

Research Article

Reduction of Hexavalent Chromium by Viable Cells of Chromium Resistant Bacteria Isolated from Chromite Mining Environment

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Environmental contamination of hexavalent chromium [Cr(VI)] is of serious concern for its toxicity as well as mutagenic and carcinogenic effects. Bacterial chromate reduction is a cost-effective technology for detoxification as well as removal of Cr(VI) from polluted environment. Chromium resistant and reducing bacteria, belonging to *Arthrobacter*, *Pseudomonas*, and *Corynebacterium* isolated from chromite mine overburden and seepage samples of Orissa, India, were found to tolerate 12–18 mM Cr(VI) during growth. Viable cells of these isolates were also capable of growing and reducing 100 μ M Cr(VI) quite efficiently in Vogel Bonner (V.B.) broth under batch cultivation. Freshly grown cells of the most potent isolate, *Arthrobacter* SUK 1201, reduced 100 μ M Cr(VI) in 48 h. Reduction potential of SUK 1201 cells decreased with increase in Cr(VI) concentration but increased with increase in cell density and attained its maximum at 10^{10} cells/mL. Chromate reducing efficiency of SUK 1201 was promoted in the presence of glucose and glycerol while the highest reduction was at pH 7.0 and 25°C. The reduction process was inhibited by divalent cations Ni, Co, and Cd, but not by Cu. Similarly, carbonyl cyanide *m*-chlorophenylhydrazone, N,N-Di cyclohexyl carbodiimide, sodium azide, and sodium fluoride were inhibitory to chromate reduction, while 2,4 dinitrophenol promoted the process. Cells permeabilized by toluene increased the efficiency of Cr(VI) reduction and, thereby, indicate that *Arthrobacter* sp. SUK 1201, indigenous to chromite mining environment, could be used as an ideal tool for chromium bioremediation.

1. Introduction

Mining activities in and around chromite mines, in general, lead to the generation of huge amount of overburden material as well as accumulation of mine seepage waters, which are the main sources of chromium pollution of inland fresh water and farm lands in the vicinity of the mining sites. The chromite mining in the vast area of Orissa, India, is no exception to this generalization [1, 2].

In humans, several health hazards are associated with continuous exposure to Cr(VI). This is mainly because of its carcinogenic as well as mutagenic properties. Workers employed in areas highly contaminated with chromium suffer from nasal irritation and ulceration, skin irritation, eardrum perforation, lung carcinoma [3, 4], bronchial asthma, kidney necrosis, and allergic reactions in the skin. At higher level, chromium is also found to cause oxidative damage to cell

membrane, alteration of enzyme specificity, and structural deformation in DNA [5].

Conventional methods used for removal of Cr(VI) comprised of chemical reduction followed by precipitation through adjustment of pH, ion exchange, and adsorption generate large quantities of solid sludge for disposal and are expensive and lack specificity [6]. Bioremediation, on the other hand, is an ecofriendly alternative for detoxification and removal of Cr-pollutants which uses indigenous microbiota [7]. Microbial reduction of hexavalent chromium has attracted increased interest, as this process not only relieves the toxicity of chromium but also leads to the precipitation at near-neutral pH for subsequent physical removal [8]. Chromium resistant and reducing bacteria isolated from chromium polluted environments such as tannery effluent and chromite mining environments have played a key role in chromium bioremediation [9–13].

During the course of our survey of chromium resistant and reducing bacteria, four efficient chromite reducing strains, namely, *Arthrobacter* sp. SUK 1201 (MTCC 8728, GenBank accession number JQ 312665), *Arthrobacter* sp. SUK 1205 (MTCC 8731, GenBank accession number JQ 312666), *Pseudomonas putida* SKPD 1202 (MTCC 8729), and *Corynebacterium paurometabolum* SKPD 1204 (MTCC 8730) were isolated from the chromite mining environment of Orissa, India [12–14]. The main objective of the present study was to determine their chromium tolerance along with reduction of Cr(VI) by viable cells of these selected bacterial isolates in Vogel Bonner (V. B.) broth. Further, attempts have also been made to optimize the cultural conditions for Cr(VI) reduction by viable cells of the isolate *Arthrobacter* sp. SUK 1201 in V. B. broth under batch culture and to assess the ability of this isolate as an ideal tool for hexavalent chromium bioremediation.

