Assessment and Remediation of Chromium Containing Soil In and Around M/S. Tamilnadu Chromates and Chemicals Limited, Ranipet by Bioremediation

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ABSTRACT: M/s. Tamil Nadu Chromates and Chemicals Limited (TNCCCL), situated at SIPCOT Industrial Complex, Ranipet, Vellore District, Tamil Nadu manufactured chromium salts in the form of Sodium bi-chromate crystals and Chromium sulphate powder during its 25 years of operation from 1976 to 2001. During the past 25 years of operation, more than 220000 metric tons (MT) of hexavalent chromium (Cr6+) bearing wastes were generated and dumped indiscriminately by the TNCCCL, within their premises in an area spanning about 2.8 hectares. The unscientific disposal of these wastes resulted in leaching of hexavalent chromium from the dumpsite, percolation of leachate through subsurface strata and widespread contamination of soil and groundwater in the nearby areas. The contamination of soil and groundwater in terms of Cr6+ has posed significant risk to human and animal life as Cr6+ is a carcinogenic compound.

KEYWORDS: Chromium(VI), Bioremediation, Leachate, Carcinogenic compound

I. INTRODUCTION

Hexavalent chromium is widely used in many industrial processes such as electroplating, wood preservation, etc. Commercially available forms of hexavalent chromium (Cr(VI)) are potassium chromate and potassium dichromate. The chromium manufacturing industry produces a large quantity of solid and liquid waste containing hexavalent chromium. The treatment of these wastes is essential before discharging them to the environment. Cr( VI) compounds are highly water soluble, toxic and carcinogenic in mammals. In contrast, trivalent chromium is considered to be nontoxic insoluble oxides and hydroxides in soil and water systems.

The remediation of chromium contaminated sites poses a number of unique challenges. Many technologies are currently used to clean up heavy metal contaminated soils. The most commonly used ones are soil removal and land filling, stabilization/solidification, physico-chemical extraction, soil washing, flushing and phytoremediation. None of these techniques are completely accepted as best treatment option because either they offer a temporary solution, or simply immobilize the contaminant or costly when applied to large areas.

Bioremediation is one of the promising technologies that is expected to play an important role in waste site cleanup. The bioremediation strategy is to detoxify Cr(VI) in the soil to reduce it to Cr(III), so that it gets immobilized in the soil matrix. Besides eliminating the toxicity of Cr(VI) by its reduction to Cr(III) the latter forms a particularly insoluble Cr(OH)3 in the pH range of 6–9 (Ksp, 6.7×10−31) severely restricting its ability to migrate to ground water.

Many microbes were reported to reduce Cr(VI) under aerobic and anaerobic conditions. Bader et al. studied the potential of aerobic reduction of Cr(VI) by an indigenous soil microbial community and found that Cr(VI) in the soil was reduced by 33% within 21 days and suggested that Cr(VI) reducing microbial populations is widespread in soil. Studies have shown that certain bacterial species on surfaces of geologic materials can detoxify the compounds by
reducing them to relatively insoluble and hence significantly less harmful trivalent chromium compounds. Thus, treatment of Cr(VI) containing wastes consists primarily of reducing toxic and mobile Cr(VI) to nontoxic and immobile Cr(III). However, the potential of this bioremediation strategy for the cleanup of Cr(VI) contaminated soil is to be still engineered.

II. MATERIALS AND METHODS

1. Collection of soil sample
   The soil samples were collected from five different locations of the contaminated site located at Ranipet, TamilNadu, India in clean polyethylene bags and preserved it for analysis. The nature of the soil/deposited sludge was not uniform through out the area. The samples were collected from representative regions, i.e.: (1) Near open pit inside the factory premises(northern side), (2) sample from waste dump site (western side), (3) sample from waste dump site (eastern side), (4) sample from waste dump site (southern side), and (5) sample from outside the premises near drain. All these sites were highly contaminated with Cr(VI). An amount of 500 g of soil from each site is taken, mixed well and dried in the oven at 60°C for 24 h, crushed the sample using a crusher to remove the boulders. A representative sample of the soil was characterized in the laboratory as per standard procedure.

