Bioabsorption Potential of Acinetobacter sp. Strain IST 103 of Bacterial Consortium for Removal of Chromium from Tannery Effluent

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A mixed microbial community obtained from pulp and paper mill and tannery effluent was enriched in a chemostat in the presence of potassium chromate as sole source of carbon and energy. The microbial community of pulp and paper mill indicated significant reduction of chromium (70 per cent) in comparison to tannery community (60 per cent) in batch culture in the presence of potassium chromate and sodium acetate as additional carbon source. The community comprised three bacterial strains, identified as CP1, CP2 and CP3, were further applied for reduction of chromium indicated higher removal of chromium (68 per cent) by strain CP3 followed by CP2 and CP1. The strain CP3 was identified as Actinobacter sp. by biochemical test and Biolog test method. The bacterial strain, CP3, applied for the removal of chromium in tannery effluent in a sequential bioreactor indicated the reduction of chromium (80 per cent).

Keywords: Microbial community, Bioabsorption of chromium, Tannery effluent

Introduction

Most of the organic pollutants are destroyed by biological and chemical methods, but toxic metal ions released into the environment often persist indefinitely, circulating and eventually accumulating throughout the food chain, thus posing a serious threat to mankind. Pollution by chromium is of considerable concern as the metal has found widespread use in electroplating, leather tanning, metal finishing, and chrome preparation. Chromium occurs in aqueous systems in trivalent and hexavalent forms the latter is of particular concern due to its greater toxicity. Various methods employed for removal of heavy metals from effluent such as, physicochemical, precipitation of metals as hydroxide, carbonates and sulphides, adsorption on the activated carbon, use of ion exchange resins, and membrane separation processes, are responsible for the generation of pollution in addition to being expensive.

Biotransformation and biosorption are emerging technologies, which utilize the potential of microorganisms to either transform or adsorb metals. Intact microbial cells, live or dead, and their products can be efficient bio-accumulators of both soluble and particulate forms of metals. The cell surface of all microorganisms is negatively charged owing to the presence of various anionic structures. It gives bacteria the ability to bind metal cations. Microbial viability is essential for biotransformation of metals as these reactions are enzyme mediated. Generally, metal ions are converted into insoluble forms by specific enzyme mediated reactions which are then removed form the aqueous phase. Many researchers have studied live microbial systems for remediating contaminated soils and waters of heavy metals. More recently, phytoremediation has appeared as a cost-effective technology for the removal of metals from contaminated areas. Higher fungi (mushrooms), seaweed and plant bark are abundantly available in nature that can be a source of low-cost biosorbents. The use of microbial cells to serve as biosorbents of heavy metals is a potential alternative to conventional methods to decontaminate liquid wastes.

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Tanneries are responsible for the release of huge amounts of chromium into the environment. Untreated tannery effluents contaminate the land, ground- and surface-water with heavy metals like chromium. Physicochemical methods have been practiced for several decades for the removal of toxic heavy metals from such effluent. In the present investigation, bacterial consortia and bacterial strains have been developed by continuous enrichment and application of bacterial strains for removal of chromium as they either transform or absorb metals, as stated earlier, from industrial effluents.

Materials and Methods

Source of Microbial Community and Enrichment

Microbial population was obtained from sediment samples together with effluent (1:10, w/v) collected from sites of Century Pulp and Paper Mill Ltd, Lalkuan (CP), Uttarakhand, and main channel of tannery effluent, located at Jazmua, Kanpur (TC). The microbial population was enriched in mineral salt medium (MSM) containing (g/L) Na₂HPO₄, 2H₂O, 7.8; KH₂PO₄, 6.8; MgSO₄, 0.02; Fe₃(PO₄)₂, 0.01; Ca(NO₃)₂, 0.05; NaNO₃, 0.085, the trace element solution, 1 mL; and potassium chromate, 0.1 in the chemostat and the pH was maintained at 7.0-7.5 throughout the course of enrichment.

Structural Characterization of the Community

The medium was removed from the culture flask under aseptic conditions, and growth of bacterial community was determined by optical density (OD) at 540 nm. The culture medium was diluted serially in ten-folds, and 0.1 mL diluted culture medium was spread on nutrient agar plates and mineral salt as described above containing potassium chromate (100 mg/L) as a sole source of carbon and energy. The plate was incubated at 30 °C, and colony forming units (cfu) were determined after 24 h on nutrient agar plate and 72 h on mineral salt agar plates.