2. Materials and Methods

2.1. Source and Maintenance of Bacterial Cultures. Four chromate reducing bacterial strains, namely, *Arthrobacter* sp. SUK 1201 (MTCC 8728, GenBank accession number JQ 312665), *Arthrobacter* sp. SUK 1205 (MTCC 8731, GenBank accession number JQ 312666), *Pseudomonas putida* SKPD 1202 (MTCC 8729), and *Corynebacterium paurometabolum* SKPD 1204 (MTCC 8730) isolated from chromite mining environment of Orissa, India [12–14], were used in this study. *Arthrobacter* sp. SUK 1201 and *Arthrobacter* sp. SUK 1205 were isolated from chromite mine overburden samples, whereas *Pseudomonas putida* SKPD 1202 and *Corynebacterium paurometabolum* SKPD 1204 were isolated from chromite mine seepage samples. For maintenance, the bacterial strains were grown on slopes of peptone yeast extract glucose (PYEG) agar medium supplemented with 2 mM Cr(VI) [15]. The medium contained (g/L) peptone, 10.0; yeast extract, 5.0; glucose, 3.0, and agar agar, 20.0 (pH 7.0). Overnight grown cultures were stored at 4°C for future use.

2.2. Chromate Tolerance. Bacterial tolerance to hexavalent chromium was evaluated following broth dilution method of Calomiris et al. [16]. V. B. broth supplemented with different concentrations of Cr(VI) (2–20 mM) was inoculated with overnight grown cultures and incubated at 35°C for 4 days under continuous shaking (120 rpm) in a rotary shaker. Tolerance to Cr(VI) was calculated by determining the relative growth of the isolates with respect to the growth in control [Cr(VI)-free medium], which was considered as 100. Growth of the isolates was measured by determining the optical density at 540 nm.

2.3. Preparation of Cell Mass. Cell mass for chromate reduction studies was obtained by growing the isolates in PYEG medium at 35°C for 24 h under continuous shaking at 120 rpm. The cell mass of the isolates was harvested aseptically by centrifugation (10,000 ×g) at 4°C for 10 min, washed 2–3 times with sterile ice cold Tris buffer (pH 7.0), and suspended in the same buffer following the method of Wang and Xiao [15]. The cell mass was adjusted to a final cell density of 10⁹

cells/mL of reduction medium and used for reduction studies. Viability of cells was determined by dilution and plating of the cell suspension on PYEG agar plates. During the course of hexavalent chromium reduction, changes of cell numbers in the reduction medium were determined by counting the total number of cells/mL by using a haemocytometer (Neubauer, Fein-Optik Jena, Germany) and a phase contrast microscope (Zeiss Winkel Model number 148786, Germany).

2.4. Chromate Reduction Assay. Reduction of Cr(VI) by freshly grown cells of the selected bacterial strains was determined in V. B. broth. Vogel Bonner broth was made up of 2.0% sterile stock solution of V. B. concentrate. The V. B. concentrate contained (g/L) K₂HPO₄, 500.0; Na(NH₄)HPO₄·4H₂O, 175.0; citric acid, 100.0; MgSO₄·7H₂O, 10.0, and 2.0% of 25% D-glucose 20.0 (pH 7.0) [15]. Medium (20 mL/100 mL flask) was supplemented with separately sterile solution of 100 μM Cr(VI). Flasks were inoculated with viable cells at a density of 10⁹ cells/mL under aseptic condition and incubated at 25°C under continuous shaking (120 rpm). The cell number and the viability of cells were determined following the same procedure as described in Section 2.3. Control (without cells) and autoclaved cells were also used for each Cr(VI)-reduction assay to monitor any abiotic Cr(VI)-reduction and biosorption of Cr(VI) by the cell mass.

Reduction of chromium was estimated by measuring the decrease of Cr(VI) contents in the reaction mixture following 1,5-diphenylcarbazine method [17]. Changes in the population of viable cells, if any, during the process of Cr(VI) reduction were also monitored by dilution and plating method on PYEG agar.

2.5. Effect of Electron Donors on Chromate Reduction. Chromate reduction by viable cells of *Arthrobacter* sp. SUK 1201 was studied in presence of various electron donors, such as glucose, glycine, glycerol, acetate, peptone, sucrose, propionate, yeast extract, benzoate, and tryptone. The reduction medium (20 mL of V. B. broth/100 mL) was supplemented with sterile 100 μM Cr(VI) along with the electron donors at 0.1% (w/v). Conditions of incubation, measurement of growth, harvesting of cells, and estimation of residual hexavalent chromium in the reduction medium were the same as described in earlier sections.

2.6. Effect of Temperature and pH on Chromate Reduction. Effect of different temperature (20°–40°C) and pH (6.0–8.0) on the Cr(VI) reducing capability of viable cells of *Arthrobacter* sp. SUK 1201 was determined. The effect of pH was determined at a wide range (pH 6.0–8.0) using citrate, phosphate, and Tris-HCl buffers with overlapping pH range. Chromate concentration, cell density/mL, and incubation conditions were the same as described earlier.

