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Full Length Research Paper

Detoxification of hexavalent chromium by an indigenous facultative anaerobic *Bacillus cereus* strain isolated from tannery effluent

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A chromate resistant facultative anaerobic bacterial strain (FA-3) was isolated from the treated tannery effluent of Jajmau, Kanpur (India) and was identified as *Bacillus cereus*. FA-3 was tolerant to 1400 µg/ml of Cr (VI) and reduced a maximum of 72% Cr (VI) at 1000 µg/ml chromate concentration. The rate of growth of *B. cereus* decreased with the increase in Cr (VI) concentration of the medium and the chromate reduction was directly correlated to the growth of the strain. The strain FA-3 was capable of reducing Cr (VI) under a wide range of temperatures (25 to 40°C) and pH (6 to 10) with optimum at 37°C and initial pH 8.0. Glucose (0.5%) slightly increased the Cr (VI) reduction (78%). Heavy metal ions such as Zn^{2+}, Ni^{2+} and Co^{2+} slightly affected the Cr (VI) reduction, while arsenic (As^{3+}) significantly affected the Cr (VI) reduction. However, in the presence of Hg^{2+}, the reduction of Cr (VI) was totally inhibited. Since, the strain has potential to survive and multiply in a wide range of environmental conditions and due to its high Cr (VI) reduction efficiency in the presence of other metal cations, it can be exploited for the bioremediation of chromate containing wastes.

Key words: Treated tannery effluent, chromate resistant bacteria, minimal inhibitory concentration, heavy metal ions.

INTRODUCTION

Heavy metals are ubiquitous and persistent environmental pollutants that are introduced into the environment through anthropogenic activities (Teitzel and Mathew, 2003; Achal et al., 2011). These metals exert a deleterious effect on the flora and fauna that grow in lakes and streams. The pollution caused by heavy metals in wastewater has always been a very serious problem because these elements are not biodegradable and can accumulate in living tissues (Deng et al., 2006). Chromium (Cr) is one of the toxic environmental pollutants due to its wide use in industries such as tanning, corrosion control, plating, pigment manufacture and nuclear weapon production. The extensive industrial usage of Cr compounds and subsequent release of effluents, without proper treatment in the environment contaminated the ecosystem. Chromium exists in several oxidation states, but the most stable forms are trivalent chromium [Cr (III)] and hexavalent chromium [Cr (VI), chromates] species, with different chemical characteristics and biological effects (Cervantes et al., 2001; Avudainayagam et al., 2003; Nath et al., 2009). Trivalent chromium is considered to be relatively innocuous, compared with bioavailable Cr (VI) compounds in the form of chromate (CrO_4^{-2}) and dichromate ($Cr_2O_7^{-2}$), that are highly toxic and have been shown to be mutagenic and carcinogenic (Zayed and Terry, 2003). In recent years, contamination of the environment by Cr especially hexavalent chromium has become a major area of concern. Towards this direction, several conventional wastewater treatment technologies were developed and are used successfully at large scale,

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to reduce the hazardous compounds concentration in effluents from higher to lower levels (Verma and Rahal, 1996).

The conventional methods to detoxify and remove Cr (VI) from the environment involve chemical reduction followed by precipitation, ion exchange and absorption on coal, activated carbon, alum, kaolinite and flyash (Ohtake and Silver, 1994; Arundhati and Paul, 2004; Ahluwalia and Goyal, 2007). Application of such traditional treatment techniques need enormous cost and continuous input of chemicals which becomes impracticable and uneconomical and causes further environmental damage. Hence, easy, economic and eco-friendly techniques are required for fine tunning of effluent/wastewater treatment. This problem has stimulated interest in microorganisms and biological methods are now being explored for metal decontamination as alternatives to conventional methods due to their eco-friendly nature. Biological reduction of Cr (VI) using indigenous microorganism offer a new cost-effective and environmentally compatible technology (Camargo et al., 2005; Liu et al., 2006).

Several reports have indicated biological reduction of Cr (VI) by microorganisms, both by aerobes and anaerobes (Wang and Shen, 1995; Philip et al., 1998; Pal and Paul, 2004; Ma et al., 2007; Farag and Zaki, 2010; Sharma and Adholeya, 2012). However, there is scanty information on Cr (VI) detoxification by facultative anaerobic bacteria (Francis et al., 2000; Srinath et al., 2001; Ge et al., 2012). In facultative anaerobes, both soluble and membrane associated reductase enzymes were found to mediate the process of Cr (VI) reduction (Cheung and Gu, 2007). Unlike the Cr (VI) reductases isolated from aerobes, the Cr (VI) reducing activity of facultative anaerobes are associated with their electron transfer system ubiquitously catalyzing the electron shuttle along the respiratory chains. The widespread occurrence of facultative anaerobes possessing Cr (VI) reducing activities will offer great potential for *in situ* bioremediation of Cr (VI) contaminated sediments; which would only require the supplementation of nutrients and the modulation of physical conditions to facilitate the reaction (Turick et al., 1996). Therefore, the present investigation was aimed to isolate and characterize the potent facultative anaerobic bacteria from tannery effluent and to evaluate its potential for the detoxification of Cr (VI) to Cr (III) under wide environmental conditions.

MATERIALS AND METHODS

Sampling and physico-chemical analyses of the treated tannery effluent

The treated tannery effluent (TTE) was collected from common effluent treatment plant (CETP), Unnao, U.P. (India) in sterile glass bottles, was transported on ice to the laboratory and processed within 6 h of collection. The effluent was analyzed for different physico-chemical properties viz. pH, temperature, BOD, COD, sulphate, phosphate and presence of different heavy metal ions. The concentration of each of the component was determined as per the procedure outlined in APHA (2005).

Isolation of chromate resistant bacterial strains

Chromate resistant bacterial strains were isolated from treated tannery effluent on facultative anaerobic agar plates supplemented with 0.22 µm membrane filter sterilized Cr (VI) (50 to 300 µg/ml) as $K_2Cr_2O_7$ (E-Merck, Mumbai, India). The inoculated plates were incubated at 37±1°C up to 4 to 5 days. The colonies of different morphologies were then selected and purified by repeated streaking on anaerobic agar plates supplemented with Cr (VI). All the isolates were maintained on slants at refrigeration temperature of 4°C.

