World J Microbiol Biotechnol (2014) 30:3211–3219
DOI 10.1007/s11274-014-1748-3

ORIGINAL PAPER

Inhibitory and stimulating effect of single and multi-metal ions on hexavalent chromium reduction by *Acinetobacter* sp. Cr-B2

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Received: 5 March 2014 / Accepted: 22 September 2014 / Published online: 8 October 2014

Abstract Potential application of chromium reducing bacteria for industrial scale wastewater treatment demands that effect of presence of other metal ions on rate of Cr(VI) reduction be investigated, as industrial wastewaters contain many toxic metal ions. In the current study, the effect of different heavy metal ions (nickel, zinc, cadmium, copper, lead, iron) on chromium reduction by a novel strain of *Acinetobacter* sp. Cr-B2 that shows high tolerance up to 1,100 mg/L and high Cr(VI) reducing capacity was investigated. The alteration in Cr(VI) reduction capacity of Cr-B2 was studied both in presence of individual metal ions and in the presence of multi-metal ions at different concentrations. The study showed that the Cr(VI) reduction rates decreased in presence of Ni^{2+}, Zn^{2+} and Cd^{2+} when present individually. Pb^{2+} at lower concentration did not show significant effect while Cu^{2+} and Fe^{3+} stimulated the rate of Cr(VI) reduction. In the studies on multi-metal ions, it was observed that in presence of Cu^{2+} and Fe^{3+}, the inhibiting effect of Ni^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} on Cr(VI) reduction was reduced. Each of these metals affect the overall rate of Cr(VI) reduction by Cr-B2. This work highlights the need to consider the presence of other heavy metal ions in wastewater when assessing the bioreduction of Cr(VI) and while designing the bioreactors for the purpose, as rate of reduction is altered by their presence.

Keywords Hexavalent chromium · Chromium reduction · *Acinetobacter* · Heavy metals · Inhibition

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Introduction

Hexavalent chromium is present in effluents discharged from various industries such as metallurgy, metal electroplating, wood preserving, iron and steel, leather tanning and textile. Chromium is unique among regulated toxic elements, sustaining in the environment in different forms of chromium, specifically trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)]. Hexavalent chromium being highly soluble is easily bioavailable. The solubility of Cr(VI) also promotes the active transport of chromate across biological membranes, and once internalized by cells, Cr(VI) exhibits a variety of toxic, mutagenic, and carcinogenic effects (Chaturvedi 2011; Singh et al. 2005). Cr(III), on the other hand is less toxic and insoluble under normal environmental conditions (Richard and Bourg 1991); therefore some 1,000-fold less mutagenic than Cr(VI) (Lofroth and Ames 1978). Hence the release of Cr(VI) represents the serious problem for environment and human life.

The traditionally used methods for decontamination of Cr(VI) compounds such as ion exchange (Maksin et al. 2012), precipitation (Esmaeili et al. 2005; Fu and Wang 2011), ultrafiltration (Harker et al. 2002), reverse osmosis and electrodialysis (Peric et al. 2004; Rengaraj et al. 2007) generate a huge amount of toxic waste, they are energy intensive processes and involve the use of sophisticated equipment and personnel for maintenance. The refuse generated by these processes generally requires to be neutralized by the employment of several energy intensive steps, thereby reducing the overall efficacy and cost effectiveness of the technology involved. In order to meet the necessity to develop simple and effective methods to reduce the toxic levels of Cr(VI) in the ecosystem, microbial bioremediation opens a new arena by

exploitation of microbial strains that can effectively bring about the reduction of Cr(VI) to Cr(III). Bioremediation describes several technologies and strategies that take advantage of biological entities for bringing transformation, removal and stabilization of hazardous pollutants.

Previously many bacterial strains such as *Pseudomonas olevorans* (Mistry et al. 2009), *Cellulosimicrobium cellulans* (Chatterjee et al. 2009), *Enterobacter cloacae* (Wang et al. 1989), *Leucobacter* sp.(Zhu et al. 2008), *Bacillus circulans* (Chaturvedi 2011), *Pannonibacter phragmitetus* LSSE-09 (Xu et al. 2012), *Arthrobacter* sp. SUK 1201 (Dey and Paul 2012) and *Bacillus cereus* (Sundar et al. 2010) that can potentially reduce hexavalent chromium to non-toxic trivalent form were reported. Recently, Narayani and Shetty (2012) have isolated bacterium, Cr-B2 an *Acinetobacter* sp. from aerator water of activated sludge process of a dye and pigment based chemical industry. Cr-B2 has been reported to efficiently reduce hexavalent chromium and also to exhibit high tolerance capacity for up to 1,100 mg/L of Cr(VI). This *Acinetobacter* sp. was found to be very efficient and tolerant to several other metal ions in addition to chromium. The Minimum Inhibitory Concentrations for Cr-B2 towards other heavy metal ions was reported as: 800 mg/L of Ni^{2+}, 700 mg/L of Zn^{2+}, 350 mg/L of Cd^{2+}, 600 mg/L of Cu^{2+}, 1,100 mg/L of Pb^{2+} and 1,000 mg/L of Fe^{3+} (Narayani and Shetty 2012). As the effluent from industries contain both toxic and non-toxic metals, studies on the reduction capacity and tolerance level of chromium reducing bacteria cannot be justified solely in the presence of chromium. The overall bioreduction process is also regulated by the type and concentration of other heavy metal ions (Srinath et al. 2001). A number of studies have been carried out to investigate the effect of various heavy metal ions on bioreduction capacity of chromium reducing bacteria (Camargo et al. 2003; Pal and Paul 2004; Zakaria et al. 2007; Zhiguo et al. 2009; Ge et al. 2013). Only few studies deal with the effect of presence of single metal ion as well as multi-metal ions on the reduction capacity of Cr(VI) reducing microorganisms (Zakaria et al. 2007). Therefore the aim of this study was to evaluate the effect of various single metal ions and multi-metal ions on the Cr(VI) reduction capacity of the strain *Acinetobacter*, Cr-B2 to determine its true potential for remediating chromium contaminated effluents.

