Biosorption of Chromium from Spent Semi-Chrome Liquor: Part 1 Effective Pollution Abatement using *Bacillus cerus*

by

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Abstract

The current study focuses on the isolation of *Bacillus cerus* from mangrove rhizosphere and its ability to treat semi-chrome process liquor of upper leathers. This strain has been identified by its molecular characteristics (16s rRNA sequencing) and confirmation has been obtained from neighbor joining tree. Minimum inhibitory concentration of the strain has been found to be 50 ppm. The growth pattern of this organism has been investigated in the presence of chromium, which showed the bacterial strain can grow luxuriantly at 50 and 100 ppm concentration of chromium. Biosorption study has been conducted at different concentrations (50, 100, 150, 200 and 250 ppm) of chromium. The biosorption capability of *Bacillus cerus* has been found to be 80.78, 73.19, 65.86, 59.44 and 39.27% for 50, 100, 150, 200 and 250 ppm respectively. Chromium sorption from the semi-chrome process liquor by *Bacillus cerus* has also been investigated, which showed a reduction of 76.15, 68.56, 61.63, 56.29 and 36.51% against 50, 100, 150, 200 and 250 ppm of chromium. Sorption characterization has been carried out by FTIR (Fourier Transform Infra-Red spectroscopy) and SEM (Scanning Electron Microscopy) analyses and the results confirmed the presence of sorption of chromium in *Bacillus cerus*.

Introduction

The leather industry has been considered as one of the most promising export industries worldwide for the past four decades. Vegetable tanning and chrome tanning are the major conventional tanning methods. However, chromium and vegetable tannins discharged from the tannery have become crucial despite several developments that have been studied in the tanning industry. The leather sector has developed several technologies for the uptake of chromium during tanning from 40-50% to near 100% in chrome tanning process.[1] However, the semi-chrome process wastewater poses a challenge in separating chromium from vegetable tannins by conventional methods. The footwear industry continues to follow the vegetable tanning processes with the traditional semi chrome processes due to the need for specific properties in these leathers, pastel shades and light weight leathers.[2] The vegetable tanned leathers are treated with basic chromium sulphate in a conventional manner during the semi-chrome tanning process. Vegetable tannins and higher molecular weight polyphenols, sugars etc. are present in the spent effluent in addition to chromium.[3] The conventional precipitative recovery of chromium involves formation of stable complexes with metal ions (o-dihydroxyphenyl chelating functional groups in the tannin molecules to form stable complexes with metal ions and settle in the spent liquor.[4] The acid used for treatment and reuse of chromium in effluent treatment of semi-chrome process generates quinonoid and other degraded organic compounds, which cause stains in the leathers when reused in tanning.[2] The introduction of metal ions like zinc and barium in the form of zinc tannate and barium tannate result when chemical methods are used.[2,3] Precipitation methods with heavy metal ions, polyvinylpyrrolidone, adsorption onto zirconium pillared clay, gelatine, collagen as well as electrochemical treatments have been reported for the removal of vegetable tannins.[4-9] Hence, there is a need to develop a low cost biological material which can potentially remediate the chromium from the semi-chrome process liquor.

Biosorption is a technique where the living organism encompasses itself in the accumulation of heavy metals from the wastewater.[10] Bacteria is one of the promising candidates to adsorb the heavy metals. Bacterial cell wall has the peptidoglycan layer, which has potential binding sites to accumulate heavy metals.[11] Hence, the present study focused on *Bacillus cerus,* a marine bacteria isolated from mangrove rhizosphere, to bring about selective biosorption of chromium from vegetable tanning containing liquor.

Experimental

Collection of effluent

The spent semi-chrome liquor after basification from the semi-chrome tanning process has been collected from a leading commercial tannery manufacturing upper leathers in Chennai, India. The process of tanning employed for making upper leathers has been wattle for vegetable tanning followed by chrome tanning after stripping process. The spent semi-chrome liquors have been analysed for chromium content, vegetable tanning content and chemical oxygen demand (COD).[12,13]

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Manuscript received June 3, 2021, accepted for publication July 5, 2021.