Evaluation of chromate resistance

Resistance of the isolated strains to Cr (VI) was measured by determining the minimum inhibitory concentration (MIC) values of Cr (VI) for each strain. The MIC was determined by agar dilution method. Anaerobic agar plates supplemented with different concentrations of Cr (VI) (100 to 1500 µg/ml) were inoculated aseptically with about 2.9×10^7 colony forming units (cfu)/ml bacterial cells of exponential phase from overnight grown cultures. Plates were incubated for 4 to 5 days at 37±1°C. Minimum concentration of Cr (VI) inhibiting the complete growth was taken as the MIC of the isolate. The strains showing higher MIC values of Cr (VI) were selected for Cr (VI) reduction experiments.

Chromate reduction assay

Selected bacterial strains were inoculated into 100 ml screw caped glass vials containing 85 ml of broth (pH 8.0 ± 0.2) containing (g/l): sodium thioglycollate (0.5), L-cysteine (0.25), peptone (5.0), NaCl (2.5), sodium sulphite (0.1), supplemented with 0.22 µm membrane filter sterilized Cr (VI) (100 to 1400 µg/ml) and incubated at 37±1°C for 108 h. Un-inoculated media containing Cr (VI) served as controls. Samples were collected at different time intervals up to 108 h, and centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant obtained after centrifugation was used to measure Cr (VI) concentration. The Cr (VI) content in each supernatant was determined by measuring absorbance of the purple complex of Cr (VI) with 1,5-diphenylcarbazide (DPC, Sigma-Aldrich) at 540 nm using UV-Vis spectrophotometer (Shimadzu 1601, Japan). The reaction mixture was set up in 10 ml volumetric flask as follows: 200 µL sample volume was made to 1 ml using glass distilled water followed by addition of 330 µL of 6 M H_2SO_4 and 400 µL of DPC and final volume was made to 10 ml using glass distilled water (Thacker et al., 2006). The Cr (VI) concentration was calculated by the standard curve of $K_2Cr_2O_7$ (100 to 1400 µg/ml). The efficiency of chromate reduction was determined in terms of % Cr (VI) reduction by measuring the difference between Cr (VI) concentration at 0 and 108 h of growth. Total chromium in the supernatant was determined by (AAS) at 357.9 nm.

Characterization and identification of bacterial isolate

On the basis of higher MIC as well as chromate reduction percentage values, the most promising strain (FA-3) was initially identified on the basis of morphological and biochemical characteristics using standard microbiological methods (Cowan and Steel, 1993) and the isolate's identity was further authenticated from Institute of Microbial Technology (IMTECH), Chandigarh, India.

Table 1. Physico-chemical analyses of the treated tannery effluent.

Parameter	Effluent	Permissible limit
pH	8.3	6.0 - 8.0
Conductivity (moles/cm)	11 300	850
Alkalinity	740	500
Total solid (TS; mg/l)	2 350	2 200
Total dissolved solid (TDS; mg/l)	2 090	2 100
Total soluble solid (TSS; mg/l)	260	100
DO (mg/l)	2.9	4-6
BOD (mg/l)	258	30
COD (mg/l)	451	250
Sulphur (mg/l)	2 390	1 000
Cl^- (mg/l)	362	600
Mg (mg/l)	240	200
Phosphate (mg/l)	5.2	5
Nitrate (mg/l)	12.0	10
Total nitrogen (mg/l)	221	780
Fluoride (mg/l)	3.8	2
Phenol (mg/l)	10.2	1
Oil and Grease	16.3	10
PCP (mg/l)	14.2	0.1

Measurement of cell growth

Growth of the most promising bacterial isolate, FA-3 was determined by inoculating 1% exponential growth phase bacterial culture in 100 ml glass vials containing anaerobic broth supplemented with varying concentrations of Cr (VI) (200 to 1400 µg/ml) and without Cr (VI) at pH 8.0 ± 0.2. Glass vials were sealed with parafilm to reduce the level of oxygen. Cells were grown in static condition at 37 ± 1°C for 108 h. About 1.5 ml of culture from each vials were aseptically transferred into an oven sterilized tube at a regular time interval of 6 h for a total of 108 h. Bacterial cell density was determined by measuring the optical density at 600 nm by using UV-Vis spectrophotometer (Shimadzu, 1601) and growth curve was plotted graphically.

Relationship between cell growth and chromate reduction activity

Growth as well as chromate reduction activity of strain FA-3 was determined by inoculating the culture (1%) in 100 ml glass vials containing anaerobic broth (pH 8.0 ± 0.2) supplemented with 1000 µg/ml of Cr (VI) and incubated at 37 ± 1°C for 108 h. Samples were withdrawn at regular time interval of 6 h for a total of 108 h. The chromate reduction activity was determined in the supernatant portion after centrifugation of the sample at 10, 000 rpm for 10 min at 4°C. The supernatant was analyzed using the DPC method as described above. Un-inoculated media amended with Cr (VI) served as control.

Factors affecting Cr (VI) reduction

Effect of growth factors viz. pH (5 to 11), different carbon sources (glucose, sucrose, fructose, maltose and lactose), incubation temperature (20 to 50°C), different heavy metal ions (Pb^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, As^{3+}, Co^{2+} and Hg^{2+}) and varying initial concentrations of Cr (VI) (200 to 1400 µg/ml) were evaluated for maximum Cr (VI) bioreduction.

RESULTS

Physico-chemical analyses of the treated tannery effluent

The physico-chemical characteristics of the treated tannery effluent from CETP are shown in Table 1. The results of this study show that the wastewater of tannery process is one of the most important sources of environmental pollutants as the concentration of chromium and other harmful materials in the wastewater is extremely high. The pH of yellowish brown color treated sample was 8.3 having conductivity of 11, 300 moles/cm. The level of alkalinity, BOD, COD, TS, TSS, sulphate, magnesium, phosphate, nitrate, fluoride, phenol, PCP, oil and grease were well above the permissible limits. However, concentration of total nitrogen, chloride, TDS and DO were within the permissible limits. Heavy metal content of the effluent is presented in Table 2. The levels of As^{3+}, Ni^{2+} and Fe^{2+} exceeded permissible limit, whereas, other metals such as Cu^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+} and Co^{2+} were present in significant quantities (CPCB, 2008). Total Cr and Cr (VI) concentrations in the tannery wastewater were 20.21 ± 1.01 and 3.82 ± 0.19 mg/l, respectively, which were above the statutory limit of Indian Standards IS: 2296 and IS: 2490.