Experimental

Bacteria

In this work, *Acinetobacter* sp.Cr-B2 previously isolated by Narayani and Shetty (2012) and with the GENBANK accession number JF461086 was used. According to their studies, this strain has shown tolerance of up to 1,100 mg/L of Cr(VI) and is highly capable of reducing Cr(VI) to Cr(III). Cell suspension of *Acinetobacter* sp. was prepared by growing the cells in pre-sterilized Luria–Bertani (LB) medium at 37 °C by shaking at 150 rpm for 24 h. The 5 %(v/v) of Cr-B2 culture grown in LB medium thus obtained was transferred into 250 mL flask containing 100 mL of optimized media along with 100 mg/L of Cr(VI) and variable concentration of other different metal ions.

Culture medium

The culture medium for Cr(VI) reduction optimized by Narayani (2011) for pH, carbon and nitrogen sources containing Casein hydrolysate (2 g/L) that served as the nitrogen source; sucrose (12.5 g/L) as the carbon source along with other components such as Na_2HPO_4 (6 g/L), KH_2PO_4 (3 g/L), $MgSO_4{\cdot}7H_2O$ (0.1 g/L), $CaCl_2$ (0.1 g/L) and NaCl (0.5 g/L) was used for all the experiments throughout. Deionized water was used for the preparation of the culture medium. The medium was then autoclaved for 20 min at 120 °C. The pH of the medium was adjusted to 7.6 using H_2SO_4 or NaOH.

Preparation of heavy metal stocks

10,000 mg/L stock solutions of Cr(VI), Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Fe^{3+} were prepared by dissolving the suitable quantity of $CuCl_2{\cdot}2H_2O$, $ZnSO_4{\cdot}7H_2O$, $NiCl_2{\cdot}6H_2O$, $CdCl_2{\cdot}5/2H_2O$, $Pb(NO_3)_2$ and $FeSO_4{\cdot}7H_2O$ salts, respectively in 100 mL of deionized water. Suitable volumes of these stock solutions were used for preparation of desired concentrations of these metal ions in culture medium by dilution.

Effect of single heavy metal co-ions

Shake flask experiments were conducted with 100 mL of the optimized culture medium containing 100 mg/L of Cr(VI) concentration and 50, 100, 150 and 200 mg/L of other heavy metal ion with 5 %(v/v) of inoculum with incubation at 37 °C and 150 rpm. The influence of each of the six heavy metal ion/s (Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Fe^{3+}) as single metal ion co-existing with Cr(VI), on Cr(VI) reduction by Cr-B2 was studied. To determine the effect of other metal co-ion, the samples withdrawn from shake flasks after every 12 h, were centrifuged at 10,000 rpm for 10 min at 4 °C to remove the suspended biomass and the concentration of Cr(VI) in the supernatant was determined spectrophotometrically at 540 nm using 1,5 diphenyl carbazide (DPC) reagent in acid solution as

the complexing agent for Cr(VI) (APHA 1998). The optimized media inoculated with 5 %(v/v) of *Acinetobacter* sp. Cr-B2 along with Cr(VI) without any other heavy metal co-ion acted as control. The mechanism behind removal of Cr(VI) by Cr-B2 in LB and NB medium has already been reported by Narayani and Shetty (2012) as the reduction of Cr(VI) to Cr(III). To confirm the reduction of Cr(VI) to Cr(III) by Cr-B2 in optimized media, total chromium concentration was determined by atomic absorption spectroscopy (AAS; GBC-932 PLUS). The concentration of Cr(III) was calculated from the difference between total Cr and Cr(VI) concentrations. The Cr(III) present in the supernatant (obtained after centrifugation) after Cr(VI) reduction, was also determined by oxidizing Cr(III) to Cr(VI) with potassium permanganate before reacting with diphenyl carbazide and thus the total Cr (sum of Cr(VI) and Cr(III)) as Cr(VI) was determined spectrophotometrically (APHA 1998). Soluble Cr(III) concentration was determined from the difference between total chromium and residual Cr(VI) concentration of the samples measured after every 12 h. The difference between concentration of initial total Cr and total Cr at any time during bioreduction, gave the concentration of insoluble Cr(III). To ensure if any change in Cr(VI) concentration occurs as a result of chemical reaction with the media components and/or other metal ions, abiotic controls were tested with each of the six heavy metal ions (Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Fe^{3+}) as single metal ion co-existing with Cr(VI).

