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ORIGINAL PAPER

Evaluation of biosorption potency of *Acinetobacter* sp. for removal of hexavalent chromium from tannery effluent

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Abstract Two bacterial consortia were developed by continuous enrichment of microbial population of tannery and pulp and paper mill effluent contained *Serratia mercascens*, *Pseudomonas fluorescence*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter* sp. identified by 16S rDNA method. The consortia evaluated for removal of chromate [(Cr(VI)] in shake flask culture indicated pulp and paper mill consortium had more potential for removal of chromate. *Acinetobacter* sp. isolated from pulp and paper mill consortium removed higher amount of chromate [Cr(VI)] under aerobic conditions. Parameters optimized in different carbon, nitrogen sources, and pH, indicated maximum removal of chromate in sodium acetate (0.2%), sodium nitrate (0.1%) and pH 7 by *Acinetobacter* sp. Bacteria was applied in 2-l bioreactor significantly removed chromate after 3 days. The results of the study indicated removal of more than 75% chromium by *Acinetobacter* sp. determined by diphenylcarbazide colorimetric assay and atomic absorption spectrophotometer after 7 days. Study of microbial [Cr(VI)] removal and identification of reduction intermediates has been hindered by the lack of analytical techniques. Therefore, removal of chromium was further substantiated by transmission electron microscopy (TEM), scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) which indicated bioaccumulation of chromium in the bacterial cells.

Keywords *Acinetobacter* sp. · Biosorption · Chromate · Transmission electron microscopy · Scanning electron microscopy · Energy-dispersive X-ray spectroscopy

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Introduction

Chromium is a serious environmental pollutant due to its wide use in industries such as tanning, corrosion control, plating, pigment manufacture, and nuclear weapons production. The geochemistry and toxicity of Chromium (Cr) are controlled by valence state. Chromium is a redox active 3d transition metal with a wide range (–2–+6) of possible oxidation states, of which only two are stable. Thermodynamic calculations predict that soluble [Cr(VI)] is energetically favoured for oxic conditions, while insoluble [Cr(III)] is favoured under anoxic or suboxic conditions. Hexavalent chromium [Cr(VI)] compounds are considered to be extremely toxic, mutagenic and carcinogenic (Pellerin and Booker 2000). The compounds are known to have a strong oxidizing

activities and cause various biological damage. The reduction of the hexavalent ion level of the compounds is very important for the protection against the environmental damage. Recently it is reported that some bacteria reduced the [Cr(VI)] to [Cr(III)], and resulted in significant reduction of the toxicity (Park et al. 2000; Ackerley et al. 2004). Therefore, microbial [Cr(VI)] reduction is of particular technological and biological importance because it converts a toxic, mobile element into a less toxic, immobile form.

Conventional methods as precipitation, oxidation/reduction, ion exchange, filtration, membranes and evaporation are extremely expensive or inefficient for metal removal. In this context, the biosorption process has been recently evaluated (Volesky and Holan 1995). Although living and dead cells are capable of metal accumulation, there are differences in the mechanisms involved, depending on the extent of metabolic dependence (Beveridge and Murray 1976). The physiological state of the organism, the age of the cells, the availability of micronutrients during their growth and the environmental conditions during the biosorption process (such as pH, temperature, and presence of certain co-ions), are important parameters that affect the performance of a living biosorbent. The efficiency of metal concentration on the biosorbent is also influenced by metal solution chemical features (Blake et al. 1993; Srivastava and Thakur 2006). Recent developments in the field of environmental biotechnology include the search for microorganisms as biosorbents for heavy metals. Bacteria, fungi, yeast and algae can remove heavy metals from aqueous solution in substantial quantities. The uptake of heavy metals by biomass can take place by an active mode (dependent on the metabolic activity) known as bioaccumulation or by a passive mode (sorption and/or complexation) termed as biosorption (Volesky and Holan 1995). The pH of the solution strongly affects the degree of biosorption of chromium on biomass. At the higher pH the removal of Cr(III) is more and the less removal of Cr(VI).