Isolation and identification of chromium resistant bacteria

Soil samples have been collected from mangrove rhizosphere and serially diluted and spread over nutrient agar plates. Morphologically different organisms have been selected and sub-cultured. Based on the chromium tolerance in the presence of vegetable tannins, the relevant strain has been selected for further studies. Biochemical characteristics of the strain has been evaluated by standard methodology.[14] Genomic DNA extraction has been carried out using the standard method.[15] PCR amplification has been carried out using universal primer and sequenced, and the obtained sequences have been submitted to NCBI and an accession number has been obtained.

Minimum inhibitory concentration

Minimum inhibitory concentration of chromium against the selected organism has been evaluated using agar well diffusion method. The bacterial culture has been grown in the nutrient broth and have been spread on the nutrient agar. Wells have been prepared on the nutrient agar and filled with chromium solution at different concentrations. The cultured plates have been incubated at 30°C for 24 h.

Bacterial growth evaluation

Growth pattern of the selected bacteria in the presence of chromium has been evaluated, 1% of the bacterial strain has been introduced into the nutrient broth containing micro-titer plate with different concentrations of chromium liquor as well as spent semi-chrome liquor (50, 100, 150, 200 and 250ppm). Plates have been incubated at 30°C and bacterial growth has been monitored at regular intervals at 600 nm.

Biosorption analysis

Chromium sorption capability of *Bacillus cerus* has been studied following the method of Saranya et al.[16] with minor modification. Nutrient broth has been prepared with chromium and spent semi-chrome liquor by introducing 1% *Bacillus cerus* culture into the broth. All the samples have been incubated in shaking condition (150 rpm) for 24 h at 30°C. The culture broth has been centrifuged and the incubated pellets have been separated and supernatant has been digested with nitric acid and perchloric acid. The digested samples have been analyzed using ICP-OES. Bacterial biomass has been introduced to the diluted semi-chrome liquor that has been collected from the commercial tannery along with nutrient broth and incubated at 30°C for 24 h. The biomass has been separated from the media for all the samples and used for further analysis.

Characterization

Biosorption ability of *Bacillus cerus* has been characterized by FTIR and SEM. The bacteria strain from the broth has been collected from centrifugation and dried at 60°C for 18 h. Dried powders have been used for characterization. Functional groups involved in the sorption study have been assessed using FTIR spectrometer (JASCO 4200) and morphological changes have been observed under microscopical analysis (PHENOM ProX). Bacterial culture without chromium has been maintained as control.

Results and Discussion

The present study emphasizes the assessment of the chromium sorption capability of the newly isolated chromium resistant