2.7. Effect of Additional Metal Ions on Chromate Reduction. Chromate reduction by *Arthrobacter* sp. SUK 1201 cells was studied in presence of additional metal ions such as Mn(II), Co(II), Zn(II), Cu(II), and Ni(II). The metals were used as chloride salts, sterilized separately, and added to V. B. broth

at equimolar (100 μ M) level of Cr(VI). Other experimental conditions were the same as described above.

2.8. Effect of Inhibitors on Chromate Reduction. The effect of metabolic inhibitors on chromate reduction by viable cells of *Arthrobacter* sp. SUK 1201 was investigated using the ATPase inhibitor N,N-Di cyclohexyl carbodiimide (DCC), protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), enolase inhibitor sodium fluoride, artificial electron acceptor sodium azide, and 2,4 dinitrophenol (DNP). The inhibitors were separately sterilized and added to the reduction medium at equimolar concentration. Other experimental conditions were the same as described previously.

2.9. Reduction by Permeabilized Cells. Permeabilized cells were obtained by harvesting overnight grown cultures of *Arthrobacter* sp. SUK 1201, washed, and suspended in sterile Tris-HCl buffer (pH 7.0). Toluene, Triton X100, and Tween 80 were added to the cell suspension at 0.1% (v/v) concentration and vortexed for 10 minutes to permeabilize the cells. Chromate reduction assay with these permeabilized cells was performed in the same way as described above, while the untreated cells were used as control.

2.10. Statistical Analysis. All experiments were carried out in triplicate and results represent mean \pm standard error.

3. Results and Discussion

Biotransformation of highly toxic and mutagenic hexavalent chromium [18] to relatively nontoxic trivalent Cr(III) form by chromate reducing bacteria offers an economical as well as ecofriendly option for chromium bioremediation. Four chromate reducing bacterial strains, namely, *Arthrobacter* sp. SUK, *Arthrobacter* sp. SUK 1205, *Pseudomonas putida* SKPD 1202, and *Corynebacterium paurometabolum* SKPD 1204 were previously isolated and reported from chromite mine overburden and mine seepage samples and found to reduce chromate during growth under aerobic conditions [12, 14].

3.1. Chromate Tolerance. Chromium tolerance of the isolates as evident from the relative growth of the isolates in Cr(VI) supplemented media is represented in Figure 1. A significant difference in the growth of the selected isolates was recorded in V. B. broth supplemented with 2–20 mM of Cr(VI). As the concentration of chromium increased, the growth of the bacterial isolates decreased. The growth of the isolates was reduced to almost 50% of the control [without Cr(VI)] at hexavalent chromium concentration of 2 mM and was strongly inhibited at 12 mM Cr(VI). Isolates *Arthrobacter* sp. SUK 1201 and *Corynebacterium paurometabolum* SKPD 1204 tolerated up to 18 and 16 mM Cr(VI), respectively, showing nearly 20% growth relative to control.

3.2. Chromate Reduction by Viable Cells. Reduction of Cr(VI) by viable cells has been studied using a variety of microorganisms [10, 19] in both aerobic [20, 21] and anaerobic conditions [22, 23]. Monitoring of Cr(VI) reduction and

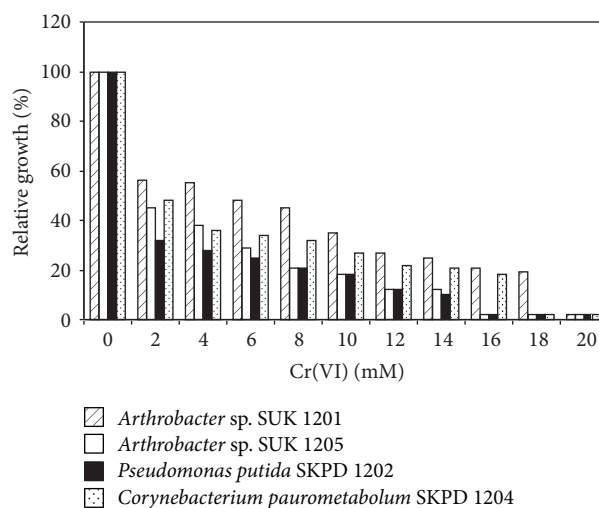


FIGURE 1: Effect of Cr(VI) concentration on relative growth of bacterial isolates [*Arthrobacter* sp. SUK 1201, *Arthrobacter* sp. SUK 1205, *Pseudomonas putida* SKPD 1202, and *Corynebacterium paurometabolum* SKPD 1204] were grown in V. B. broth supplemented with different concentrations of Cr(VI) and relative growth was calculated considering the growth in control as 100].