Effect of presence of multi-metal co-ions

In order to determine the effect of presence of multi-metal co-ions on Cr(VI) reduction, the cells of *Acinitobacter* sp. Cr-B2 were subjected to different combinations of heavy metal co-ions based on the results obtained from the experiments detailed in "Effect of single heavy metal co-ions" section on the effect of single metal co-ions. The different combinations of metal ions were made by taking a metal ion that enhances the reduction rate with the other selected metal ion that reduces the reduction rates of Cr(VI). Based on the results obtained from the experiments with single metal co-ions, following combinations were selected to determine the effect of multi metal co-ions: Cu^{2+}(50 mg/L)–Ni^{2+}(200 mg/L), Cu^{2+}(50 mg/L)–Zn^{2+}(200 mg/L), Cu^{2+}(50 mg/L)–Cd^{2+}(200 mg/L), Cu^{2+}(50 mg/L)–Pb^{2+}(200 mg/L), Cu^{2+}(50 mg/L)–Fe^{3+}(100 mg/L), Pb^{2+}(50 mg/L)–Ni^{2+} (200 mg/L), Pb^{2+}(50 mg/L)–Zn^{2+}(200 mg/L), Pb^{2+}(50 mg/L)–Cd^{2+}(200 mg/L), Pb^{2+}(50 mg/L)–Fe^{3+}(100 mg/L), Fe^{3+}(100 mg/L)–Ni^{2+}(200 mg/L), Fe^{3+}(100 mg/L)–Zn^{2+}(200 mg/L), Fe^{3+}(100 mg/L)–Cd^{2+}(200 mg/L). To determine the effect of these multi-metal co-ions, same procedure was followed as that for studying the effect of single heavy metal co-ions. To ensure if any change in Cr(VI) concentration occurs as a result of chemical reaction with the multi-metal co-ions, abiotic controls were tested with different combinations of metal co-ions in the optimize media.

Results

Effect of presence of single metal co-ion on Cr(VI) reduction

In the present study the reduction of Cr(VI) to Cr(III) by Cr-B2 in optimized media was accompanied by a fading of the color (dark yellow) specific to Cr(VI) and an approach to pale green, indicating the removal of Cr(VI). Similar observations were made by Narayani and Shetty (2012) during the Cr(VI) reduction by Cr-B2 in nutrient broth (NB) and LB medium. They have also experimentally shown that the removal of Cr(VI) from water is through reduction of Cr(VI) to Cr(III) when the media used were NB and LB broth. Very often, there arise an argument about two different instruments being used for total Cr measurement and Cr(VI) measurement followed by the calculation of difference between the two values to obtain Cr(III) concentration. To confirm the correctness of the method used by Narayani and Shetty (2012), and to test whether the reduction in Cr(VI) concentrations in optimized media during the biological process was due to sorption of Cr(VI), or due to a reduction of Cr(VI) to Cr(III), in the present study Cr(VI) and Cr(III) concentrations were analyzed by method (i): analysis of total Cr by AAS and Cr(VI) by DPC assay with spectrophotometric analysis; then determination of the difference between the two to give Cr(III) concentration, by method (ii): by oxidizing Cr(III) to Cr(VI) (APHA 1998) and analysis of Cr(VI) by DPC assay with spectrophotometer, then also analyze Cr(VI) concentration by DPC assay without converting Cr(III) as described in "Effect of single heavy metal co-ions" section; determination of the difference between the two values to give Cr(III) concentration. According to Fernández et al. (2010), the method involving oxidation of Cr(VI) to Cr(III) (method ii) is not reliable due to interference of media components during oxidation and it is determined by the oxidants used. Thus in the present work results from both the methods were compared. Table 1, shows the various states of Cr formed during the time course of Cr(VI) reduction as analyzed by method (i). Though the initial Cr (as hexavalent) taken was around 122 mg/L, the total Cr at all the times were lower than 122 mg/L as observed by AAS analysis of total Cr. Table 2, shows the various states of Cr formed during the time course of Cr(VI) reduction as analyzed by method (ii). The concentration of total Cr(VI)as analyzed by spectrophotometer after oxidation of Cr(III) at all the times were also found to be lower than the initial total Cr concentration of around 125 mg/L as Cr(VI),as observed

Table 1 Estimation of Cr(III) formed during Cr(VI) reduction in optimized media by Cr-B2 by method (i)

Time (h)	Total Cr (AAS) (mg/L)	Residual Cr(VI) (DPC assay) (mg/L)	Soluble Cr(III) (mg/L)	InsolubleCr(III) and bisorbed Cr(VI) (mg/L)
0	122.26	122.00	–	–
24	109.60	44.29	65.31	12.66
36	101.46	18.00	83.46	20.80
48	95.62	6.86	88.76	26.64
60	96.74	0.86	95.88	25.52
72	94.34	0.57	93.77	27.92
84	96.02	ND	96.02	26.24
96	96.36	ND	96.36	25.90
108	92.70	ND	92.70	29.56

Table 2 Estimation of Cr(III) formed during Cr(VI) reduction in optimized media by Cr-B2 by method (ii)

Time (h)	Total [Cr(VI) +Cr(III)] as Cr(VI)after oxidation of Cr(III) (mg/L)	Residual Cr(VI) (DPC assay) (mg/L)	Soluble Cr(III) (mg/L)	InsolubleCr(III) and biosorbed Cr(VI) (mg/L)
0	125.71 [only Cr(VI)]	125.71	–	–
24	108.57	44.29	64.29	17.14
36	102.86	18.00	84.86	22.85
48	97.14	6.86	90.29	28.57
60	91.43	0.86	90.57	34.28
72	97.14	0.57	96.57	28.57
84	94.86	ND	94.86	30.85
96	91.43	ND	91.43	34.28
108	92.57	ND	92.57	33.14

in Table 2. The variation in concentration of total Cr (by AAS) shown in Table 1 and total Cr(VI) [after oxidation of Cr(III)] shown in Table 2 at different times may be attributed to the insoluble form of Cr(III) [formed by bioreduction of Cr(VI)] that precipitated after the reduction process or may be due to the adsorption of some portion of chromium by the cells. It has been observed in Tables 1 and 2 that the total chromium (AAS) and total Cr(VI) [after oxidation of Cr(III)] content was found to decrease initially and then to reach almost a constant value as the bioreaction proceeded. Initial decrease may be due to sorption of Cr(VI) or may be due to formation of insoluble organo-metallic complexes with the Cr(III) [which is formed after the reduction of Cr(VI) to Cr(III)]. The presence of insoluble form of Cr(III) complexes in the media will not contribute to the AAS readings (total Cr) or spectrophotometric readings [Cr(VI)] as these measurement techniques involve analysis of supernatants which quantify only the soluble chromium.