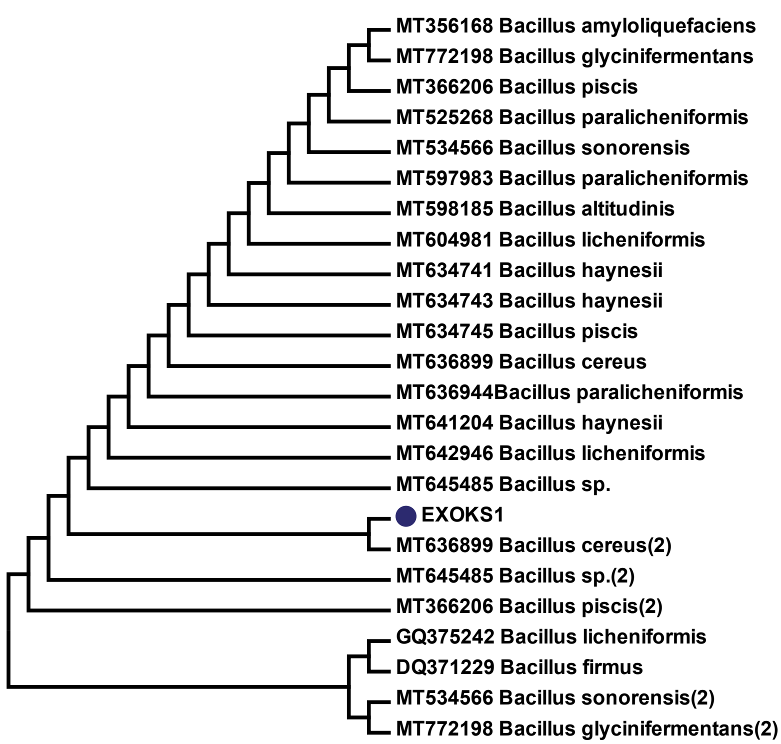

Figure 1. Phylogenetic tree of the strain *Bacillus cerus*

Table I
Characteristics of spent semi-chrome liquor

Parameter	Value
Spent semi-chrome liquor	
pH	3.5±0.1
COD (ppm)	7056±42
Total chromium as Cr (ppm)	2140±18
Vegetable Tannins (ppm)	4280±16

bacteria *Bacillus cerus.* This strain was isolated from a marine source mangrove rhizosphere and identified using biochemical and molecular characteristics. Sequence of the strain has been submitted to the NCBI and an accession number has been obtained (MW463427). Phylogenetic tree has been drawn (Figure 1) using MEGA 6.0 software to confirm the identification. *Bacillus cerus* has been identified as a gram positive, aerobic, spore forming rod shaped bacteria[17] as the potential candidate for the removal of chromium in the presence of vegetable tannins. The characteristics of spent semi-chrome liquor from the commercial tannery is given in Table I. The COD, vegetable tannin content and chrome content of the semi-chrome liquor taken for studies are 7056, 4280 and 2140 ppm, respectively. The spent semi-chrome liquor has been suitably diluted and used for the study.

In earlier days, researchers focused on the isolation of bacteria from industrial water or from the leather dump yard due to their ability to create resistance against certain pollutants for their survival.[18] In the present study, as a novel approach, we isolated the bacterial strain from the mangrove rhizosphere, which has the possibility to adsorb only chromium from the semi-chrome liquor containing vegetable tannins. Marine bacteria rather than terrestrial bacteria have been considered as efficient community due to their tolerance capability. The natural existing bacteria from marine source of mangrove rhizosphere have also been proved that they also have equal potential to resist developed organisms.

To identify chromium resistance of the strain, minimum inhibitory concentration has been investigated against different concentrations of chromium (50, 100, 150, 200 and 250 ppm). The zone of inhibitions were measured as 0.1, 0.3, 0.4, 0.5 and 0.6 cm against 50, 100, 150, 200 and 250 ppm of chromium respectively. Followed by the minimum inhibitory concentration test, growth pattern of the *Bacillus cerus* has been evaluated in the presence of same concentrations of chromium, which has been used for the minimum inhibitory concentration evaluation. Growth pattern of the bacterial strain showed increasing concentration of chromium promote inhibition of bacterial growth (Figure 2). Maximum growth has been noted at lower concentration (50 ppm) of chromium for up to 48 h and maximum inhibition has been observed at 250 ppm. Bacterial growth has been found to be higher at 24h at 100, 150 and 200 ppm concentrations, which can be attributed to contact time and binding site availability of the bacteria. A similar growth pattern has been observed in the presence of semi-chrome liquor also (Figure 3), but the bacterial growth has been comparatively lower than the above study, where only chromium resides. This can be due to the lower availability of bacterial species for the vegetable tannins present in the semi-chrome liquor at the same concentration and duration of chromium uptake.

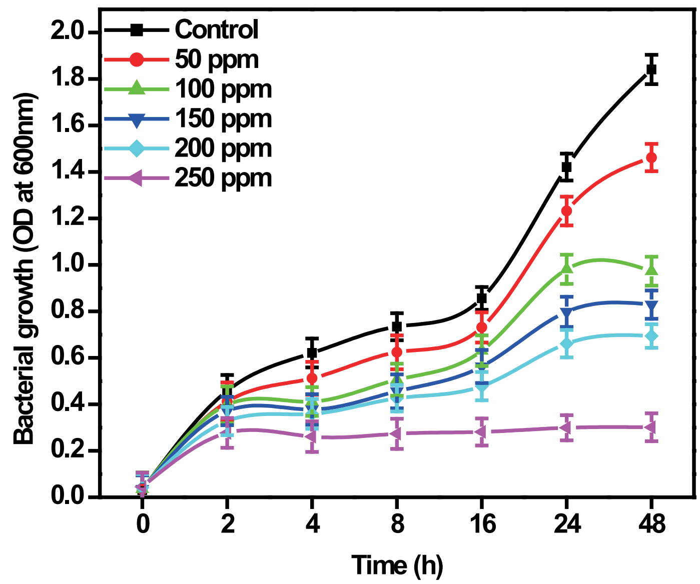

Figure 2. Growth analysis of the *Bacillus cerus* with and without presence of chromium