Table 2. Heavy metal content of treated tannery effluent.

Heavy metal	Concentration[a] (mg/l)	Permissible limit
Total Cr	20.21	2.0
Cr^{6+}	3.82	0.1
Cu^{2+}	1.78	3.0
Mn^{2+}	1.65	2.0
Zn^{2+}	3.2	5.0
As^{3+}	0.4	0.2
Pb^{2+}	0.1	0.1
Cd^{2+}	0.1	2.0
Ni^{2+}	3.0	2.5
Co^{2+}	0.35	1.5
Fe^{2+}	3.2	3.0

[a]Average of three replicates

Table 3. The MIC and % Cr (VI) reduction of selected Cr (VI) resistant bacterial isolates.

S/N	Strain	MIC of Cr (VI) (µg/ml)	Maximum concentration of Cr (VI) at which maximum Cr (VI) reduction occurred	% Cr (VI) reduction
1	FA-1	875	425	66 ± 0.025[a]
2	FA-3	1500	1000	72 ± .032[b]
3	FA-9	750	350	33 ± 0.020[b]
4	FA-16	675	515	58 ± 0.040[c]
5	FA-22	650	450	45 ± 0.034[a]
6	FA-25	500	625	34 ± 0.060[d]
7	FA-48	575	400	47 ± 0.035[c]

Isolation, MIC determination and identification of bacterial strain

A total of fifty three (53) morphologically different bacterial strains were isolated from the treated tannery effluent. Among these strains, on the basis of higher MIC values (>300 µg/ml) 18 strains were selected for the determination of chromate reduction efficiency. Out of these 18 strains, chromate reduction efficiency was observed only in seven strains (FA-1, FA-3, FA-9, FA-16, FA-22, FA-25 and FA-48). The Cr (VI) reduction capacity of these 7 strains varied with the extent of their Cr (VI) tolerance. The MIC as well as reduction percentage (%) of Cr (VI) for these strains is depicted in Table 3. Out of these, the isolate FA-3 showed a maximum MIC of 1500 µg/ml as well as maximum Cr (VI) reduction (72%) at 1000 µg/ml in 90 h, whereas, the values of total Cr was almost the same throughout the experiment (data not shown). Hence, all further studies were done using strain FA-3.

The most promising selected bacterial strain (FA-3) was sent to the IMTECH, Chandigarh, India for identification. The strain was identified as *B. cereus* on the basis of phenotypic characteristics. FA-3 is gram-positive, and an endospore producing chromate resistant rod shaped bacteria. Biochemical analyses showed that the strain FA-3 was positive for catalase, gelatin liquefaction, cytochrome oxidase, starch hydrolysis, nitrate reduction, methyl red tests and was negative for indole production, H_2S production, citrate utilization, lysine decarboxylase and Voges Proskauer tests (Table 4). FA-3 showed no growth on MacConkey agar.

Measurement of cell growth

Growth profile for *B. cereus* in anaerobic broth (pH 8.0 and temperature 37°C) in the absence and presence of Cr (VI) at different concentrations of Cr (VI) (200 to 1400 µg/ml) is shown in Figure 1. The growth curve of *B. cereus* showed that the lag phase and optical density attained by the strains greatly depend on the concentration of Cr (VI) in the medium. It was found that the Cr (VI) does not significantly delay bacterial growth up to 400 µg/ml. The growth of the cells was heavily influenced by Cr (VI) at a concentration of 1200 µg/ml, while Cr (VI) at 600, 800 and 1000 µg/ml had only slight effect on the growth. The lag phase was 12 h for all the

Table 4. Important characteristics of chromate reducing strain FA-3.

Morphological test	**Result**
Gram's reaction	+
Shape	Rod
Colony configuration	Round
Margin	Entire
Elevation	Convex
Surface	Smooth
Pigment	Cream
Arrangement	Scattered
Endospore	+
Motility	–
Physiological tests	
Growth at temperatures (°C)	
15	+
20	+
25	+
30	+
35	+
40	+
45	+
50	+
55	+
60	+
***Growth at* pH**	
4.0	–
5.0	+
6.0	+
7.0	+
8.0	+
9.0	+
10.0	+
11.0	+
12.0	+
Growth on NaCl (%)	
2.0	+
4.0	+
6.0	+
8.0	+
Biochemical tests	
Growth on MacConkey agar	–
Indole test	–
Methyl red test	+
Voges Proskauer test	–
Gelatin hydrolysis	+
Citrate utilization	-
H_2S production	–
Starch hydrolysis	+
Urea hydrolysis	+

Table 4. Continued.

Cytochrome oxidase	+
Catalase test	+
Nitrate reduction	+
Ornithine decarboxylase	+
Lysine decarboxylase	–
Arginine dihydrolase	+

+, Positive; –, negative.