growth of the suspended cells of all 4 bacterial strains, namely, SUK 1201, SUK 1205, SKPD 1202, and SKPD 1204 (Figure 2) indicates that the strains were very much resistant to Cr(VI) and reduced it effectively. Both *Arthrobacter* isolates, that is, SUK 1201 and SUK 1205, completely reduced 100 μ M Cr(VI) in 48 h. Reduction of Cr(VI) was accomplished by gradual discolouration of the medium but the pattern of increase in cell number/mL of medium was different for the different strains. In SUK 1201, cell number increased till 40 h of incubation and was followed by a decline, while in SUK 1205, there was a gradual increase till 48 h of incubation. The other two isolates, *Pseudomonas putida* SKPD 1202 and *Corynebacterium paurometabolum* SKPD 1204, could reduce about 90% of 100 μ M Cr(VI) during the same period of incubation. Therefore, based on the limit of Cr(VI) tolerance (18 mM) and the efficiency of chromate reduction [100 μ M Cr(VI) in 48 h], *Arthrobacter* sp. SUK 1201 was selected for further studies.

3.3. Effect of Initial Cr(VI) Concentration. The effect of different initial Cr(VI) concentrations (50–800 μ M) were tested on Cr(VI) reducing ability of *Arthrobacter* sp. SUK 1201. The results presented in Table 1 showed that cells of SUK 1201 completely reduced 50 and 100 μ M Cr(VI) 24 h and in 48 h respectively. The other concentrations of Cr(VI) were also significantly reduced within 48 h and about 75% of total Cr(VI) was reduced at the highest concentration (800 μ M) tested. Likewise, chromate reduction by viable cells of different chromate reducing bacterial isolates was found to be influenced by the initial Cr(VI) concentration [11, 24, 25]. The present study showed that complete reduction failed to occur at higher initial Cr(VI) concentration (Table 1) which also corroborates the findings of several others [26, 27]