Study of microbial Cr(VI) reduction, such as identification of reduction intermediates has been hindered by the lack of analytical techniques that can identify oxidation state with subcellular spatial resolution. The most common method for measuring Cr(VI) reduction in bacterial cultures is the diphenylcarbazide colorimetric assay in which Cr(VI) concentration is determined from absorbance at 540 nm by the stoichiometric oxidation products of diphenylcarbazide reagent. However, this bulk technique cannot provide the submicron-scale information necessary for understanding microbial reduction processes. Conventional transmission electron microscopy (TEM) studies of microbial reduction of metals have provided important information. Hexavalent chromium species are strong oxidants, high solubility, bioavailability, and toxicity make it a particular environmental concern. Microbial mechanisms for Cr(VI) reduction are of particular technological and biological importance because they convert a toxic, mobile element into a less toxic, immobile form (Park et al. 2000; Garcia-Arellano et al. 2004). Tanneries are mainly responsible for the release of huge amount of chromium in the environment. Pentachlorophenol (PCP) is another toxic compounds used in the leather tanning processes are refractive for the growth of microorganism and it also reduces removal of chromium in tannery effluent (Shrivastava and Thakur 2003).The microbial strains have the potency to degrade PCP and bioaccumulate chromium simultaneously could be a major biological source for removal of major contaminants from tannery effluents (Srivastava et al. 2007). Therefore, in the present study bacterial population from tannery and pulp and paper mill effluents are enriched by continuous culture in the chemostat for isolation and identification of potential bacterial strains for removal of chromium, process parameters are optimized in presence of toxic form of chromium, and finally removal of chromium was identified by analytical techniques.

Materials and methods

Sample collection and culturing of bacteria

The sediment sample together with liquid effluent (1:10 w/v) were collected from three sited of main channel of tanneries located at Jazmau,

Kanpur, Uttar Pradesh, India, and Century Pulp and Paper mill, Lalkua, Uttaranchal, India. Microorganism was extracted from sample by centrifugation at 900 rpm for 5 min. The supernatant was used for enrichment in mineral salt medium containing sodium pentachlorophenol (100 mg/l) as sole source of carbon in the chemostat (Thakur 1995). The media were prepared in a minimal base of following composition (g/l): Na_2HPO_4, $2H_2O$, 7.8; KH_2PO4, 6.8; $MgSO_4$, 0.2; ammonium ferric citrate, 0.01; $Ca(NO_3)_2$, $4H_2O$, 0.05; $NaNO_3$, 0.085, PCP, 0.1 and trace element solution, 1 ml/l (Thakur 1995; Pfennig and Lippert 1966). Samples of the culture were collected under aseptic conditions. The growth of the bacterial community was determined by measuring the optical density at 540 nm. Samples were diluted and plated on nutrient agar plate (0.1 ml/plate). The bacterial colonies appearing on nutrient agar plates were morphologically characterized and purified by repeated culturing. The morphologically distinct isolates were identified by 16S rDNA analysis (Moore et al. 1993).

Batch culture studies and removal of chromium

The bacterial isolates grew in minimal salt medium containing potassium chromate as described above (Thakur 1995). The salt of potassium chromate (500 ppm) was used as source of hexavalent chromium. The pH was maintained at 7.0, and incubated at 30°C in a rotatory shaker for 7 days. Ten ml samples from the culture flasks were drawn at 0, 1, 3, 5 and 7 days, and chromium was measured. On the basis of chromium analysis, percentage removal was studied by each isolates along with the control.

Optimization of process parameters for biosorption of chromium

Batch culture was performed in Erlenmeyer flasks containing tannery effluent for optimization of carbon source as co-substrate. Dextrose, sodium acetate, sodium citrate and sucrose were used at the rate of 0.2% (w/v) of the effluent i.e., 2g/l, pH adjusted to 7.0. It was then inoculated with 10% (w/v) of PCP3 strain. These flasks were incubated for 7 days at 30°C under shake flask condition in rotary shaker. Sampling was done at an interval of 0, 1, 3, 5 and 7 day's duration. As nitrogen source glutamate, urea and sodium nitrate were used. Similar procedure as carbon was adopted for optimization of nitrogen source. The rate of nitrogen source, inoculum size, incubation period, temperature, pH and sampling interval were also similar. The optimization of pH, for the treatment of tannery effluent was adjusted at pH 3, 5, 7 and 9 respectively and supplemented with MSM and C:N (0.1%:0.2%:dextrose:sodium nitrate). It was inoculated with 10% (w/v) inoculums and incubated at 30°C for 7 days under shake flask condition. Sampling was done at an interval of 0, 1, 3, 5 and 7 days, respectively.