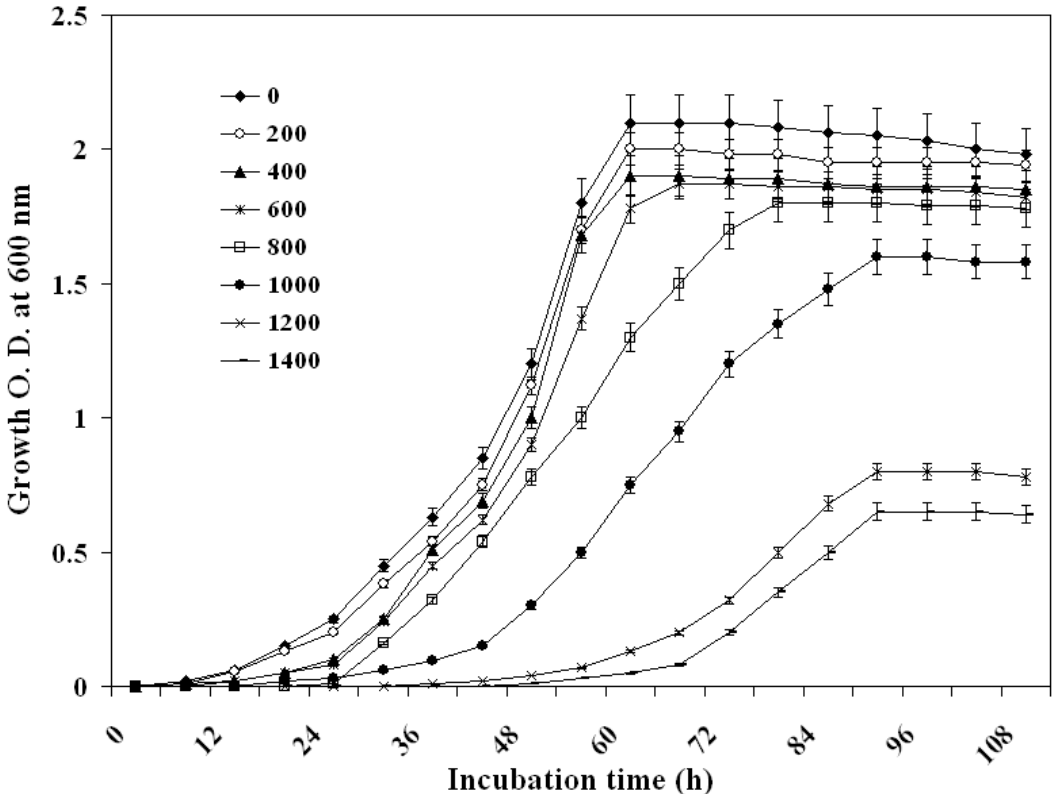

Figure 1. Growth profile of *Bacillus cereus* at different concentrations of Cr (VI).

studied Cr (VI) concentrations below 800 µg/ml. In the presence of 1000 µg/ml, a 24 h lag phase was observed and it was possible to observe a stationery phase of growth during the period of 96 h.

Relationship between cell growth and chromate reduction

Growth of *B. cereus* and chromate reduction at 1000 µg/ml (pH 8.0 and temperature 37±1°C) concentration of Cr (VI) was simultaneously examined to evaluate the relationship between chromate reduction and bacterial growth. Figure 2 shows that the Cr (VI) reduction activity was basically correlated with the bacterial growth. The concentration of Cr (VI) gradually decreased with the increase in bacterial growth, and there was significant increase in bacterial growth as Cr (VI) was reduced more efficiently. It is clear from the figure that in the 90th hour, strain obtained maximum growth simultaneously at that period strain showed maximum Cr (VI) reduction (72%). This may indicate that Cr (VI) reduction and bacterial growth affect each other.

Factors affecting Cr (VI) reduction

Effect of pH and incubation temperature on Cr (VI) reduction

Different pH and incubation temperatures remarkably

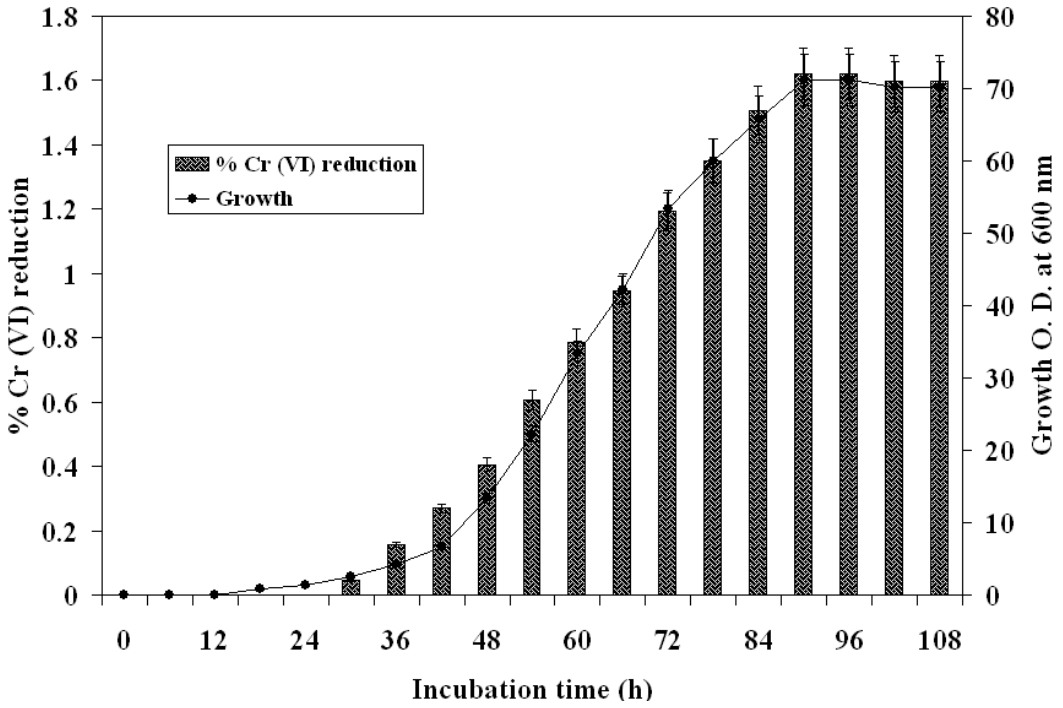

Figure 2. Growth as well as chromate reduction percentage profile of *B. cereus* at 1000 µg/ml Cr (VI) concentration.