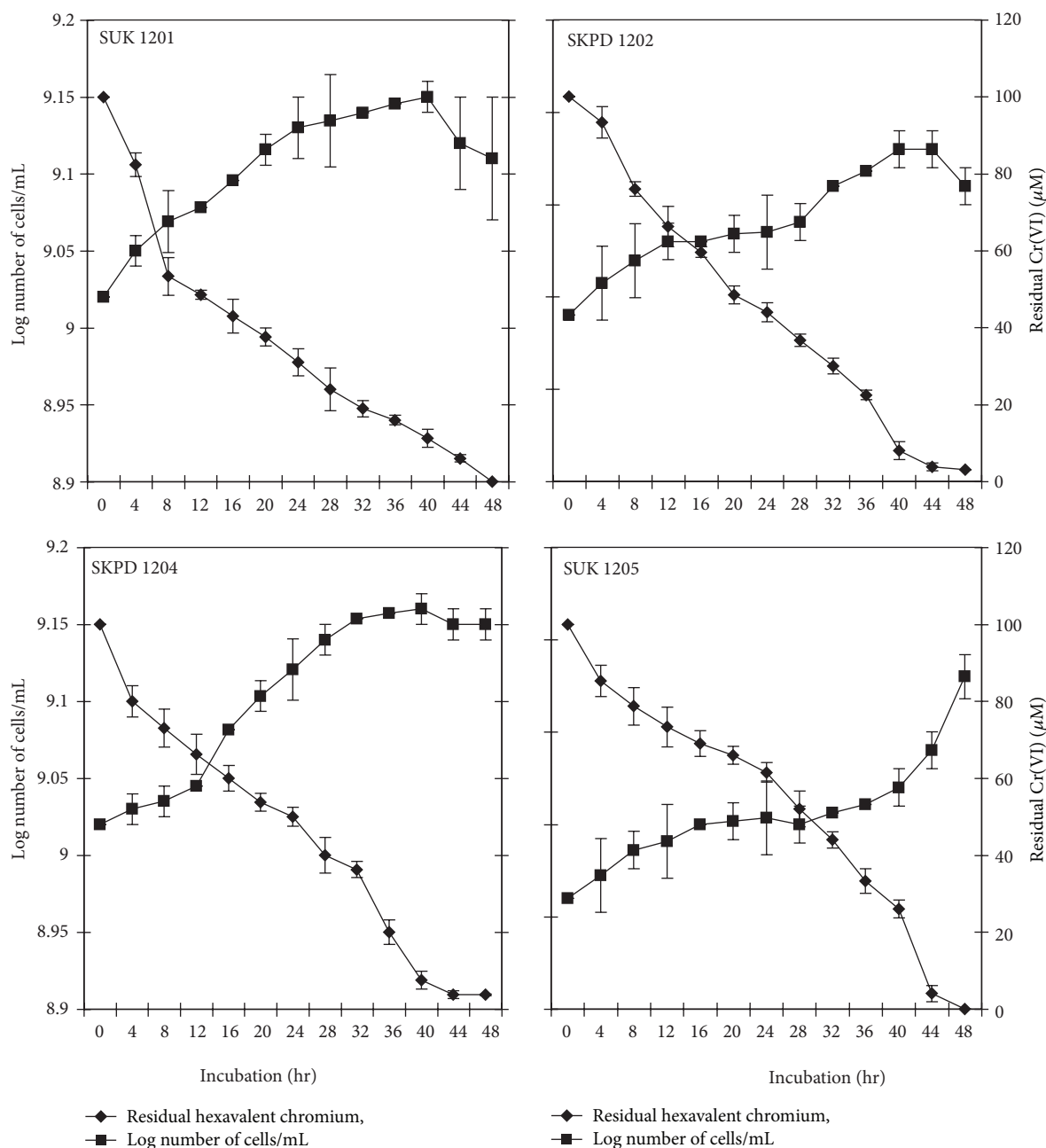


FIGURE 2: Time course of hexavalent chromium reduction by viable cells of selected bacterial isolates [*Arthrobacter* sp. SUK1201, *Arthrobacter* sp. SUK 1205, *Pseudomonas putida* SKPD 1202, and *Corynebacterium paurometabolum* SKPD 1204] in V. B. broth under batch culture.

TABLE 1: Effect of Cr(VI) concentration on Cr(VI) reduction by *Arthrobacter* sp. SUK 1201.

Incubation, hr	% Cr(VI) reduced						
	Concentration of Cr(VI), μM						
	50	100	200	300	400	600	800
12	66.0 ± 0.7	46.0 ± 1.2	16.5 ± 2.6	28.0 ± 1.2	34.4 ± 1.9	35.8 ± 2.2	35.4 ± 1.9
24	100.0 ± 0.0	60.0 ± 2.1	32.3 ± 3.1	36.0 ± 1.6	50.0 ± 2.3	46.6 ± 2.1	45.0 ± 2.6
48	100.0 ± 0.0	100.0 ± 0.0	87.5 ± 1.5	73.3 ± 2.3	71.0 ± 3.5	70.0 ± 0.8	68.0 ± 2.5

(Cr(VI) reduction was carried out in V. B. broth containing 50–800 μM of Cr(VI). The initial cell density was maintained at 10^9 cells/mL. Incubation: 48 h at 25°C under continuous shaking (120 rpm). Residual Cr(VI) was estimated by usual diphenylcarbazide method. Results represent mean of triplicate experiments ± standard error).

TABLE 2: Effect of cell density on Cr(VI) reduction by viable cells of *Arthrobacter* sp. SUK 1201.

Incubation, hr	% Cr(VI) reduced Cell density (cells/mL)				
	10^6	10^7	10^8	10^9	10^{10}
12	5.40 ± 1.2	14.1 ± 2.6	20.0 ± 1.0	41.2 ± 1.6	66.0 ± 1.4
24	19.0 ± 0.8	24.0 ± 1.6	30.0 ± 1.8	64.2 ± 2.6	76.0 ± 0.9
48	34.0 ± 2.2	48.1 ± 0.6	75.0 ± 1.0	100 ± 0.6	100 ± 0.4

(Cr(VI) reduction was carried out in V. B. broth containing $100 \mu\text{M}$ Cr(VI) and a cell density ranging from 10^6 – 10^{10} cells/mL. Incubation: 48 h at 25°C under continuous shaking (120 rpm). Residual Cr(VI) was estimated by usual diphenylcarbazide method. Results represent mean of triplicate experiments \pm standard error).

with *Arthrobacter* sp. Such decrease in chromate reduction capability with the increasing concentration of initial Cr(VI) might be due to toxicity of chromium to viable whole cells.

3.4. Effect of Initial Cell Density. Reduction of Cr(VI) increased proportionally with increase in cell density ranging from 10^6 to 10^{10} cells/mL (Table 2). *Arthrobacter* sp. SUK 1201 cells reduced $100 \mu\text{M}$ Cr(VI) in 48 h when the initial cell concentration was maintained at 10^9 cells/mL; however, with 10 fold increase in cell density, $100 \mu\text{M}$ Cr(VI) was completely reduced in 30 h. Increase in cell density stimulated Cr(VI) reduction process (Table 2) as has been reported with *Bacillus sphaericus* AND 303 [28], *Pseudomonas* CRB5 [29], *Ochrobactrum intermedium* SDCr-5 [30], *Lysinibacillus fusiformis* ZC1 [25], and *Arthrobacter* sp. SUK 1205 [27].