Laboratory scale removal of chromium in tannery effluents by bacteria

A laboratory scale bioreactor was made from 2 l glass column. The column was equipped with stirring and aeration facilities. The column was loaded with tannery effluent, and inoculated by PCP3 bacterial strain. The flow inlet-outlet rate 20.8 ml/h was maintained in bioreactor. The pH was maintained between 7 and 7.5 throughout the course of treatment. Samples of treated tannery effluent were removed at 0, 1, 3, 5, 7 and 15 days interval, samples were centrifuged, and both supernatant and pellets was used for the determination of chromium. In supernatant percent removal of chromium was analysed while in bacterial pellets uptake of chromium was estimated.

Analysis of chromium from the effluents and bacterial cell biomass

Sample from each experiment flask(s) was drawn on the following 0, 1, 3, 5 and 7 days. Sample was centrifuged at 10,000 rpm for 10 min at 4°C. The presence of chromium was determined in supernatant and bacterial cell. Supernatant (50 ml of sample) was mixed with concentrated HNO_3 (5 ml) and boiling chips. The content was boiled and evaporated to 16–20 ml on hot plate.

Concentrated HCl (5 ml) was added and boiled again. The solution was boiled till sample become clear and brownish fumes were evident. Finally it was cooled and diluted up to 50 ml with distilled water. Aliquot of this solution was used for determination of the concentration of total chromium with the help of flame atomic absorption spectrophotometer (GBC, Avanta–Sigma, USA) and chromium (VI) by spectrophotometer (Greenberg et al. 1995).

Pellets were transferred in the known weight sterile crucible and it was dried overnight at 60°C in the oven. Dried pellets were acid lysed overnight by 5 ml aquaregia. Acid lysed pellets were incinerated on sand bath for 6 h at slow heating (40–45°C) till the white residue formed. One ml of concentrated HNO_3 and perchloric acid (60%) in the ratio 6:1 was added and kept for incineration on sand bath for 3 h. The concentration of chromium ions (ppm) in the respective samples, pellet as well as supernatants, were analyzed with the help of flame atomic absorption spectrophotometer at 357.9 nm wave length and having optimum working range 0.2–10 ppm. The used flame type was air-acetylene (oxidizing) with lamp current 4 mA (Greenberg et al. 1995).

Scanning electron microscope and energy dispersive X-ray spectrometer

Cells were fixed as described above were smeared over the cover slip coated with poly-L-lysin for 30 min in wet condition (Baldi et al. 1990). The specimen was washed with buffer, dehydrated in a series of ethanol-water solution (30, 50, 70 and 90% ethanol, 5 min each), and critical point dried under a CO_2 atmosphere for 20 min. Mounting was done on aluminium stubs, and cells were coated with 90 Å thick gold-palladium coating in polaron Sc 7640 sputter coater (VG Microtech, East Sussex, TN22, England) for 30 min. Coated cells were viewed at 15 kV with scanning electron microscopy (Leo Electron Microscopy Ltd., Cambridge). Dx4 Prime Energy Dispersive X-ray spectrometer (EDAX, USA) was performed at 20 kV for confirming the bioaccumulation of chromium in the bacterial cell. X-ray absorption spectroscopy provides information on the electronic and structural state of an element.

Transmission electron microscopy

Transmission Electron Microscopy was performed in bacterial cells fixed in glutaraldehyde (1% solution) and paraformaldehyde (2%) buffered with sodium phosphate buffer saline (0.1 M, pH 6.8). Fixation was for 12–18 h at 4°C temperature, after which the cells were washed in fresh buffer, and post fixed for 2 h in osmium tetraoxide (1%) in the same buffer at 4°C. After several washes in buffer, the specimens were dehydrated in graded acetone solutions and embedded in CY 212 araldite. Ultrathin section of 60–80 nm thickness were cut using an ultracut E, Ultramicrotome and the sections were stained in alcoholic uranyl acetate (10 min) and lead citrate (10 min) before examining the grids in a transmission electron microscope (Morgagni 268 D TEM, Fei company, Neitherland) operated at 60–80 kV (David and Herbert 1973)

Results

Biosorption of chromium in batch culture experiments

The microbial population was enriched in the chemostat to obtain bacterial strains having the potency for degradation and removal of PCP and chromium, respectively from industrial effluent. The members of bacterial consortia, tannery and pulp and paper, were purified by repeated culturing on LB-agar plates and strains were identified by 16S rDNA analysis. The tannery consortium have four members which named as TE1, TE2, TE3 and TE4 were identified as *Serratia mercascens* and *Pseudomonas fluorescens*, and Pulp and paper mill consortium have three members which named as PCP1, PCP2 and PCP3, identified as *E. coli*, *Pseudomonas aeruginosa* and *Acinetobacter* sp. (Table 1).The strains isolated from the consortium were resistant, and had degradation potency to PCP. The pulp and paper mill consortium had higher utilization to PCP as well as biosorption of chromate. Due to inherent problem of chromium and PCP in tannery effluents, methods are adopted to isolate suitable consortium for removal of chromium (Srivastava et al. 2007).