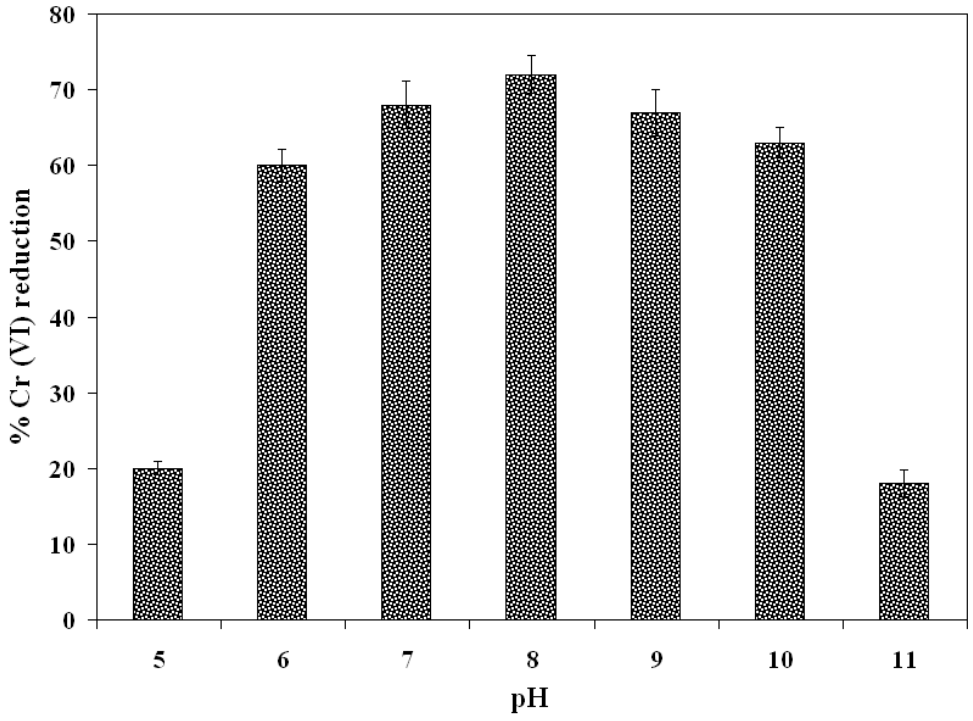

Figure 3. Effect of pH on Cr (VI) reduction by *B. cereus* at 1000 µg/ml Cr (VI) concentration.

affected the microbial Cr (VI) reduction. Figure 3 shows the result of Cr (VI) reduction experiment using the bacterial isolate *B. cereus* in the medium at initial Cr (VI) concentration of 1000 µg/ml at different pH from 5 to 11. No significant difference in Cr (VI) reduction was observed in a pH range between 6.0 to 10.0 with a

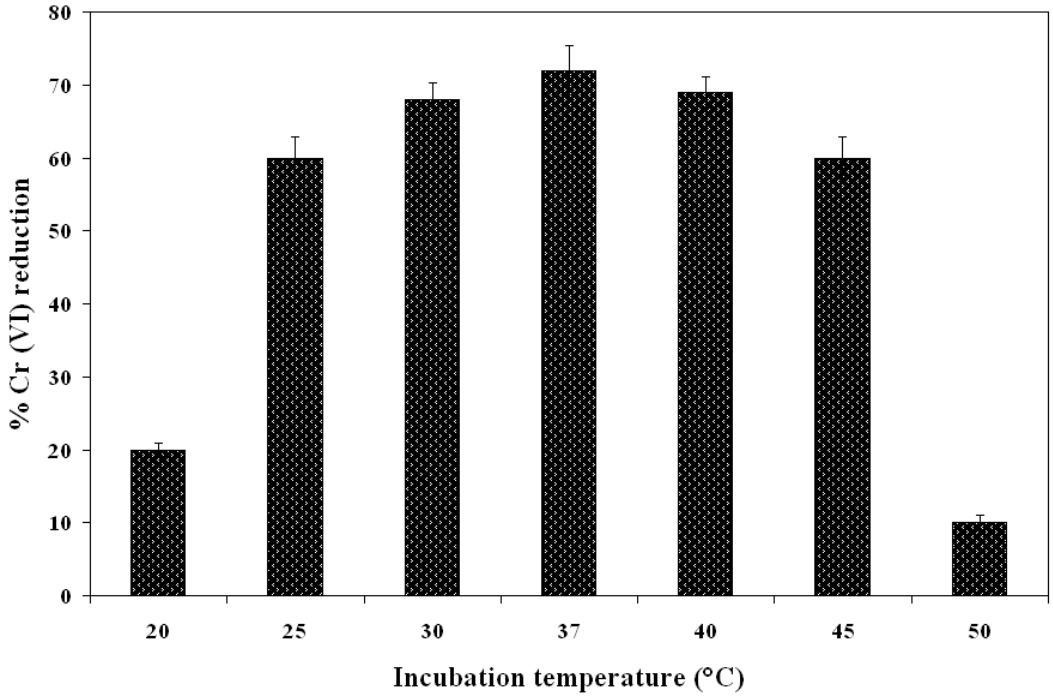

Figure 4. Effect of incubation temperature (°C) on Cr (VI) reduction at 1000 µg/ml Cr (VI) concentration.

maximum at pH 8.0. More than 70% of the reduction activity at optimum pH (8.0) was lost when the pH was changed to 5.0 or 11.0. Thus, the reduction of Cr (VI) slightly decreased with either increase or decrease of pH from 8.0. Temperature is another important factor that has an effect on microbial Cr (VI) reduction. The *B. cereus* strain could reduce Cr (VI) at an extensive temperature range of 20 to 50°C. No significant difference in Cr (VI) reduction was thus evident between 25 to 45°C (Figure 4). Further, decrease or increase in incubation temperature lowered the rate of Cr (VI) bio-reduction.

Effect of carbon sources on Cr (VI) reduction

In the present study, glucose (0.5 %) decreased Cr (VI) concentration from 1000 µg/ml to 220 µg/ml (78% reduction of Cr (VI)), while with sucrose, the Cr (VI) concentration decreased from 1000 µg/ml to 310 µg/ml [69% reduction of Cr (VI)]. However, the reduction of Cr (VI) was observed in the presence of all five carbon sources, although the extent of reduction varied significantly (Figure 5); with glucose was the most effective and lactose was the least effective carbon source for Cr (VI) reduction.

Effect of initial Cr (VI) concentration on Cr (VI) reduction

Reduction of Cr (VI) has been shown to be profoundly influenced by the Cr (VI) concentration. Figure 6 presents effect of initial Cr (VI) concentration (200 to 1400 µg/ml) on Cr (VI) reduction. Figure reveals that the reduction of Cr (VI) occurred even at the highest concentration of 1400 µg/ml of Cr (VI), but complete Cr (VI) reduction was not observed at this initial concentration in 90 h. Complete Cr (VI) reduction was observed for 200 and 400 µg/ml in 54 h. At initial concentration of 600 and 800 µg/ml of Cr (VI), strain reduced 94 and 89% in 66 and 72 h, respectively. However, 1000, 1200 and 1400 µg/ml of Cr (VI) concentration was reduced to 72, 52 and 20% of Cr (VI) in 90 h, respectively.