3.5. Effect of Different Electron Donors. The effect of different electron donors on Cr(VI) reduction by *Arthrobacter* sp. SUK 1201 cells was studied. The electron donors include glucose, glycine, glycerol, acetate, peptone, sucrose, propionate, yeast extract, benzoate, and tryptone, which were added at 0.1% concentration to the reaction mixture. Cells of SUK 1201 completely reduced $100 \mu\text{M}$ Cr(VI) in 12 h when glucose was used as the electron donor (Figure 3). Glycerol, acetate, and peptone as electron donors could have completely reduced the added Cr(VI) in 24 h, while glycine and yeast extract appeared to be less efficient electron donors for reducing Cr(VI). Likewise, chromate reducing organisms are reported to utilize a variety of organic compounds as electron donors for Cr(VI) reduction [27, 31]. Whole cells of *Arthrobacter* sp. SUK 1205 [26], *Ochrobactrum* sp. strain CSCr-3 [24], and *Bacillus cereus* [32] were also found to utilize glucose as electron donor for efficient chromate reduction.

3.6. Effect of Temperature and pH. Environmental factors, such as temperature and pH were found to influence the chromate reducing potential of the viable cells of SUK 1201 (Figures 4(a) and 4(b)) as these two factors, in general, regulate the metabolic activities of the cells. The optimum temperature and pH for Cr(VI) reduction were 25°C (Figure 4(a)) and 7.0 (Figure 4(b)), respectively. On either side of the pH and temperature scale, the reduction capability of the cells was impaired. Optimum temperature was found to range between 35°C – 37°C with *Ochrobactrum intermedium* Rb-2

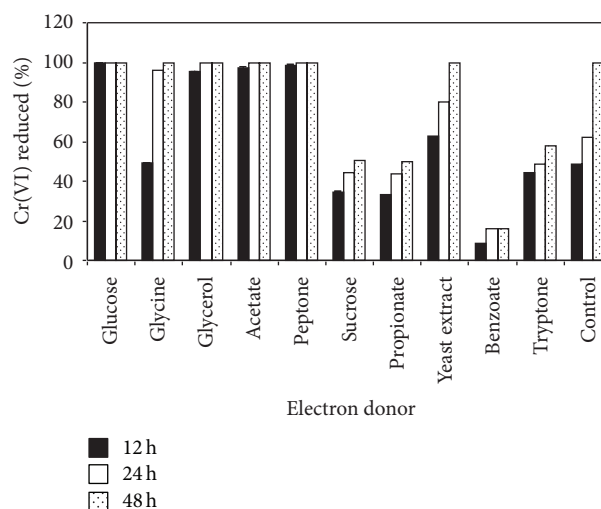


FIGURE 3: Effect of electron donor supplementation on Cr(VI) reduction by viable cells of *Arthrobacter* sp. SUK 1201. [Cr(VI) reduction was carried out in V. B. broth containing $100 \mu\text{M}$ Cr(VI). The initial cell density was maintained at 10^9 cells/mL. The electron donor was present at 0.1% w/v level, and 0.01% w/v glucose was present in control set. Incubation: 48 h at 25°C under continuous shaking (120 rpm). Residual Cr(VI) was measured by diphenylcarbazide method. Results represent mean of triplicate experiments \pm standard error].

[33], *Ochrobactrum* sp. CSCr-3 [24], *O. intermedium* SDCr-5 [30], and *Nesterenkonia* sp. MF2 [34]. It is presumed that deviation of these factors from their optima might alter the chromate reductase activity possibly due to change in the conformation and/or ionization of the enzyme [35].

3.7. Effect of Metal Ions. The process of chromate reduction is adversely affected by the presence of additional metal ions possibly due to metal toxicity and inhibition of the Cr(VI) reduction process [36]. Chromate reduction by viable cells of *Arthrobacter* sp. SUK 1201 was in general negatively affected when the reduction medium was supplemented with different heavy metals such as Ni(II), Zn(II), Mn(II), and Co(II) at equimolecular concentration. As compared to control, presence of Ni(II), Zn(II), Mn(II), and Co(II) showed nearly 66%, 74%, 60%, and 64% reduction, respectively (Table 3). However, Cr(VI) reducing capability of the isolate was enhanced when Cu(II) was present in the medium along with Cr(VI).