Table 1 Members of the bacterial consortia developed by continuous enrichment of microbial population of pulp and paper mill effluent (pulp and paper mill consortium) and tannery effluent (tannery consortium)

S.No.		Gene Bank Accession No.
16S rDNA sequencing of members of pulp and paper mill consortium		
1.	*Escherichia coli* (PCP1)	AY131333
2.	*Pseudomonas aeruginosa* (PCP2)	AY131332
3.	*Acinetobacter* sp. (PCP3)	AF291188
16S rDNA sequencing of members of tannery consortium		
1.	*Serratia marcescens* (TE1)	AF536218
2.	*Serratia marcescens* (TE2)	AF536219
3.	*P. fluorescens* (TE3)	AF137203
4.	*Serratia marcescens* (TE4)	AF536220

In this study, initially both PCP consortium and tannery consortium was tested in MSM containing potassium chromate (as source of chromate) for removal of chromium. Percent chromium removal and uptake of chromium by tannery and paper mill consortium was observed during the period of 7 days. There was very less removal of chromium (5%) in control at day 7. The result of the study indicated PCP consortium and tannery consortium removed 86% and 60% chromium, and uptake of chromate in cell biomass was 7.9 and 3.2 mg/g respectively from MSM-potassium chromate solution (Fig. 1).

Batch culture study was further performed for the chromate biosorption by bacterial strains of PCP consortium. Results of comparative study of all three strains for biosorption of chromate show significant removal of chromate. Percent chromium removal by PCP3 strain was 86%, while PCP1 and PCP2 removed 45% and 55%, respectively at day 7. Uptake of chromate in cell biomass was 19.7, 10.6 and 3.2 mg/g of dry wt. of cells for PCP3, PCP2 and PCP1, respectively. The results were interpreted with abiotic control data, which indicated less removal (8%) of chromate (Fig. 2).

Optimization of process parameters in tannery effluent

Process parameters for removal of chromate were performed in presence of carbon, nitrogen and pH. Four carbon sources, dextrose, sucrose, sodium citrate and sodium acetate were taken for the optimization of process parameters. The results of the study indicated that percent removal of chromium in presence of sodium

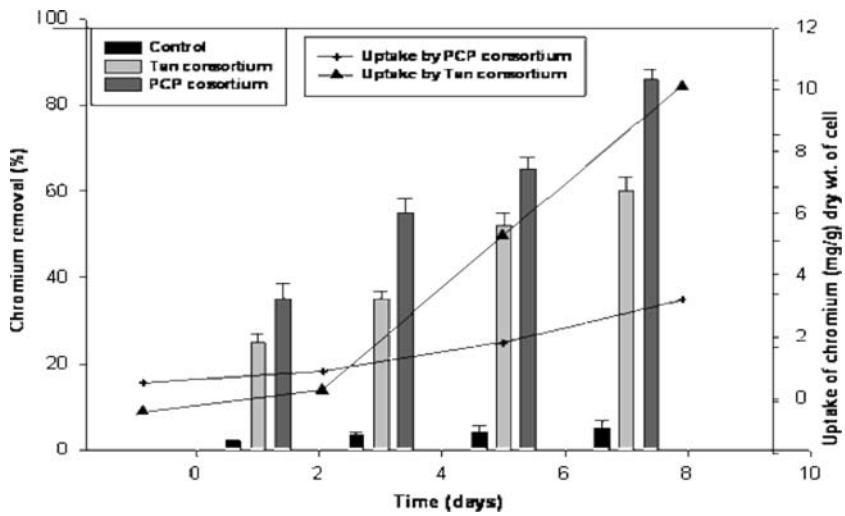

Fig. 1 Percent chromium removal and uptake of chromate by the consortium developed after continuous enrichment of microbial population from tannery and paper mill effluent in the batch culture (Error bars are standard deviation)

