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Biological removal of carcinogenic chromium(VI) using mixed *Pseudomonas* strains

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The contamination of soil and wastewaters with Cr(VI) is a major problem. It has been suggested that microbial methods for Cr(VI) reduction are better than chemical methods, as they do not add other ions or toxic chemicals to the environment. In this study an aerobic reduction of Cr(VI) to Cr(III) by employing mixed *Pseudomonas* cultures isolated from a marshy land has been reported. The role of chromium concentration, temperature, pH and additives on the microbial reduction of Cr(VI) has been investigated. NADH was found to enhance the rate of reduction of Cr(VI). Complete reduction of chromium(VI) has been possible even at chromium(VI) concentrations of 300 ppm. Ions like SO_4^{2-} and poly-phenols inhibited the metabolic activity relating to Cr(VI) reduction. Under optimal conditions 100 mg/L of Cr(VI) was completely reduced within 180 min.

Key Words—chromium(VI); marshy land; NADH; *Pseudomonas* sp.; tannery soil

Introduction

The environment is sensitive to heavy metals due to their longevity and toxicity. Chromium is a heavy metal that has many uses in the metallurgic, refractory, chemical and tanning industries. While Cr(VI) complexes were one of the first classes of chemicals to be implicated as human carcinogens, Cr(III) has generally been considered as one of the least toxic of the transition metals (Nriagu and Nieboer, 1988). The acute effect of chromium(VI) in humans is well documented and this includes skin dermatitis, chromosome aberrations, liver damage, internal haemorrhage, respiratory disorders, etc. Cr(VI) is a classified group A carcino-

gen based on its chronic and subchronic effects (Anderson, 1998; IARC, 1990).

Chromium contamination is common in soil, ground and surface waters in industrial areas. Chromium compounds have been released to the environment due to improper disposal and leakage, e.g., in ore processing, discharge of unutilized chromium from galvanic, dye processing units, etc. (Kimbrough et al., 1999). In the soil environment, chromium can exist as non-hazardous Cr(III) or as a toxic and carcinogenic Cr(VI) form (Vitale et al., 1997).

The conventional management of chromium includes a) reduction of Cr(VI) to Cr(III), b) the precipitation of Cr(III) as $\text{Cr}(\text{OH})_3$ under high pH conditions, c) the settling of insoluble metal hydroxide and finally d) the disposal or reuse of the dewatered sludge. The tanning industry, in particular, has adopted a recovery/reuse strategy for management of chromium used in tanning. Near-zero discharge concepts have been developed to ensure complete utilization of

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chromium in the tanning process (Sreeram and Ramasami, 2003). In the case of other industries like electroplating, chromite ore processing, dye manufacturing units, etc., the major shortcomings of the conventional treatment systems include safe disposal of the toxic sludge, high cost of chemicals used for Cr(VI) reduction and incomplete reduction of Cr(VI) (Kratochvil et al., 1998). Wastewater containing relatively low concentrations of Cr(VI) is usually treated with ion exchange resins which offer the advantage of the recovery of chromic acid but at a high cost brought about mainly by the high cost of resins (Rao et al., 1989). Recently, chromium sequestering capabilities of inexpensive biomasses including peat moss, maize cob, sawdust, beet pulp, sugarcane bagasses, pine bark, waste fertilizer slurry, shrimp chitin, dead cells of bacteria and fungi as well as seaweed have been studied (Aravindhan et al., 2004; Kratochvil et al., 1998).

Biotechnological options offer advantage as they do not add chemical reagents and are not degradatory to the environment (Ganguli and Tripathi, 2002). The two major processes being investigated are adsorption onto biological materials and dissimilatory reduction of metal ions from a higher valent state to a lower through enzymatic reaction or indirectly with metabolites produced (Lovley and Philips, 1994). The potential of microbial Cr(VI) reduction has been visualized by several authors for a long time; however, biosorption and bioaccumulation of Cr(VI) has gained attention only recently. Bioaccumulation of heavy metals often comprises two phases: an initial rapid phase involving physical adsorption or ion exchange at cell surface and a subsequent slower phase involving active metabolism-dependent transport of metal into bacterial cells (Gadd, 1990). Recently, heavy metal accumulating strains have sought a new role as small factories for production of 'nano particles' (Joerger et al., 2001). Enzymatic reduction of chromate is reported in many bacteria. Early investigations demonstrated that facultative anaerobic bacteria remove chromium(VI) from solution by the formation of a chromium(III) precipitate, presumably chromium hydroxide (Ohtake and Silver, 1994).

