): yeast extracts 3.0; peptone 5.0; Nacl 2.5; MgSO₄. 7H₂O 0.5; was adjusted pH 7. The above culture medium was inoculated with 1ml of the seed culture at 30⁰C at constant shaking condition.

2.3. Biosynthesis of chromium nanoparticle

The solution of K₂Cr₂O₇ for 1000 mg L⁻¹ was prepared using sterile distilled water and it was filter sterilized. The chromium reduction performed at media containing 100mg L⁻¹ of Cr and rest of the culture condition was same as followed in the above section. The process of reduction was carried out in a continuous shaking condition at 30⁰C. The samples were withdrawn aseptically at regular intervals and centrifuged at 14,000 rpm for 10min. The supernatant was taken for further analysis like estimation of

residual chromium concentration was determined spectrophotometrically by diphenyl carbazide method and the absorbance was measured at 540 nm. The absorbance of the bacterial growth was measured at 660nm in spectrophotometer (Shimadzu UV-1700).

2.4. Effect of varying culture condition in removal and nanoparticle synthesis

The Effect of different parameters like pH, inoculum dosage and metal concentration on the growth, bioaccumulation and nanoparticle synthesis by *Bacillus sp.*, was studied.

2.4.1. Effect of pH

The culture media was maintained as described in the above section except the pH. For pH the media was prepared in a 250 ml conical flask with different pH 2.0- 9.0. The pH was adjusted with 0.1 N HCL or NaOH prior to the inoculation.

2.4.2. Effect of biomass dosage

For dosage the media was prepared and adjusted to pH 7 and then it was inoculated with different amount of inoculum (1-5 ml) was added and kept for incubation.

2.4.3. Effect of chromium Concentration

The effect of chromium concentration was monitored in the range of 50-250 mg/L carried at pH 7. Absorbance was measured at regular intervals for metal reduction. The cells harvested with the above culture condition were used for further characterization. All analyses were performed in triplicates.

2.5. Characterization of synthesized Chromium nanoparticle

The geometry of the chromium nanoparticle was confirmed using the UV-Vis spectrophotometer, FTIR, XRD and SEM

2.5.1. UV-Vis spectrophotometer analysis

The chromium nanoparticle synthesis was monitored using the supernatant collected after the centrifugation and it was analyzed in the UV-VIS Spectrophotometer was recorded in UV-1700 series.

2.5.2. Fourier Transfer Infrared Spectroscopy (FTIR) analysis

The bacterial cells grown in the presence of chromium were then centrifuged at 8000rpm for 10min. After that the pellets was collected and washed with sterile distilled water. It was made into a

fine powder and subjected to FTIR analyses using KBr pellet method. The spectra were recorded in the range of 400-4000 cm^{-1} with a Bruker model IFS-55 FTIR spectrometer coupled to a Bruker IR microscope fitted with an IBM compatible PC running OPUS, Version 2.2 software.

2.5.3 X-ray Diffraction (XRD) analysis

The samples were characterized by X-ray diffraction, recorded on a Siemens D500 instrument with Ni-filtered $\text{Cu K}\alpha$ (40 KV, 40 mA). Every scan was recorded from 0 to 90° (for 2 θ) in step-by-step mode with intervals of 5° between each scan and the intensities were recorded.

2.5.4. Scanning electron microscope (SEM) analysis

The samples obtained after the centrifugation were washed three times with PBS to remove the impurities the size of the nanoparticle was confirmed using the SEM (Hitachi; Japan) with 0.3 – 30 kV.

3. RESULTS AND DISCUSSION

3.1 Chromium biosorption by *Bacillus subtilis*

The growth of the organism was not significantly affected by the presence of chromium when compared to the control with zero chromium was shown in Figure. 1. But the lag phase was extended as compared to the control [7]. Since the bacterial surface was affected by the chromium reduction and uptake process [3]. The complete biosorption of chromium is found to occur during the exponential phase and the remediation would occur within 48 h at low concentration. Thus microbial system according to their adaptive and physiological mechanisms can detoxify the metal by effluxing it out and accumulating in the cytoplasm or by converting it into the less toxic form. The synthesis of chromium nanoparticle would be mediated through some enzyme followed by aggregation with other cellular proteins.