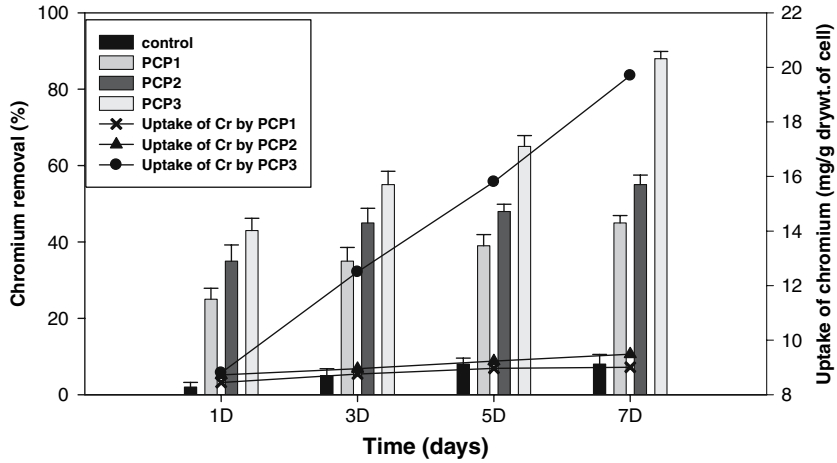

Fig. 2 Percent chromium removal and uptake of chromate by bacterial strains of the PCP consortium (PCP1, PCP2 and PCP3) in batch culture study. Within each group, values not followed by the same letter are significantly different at $P \leq 0.05$ (Error bars are standard deviation)

acetate was higher than the other. Data indicated 89% chromate removal in presence of sodium acetate as compare to control (i.e., untreated where 5% chromium was removed at day 7), and uptake of chromate was 13.3 mg/g dry wt. of cells. Dextrose was observed second most significant carbon sources where 70% removal of chromate and uptake of chromate 7.58 mg/g dry wt. of cells was observed (Fig. 3a).

Three nitrogen sources, sodium nitrate, urea and glutamate were taken for the optimization of process parameters for the chromate removal. The results in Fig. 3b shows that percent removal of chromium in presence of sodium nitrate was 85% and uptake of chromate was 13.5 mg/g dry wt. of cells followed by glutamate that showed 78% reduction of chromate as compare to control (i.e., untreated where removal of chromium, 5%, at day 7) and uptake of chromate was 6.7 mg/g dry wt. of cells. Optimization of pH for chromate removal by PCP3 strain was studied in batch culture in pH range 3–10. The result of the study indicated higher removal of chromium (88%) and uptake of chromate in cells was 12.3 mg/g dry wt. of PCP3 strain at pH 7.0 as compare to untreated (removal of chromium, 5%, in control at day 7). However at pH 5.0, removal of chromate was 78% as compare to control (untreated, removal of chromium 5% at day 7) and uptake of chromate in cells was 9.28 mg/g dry wt. of cells was observed (Fig. 3c).

Potential of bacteria for removal of chromium in tannery effluent

In order to compare the results of batch scale, bioreactor studies were carried out in a glass column of capacity 2 l with all necessary arrangement. During the startup, 1,300 ml of fresh effluent sample with all contaminants was adjusted to pH 7.0. The effluent to be treated was enriched with (0.2%) sodium acetate and (0.1%) sodium nitrate. The total concentration of chromium initially in tannery effluents was 557 ppm. The results of chromate removal by PCP3 strain is presented in Fig. 4. During the treatment process in bioreactor, removal of chromium by bacteria was 80% (112 ppm) and uptake in bacterial cell was 19 mg/g dry wt. of cell at 15 days, where as in control (tannery effluents without bacterial inoculums) only 12% chromate was removed at day 15.

Scanning electron microscopy and energy dispersive X-ray analysis

Assessment of morphological changes in response to chromium accumulated in bacterial strain, *Acinetobacter* sp., and quantification of chromium within bacterial strains was performed by SEM and Energy Dispersive X-ray analysis (EDX) analysis. SEM analysis of bacteria (*Acinetobacter* sp.) was shown at 24 h incubation without chromate exposure (Fig. 5a). The bacterial cells were