The increase in total chromium observed by AAS analysis and total Cr(VI) observed by spectrophotometer may be either due to desorption of sorbed Cr by the cells or if insoluble form of Cr(III) had been formed, then this insoluble form would have been converted into soluble form. If the process of Cr(VI) removal is biosorption and if desorption of Cr(VI) at later time is the reason behind the increase in total chromium concentration, then it should be also accompanied by the increase in Cr(VI) concentration. But in the present study, it was observed that the Cr(VI) concentration did not increase over time indicating that increase in total Cr is not attributed to the desorption of biosorbed Cr(VI). So the residual Cr, after deducting total Cr at any time from the initial Cr, may be largely attributed to insoluble form of Cr(III). During the reduction process, along with the formation of soluble Cr(III), insoluble organo-Cr(III) complexes would have also been formed by complexing of produced Cr(III) with certain organic constituents or metabolites present in the media. Similar observations have been reported by Narayani and Shetty (2012). Thus it is clearly understood from Tables 1 and 2, that Cr(VI) is being reduced to Cr(III) during Cr(VI) removal. Studies on hexavalent chromium reduction as a function of time was carried out in the presence of different metal co-ions and the results of these studies were compared with the reduction rates of Cr(VI) in the absence of other heavy metal co-ion. The results obtained from this study are discussed in the following sections. The presence of metal co-ions solely in the optimized media (abiotic control) was found to show no change in Cr(VI) concentration in the media indicating that the biological process controls the rate of Cr(VI) reduction, which may be altered by metal-microbe interaction.

Effect of presence of Nickel on Cr(VI) reduction

As shown in Fig. 1, even in the presence of low concentration of Ni^{2+} (50 mg/L) the reduction rate is significantly lower than the rate in the absence of Ni^{2+}. But more than 90 % of 100 mg/L of Cr(VI) was reduced in 72 h as compared to around 95 % removal which occurred in 48 h in the absence of Ni^{2+}. As the concentration of Ni^{2+} was increased to 100 mg/L, the rate of reduction was further reduced with only 80 % of Cr(VI) reduction being achieved in 72 h. A drastic decrease in rate of Cr(VI) reduction was observed with further increase in the concentration of Ni^{2+} to 150 mg/L and 200 mg/L. The Cr(VI) reduced at these high concentrations of Ni^{2+} was approximately 50 % in 72 h. Presence of Ni^{2+} reduces the rate of Cr(VI) reduction.