The microbial cells that appeared on the nutrient agar plate were characterized depending upon morphology of colonies based on diameter, colour, opacity, form elevation, margin smoothness, texture and spreading nature. Different colonies that appeared on nutrient agar plates, were streaked on another nutrient agar plates (Table 1). The process was repeated three times to ensure purity of each isolate. The morphologically distinct isolates were identified biochemically and by the Biolog test method.

Absorption of Chromium by Bacterial Community

The bacterial community was inoculated in MSM and potassium chromate (100 mg/L) in an Erlenmeyer flask for enrichment as described earlier and the pH was maintained at 7.5. Bacterial strains were also inoculated in MSM, potassium chromate (100 mg/L) and an additional carbon source (0.2 per cent) as sodium acetate, sodium citrate, L-glutamate, or glucose. Flasks were incubated at 30 °C on an orbital shaker at 175 rpm. Samples were collected on 0, 1, 3, 5, 7 and 15 d, and the growth of microbial cells was determined initially by measuring OD at 540 nm, and chromium was determined. The potency of bacterial strains purified from the nutrient-agar plates was also evaluated by the inoculation of strains in MSM containing potassium chromate as described earlier.

Bioreactor and Chromium Removal in Tannery Effluent

A laboratory scale sequential bioreactor was fabricated from 2 L glass column connected with another 2 L glass fractionation column sequentially as described earlier. The column was equipped with stirring (250 rpm) and aeration (500 mL/min). The enriched bacterial strain of the microbial community from the chemostat was applied for removal of chromium from tannery effluent in the immobilized

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Table 1—Morphological characterization of the bacterial strains capable of removing chromium

<table>
<thead>
<tr>
<th>Morphological characteristics</th>
<th>Isolates present in the bacterial community of pulp and paper mill</th>
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<tbody>
<tr>
<td>Shape</td>
<td>Isolate CP1</td>
</tr>
<tr>
<td>Size, mm</td>
<td>Circular</td>
</tr>
<tr>
<td>Colour</td>
<td>Yellow</td>
</tr>
<tr>
<td>Opacity</td>
<td>Opaque</td>
</tr>
<tr>
<td>Colony elevation</td>
<td>Raised</td>
</tr>
<tr>
<td>Colony margin</td>
<td>Entire</td>
</tr>
<tr>
<td>Texture</td>
<td>Not viscous</td>
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<tr>
<td>Spreading nature</td>
<td>Not</td>
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bioreactor comprising 3 cm layer of gravel placed at bottom of the columns as solid support for immobilization of bacterial cells. Retention time of 3 d was maintained under both treatment and control conditions. The flow inlet-outlet rate, 20.8 mL/h, was maintained in the bioreactor and pH was maintained at 7-7.5 throughout the course of the treatment.

The chromium removal in flask and bioreactor was determined, as described by Greenberg et al.\(^2\). The chromate medium was digested with HNO\(_3\)-H\(_2\)SO\(_4\) mixture, trivalent compound was oxidised, finally colour was developed and chromium was measured. A blank was prepared in the same manner by adding all reagents in distilled water, and colour developed was read at 540 nm against the blank. Standard curve for the determination of chromium was prepared using different concentrations of chromium.

**Results and Discussion**

Tanneries are mainly responsible for the release of huge amounts of chromium in the environment. There are about 3000 tanneries in India and they discharge a total wastewater of \(10^5\) m\(^3\)/d. The chromium in raw waste-water is 150-5000 mg/L. The present investigations concern with the removal of chromium from the wastewater by the isolated bacterial strain.

The bacterial population isolated from the sediment core of paper mill, CP and tanneries, TC were enriched in the chemostat containing MSM and potassium chromate (100 ppm). Both the bacterial communities were further enriched in shake flasks containing potassium chromate and additional carbon source. The growth pattern of the communities was recorded at different durations. Sorption of chromium by the consortia and the residual chromium were determined up to day 15 after digestion of chromium from the culture medium. The residual chromium after day 15 was not determined in this study. It was observed that there was increase in absorbance at different time intervals, showing chromium removal efficiency of both the communities. However, total chromium removed by PC consortium was 70 per cent, but the TC consortium was able to remove 40 per cent in the presence of co-metabolites (Figure 1). Data of the study indicated that removal of chromium by PC consortium was higher right from the beginning, however the potency of TC consortium was slow initially which reached to the highest on day 15 (Figure 2).