2. Isolation of bacteria from the soil
   Bacterial colonies were isolated from chromium contaminated soil by soil dilution method using nutrient agar media and mineral salt media. Bacterial colonies isolates were identified based on cultural and morphological characteristics.

3. Extraction and analysis of Cr(VI) and total chromium
   For the extraction of Cr(VI) and total chromium from soil, an alkaline digestion method and nitric acid/sulfuric acid digestion method as per Standard Methods were used, respectively. The hexavalent chromium was measured colorimetrically at 540 nm by reaction with diphenyl carbazide in acidic conditions. In the case of Cr(III), potassium permanganate was used to oxidize Cr(III) to Cr(VI).

4. Measurement of cell density
   Overnight cultures were centrifuged, and the cell pellets was washed with physiological saline water thrice, resuspended in saline water, homogenized and was used as stock solution. Different dilutions were made from the stock solution. A known volume of these solutions were filtered through 0.45µm filter paper (Millipore, USA) to find out the dry weight of cells. Corresponding absorbance was measured at 440 nm using a spectrophotometer. This information was used to prepare a calibration curve, dry weight versus absorbance. For unknown samples, the absorbance was measured at 440 nm and was converted to dry weight using absorbance versus dry weight calibration curve.

III. EXPERIMENTAL METHODS

1. Enrichment of the Cr(VI) reducing bacterial strains
   Bacterial strains were isolated from the soil samples collected in and around the contaminated site. About 1 g of soil sample was added to 100 ml of growth medium (N1) and incubated for 24 hour in facultative condition. The shake flask cultures were closed using Teflon stoppers. Then, 1ml of this was transferred to 100 ml nutrient broth (N1) containing 100 ppm Cr(VI). This procedure was repeated by progressively increasing Cr(VI) in the nutrient medium up to 500 mg/l. A loopful from the above mixture was streaked on agar slants, incubated for 24 hour and stored in the freezer at 4°C for further use.

2. Screening of the Enriched cultures
This study was carried out in shake flasks with nutrient medium (N1) spiked with a Cr(VI) concentration of 50 mg/l. The enriched cultures obtained from seven locations of the contaminated site were evaluated for their chromium(VI) reduction potential based on the kinetics of Cr(VI) reduction and their specific Cr(VI) reduction potential. Specific Cr(VI) reduction potential was calculated as Cr(VI) reduction by unit biomass in unit time. Further studies were conducted using the most promising bacterial strain emerging in the screening test. For the screening test, eight conical flasks with 100 ml of autoclaved nutrient medium (N1) spiked with 50 mg/l Cr(VI) were inoculated with same quantity (measured as optical density) of previously grown bacterial cultures isolated from seven locations.

The residual Cr(VI) concentration in the liquid phase was measured with respect to time. Further studies to evaluate the effect of initial Cr(VI) concentration, Cr(VI) inhibitions, etc. were conducted using the selected bacterial isolate. For this, six conical flasks with 100 ml autoclaved nutrient medium (N1), were inoculated with the previously grown bacterial culture in log growth phase and incubated at 35°C for 24 h. Then stock potassium dichromate solution (stock solution) was added to the mixture so that the Cr(VI) concentration in conical flasks was 50, 100, 150, 200, 300 and 400 mg/l, respectively. Initial bacterial concentration was also measured. The Cr(VI) concentration, COD and bacterial concentrations were determined at regular intervals. A control (all conditions were same except there was no bacterial addition) was always employed to quantify the abiotic reduction of Cr(VI).

3. Effect of pH
The effect of pH on Cr(VI) reduction was studied by varying the reaction mixture pH from 4 to 10. The experiments were carried out with an initial Cr(VI) concentration of 50 mg/l.