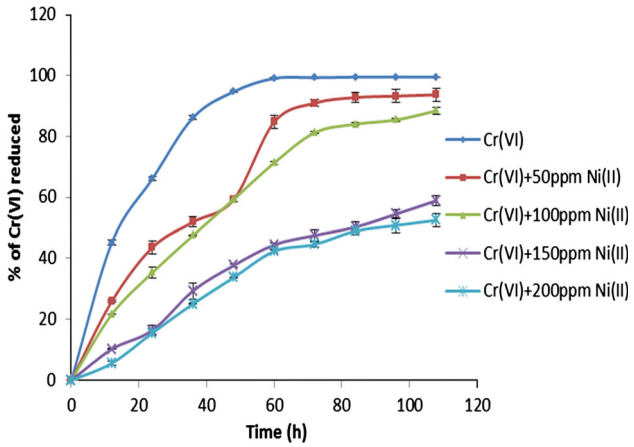

Fig. 1 Effect of Ni(II) on Cr(VI) reduction

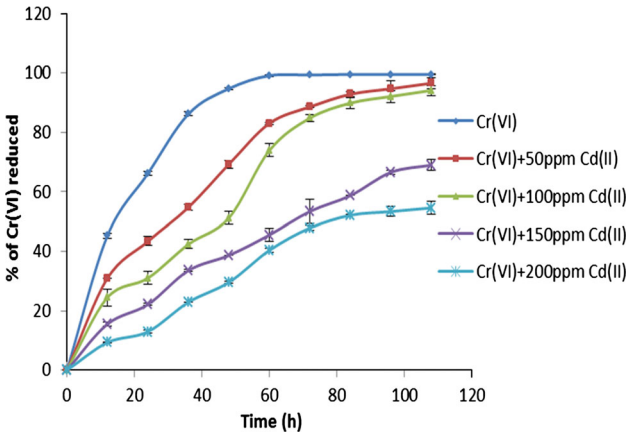

Fig. 3 Effect of Cd(II) on Cr(VI) reduction

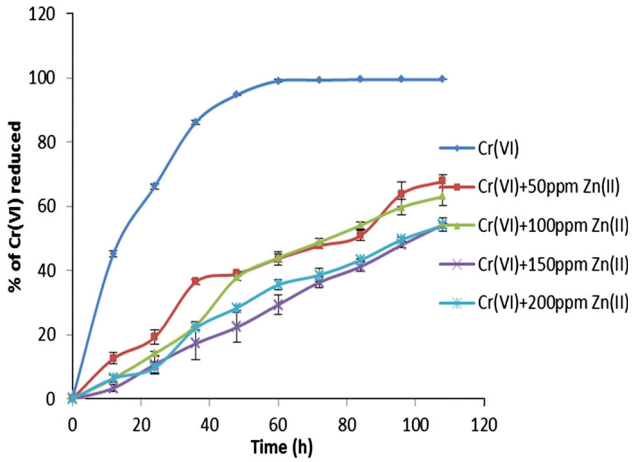

Fig. 2 Effect of Zn(II) on Cr(VI) reduction

Effect of presence of Zinc on Cr(VI) reduction

In the presence of Zn^{2+}, a substantial decrease in the rate of Cr(VI) reduction was observed (Fig. 2). Zinc at a concentration of 50 mg/L reduced the efficiency of Cr(VI) reduction by approximately 50 %. As shown in Fig. 2, the reduction of Cr(VI) achieved after 96 h was only 63.90 % in the presence of 50 mg/L of Zn^{2+}. From Fig. 2, it can also be depicted that the rate of Cr(VI) reduction is tremendously reduced when the concentration of Zn^{2+} is increased to 100, 150 and 200 mg/L. In the presence of highest concentration of 200 mg/L Zn^{2+}, the strain was able to reduce only 49.61 % of Cr(VI) in 96 h.

Effect of presence of Cadmium on Cr(VI) reduction

The presence of Cd^{2+}has also reduces the rate of Cr(VI) reduction (Fig. 3). But the decrease in rate of Cr(VI) reduction was not as high as that in the case of Zn^{2+}. In the presence of 50 mg/L of Cd^{2+}, *Acinetobacter* sp. Cr-B2 was able to reduce 90 % of 100 mg/L of Cr(VI) in 72 h. A little decrease was observed in the reduction rate of Cr(VI) when the concentration of Cd^{2+} was increased to 100 mg/L, with approximately 85 % reduction of Cr(VI) in 72 h. On further increasing the concentration of Cd^{2+} to 150 and 200 mg/L a significant decrease in the rate of Cr(VI) reduction was seen. But the strain showed its potential to reduce up to 50 % of Cr(VI) in 72 h even in the presence of high concentrations of Cd^{2+}.

Effect of presence of copper on Cr(VI) reduction

The effect of presence of Cu^{2+} at different concentrations on Cr(VI) reduction is shown in Fig. 4. Presence of Cu^{2+} has shown a significant stimulatory effect on Cr(VI) reduction. When present in low concentration of 50 mg/L, Cu^{2+} increased the rate of Cr(VI) reduction to an extent of 98.21 % of reduction which took place in 24 h. But in the absence of Cu^{2+}, around 66.18 % reduction occurred in 24 h. On increasing the concentration of Cu^{2+} to 100 mg/L it was found that that rate of Cr(VI) reduction was still very high with 96.49 % of Cr(VI) being reduced in 24 h. With higher concentration of Cu^{2+} of up to 150 mg/L, it was found that the presence of Cu^{2+}could still stimulate the rate of Cr(VI) reduction. Though Cu^{2+} stimulated the rate of Cr(VI) reduction at concentrations of up to 150 mg/L, extent of stimulation decreased with the increase in Cu^{2+} concentration, the maximum being at 50 mg/L and minimum at 150 mg/L concentration of Cu^{2+}. But on further increasing the Cu^{2+} concentration to 200 mg/L, the rate of reduction was slightly lower as compared to that in the absence of Cu^{2+}. So Cu^{2+} at concentration of 200 mg/L inhibits the Cr(VI) reduction rate.