Effect of heavy metal ions on Cr (VI) reduction

Figure 7 represents the effect of heavy metal ions, Pb^{2+}, Cu^{2+} and Mn^{2+} (600 µg/ml), Zn^{2+} Ni^{2+}, Co^{2+} (400 µg/ml), As^{3+} (200 µg/ml) and Hg^{2+} (25 µg/ml), on Cr (VI) reduction. Presence of heavy metal ions (Pb^{2+}, Cu^{2+} and Mn^{2+}) in combination with Cr (VI) (1000 µg/ml), did not significantly affect the reduction potential of *B. cereus*. However, reduction of Cr (VI) was slightly inhibited in the presence of Zn^{2+}, Ni^{2+} and Co^{2+} metal cations in combination with Cr (VI) (1000 µg/ml). Arsenic (As^{3+}) significantly affected the Cr (VI) reduction while, in the presence of Hg^{2+}, the reduction of Cr (VI) was totally inhibited.

DISCUSSION

Agricultural fields in the Jajmau area (Kanpur) have been

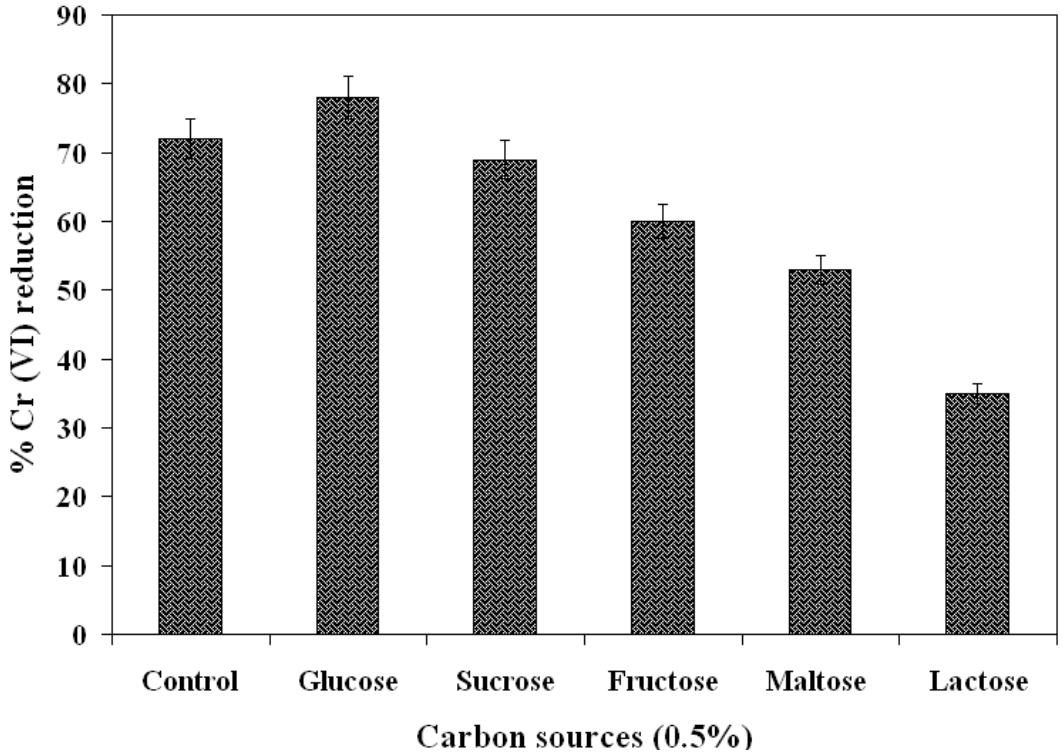

Figure 5. Effect of different carbon sources (0.5%) on Cr (VI) reduction at 1000 µg/ml Cr (VI) concentration. Control does not include any carbon source.

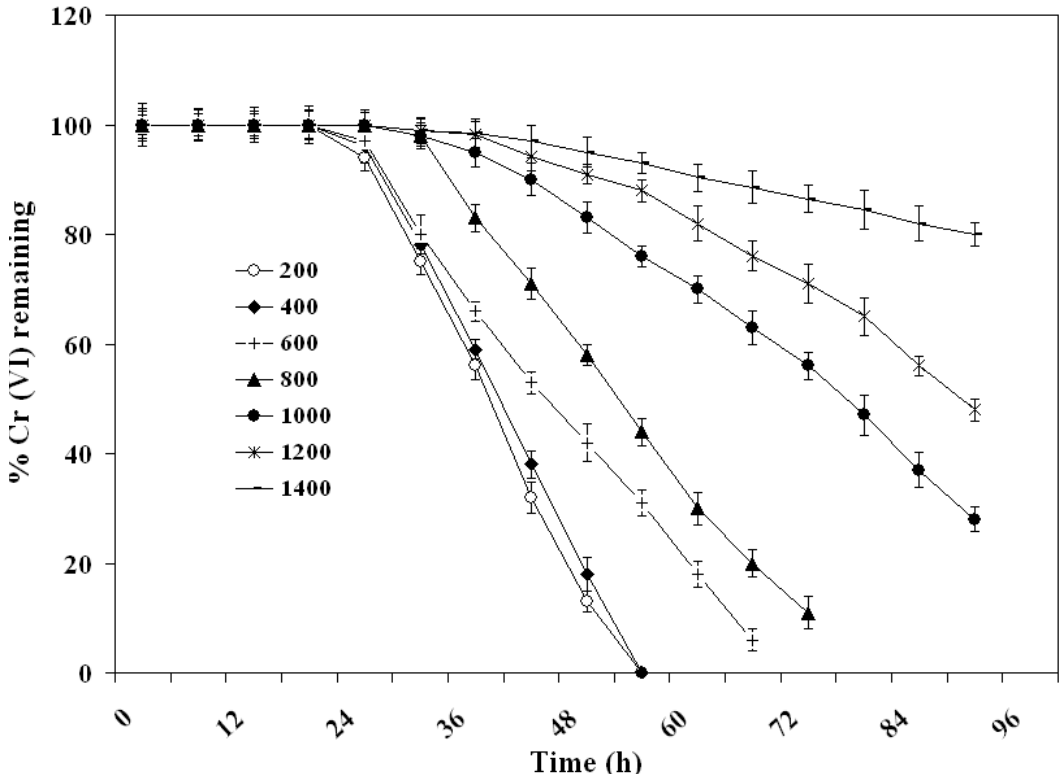

Figure 6. Effect of different initial Cr (VI) concentrations on chromate reduction by *B. cereus*.