The microbial reduction of Cr(VI) to Cr(III) has been one of the most widely studied forms of metal bioremediation (Lovley, 1995). A wide diversity of heterotrophic organisms is known to carry out this reaction, which, depending upon the organism, can take place anaerobically or aerobically (Wang and Shen, 1995). A sur-

vey of the literature indicates that specific strains of aerobic and anaerobic micro-organisms are capable of reducing Cr(VI). For industrial applications of the microbial reduction of Cr(VI), an aerobic route with mixed cultures is more adaptable than those employing anaerobic routes and individual strains. Camargo et al. (2003) have studied the chromium reduction by bacterial consortium isolated from soils contaminated with dichromates. This work focuses on the development of a microbial route to Cr(VI) reduction by employing easily available mixed cultures commonly found in waste and marshy lands. The work also focuses on methodologies to enhance the rate of reduction of Cr(VI) by microbes.

Materials and Methods

Materials. Wet soil samples were obtained from a marshy land in the outskirts of Chennai (labelled as A) and from a tannery in Chennai (labelled as B). Chromate reducing bacteria were isolated from them. All chemicals used in the study were of analytical grade and sourced from M/s. SD Fine Chemicals, Ltd., India and M/s. Ranbaxy India. Spectrophotometric studies were carried out on a UV-Vis Spectrophotometer (Shimadzu, UV160A).

Constituents of culture media. Aerobic nutrient agar medium comprised peptone (5 g/L); yeast extract (1.5 g/L); beef extract (1.5 g/L); sodium chloride (5 g/L) and agar (30 g/L) in distilled deionised water (1 L). The pH was adjusted to 7.0. Anaerobic media was prepared by the procedure described by Vainstein et al. (2003). This media was specific for sulphate-reducing bacteria (SRB), which can reduce chromium indirectly by production of hydrogen sulphide, a strong reducing agent. The cetrinide agar, which is a specific medium for *Pseudomonas* sp. comprised peptone (2.0 g/L); magnesium chloride (0.14 g/L); potassium sulphate (1.0 g/L); cetrinide (0.3 g/L) and agar (35 g/L) in deionised distilled water. The pH was adjusted to 7.0.

Inoculation of bacteria. The quadrant streaking method was followed to inoculate the bacteria to the agar plates (Cappuccino and Sherman, 1996). The prepared media was autoclaved at 15 lbs pressure for 15 min. The sterilized media was allowed to cool to 60°C. Clean petri plates, sterilized in a hot air oven at 160°C for 2 h, were used. After cooling, the media was poured in each petri plate by keeping it inside the inoculation chamber. The plates were allowed to solidify.

After solidification, a sterile inoculation loop was used to streak the sample over the agar plate. The streaked plates were marked appropriately and kept inside the incubator at 37°C for 24–48 h. After incubation the plates were examined for growth.

Isolation and identification. Isolation and identification of bacteria were carried out as per standard microbiological procedures (Cappuccino and Sherman, 1996). Anaerobic nutrient broth was prepared, autoclaved at 15 lbs pressure for 15 min and cooled around 50°C. From anaerobic nutrient agar plates colonies were picked and dispersed in 1% saline suspension (0.1 mg NaCl in 10 ml distilled water) and 5 ml of the sample was transferred to the broth (100 ml) and incubated at 37°C for 48 h. Nitrogen was purged periodically to maintain anaerobic conditions. Cetrimide broth (aerobic) was prepared and autoclaved at 15 lbs pressure for 15 min and cooled below 50°C. Colonies from cetrimide agar plates were picked and dispersed uniformly in 1% saline suspension. Five milliliters of the sample was transferred to the total volume (100 ml) of cetrimide broth.

Cell density measurement. Cell density of the broth cultures was determined by measuring the absorbance with a 1-cm cuvette at 610 nm with Shimadzu UV-160A UV visible spectrophotometer.

Chromium(VI) estimation. A known volume (1 ml) of cultured broth was taken in a centrifuge tube and centrifuged at 10,000 rpm for 10 min. Zero point five milliliter of supernatant was taken and 1 ml of 3 M H_2SO_4 was added to make the concentration of acid about 0.1 M when diluted to 10 ml. Zero point five milliliter of diphenylcarbazide (0.25% solution in 50% acetone) was added and the final volume made up to 10 ml. The absorbance was measured at 540 nm against a reagent blank. A calibration curve had been prepared from solutions of authentic samples of potassium dichromate at different dilutions. From the standard calibration graph, the concentration of chromium in the solution was estimated. The chrome content in the solution was determined after providing appropriate allowances for dilution factors (Jeffery et al., 1994).

Studies on factors affecting chromium(VI) reduction. Nature of bacteria: After attaining the desired cell density ($\text{O.D.}_{\lambda=610\text{ nm}}=1.2\text{--}1.3$) in the respective media, the bacteria were used for reduction of Cr(VI). Cultures grown in anaerobic broth and cetrimide broth (aerobic broth) were separately treated with 25 mg/L and 50 mg/L of Cr(VI). In both the cases the reduction was

monitored at regular time intervals by the diphenyl carbazide method.