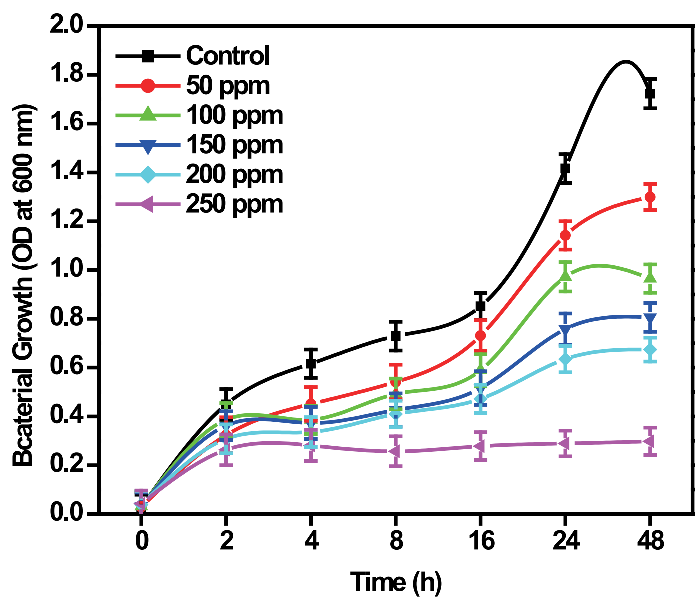

Figure 3. Growth analysis of the *Bacillus cerus* with and without presence of semi-chrome liquor at different concentrations of chromium

The removal of Chromium by the bacterial strain has been centered on the variation in concentration of chromium. The observed chromium removal percentage has been 80.78, 73.19, 65.86, 59.44 and 39.27% for 50, 100, 150, 200 and 250 ppm concentrations of the chromium respectively (Figure 4). Chromium removal percentage of the strain *Bacillus cerus* has been gradually increased from high to lower concentrations that could be due to availability of more reactive sites, which could have fastened up the sorption process. This can be supported by the previous study, which indicated that increasing metal concentration can increase the biosorption capability of bacteria up to certain threshold level.[19] Removal of chromium from semi-chrome liquor has also been evaluated at the concentration of 50, 100, 150, 200 and 250 ppm. Biosorption percentage calculated on post treatment of semi-chrome liquor, resulted in 76.15, 68.56, 61.63, 56.29 and 36.51% of chromium absorption at respective concentrations of 50, 100, 150, 200 and 250ppm (Figure 5). Maximum biosorbed biomass was taken for further characterization (50ppm).

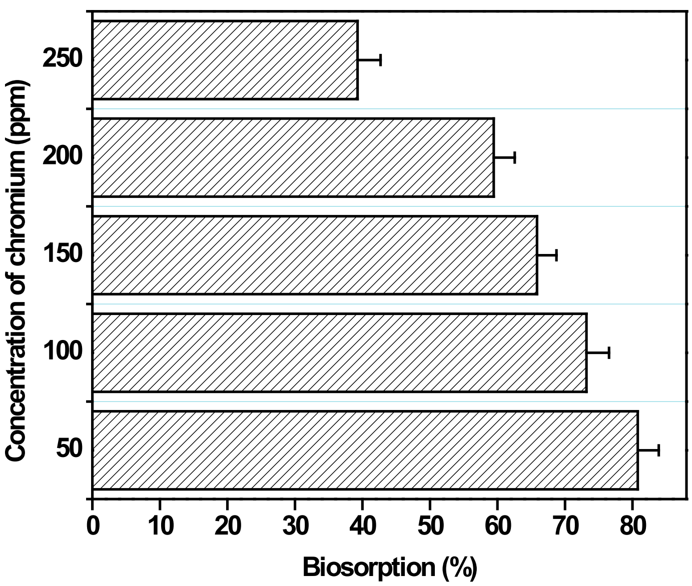

Figure 4. Biosorption capability of *Bacillus cerus* at different concentration of chromium