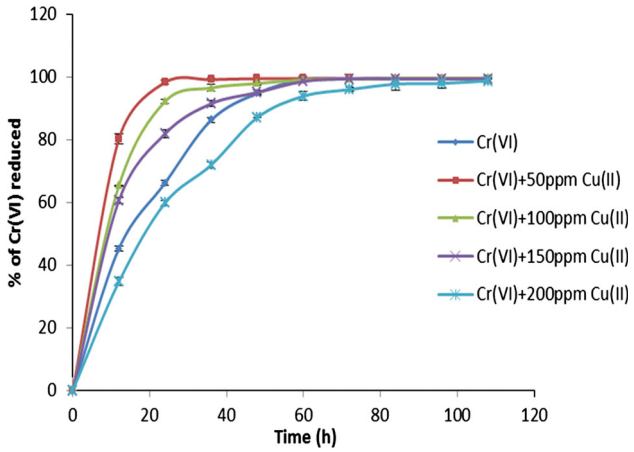

Fig. 4 Effect of Cu(II) on Cr(VI) reduction

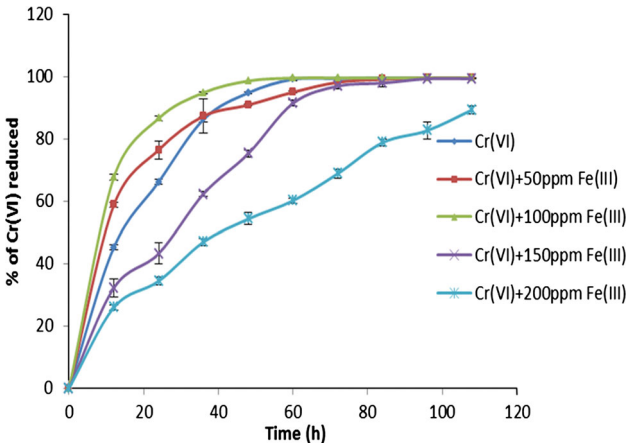

Fig. 6 Effect of Fe(III) on Cr(VI) reduction

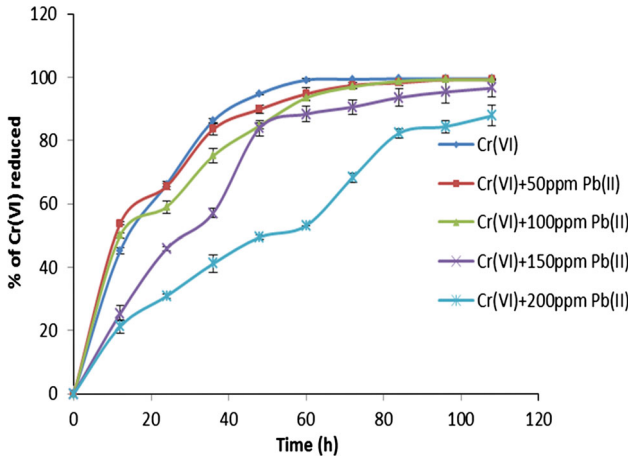

Fig. 5 Effect of Pb(II) on Cr(VI) reduction

Effect of presence of Lead on Cr(VI) reduction

From Fig. 5, it can be observed that, when Pb^{2+} was involved in the reduction medium at low concentrations of 50 mg/L, it did not show significant effect on the rate of Cr(VI) reduction. But on increasing the concentration of Pb^{2+} to 100 mg/L or above, it was found that Pb^{2+} also lowers the rate of Cr(VI) reduction. The strain was able to reduce 90.61 % of Cr(VI) in 72 h in presence of 150 mg/L of Pb^{2+}, while in presence of 200 mg/L of Pb^{2+} it was able to reduce only 68.23 % of Cr(VI) in 72 h, as compared to 95 % reduction achieved in 48 h, in the absence of Pb^{2+}.

Effect of presence of Iron on Cr(VI) reduction

It is observed from Fig. 6 that, an addition of 100 mg/L of Fe^{3+} was favorable for reduction of Cr(VI).On comparison of Cr(VI) reduction obtained in 12th h in the presence or absence of iron, it is found that 20 % enhancement in Cr(VI) reduction is achieved with 100 mg/L of Fe^{3+}. In the presence of Fe^{3+} at concentration of 50 mg/L there was no significant effect on rate of Cr(VI) reduction while at higher concentration of Fe^{3+} (150 and 200 mg/L) the rate of reduction were slightly decreased as compared to that in the absence of Fe^{3+}. However the strain *Acinetobacter* sp. Cr-B2 was able to reduce 91 % of Cr(VI) in 60 h in presence of Fe^{3+} at 150 mg/L concentration.

Effect of presence of multi-metal co-ions

These studies on the effect of multi metal co-ions on Cr(VI) reduction focused on the presence of dual metal ions. No changes in the Cr(VI) concentration were observed in the abiotic controls with different combinations of multi-meta ions, indicating that the biological process controls the rate of Cr(VI) reduction, which may be altered by metal-microbe interactions. Combinations of stimulatory metals (Cu^{2+} and Fe^{3+} at their stimulatory concentrations) with other metal ions were chosen. Combinations of Pb^{2+} (concentration at which it did not show any effect) with other metals also was used. The effect of presence of other heavy metal co-ions (Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} and Fe^{3+}) on Cr(VI) reduction was studied in the presence of 50 mg/L of Cu^{2+} (that was found to highly enhance the rate of Cr(VI) reduction as reported in "Effect of presence of copper on Cr(VI) reduction" section). As shown in Fig. 7, it was observed that when Cu^{2+} at this concentration was introduced along with 200 mg/L of Pb^{2+}, the rate of reduction were slightly increased as compared to the control [only Cr(VI)] while in absence of Cu^{2+}, Pb^{2+} at concentration of 200 mg/L reduces the rate of Cr(VI) reduction. It indicates that the degree of stimulating effect of Cu^{2+} is more, as compared to the degree of inhibition effect of Pb^{2+} resulting in net increase in Cr(VI)

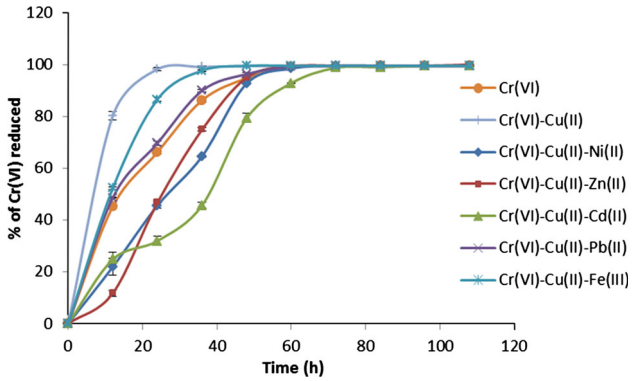

Fig. 7 Effect of other metal ions on Cr(VI) reduction in presence of Cu(II)