4. Cr(VI) reduction in aerobic and anaerobic conditions
Cr(VI) reduction potential was studied under aerobic and anaerobic conditions with an initial Cr(VI) concentration of 50 mg/l in nutrient medium (N1). Two conical flasks were filled with 100 ml of nutrient medium (N1), autoclaved, and 1ml of previously grown H1 bacterial strains were added in each conical flasks, and incubated at 35°C for 24 h. Then stock potassium dichromate solution (stock solution) was added so that the Cr(VI) concentration in each conical flask was 50 mg/l. To achieve anaerobic condition one of the conical flasks with the reaction mixture was flushed with N2 through a filter for 5 min. Air tight caps were employed. The samples were withdrawn from this flask using syringes.

5. Evaluation of optimum conditions using miniature reactor
To evaluate the optimum conditions for bioremediation, miniature reactors were employed. Soil employed for all the biotransformation studies were sterilized in a hot air oven by keeping it at 150°C for 2 h. The miniature reactors were of 500 ml capacity glass beakers containing 25 g of contaminated soil. These received different concentrations of molasses and were inoculated with bacteria. The performance of the reactors was monitored regularly under anaerobic conditions. The schematic diagram of miniature reactor is shown in Fig.1.

![Schematic diagram of miniature soil bioreactor](image-url)
IV. RESULT AND DISCUSSION

1. Screening of microbes for Cr(VI) reduction

Bacterial cultures isolated from seven locations of the contaminated site were screened based on their Cr(VI) reduction capacity. Kinetics of Cr(VI) reduction was carried out under aerobic conditions with an initial Cr(VI) concentration of 50 mg/l for all the seven strains and the results are presented. Thus the strain was used for further studies. The strain which had a history of exposure to high Cr(VI) concentration has exhibited an excellent Cr(VI) reduction potential. The environmental stress might have forced the strain to develop an effective detoxification mechanism to survive in the adverse condition. It was also reported that many microorganisms isolated from the contaminated sites showed high resilience to a toxicant and hence, better pollution remediation potential. The specific Cr(VI) reduction potential was also higher for bacterial strain. Bacterial strains B and C also showed high specific chromium reduction.

Table 1: Bacterial strains employed for the present study

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Bacterial strains</th>
<th>Strain Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacillus cereus</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas fluorescens</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>Actinobacter species</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>Enterobacter species</td>
<td>D</td>
</tr>
<tr>
<td>5</td>
<td>Klebsilla species</td>
<td>E</td>
</tr>
</tbody>
</table>

2. Effect of pH

pH plays an important role in most of the biological systems. The preferable pH range in such systems is 6–8. The effect of pH on Cr(VI) reduction was studied for a wide range of pH 4–10 under aerobic conditions. The results are shown in Fig. 2. At neutral pH, the Cr(VI) reduction was found to be 100% within 3 h when an initial Cr(VI) concentration of 50 mg/l was exposed to a bacterial concentration of 670 mg/l. The reduction decreased with either increase or decrease of pH from neutral. Here, entire Cr(VI) was in liquid phase and when bioremediation of Cr(VI) for cleanup of contaminated soils is opted, then, adsorption and desorption characteristics of Cr(VI) as influenced by pH may determine the performance of the system. It was reported that Cr(VI) could be desorbed from soil at a faster rate at elevated pH values.

3. Cr(VI) reduction in aerobic and anaerobic conditions

Bioremediation of Cr(VI) was studied under aerobic and anaerobic conditions with an initial Cr(VI) concentration of 50 mg/l and the results are shown in Fig. 2. It was observed that under aerobic conditions, Cr(VI) reduction was high. However, as large areas have to be bioremediated under field condition, provision of aeration may not be economically feasible. Therefore, the anaerobic reduction/bioremediation option is evaluated further though it is slightly less efficient (90%) than the aerobic system.