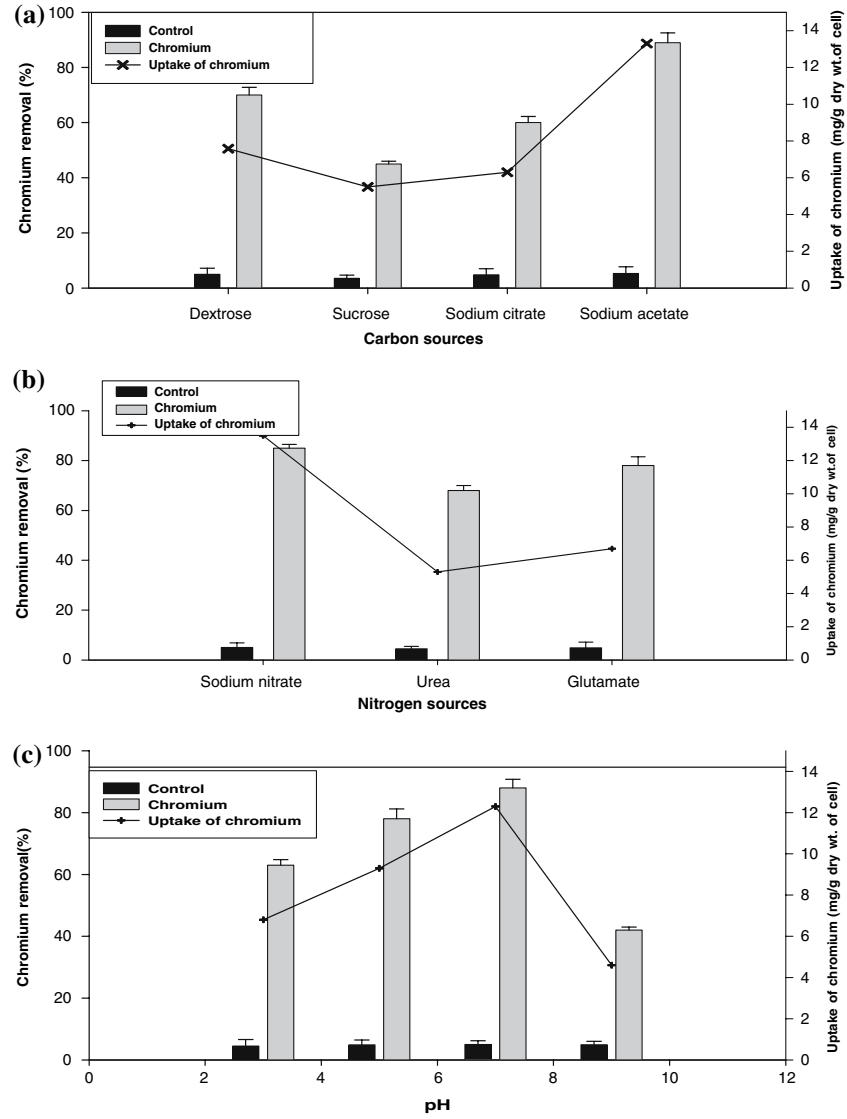

Fig. 3 Optimization of process parameters for removal of chromate in presence of carbon (**a**) and nitrogen source (**b**) and pH (**c**) by PCP3 strain (Error bars are standard deviation)

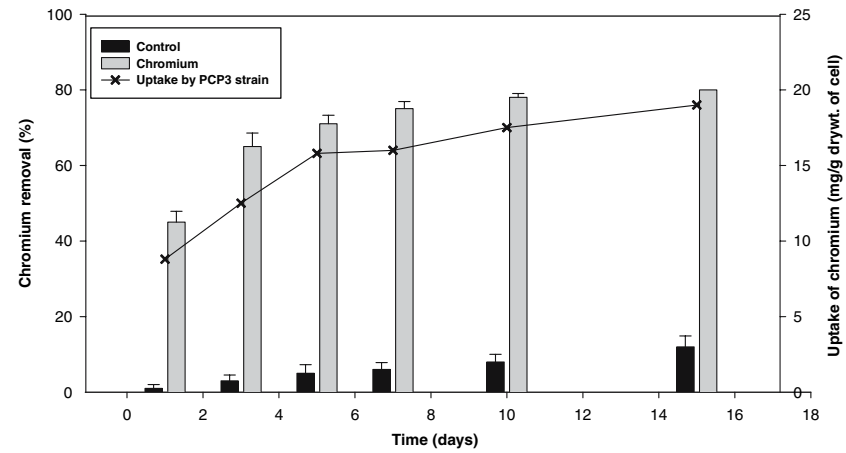

Fig. 4 Removal of chromium in tannery effluent by PCP3 strain (*Acinetobacter* sp.) in the glass column bioreactor (Error bars are standard deviation)