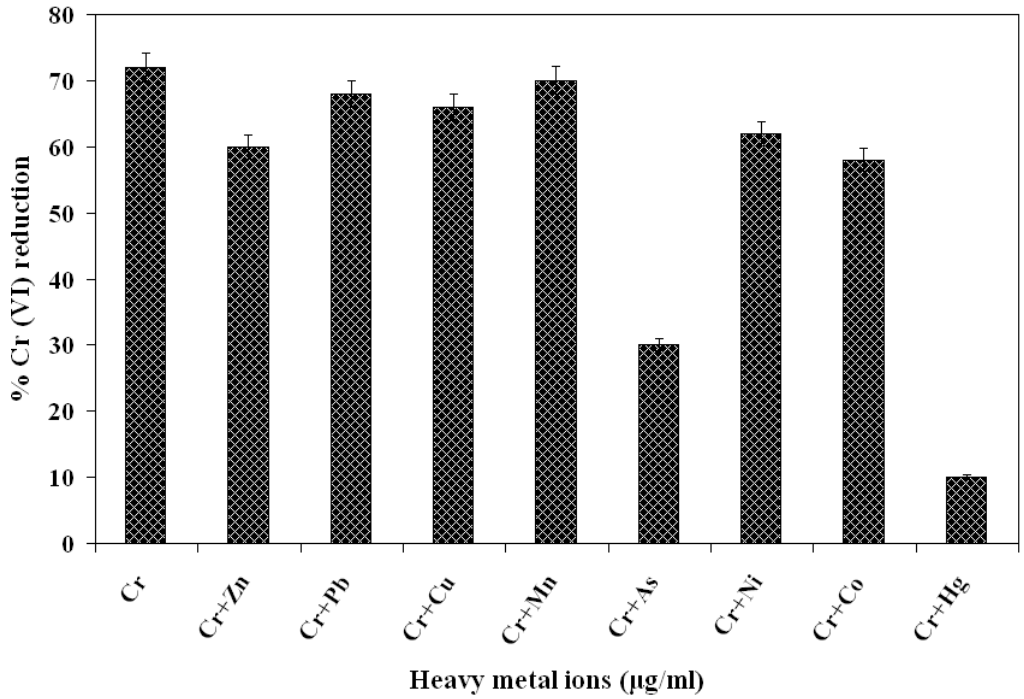

Figure 7. Effect of different heavy metal ions on Cr (VI) reduction by *B. cereus*.

receiving industrial wastewater rich in tannery effluent for a long time. For this reason, they are contaminated with different types of heavy metals. The concentration of chromium remain very high in the soil as these agricultural lands are irrigated with wastewater rich in tannery effluents which contain high concentration of chromium sulphate. Thus, it is clearly desirable to reduce the level of chromium in the treated wastewater. Other researchers have also reported high concentrations of chromium in the same area (Khwaja et al., 2001; Singh et al., 2004). Serious and extensive chromate pollution at several other sites receiving discharges from the tannery has been described (Basu et al., 1997; Achal et al., 2011). Thus, the ever increasing concern about the toxicity of chromate in industrial effluents coerce the isolation of chromate reducing bacteria and their use in detoxification of toxic chromate from industrial effluents. In order to achieve this, in the present study, *B. cereus* strain was isolated from the treated tannery effluent. The tolerance to Cr (VI) of the *B. cereus* isolated in this study is similar, and in some cases even higher to that of the strains reported previously, although an accurate comparison among the different isolates is difficult given that the tolerance can vary greatly depending on the medium and conditions used for each assay (Megharaj et al., 2003).

Nevertheless, the capacity of this strain to survive at higher Cr (VI) concentrations, coupled to its tolerance to other heavy metals and its Cr (VI) reduction ability under wide environmental conditions, makes it a promising strain for use in bioremediation of Cr (VI)-contaminated environments.

In our experiments, the growth of *B. cereus* decreased with the increase in Cr (VI) concentration and a lag period of more than 12 h were observed as *B. cereus* was exposed to Cr (VI) concentration higher than 800 µg/ml. The Cr (VI) concentration from 600 to 1000 µg/ml had only a slight effect on growth of the strain. As Cr (VI) concentration increased up to 1200 µg/ml, highly decreased growth rate was observed (Figure 1). This indicates that the growth of *B. cereus* was heavily inhibited due to the toxicity of Cr (VI) at higher concentrations. Our observations are in agreement with the work reported by Soni et al. (2012). Bae et al. (2000) reported that the specific growth rate of *E. coli* decreased and lag phase increased as Cr (VI) concentration in culture medium increased. It was basically due to the inhibitory effect of higher Cr (VI) concentration on the growth of organism because each organism has a specific resistance at a specified growth conditions. As the initial age of the inoculums remains fixed at 24 h, the acclimatization period at varying Cr (VI) concentrations would not remain the same. Hence, the following behavior was observed.

The time course of Cr (VI) reduction and growth of the *B. cereus* were simultaneously investigated in the presence of an initial Cr (VI) concentration of 1000 µg/ml to evaluate the relationship between Cr (VI) reduction and bacterial growth. Figure 2 shows that the Cr (VI) reduction activity and the growth of the *B. cereus* in the

present study were dependent on each other. The growth of the cells stimulates Cr (VI) reduction and efficient Cr (VI) reduction conversely promotes the bacterial growth (Wei-hua et al., 2009; Soni et al., 2012). This may be due to the nature of Cr (VI) resistant bacteria that create a reducing environment to detoxify Cr (VI) for their own growth.