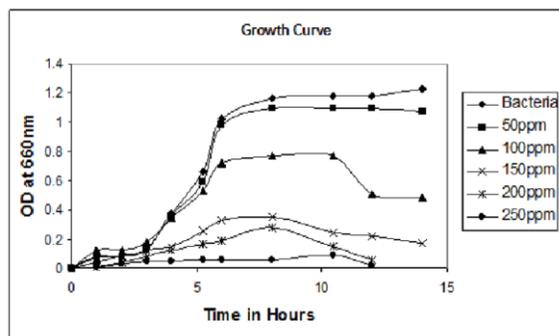
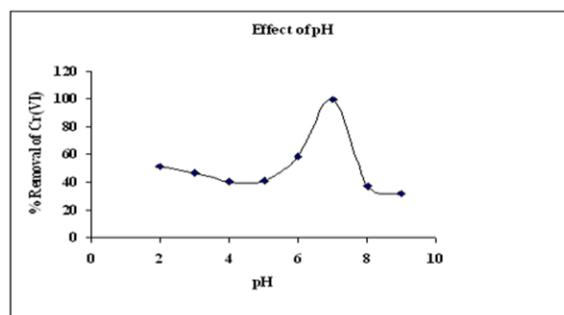


Figure 1. Growth curve of *B. subtilis*

3.2 Effect of pH, biomass dosage and metal concentration

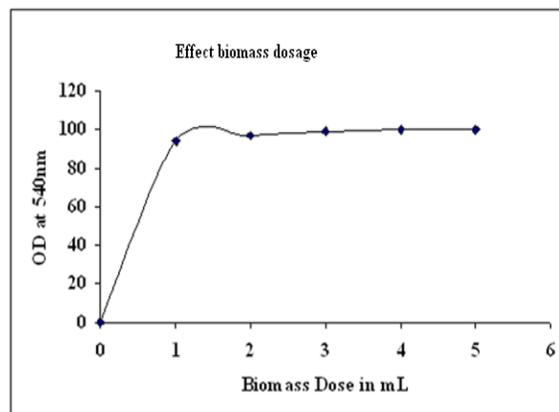
A series of experiments were carried out to determine the chromium reduction like effect of pH, dosage and metal concentration. pH is one of the important parameter in metal recovery and nanoparticle synthesis. In the present study pH range of 2-9 was studied. Figure.2. shows high biosorption of chromium was believed to occur in the neutral pH 7 this is due to the ligands like carboxyl, phosphate, imidazole and amino group would be exposed and carry negative charge with a subsequent attraction of metallic ions with positive charge and biosorption on to the cell surface [8].

Figure 2. Effect of pH (biosorption of chromium)



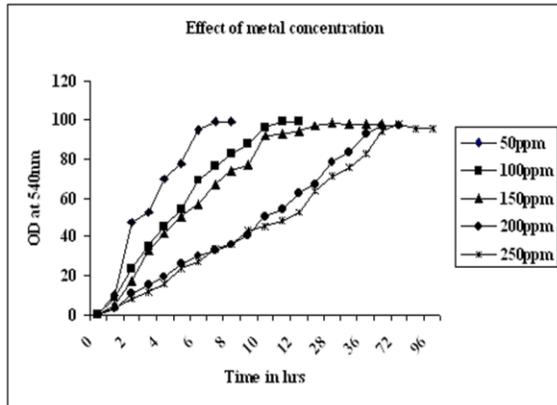
For biomass dosage the medium with 100ppm of chromium was inoculated with 1-5ml of the biomass. The biosorption and nanoparticle synthesis was increased with increase in biomass was shown in the figure 3. Increase in biomass concentration generally increases the level of biosorption of Cr (VI) ions because of an overall increase in surface area of the biosorbent, which in turn increases the number of binding sites [9].

Figure 3. Effect of biomass dosage



Finally the effect of metal concentration with different concentrations like 50-250 ppm was carried out figure 4. In the given duration of 48h maximum removal was observed in low concentration were as gradual decrease in the percentage of removal with increase in chromium concentration.

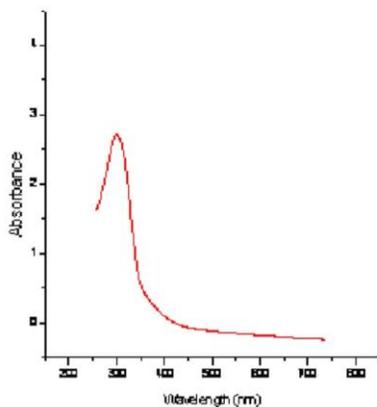
Figure 4. Effect of metal Concentration



3.3. Characterization of synthesized chromium nanoparticle

The UV visible spectrum demonstrates the novel technique to determine the metallic nature and the absorption band for Cr nanoparticle which is a potential application for subsequent processing of materials and the absorption peak focused at 300 nm which is in good agreement with the previous work [13] shown in figure 5.