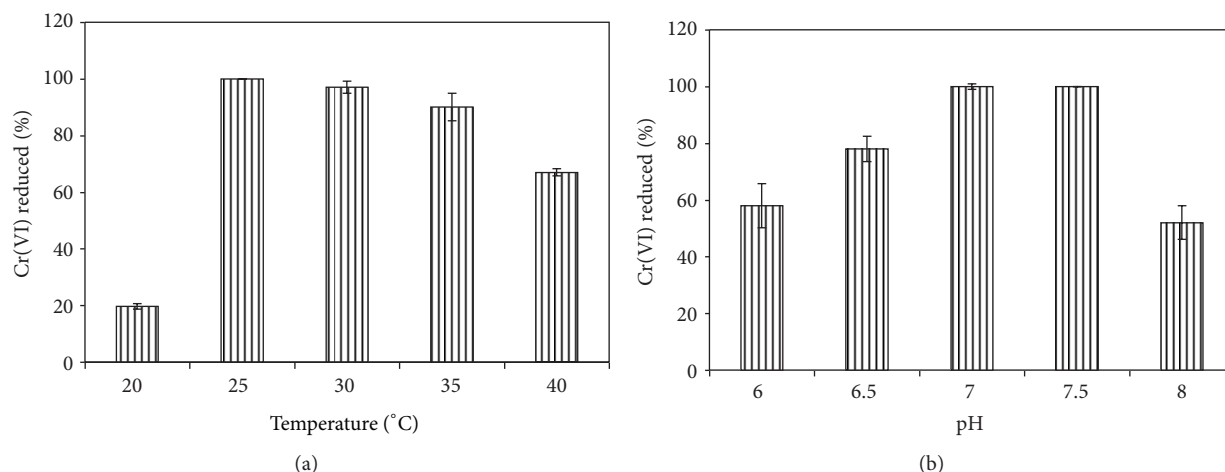


FIGURE 4: Effect of temperature (a) and pH (b) on chromate reduction by viable cells of *Arthrobacter* sp. SUK 1201. [Cr(VI) reduction was carried out in V. B. broth containing 100 μ M Cr(VI). The initial cell density was maintained at 10^9 cells/mL. The initial temperature was maintained from 20–40°C and pH of the reaction medium was maintained in the range of pH 6.0 to 8.0. Residual Cr(VI) was measured by diphenylcarbazide method. Results represent mean of triplicate experiments \pm standard error].

TABLE 3: Effect of metal ions on chromate reduction by viable cells of *Arthrobacter* sp. SUK 1201.

Incubation, hr	% Cr(VI) reduced					
	Control (-Additional metal ions)	Metal ions, 100 μ M				
		Cu(II)	Ni(II)	Co(II)	Mn(II)	Zn(II)
12	46.2 \pm 1.8	81.4 \pm 3.2	44.1 \pm 2.6	53.0 \pm 2.0	54.1 \pm 0.6	48.0 \pm 0.4
24	60.2 \pm 2.6	100 \pm 0.8	53.0 \pm 1.6	57.0 \pm 0.8	60.0 \pm 1.6	57.0 \pm 1.9
48	100 \pm 0.6	100 \pm 0.2	66.0 \pm 2.6	74.0 \pm 1.0	60.0 \pm 0.6	63.4 \pm 2.4

(Cr(VI) reduction was carried out in V. B. broth containing 100 μ M Cr(VI). The initial cell density was maintained at 10^9 cells/mL. Separately sterilized metal solutions were added to V. B. broth at equimolar (100 μ M) level of Cr(VI).

Incubation: 48 h at 25°C under continuous shaking (120 rpm). Residual Cr(VI) was estimated by usual diphenylcarbazide method. Results represent mean of triplicate experiments \pm standard error).

Such stimulatory effect of Cu(II) on Cr(VI) reduction activity has also been reported for Cr(VI)-reduction by *Bacillus* sp. ES 29 [7], *O. intermedium* strain SDCr-5 [30], *Ochrobactrum* sp. strain CSCr-3 [24], *Amphibacillus* sp. KSUCr3, *Bacillus* sp. KSUCr9a [37, 38], and *Arthrobacter* sp. SUK 1205 [27]. The function of Cu(II) is either related to electron transport protection or to act as an electron redox centre, in some cases, as a shuttle for electrons between protein subunits [7, 24, 39].

3.8. Effect of Inhibitors. Inhibitors of different type such as sodium azide (NaN_3), sodium fluoride (NaF), 2,4-dinitrophenol (DNP), carbonyl cyanide-m-chlorophenyl hydrazone (CCCP), and N,N-Di cyclohexyl carbodiimide (DCC) (DCC) were used at equimolecular concentration to assess their influence on chromate reduction by viable cells of *Arthrobacter* SUK 1201. The influence of DNP was exceptionally different from the rest. In presence of DNP, cells of SUK 1201 could reduce nearly 80% of the 100 μ M Cr(VI) as compared to 50% reduction in the control in 24 h of incubation (Figure 5). On prolonged incubation (48 h), control and DNP treated cells completely reduced the 100 μ M of Cr(VI). Such promoting effect of DNP has also been reported in *Burkholderia cepacia* [40] and

Staphylococcus gallinarum [11] and *Arthrobacter* sp. SUK 1205 [27]. Further, it has been pointed out that DNP, being an uncoupler, might have accelerated the respiratory chain linked electron transport mechanism [40]. Amongst the rest, DCC was most inhibitory showing only 53% Cr(VI) reduction and was followed by NaN_3 and NaF (57% in both) and CCCP (71%). These inhibitors are known to inhibit the activity of cytochrome oxidase and enolase [9], disrupt chemiosmotic gradient, and inhibit the ATPase activity.