Temperature and pH were considered as a major factor for growth as well as Cr (VI) reduction ability of *B. cereus*. At low (20°C) and high temperature (50°C), metabolic activities of bacterial cells were affected adversely; hence a sharp decrease in Cr (VI) reduction was noticed at these temperatures. Generally, the optimal Cr (VI) reduction depends mostly on the optimum growth temperature of the organism. It has been reported that the optimal temperature for Cr (VI) reduction is in the range of 30 to 37°C (Cheung and Gu, 2007). Ibrahim et al. (2012) also reported that the optimal temperature of Cr (VI) reduction could be in the range of 25 to 37°C. Maximum Cr (VI)-reduction by *Nesterenkonia* sp. strain MF2 (Amoozegar et al., 2007) and *Ochrobactrum* sp. CSCr-3 (He et al., 2009) was found to be 35°C, whereas, for *Bacillus* sp., (Wang and Xiao, 1995) and *Pseudomonas* strain CRB5 (McLean et al., 2000), it was reported to be 30°C.

Moreover, the *B. cereus* in the present study reduced Cr (VI) fairly well (60 to 72 %) within the pH range of 6 to 10 (Figure 3) and temperature 25 to 45°C (Figure 4), indicating the FA-3 was more prevalent under alkaline conditions. The maximum Cr (VI) reduction (72%) was observed at pH 8.0 and temperature 37°C, on the other hand no significant difference was recorded below and above pH 8.0. However, a sharp decline in Cr (VI) reduction was recorded below pH 6.0 and above 10.0. Thus, the results suggest that a neutral to slight alkaline pH was a best suited range for maximum Cr (VI) reduction. This may be probably due to the highly stable nature of Cr (VI) reductase enzyme as changes in pH and temperature is not probably affecting the protein confirmation as well as the enzyme activity. Results of this study are in agreement with the research of Wang et al. (1990), who reported that, the Cr (VI) reduction by *Enterobacter cloacae* occurred at pH 6.5 to 8.5 and were strongly inhibited at pH 5.0 and 9.0. Shakoori et al. (2000) and Mangaiyarkaras et al. (2011) reported that the optimum pH was 9.0 for Cr (VI) reduction by a Gram-positive bacterium and alkaliphilic *B. subtilis*, respectively.

A variety of organic compounds can serve as electron donors for Cr (VI) reduction by chromate reducing bacteria (Wang and Shen, 1995), with the majority of known electron donors being natural aliphatic compounds, many low molecular weight carbohydrates, amino acids and fatty acids. The addition of exogenous carbon source as electron donors greatly increased the Cr (VI) reduction ability, but was not obligatory for Cr (VI) reduction. In our study, glucose increased the reduction of Cr (VI) only by 6% (Figure 5). This indicates that the glucose in this study have stimulatory effect on Cr (VI) reduction. These results are in consistence with other reports indicating requirement of glucose as electron donor for Cr (VI)-reduction. Glucose has been reported to act as an electron donor and demonstrated to significantly increase Cr (VI) reduction by *Bacillus* sp. (Pal et al., 2005; Liu et al., 2006), *Ochrobactrum* sp. CSCr-3 (He et al., 2009) and *Streptomyces griseus* (Poopal et al., 2009). An increase in Cr (VI) reduction by the soluble fraction of cell free extracts of *B. subtilis* was found in the presence of glucose (Garbisu et al., 1998).

The effect of the initial concentration of Cr (VI) on the reduction of Cr (VI) was investigated over a range of 200 to 1400 µg/ml. Figure 6 reveals that Cr (VI) reduction occurred even under the highest Cr (VI) concentration. The maximum concentration of Cr (VI) for which maximum reduction occurred was 1000 µg/ml, at this concentration 72 % of Cr (VI) reduction was obtained in 90 h of incubation. Above this concentration, the Cr (VI) reduction was decreased due to increasing Cr (VI) concentration. The rate of Cr (VI) reduction decreased with time and eventually ceased at higher initial Cr (VI) concentration. This indicated the existence of a finite Cr (VI) reduction capacity that is possibly due to Cr (VI) toxicity to cells (Philip et al., 1998). Moreover, these results also show that the *B. cereus* was able to sustain a Cr (VI) concentration in the range of 1000 µg/ml without much adverse effect. This is an important observation especially when *in situ* bioremediation is contemplated (Singh and Philip, 2005).

Natural habitats are generally characterized by the co-existence of a large number of toxic and nontoxic cations and therefore, it is necessary to study the effect of multiple metal ions on Cr (VI) resistance and reduction. Our results revealed that different heavy metals at low concentration did not significantly affect the reduction potential. However, they might be problematic at higher concentration. The degree of inhibition in Cr (VI) reduction caused by the tested metal cations was Hg^{2+}> As^{3+}> Ni^{2+}> Co^{2+}> Zn^{2+}> Pb^{2+} >Cu^{2+}> Mn^{2+}. Other researchers have also reported strong inhibition caused by different heavy metal cations on the chromate reduction for *B. sphaericus* (Pal and Paul, 2004), *Streptomyces sp*. (Morales et al., 2007), *Ochrobactrum sp*. (He et al., 2009) etc. Heavy metals can be toxic to microorganisms due to their strong affinity to form complexes with the cell membrane constituents, causing loss of integrity and impairment of their functions. However, microbial resistance to heavy metals is attributable to a variety of detoxifying mechanisms developed by resistant microorganisms. Thus, the heavy metal resistant organisms could be potential agents for bioremediation of heavy metal pollution.

In summary, the present study focuses on the ability of a *B. cereus* strain isolated from treated tannery effluent to biotransform Cr (VI), which will provide a wider application in the bioremediation of Cr (VI) contaminated

soils, sediments or groundwater. The chromate-reducing capacity of the strain was not significantly affected by the various environmental factors such as pH, temperature and other heavy metal ions present in the effluent. Supplementation of glucose (0.5 %, v/v) in the medium supported a trivial increase (6.0 %) in bioremediation efficiency of the strain, which is not significant at industrial level. Thus, fishing out a bacterium exhibiting capability to reduce 72% Cr (VI) at 1000 µg/ml of initial concentration without addition of any carbon source could offer a great advantage in bioremediation of chromium contaminated tannery wastes.

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