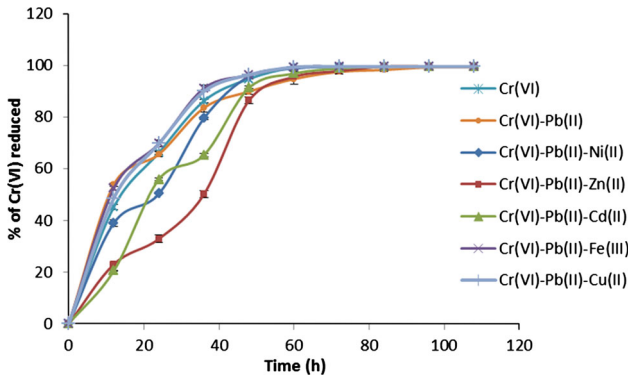

Fig. 8 Effect of other metal ions on Cr(VI) reduction in presence of Pb(II)

reduction. When Ni^{2+}, Zn^{2+} and Cd^{2+} were introduced in reduction medium along with Cu^{2+}, the inhibiting effects of Ni^{2+}, Zn^{2+} and Cd^{2+} were found dominant as the rate of Cr(VI) reduction was lowered as compared to the control [Cr(VI) alone]. However, due to presence of Cu^{2+} the rates were slightly improved as compared to that in presence of Ni^{2+}, Zn^{2+} and Cd^{2+} alone in the Cr(VI) reducing medium (Figs. 1, 2, 3). In the presence of both Cu^{2+} and Fe^{3+} (100 mg/L), a cumulative stimulatory effect was observed with 97.70 % of Cr(VI) being reduced in 36 h that was very high in comparison to the control [only Cr(VI)] where only 86.22 % of Cr(VI) was reduced in the same time period. The rate of Cr(VI) reduction in presence of Fe^{3+} individually in reduction medium was found to be 94.80 % in 36 h (Fig. 6), that was lower than the reduction found in presence of Cr(VI), Cu^{2+} and Fe^{3+} together.

The influence of other metal ions (Ni^{2+}, Zn^{2+}, Cd^{2+} and Fe^{3+}) was also studied in presence of 50 mg/L of Pb^{2+} (that was found to have no effect on rate of Cr(VI) reduction), to determine whether in presence of other metal ions also, it does not affect the rate of Cr(VI) reduction as

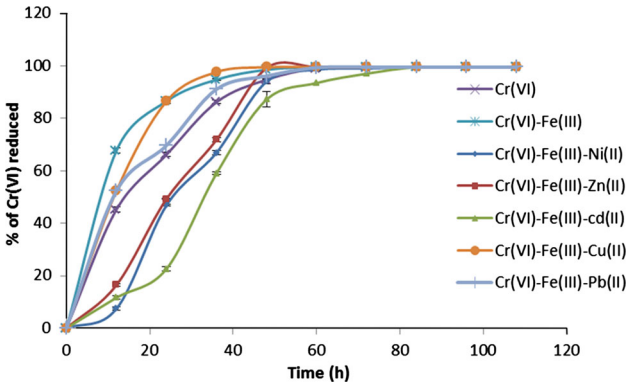

Fig. 9 Effect of other metal ions on Cr(VI) reduction in presence of Fe(III)

observed in Fig. 5 and "Effect of presence of lead on Cr(VI) reduction" section. As observed in Fig. 8, the presence of $Ni^{2+}$and Cd^{2+} along with Pb^{2+} reduce the rate of Cr(VI) reduction marginally, while with the addition of Zn^{2+} with Pb^{2+}, significant decrease in Cr(VI) reduction rate is observed when compared to the control [medium containing only Cr(VI)]. The presence of Fe^{3+} (100 mg/L) along with Pb^{2+} in the medium showed higher rate of chromium reduction with 92 % of Cr(VI) reduced in 36 h.

The effect of other metal ions (Ni^{2+}, Zn^{2+} and Cd^{2+}) along with 100 mg/L of Fe^{3+} [that was found to significantly increase the rate of Cr(VI) reduction] was also determined. Iron predominantly increased the rate of Cr(VI) reduction. In the presence of other heavy metal ions at high concentration of 200 mg/L along with 100 mg/L of Fe^{3+}, the rate of Cr(VI) reduction was lesser compared to the control [only Cr(VI)] during the initial periods of time. But in cases with presence of Ni^{2+} and Zn^{2+}, the strain *Acinetobacter* sp. Cr-B2 was able to reduce more than 90 % of Cr(VI) in 48 h (Fig. 9), which is similar in magnitude as that in control, indicating that the inhibiting effect of these metal ions were suppressed by Fe^{3+}. Inhibition effect of Ni^{2+} and Zn^{2+}, were found to be dominant over the stimulatory effect of Fe^{3+} at initial reduction period, but later the stimulatory and inhibitory effects were found to get balanced. But presence of Cd^{2+} with Fe^{3+} has shown significant inhibition effect on Cr(VI) reduction, indicating that stimulation effect of Fe^{3+} is masked by the inhibition effect of Cd^{2+}.

Discussion

The effluents from the industries contaminated with Cr(VI) contain other heavy metal ions that may affect the Cr(VI) reduction capacity of the bacteria. If the strain *Acinetobacter* sp. Cr-B2 is to be employed for the reduction of

Cr(VI) in wastewater treatment facilities of industries, the effect of presence of other heavy metal ions on Cr(VI) reduction capacity of the bacteria is to be tested. The effect of six different heavy metal ions (Ni^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, Fe^{3+}) to which *Acinetobacter* sp. Cr-B2 showed tolerance, were studied and it was found that some of these metal ions reduce rate of Cr(VI) reduction while some metal ions increases it significantly. Moreover, industrial discharges may contain different combination of these metal ions, therefore, depending on the results obtained from the study of effect of presence of single metal co-ions on Cr(VI) reduction, the effect of presence of different combinations of these heavy metal ions were investigated to determine the true potential of *Acinetobacter* sp. Cr-B2 for bio-reduction of hexavalent chromium.