4. Performance evaluation of soil reactor under optimum conditions

The soil samples at different depths were taken from the bioreactor and the control reactor and analyzed for Cr(VI). The results are presented in Figs. 3 and 4. The Cr(VI) reduction rate decreased with increase of depth. The soil samples collected from the bioreactor revealed that there was no Cr(VI) presence at different depths after 20 days. There was slight reduction of Cr(VI) observed in the control reactor. This might be due to abiotic reduction. The mass balance of Cr(VI) and total Cr is presented in Table 2. It was observed that 97±1.2% reduction of Cr(VI) occurred in the bioreactor. The initial mass of Cr(VI) present in the bioreactor was 4320 mg (2.7 mg/g) of soil in 1600 g of contaminated soil and the total Cr was 18 400 g (11.5 mg/g of soil). The amount of Cr(VI) and total Cr leached out along with the mineral medium was 123±1.5 and 127±1.7 mg, respectively. After 20 days the Cr(VI) present in the bioreactor was nil and total Cr was found to be 18 272 mg (average). It revealed that the Cr(VI) had converted to Cr(III). Hence percentage reduction of Cr(VI) to Cr(III) was observed as 97±1.2%. Similarly, the
amount of Cr(VI) and total Cr present in the control reactor were observed and the reduction of Cr(VI) to Cr(III) was 12±0.8%.

![Fig.2 Cr(VI) reduction in aerobic and anaerobic conditions](image1.png)

![Fig.3 Cr(VI) reductions in the soil with respect to depth in the control reactor](image2.png)

![Fig.4 Cr(VI) reductions in the soil with respect to depth in](image3.png)
Table 2: Mass balance of reactor under optimum condition, period of study: 20 days

<table>
<thead>
<tr>
<th>Initial mass of Cr(VI) (mg)</th>
<th>Initial mass of Total Chromium (mg)</th>
<th>Cr(VI) remained in the soil at the end of 20 days (mg)</th>
<th>Total Cr remained in the soil at the end of 20 days (mg)</th>
<th>Mass of Cr(VI) converted to Cr(III) (mg)</th>
<th>Reduction of Cr(VI) to Cr(III) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4305</td>
<td>15600</td>
<td>2546</td>
<td>15264</td>
<td>4196</td>
<td>97.47</td>
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V. CONCLUSIONS

A watershed area of 40 Sq.km covering the TNCCCL, Ranipet and its surrounding upto Palar river is beset with Hard Rock and Alluvium. The detail geophysical investigations, drilling of bore wells, chemical analysis of soil/water samples, tracer injection experiments and groundwater flow and mass transport modeling has helped in arriving a long term remediation plan to control/arrest the chromium contamination in and around TNCCCL dump site and its downstream upto Palar river.

The present study focuses on the ability of a mixed population of microbial culture isolated from hexavalent chromium contaminated site to biotransform Cr(VI). Batch and continuous experiments were carried out to study the biotransformation pattern of Cr(VI) with molasses as the electron donor. Among the bacterial strains isolated from five different locations of the contaminated site and made it to representative sample, the bacterial strain (B) which was isolated from a clay mat near the effluent treatment plant (ETP) showed high Cr(VI) reduction potential. A total of 56 isolates belong to five bacterial species were recorded. Isolated bacterial strains were listed here

- Bacillus cereus
- Pseudomonas fluorescens
- Actinobacter sp.
- Enterobacter sp.
- Klebsilla sp.

Optimum concentration of bacteria and molasses for the reduction of Cr(VI) to Cr(III) was observed as 15 and 34 mg/g of soil, respectively. The 97.47% reduction of Cr(VI) was achieved within 20 days under optimum concentration of molasses and bacteria in anaerobic conditions. The results of the present study suggest that indigenous microorganisms are capable of anaerobic reduction of high concentration of Cr(VI) in contaminated soil. Reduction of Cr(VI) to Cr(III) is a microbially mediated process, and provision of a suitable electron donor to contaminated soils shall greatly speed up this reaction, thereby decreasing Cr(VI) toxicity and mobility. The biotransformed Cr(VI) remained in the soil as Cr(III).

REFERENCES