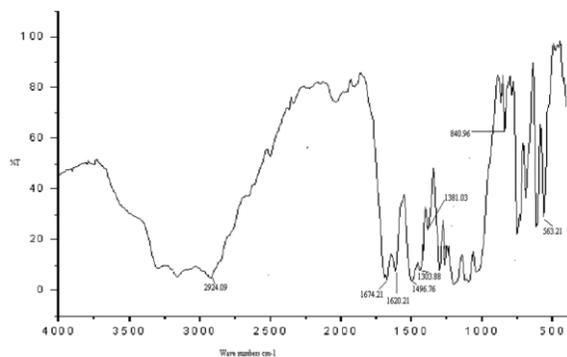
Figure 5. UV-Visible spectra for chromium oxide nanoparticle by *Bacillus* sp.



The characteristics of chromium nanoparticle were observed in the FTIR spectrum. Figure 6 shows the vibration band at 840.96 cm⁻¹ delineates the

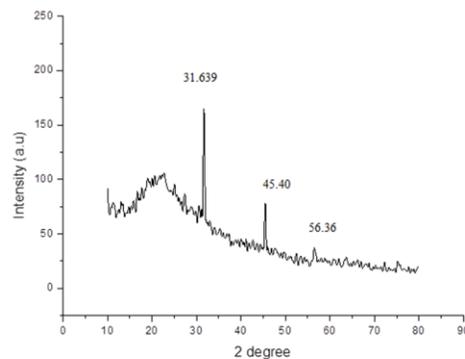
transportation of chromate into the cytoplasm [2]. The peak at 563.21 cm⁻¹ indicates that Cr=O and Cr-o vibration of Cr nanoparticle and the peak at 1303 cm⁻¹ indicating the presence of (-COO-) carboxylate ions, responsible for stabilizing the Cr nanoparticle [10]. The carbonate contaminants were seen in the 1381 cm⁻¹ and 1496.76 cm⁻¹ [1]. The band obtained at 1620.21 cm⁻¹ is due to the bending modes of nondissociated water molecules [11]. Bands at 2924.09cm⁻¹ indicative of the presence of methoxyl group, while the peak at 1674.21 cm⁻¹ corresponds to the bending vibration of hydroxyl group [12].

Figure 6. FTIR Spectra for chromium oxide nanoparticle



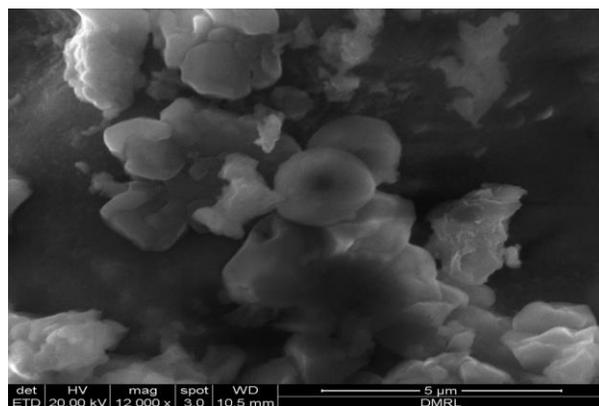
The X-ray diffraction was taken for the Cr nanoparticles for its conformation. The XRD patterns of the obtained nanoparticles of Cr are shown in the figure 7. The spectrum obtained from the XRD analysis shows three peaks that are clearly distinguishable. The 2θ peaks at 31.63°, 45.40° and 56.36° were identified and shows the presence of Cr in the sample [13]. The particle size were calculated using the Scherrer formula which were applied to the major intense peaks and it was found to be in the range of 50-78 nm.

Figure 7. XRD pattern for the chromium oxide nanoparticle



The SEM images were measured and it was analysed based on the surface of the particle. Normally the SEM image provides detailed information about the morphology, size of the particle etc. At different magnification particle formed were observed spherical in shape figure 8. Similar results in the shape of the Cr nanoparticle were reported in the previous studies in the Cr nanoparticle using the chemical method [12]. Thus the various analytical results conclude that the chromium oxide nanoparticles were intracellularly synthesized.

Figure 8. SEM image of the synthesized chromium oxide nanoparticle



4. CONCLUSION

The study proves the biological removal of Cr (VI) is an attractive technique. The *B. subtilis* is a novel strain for the remediation of chromium from aqueous solution and nanoparticle synthesis with good monodispersity. The maximum removal of chromium using *B. subtilis* was found at pH 7. The formation of the nanoparticle was confirmed using UV Spec, FTIR and SEM. The chromium nanoparticles synthesized intracellularly were recoverable. This study was mainly focused to solve the chromium pollutant from different sources and alternative method to biosynthesis the nanoparticle. It can be viewed as an eco-friendly method to remediate the waste water and to synthesis nanoparticle.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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