The metal ions like (Ni^{2+}, Zn^{2+}, $Cd^{2+}$and Pb^{2+}), were found to inhibit the rate of Cr(VI) reduction. Though Cr-B2 was found resistant up to 800 mg/L of Ni^{2+} (Narayani and Shetty 2012), Ni^{2+} reduces the reduction rates of Cr(VI), therefore it can be deduced that Ni^{2+} may act as inhibitor of chromate reductase enzyme by binding onto the active sites for Cr(VI) (competitive) or by binding to other functional groups outside the active sites on the enzyme forming a complex which changes the conformation of active sites (noncompetitive or uncompetitve). The inhibitory effect of Ni^{2+} is also reported for Cr(VI) reduction by *B. sphaericus* (Pal and Paul 2004) and *Orchobactrum* sp. CSCr-3 (Zhiguo et al. 2009).

Presence of Zn^{2+} also significantly reduces the rate of Cr(VI) reduction by Cr-B2. The inhibition of enzyme activity observed in this study is in accordance with the previous report on toxic effect of Zn^{2+} at high concentration that could involve masking the catalytically active subunits of the enzyme or substrate proteins, changing the conformation of the enzyme structure and competing with cation activators connected with the formation of a substrate enzyme complex (Silver and Ji 1995). Inhibition of Cr(VI) reduction in presence of Zn^{2+} has also been reported for *Bacillus* sp. strain KSUCr5 (Ibrahim et al. 2013) and *Pseudomonas aeruginosa* (Wei-hua et al. 2009). The mechanism behind inhibiting effect of Cd^{2+} may be due to the modification of cell structure and cell density or inhibition of the enzymes responsible for Cr(VI) reduction (Dogan et al. 2011; Mabbett et al. 2002). The inhibition of chromate reduction in presence of Pb^{2+} at concentrations greater than 50 mg/L as reported in case of Cr-B2, was also reported in *B. sphaericus* (Pal and Paul 2004) and *A. haemolyticus* (Zakaria et al. 2007). The inhibiting effect of Pb^{2+} may be due to either its adsorption on the bacterial cell wall or due to formation of complex with Cr(VI) reducing enzymes leading to inactivation of chromate reductase enzymes or sites responsible for Cr(VI) reduction (Dogan et al. 2011; Mabbett et al. 2002).

While Ni^{2+}, Zn^{2+}, $Cd^{2+}$and Pb^{2+} were found to suppress the chromate reduction ability of Cr-B2, metal ions like Cu^{2+} and Fe^{3+} significantly enhanced the chromate reduction up to a certain concentration. The mechanism behind the stimulatory effect of Cu^{2+} is still not clear. The stimulatory effect of Cu^{2+} has been reported by few authors (Zhiguo et al. 2009; Camargo et al. 2003; Ibrahim et al. 2013) while many other studies have also reported the inhibitory effect of Cu^{2+} (Shen and Yi-Tin 1994; Ohtake and Silver 1994). Cu^{2+} can stimulate Cr(VI) reduction up to a concentration of 150 mg/L, beyond which it inhibits. However, Cu^{2+} is a prosthetic group for many reductase enzymes. In addition, it has also been reported that function of Cu^{2+} is related to electron transport protection or to act as an electron redox center and in some cases as shuttle for electrons between protein subunits (Abe et al. 2001). When $FeCl_3$ was added, a significant increase in chromium reduction was observed up to a concentration of 100 mg/L, as mentioned in "Effect of presence of Iron on Cr(VI) reduction" section. In a recent study, an "electron shuttle" mechanism was observed, whereby the Cr(VI) was reduced to trivalent chromium (Cr(III)) by Fe^{3+} (Yarong et al. 2009). Fe^{3+} was reduced to Fe^{2+} through microbial respiratory activity, and the reduced Fe^{2+} then served as the catalyst for chromium reduction. The results of the present study are consistent with the conclusion drawn with Cr(VI) reduction by *Shewanella* alga (Wielinga et al. 2001), *Cellulomonas flavigena* (Wei-hua et al. 2005) and *E.coli* (Tang et al. 2013).

Based on the studies conducted for multi metal co-ion effects on Cr(VI) reduction, it was fund that the presence of Cu^{2+} or Fe^{3+} in the reduction medium suppress the inhibitory effect of Ni^{2+}, Zn^{2+}, $Cd^{2+}$and Pb^{2+}, while in the presence of both Cu^{2+} and Fe^{3+} (100 mg/L), a cumulative stimulatory effect was observed.

Conclusion

In the present study, *Acinetobacter* sp. Cr-B2 was found to be a potential biological reducing agent of Cr(VI). This strain can tolerate high concentrations of other heavy metal ions along with Cr(VI) and bio-reduction of Cr(VI) is only moderately affected by the presence of other heavy metal ions. Cu^{2+} and Fe^{3+} stimulated the Cr(VI) reduction rates. Presence of metal ions like Ni^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} inhibit the Cr(VI) reduction rate. In accordance with the study of multi-metal ions, Cu^{2+} and Fe^{3+} were predominantly found to suppress the inhibiting effect of Ni^{2+}, Zn^{2+}, and Cd^{2+}, thereby enhancing the rate of Cr(VI) reduction. These findings provide important information regarding bioremediation of Cr(VI) in presence of wide range of heavy metal ions and can help in developing industrial scale process for Cr(VI) removal from multi-metal contaminated industrial effluents.

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