3.9. Effect of Permeabilized Cells on Reduction. Freshly grown cells of *Arthrobacter* sp. SUK 1201 were permeabilized in presence of triton, toluene, and tween 80 and used for chromate reduction studies. Efficient reduction of hexavalent chromium was achieved with toluene treated cells in 24 h and was followed by cells treated with triton and tween 80 which took 42 h to reduce the total Cr(VI) in the media as against 48 h in the control (Figure 6). Permeabilized cells of *Arthrobacter* sp. SUK 1201 as induced by triton X-100, toluene, and tween 80 have enhanced the reduction of chromate (Figure 6), which might indicate that the Cr(VI) reduction is mediated by soluble protein of the cell [6]. Similar enhancement in Cr(VI) reduction rate was observed with *Providencia* sp. [41].

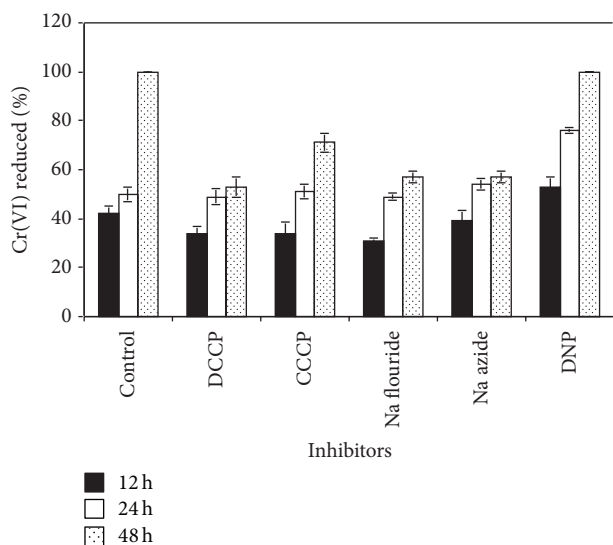


FIGURE 5: Effect of inhibitor on chromate reduction by viable cells of *Arthrobacter* sp. SUK 1201. [Cr(VI) reduction was carried out in V. B. broth containing 100 μ M Cr(VI). The initial cell density was maintained at 10^9 cells/mL. Incubation: 48 h at 25°C under continuous shaking (120 rpm). All inhibitors were sterilized separately and added to V. B. broth at equimolar (100 μ M) level of Cr(VI). Residual Cr(VI) was estimated by usual diphenylcarbazide method. Results represent mean of triplicate experiments \pm standard error].

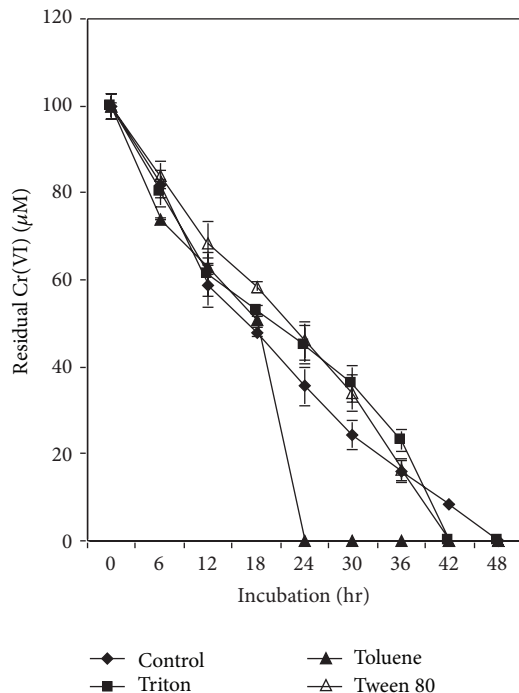


FIGURE 6: Hexavalent chromium reduction by permeabilized cells of *Arthrobacter* sp. SUK 1201. [Cr(VI) reduction was carried out in V. B. broth containing 100 μ M Cr(VI). The initial density of the permeabilized cells was maintained at 10^9 cells/mL. Incubation: 48 h at 25°C under continuous shaking (120 rpm). Residual Cr(VI) was estimated by usual diphenylcarbazide method. Results represent mean of triplicate experiments \pm standard error].

4. Conclusion

The optimization of different conditions of Cr(VI) reduction by viable cells of *Arthrobacter* SUK 1201 has categorically established the biotechnological potential of this bacterial strain for transformation of highly toxic and mutagenic Cr(VI) to less toxic Cr(III) and, thus, could be used in detoxification of chromium pollutants.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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