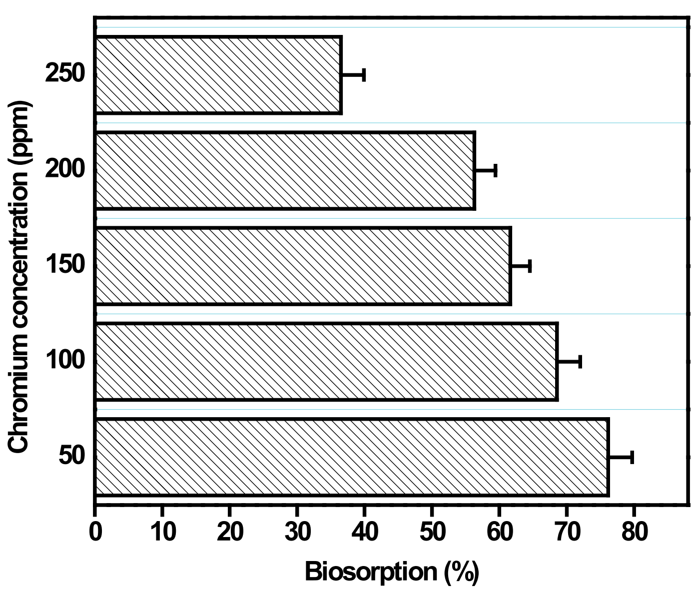

Figure 5. Biosorption capability of *Bacillus cerus* from semi-chrome liquor

The probable mechanism of biosorption is that the bacterial cell walls consist of various charged functional groups which act as binding sites for heavy metals. Hence, in order to identify the functional groups which play a role in the sorption process, FTIR analysis has been carried out. The FTIR spectrum analysis has been investigated for *Bacillus cerus* with and without chromium in the range of 400-4000cm^{-1} to determine the possible functional groups involved in the sorption process (Figure 6). The functional groups responsible for biosorption and the shift in spectrum have been analyzed and given in Table II. The change in shift of peak is at 3647, 1839 and 13396 from 3282, 1640 and 1366 cm^{-1} respectively, which are responsible for –OH, C-O/C-C and –CO/-OH stretching bands. One more peak has been observed at 801 cm^{-1} in metal absorbed biomass, which arise due to metal ligand vibration and the presence chromium. Vibrational spectrum between control and experimental samples concludes the presence of sorption on the bacterial biomass.

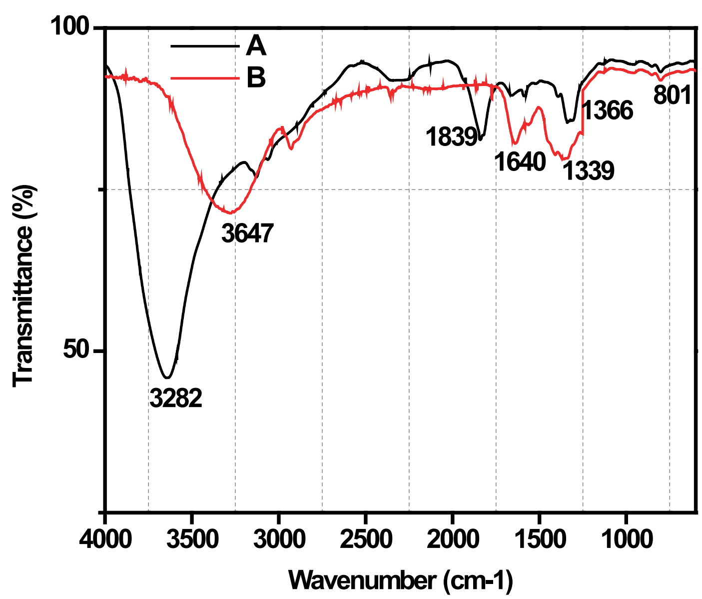

Figure 6. FTIR of the bacterial strain **(A)** Control **(B)** After Experiment

Table II
IR peaks of responsible functional groups involved in biosorption

S.No.	With chromium	Without chromium	Corresponding Functional Group
1.	3647	3282	-OH stretching
2.	1839	1640	C-O/C-C stretching
3.	1339	1366	-CO/-OH Stretching
4.	–	801	Metal ligand vibration