The physiological state of organism the age of cells the availability of micronutrients during their growth and the environmental conditions during the sorption process such as, pH, temperature, and presence of certain co-ions, and co-metabolites are important parameters that affect performance of a living sorbent\(^{18,19}\). The efficiency of metal concentration on the biosorbent is also influenced by metal solution chemical features\(^7\). Additional carbon source as co-metabolites has been used for bioremediation and bioabsorption of heavy metals from the industrial effluents. The most significant
ABSORPTION POTENTIAL OF ACINETOBACTER SP

finding emerging from the study was that more than 20 per cent growth of bacterial consortium and reduction of chromium in the presence of sodium acetate, 15 per cent in presence of glucose, 10 per cent in the presence of sodium citrate and 5 per cent in L-glutamate on day 15 in comparison to without the supply of additional carbon source. The data demonstrated that co-metabolites increased biomass initially which enhances the biosorption potential of bacterial cells employed, and helped in further reduction of chromium (Figure 1 and 2). The results of the study indicated that the potential for utilization of chromium by CP consortium was better than that of TC consortium, therefore, CP consortium was selected for enumeration of bacterial strains.

CP consortium was plated on nutrient agar plates. Morphologically, three bacterial strains were distinguishable on agar plates, designated as CP1, CP2 and CP3. They were partially purified by further streaking on nutrient agar plates, and finally on minimal salt agar plate containing potassium chromate (100 mg/L) for evaluation of absorption potential of the strains. The bacterial strains isolated from the CP community were separately grown on MSM and potassium chromate (100 mg/L) in batch culture. Conventional methods for metal removal from industrial effluent are either extremely expensive or inefficient from dilute solutions containing 1-100 mg/L dissolved metal\(^6\). In this context the biosorption process has been recently evaluated and optimized for pilot and scale up processes. In the present study bacterial strains were isolated, and potency for chromium removal was optimized in the presence of less chromium for the large-scale removal from industrial effluents.

Figure 3 shows the growth pattern of bacterial strains in terms of OD at 540 nm and removal of chromium at different durations. It is apparent that the growth of CP3 was much higher than the other two strains, CP2 and CP1. The absorption of chromium by the three strains also indicated that CP3 had higher chromium absorption capability than the other two. CP3 absorbed about 70 per cent chromium but CP2 and CP1 strains had capability to remove 45 per cent chromium, respectively (Figure 3). The actual reason for the biosorption of chromium is not known, but higher growth of CP3 strain may have absorbed chromium more efficiently than CP2 and CP1 mainly due to the biomass advantage. The mechanisms by which microorganisms remove metals from solutions are the extracellular accumulation/precipitation, cell-surface sorption or complexation and intracellular accumulation\(^4\).

It is believed that the uptake of chromium by bacterial strains has been complicated by the nature of both the absorbent and the metal species in aqueous solutions\(^6\). The cell wall of bacterial strains
ABSORBANCE AT 540 nm (Q)

Figure 3 — Growth pattern of bacterial strains of pulp and paper mill community and absorption of chromium in batch culture in mineral salt medium containing potassium chromate and sodium acetate. In figure growth is indicated as CP3, x-x, CP2, and CP1 ax. The absorption of potassium chromate indicated as CP3, CP2 and CP1 as

contain many potential sites for the uptake of ions and it is unlikely that any one type of molecule or functional group is responsible for the absorption of the metal\(^6,18\). Biosorption of metal ions primarily occurs by surface binding, including ion exchange reactions and complexation with the functional groups present on the cell surface of microbes\(^6,18,19\). Various functional groups believed to be involved in metal binding include carboxyl amine, hydroxyl phosphate, and sulphahydryl groups\(^6\).