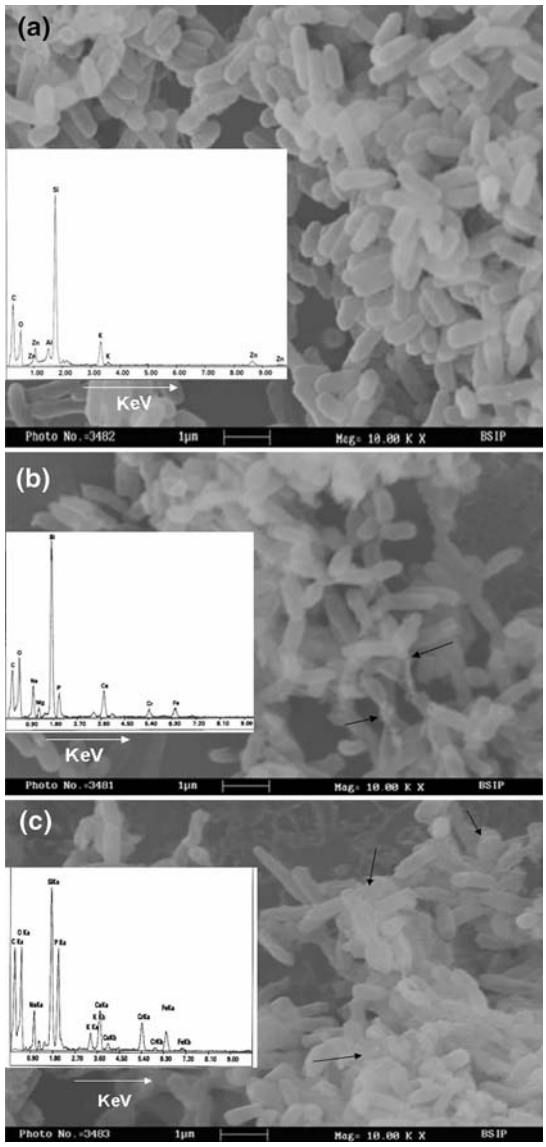

Fig. 5 Scanning Electron Microscopy and Energy Dispersive X-Ray analysis of bacterial strain, *Acinetobacter* sp. no chromium, control (**a**) biosorption of chromium in the cells after 24 h incubation, arrow indicated precipitates of chromium (**b**) biosorption of chromium in the cells after 48 h incubation (**c**) arrow indicated precipitates of chromium

short rod shaped having smooth surface, and there was no peak of chromate at 5.4 keV after 24 h incubation determined by EDX (Fig. 5a). However, when chromate exposed (100 ppm) bacterial cell at 24 h and 48 h incubation was applied for SEM–EDX, it revealed that chromium was uniformly bound to the *Acinetobacter* sp. cells wall having ridges and changes in morphology due to exposure of chromate to the cells was observed (Fig 5b, c). In addition, Electron micrograph presented in Fig. 5b after 24 h incubation have shown some round shiny uniformly distributed bodies over the surface of bacterial cell wall. It is predicted to the metal precipitate on the surface of the cell wall of bacteria.

Transmission Electron Microscopy (TEM)

Chromium accumulation in bacterial cell was observed by TEM for the identification of chromate accumulation within cells of microorganism. TEM of *Acinetobacter* sp. was also performed in control (Fig. 6a) and exposed cells (Fig. 6b, c). TEM photograph of morphological changes in PCP3 strain due to expose of chromate revealed circular electron dense (dark black point) inclusion within the cell cytoplasm, indicated that Cr (III) was adsorbed on surface of microbial cells, and precipitate was also dispersed in the bulk solution.

Discussion

The chromium hexavalent compounds are known to have a strong oxidizing activities and cause various biological damage. The removal of the hexavalent ion level of the compounds is very important for the protection against the environmental damage. The enzyme of Cr (VI) reductase reduces the compounds to the relative stable and non toxic Cr (III) state. The bacteria strain was known in the mechanism of aerobic Cr (VI) reduction based on the subcellular location and kinetic parameters of NADH-dependent Cr (VI) reductase activity (Ackerely et al. 2004; Gonzalez et al. 2005). The chromate reductase-encoding gene were cloned, purified and characterized a novel soluble chromate reductase from *Pseudomonas putida* (Park et al. 2000).In this study efforts have been made to isolate genetically potential bacteria for removal of the hexavalent chromium. PCP is highly toxic and recalcitrant compounds used in tannery as biocide. Most of the microorganism has not the capability to grow on PCP and adsorb