The results of the present study are also supported by some earlier investigations with respect to metal uptake by bacteria.[20,21] The morphological changes of the bacterial strain due to the sorption has been analyzed using scanning electron microscopy. *Bacillus cerus* exposure to the chromium in the presence of vegetable tannins resulted in morphological changes as seen in Figure 7. Due to the production of extracellular compounds by the bacterial strain, there are distinguished changes in communal structure, showing that the cell wall has been wrapped with heavy metals.

Conclusion

Chromium removal from the spent semi-chrome effluent is a challenging issue in wastewater management. The present study concludes that the newly isolated culture *Bacillus cerus* from mangrove rhizosphere (a marine bacteria) showed the minimum inhibitory concentration at 50 ppm and the growth analysis study determined that the strain could grow rapidly for up to 200 ppm concentration. The strain efficiently removed chromium up to 80.78% at 50 ppm concentration of chromium. Biosorption capacity of *Bacillus cerus* has been observed at 76% from the semi-chrome liquor at a concentration of 50 ppm and has been supported by the characterization evidence of FTIR and SEM results. The study concludes that the strain *Bacillus cerus* can be a potential candidate for the treatment of chromium from vegetable tannin containing semi-chrome process effluent.

Acknowledgment

The authors thank authorities of CSIR-CLRI, Adyar, Tamil Nadu, India for providing necessary facilities during the period of research. CSIR-CLRI Communication number is A/2021/CHR/DST-SERB/1553. We are also thankful to DST-SERB (File number: PDF/2019/003239) for the financial support.

Author Contributions

All the authors contributed to frame the manuscript. SK: Experimental performance and writing original manuscript; KB: Writing review; SVK: Technical review, editing, supervision.

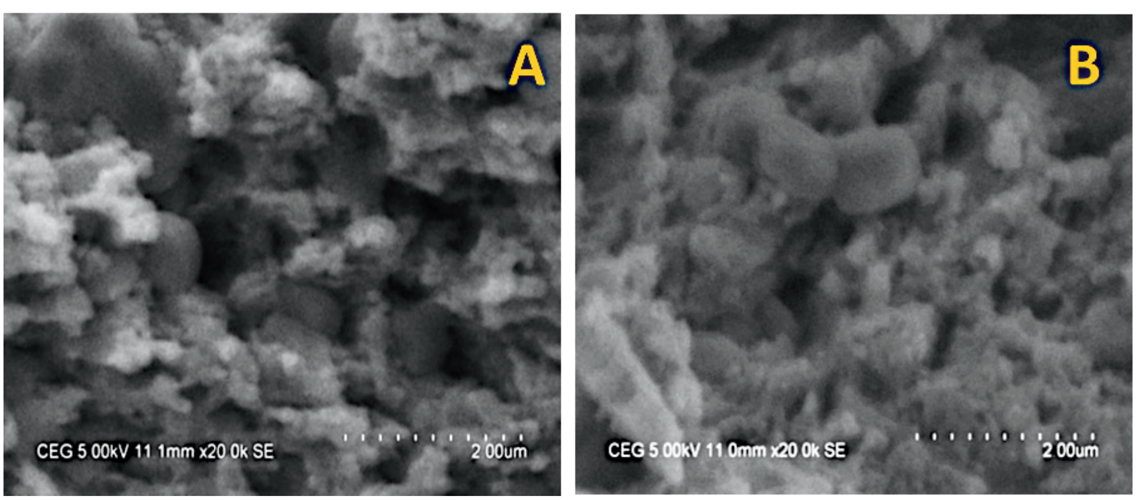

Figure 7. SEM micrograph of *Bacillus cerus* **(A)** Control bacteria **(B)** Experimental bacteria

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