The isolate CP3 had higher potency to remove chromium was identified by biochemical, and Biolog test methods. The bacterial isolate was gram negative, rod shaped, slightly curved cocci. Table 2 gives the biochemical characterization of CP3, which showed negative test for urease, casein hydrolysis, gelatin hydrolysis and oxidase, however, catalase, methyl red

citrate and indole production tests were positive. Based on biochemical tests the isolate was identified as \textit{Acinetobacter} sp. The isolates were further identified by commercial Biolog test method. On the basis of utilization of 95 carbon sources of Biolog test method the isolate indicated similarity with \textit{Acinetobacter calcoaceticus}. Bacterial strains, previously isolated from a chromium-polluted soil, were identified on the basis of Gram reaction and biochemical characteristics, and Biolog system tests\(^20\). The most Cr(VI)-resistant isolates were identified as \textit{Corynebacterium hoagie}, \textit{Pseudomonas} sp., \textit{Alcaligenes eutrophus}, \textit{Escherichia}, \textit{Aeromonas}, \textit{Enterobacter}, and \textit{Pseudomonas fluorescens}\(^20,21\). Isolates were also screened for the presence of plasmid DNA and it was observed that \textit{Pseudomonas} sp. harbored one and two plasmids of high molecular mass\(^20\).

In tannery effluent, higher amount of hexavalent chromium is present, which is toxic to living beings\(^21\). Microbial methods have been developed for the removal of chromium from effluents. Tannery effluent was collected from Jazmou, Kanpur, and solid particle was removed by screening with muslin cloth. Bacterial strain, CP3, was inoculated in the bioreactor. The bioreactor was designed for studying the biotreatability of chromium in tannery effluent by CP3. Several reports are available on principle and design of bioreactor\(^16\). To provide conditions resembling the nature the bioreactor consisted of

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Result of the test</th>
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<tbody>
<tr>
<td>Gram test</td>
<td>-ve</td>
</tr>
<tr>
<td>Gelatin hydrolysis</td>
<td>+ve</td>
</tr>
<tr>
<td>Catalase test</td>
<td>+ve</td>
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<tr>
<td>Urease test</td>
<td>-ve</td>
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<tr>
<td>Oxidase test</td>
<td>+ve</td>
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<tr>
<td>Methyl red test</td>
<td>+ve</td>
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<tr>
<td>Citrate test</td>
<td>+ve</td>
</tr>
<tr>
<td>Indole production test</td>
<td>+ve</td>
</tr>
<tr>
<td>Casein hydrolysis test</td>
<td>-ve</td>
</tr>
<tr>
<td>Isolate identified as</td>
<td>\textit{Acinetobacter calcoaceticus}</td>
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</table>
gravel in order to immobilize the biomass. Stirrer was applied for mixing inoculum in the effluent. The aeration made the reactor aerobic.

The growth of bacterial strain CP3 in bioreactor is shown in Figure 4. The figure shows that initially the growth increased and reached to the highest on 7th day. The efficiency of chromium removal by CP3 was better than the batch culture medium (Figure 4). As can be seen in the figure, as the growth of bacterial strain increased the potential of chromium removal also increased and reached 80 per cent on 15th day. In the bioreactor, bacterial strain achieved natural conditions and proper aeration and regeneration due to the presence of sodium acetate as additional carbon source. It is well known that the microorganism thrive under extreme conditions. The aeration made the reactor aerobic, hence suitable for the bacterial growth.

In addition, CP3 got immobilized onto the gravel substratum of bioreactor and formed microbial film over the gravel layer and this film reacted with the effluent. In bioreactors microbial cells act as metal-binding agents and hence they absorb metals. The main requirement of an industrial sorption system is that the microbes can be utilized as a fixed or expanded bed and it should not cause much pressure drop across the bed. Therefore, immobilized biomass for bioreactor may enhance metal-specific binding sites and help in the absorption of chromium. Immobilized biomass in bioreactor offers many advantages including better reusability, higher biomass loading and minimal clogging in continuous flow systems. Many matrices have been employed for immobilization of cells. One such matrix of insoluble Ca-alginate fluidized beds of Ca-entrapped cells of insoluble Ca-alginate was applied. Fluidized beds in bioreactor of Ca-entrapped cells of Chlorella vulgaris and Spirulina platensis were successfully used to recover gold from simulated gold bearing process. Therefore, in this study sequential bioreactor was applied for the removal of chromium from tannery effluent. In the present study we have evaluated the potency of isolated bacteria in biosorption of chromium. We could not determine the toxic effects of chromium at this stage. Chromium is toxic at higher concentrations. In addition fungal strains are isolated from tannery effluent for removal of chromium in our laboratory (data not shown). After proper evaluation of bacteria and fungi we will be in a position to demonstrate the two-step sequential bioreactor for removal of chromium in tannery effluent.

Acknowledgement

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References