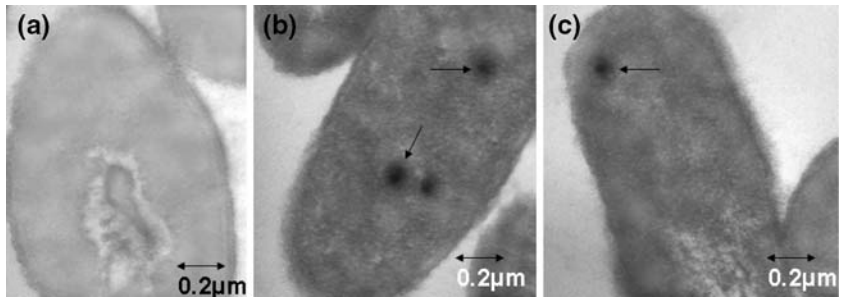

Fig. 6 Transmission Electron Microscopy of *Acinetobacter* sp. (**a**) chromium (Potassium chromate) uptake by *Acinetobacter* sp., no chromium, control (**b**) and (**c**) indicated longitudinal section of *Acinetobacter* sp. cells (0.2 μm). Arrow indicated intracellular accumulation of chromium

the chromium; therefore, biological treatment method of biosorption of chromium has not been successful.

The bacterial community of tannery and pulp and paper mill effluent enriched in presence of PCP as sole carbon source in the chemostat had potency to biosorb and remove chromate. The result of the study indicated enrichment of bacterial strain and isolation and identification of *Acinetobacter* sp. which had higher potency to remove chromium. Bacterial strains having the potency to degrade PCP and biosorption potency of chromium would be most successful biological entities for removal of contaminants from tannery effluent. Therefore, microbial population was obtained after enrichment in the chemostat from tannery effluent which is contaminated by chromium, and pulp and paper mill effluent contaminated by chlorinated phenol including PCP.

Process parameters for removal of chromate were performed in presence of carbon, nitrogen and pH. The purpose of this experiment was determination of the growth of bacterial cells and finally removal of chromium in presence of carbon and nitrogen source. In this study in presence of sodium acetate and sodium nitrate bacteria successfully removed significant amount of chromium. The other parameter for evaluation of the effect on biosorption is pH. The result of the experiment indicated higher removal of chromium at pH 7. The effects of pH on metal biosorption have been studied by many researchers, and the results demonstrated the increasing cation uptake with increasing pH values (Kapoor and Viraraghvan 1995).

The biosorbed chromate was assumed to be Cr(III), as Cr(VI) is reduced to Cr(III) in the living cells due to reducing environment and enzymes present inside the cell. Cr(III) is free to bind ionic sites and once bound act as a template for further heterogeneous nucleation and crystal growth. The result of the study indicated enrichment of bacterial strain cells performed by SEM–EDX and TEM analysis, gave confirmation of chromate accumulation with in bacterial cells. This could be due to precipitation of Cr(III) inside the cells in the form of hydroxyl and carboxyl group (McLean and Beveridge 2001). The mechanisms of metal binding are not well understood due to the complex nature of microbial biomass, which is not readily amenable to instrumental analysis (Kapoor and Viraraghavan 1995). However, localization of metals has been carried out using electron microscopic and X-ray energy dispersive analysis studies. X-ray photo electron spectroscopy for chemical analysis studies is a relatively new technique for determining of binding energy of electrons in atoms/molecules which depends on distribution of valence charges and thus gives information about the oxidation state of an atom/ion. The binding of chromate to the cell wall due to presence of electronegative surface functional groups i.e., carboxyl, hydroxyl and phosphoryl (Leusch et al.1995).

Electron microscopic observation carried out by Mullen et al. (1989) revealed the presence of Ag^{2+} as discrete particles at or near the cell wall of both gram positive and gram negative bacteria and the presence of silver was confirmed by EDX. McLean and Beveridge (2001) have reported that

Pseudomonad (CRB5) was reduced toxic chromate to insoluble Cr (III) precipitation under aerobic and anaerobic condition. De Leo and Ehrlich (1994) reported that formation of Cr(III) precipitates was not evident in batch and continuous cultures of *P. fluorescens* LB300 whereas *Pseudomonas chromatophillica*, *P. ambigna* and *Pseudomonas maltophilia* all induce the formation of precipitates due to accumulation of metal. Microorganisms have excellent nucleation sites for grained mineral formation, due to their high surface area and volume ratio and the presence of electronegative charges on the cell wall (Beveridge 1988). Surface functional groups (e.g., carboxyl, phosphoryl and hydroxyl) play major role in bioaccumulation of metals and significantly removed chromate which is toxic.

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