Bacterial source: *Pseudomonas* cultures from plate A and B adjusted to appropriate cell density ($\text{O.D.}_{\lambda=610\text{ nm}}=1.2\text{--}1.3$), were treated with Cr(VI) concentrations of 50 and 100 mg/L and the reduction profile of Cr(VI) was monitored.

Initial concentration of Cr(VI): The ability of *Pseudomonas* cultures to reduce Cr(VI) in concentrations ranging from 2.5–300 mg/L was studied. Rate of reduction of Cr(VI) with increasing concentration of chromium was monitored by keeping the initial cell density constant.

Energy source: In order to study the growth profile and rate of reduction of Cr(VI) under varying conditions the *Pseudomonas* cultures grown on cetrimide agar plates were transferred to broth containing glucose, glycerol or phenol. In the preparation of culture broth glycerol (1 ml) was replaced by either glucose (2.52 g) or phenol (1.32 g). The cell density was monitored with time and after attaining specified growth ($\text{O.D.}_{\lambda=610\text{ nm}}=1.2\text{--}1.3$), they were treated with a Cr(VI) concentration of 100 mg/L and the rate of reduction of Cr(VI) was monitored.

Additives: Commercial effluents are often contaminated with other ions. In order to study the influence of some commonly found ions on the reduction profile of Cr(VI), as well as to study the influence of electron donors like NADH, the *Pseudomonas* cultures grown in glycerol media were subjected to varying concentrations (0.7, 1.4, 2.8, 4.2 mM) of NADH, SO_4^{2-} (as Na_2SO_4) and tannic acid. The rate of reduction of Cr(VI) with an initial Cr(VI) concentration of 25 mg/L was monitored. In the case of NADH the studies were also carried out using 100 mg/L Cr(VI).

Temperature: Employing a cetrimide broth the influence of temperature on bacterial growth and chromate reduction was assessed. The effect of temperature was studied by adjusting the autoclaved culture medium to temperatures of 35, 45, 55, 65 and 75°C, in an incubator.

pH: Employing a cetrimide broth the influence of pH on bacterial growth and chromate reduction was assessed. The pH of the cetrimide broth was adjusted to 4.0, 5.8, 7.0 and 8.0 with pre-determined amounts of filtered and sterilized 1 M HCl or 1 M NaOH and incubated at 30°C. The inoculum used was 5 ml sample/100 ml of cetrimide broth.

Results and Discussion

Isolation and identification of cultures

Bacteria were isolated from a marshy land in the outskirts of Chennai, India (consortium A) and from a soil sample sourced from the premises of a tannery (consortium B). Isolation of cultures was carried out as per standard procedures (Cappuccino and Sherman, 1996). Both the bacterial consortia had aerobic and anaerobic bacteria. *Pseudomonas* species were identified and isolated from cultures. Table 1 presents salient observations from the biochemical tests carried out on the bacterial consortia A and B.

Bacterial reduction of chromium(VI)

Cr(VI) reduction was observed when bacterial consortia, along with some organic substrate, were added to a solution of Cr(VI). No chromium reduction occurred when one of these components (bacterial consortium or an additional organic substrate) was absent. This means that organic compounds present in culture media are recalcitrant and not used for Cr(VI) reduction under experimental conditions employed. Bacterial reduction of Cr(VI) has been reported to occur under both anaerobic as well as aerobic conditions. In order to determine the conditions for maximum reduction of Cr(VI), the reduction of hexavalent chromium was carried out under both aerobic and anaerobic conditions by employing the bacterial consortium A and B along with suitable organic substrates. The reduction profile was monitored for 100 mg/L of Cr(VI). The reduction of Cr(VI) with time was monitored by analyzing the residual Cr(VI) by diphenyl carbazide method. No reduction of Cr(VI) was observed over a period of 72 h under anaerobic conditions, while a complete reduction was

possible under aerobic conditions for an initial Cr(VI) concentration of 100 mg/L. Therefore, further studies have been carried out under aerobic conditions.

Chromium(VI) tolerance studies

The bacterial consortia were able to grow in the medium containing chromium(VI). Considerable reduction in Cr(VI) has been observed up to an initial concentration of 300 mg/L (Data not presented); this is much higher than earlier reports for similar bacterial sources (Ganguli and Tripathi, 2002; Laxman and More, 2002). It is also observed that the color of the medium changed from yellow to greenish blue indicating the conversion of hexavalent chromium to trivalent form. Sub-culturing of the bacteria from the broth used for treating 300 mg/L of Cr(VI) was possible, indicating the tolerance levels of the bacterial consortia used in this study.

Studies on the effect of various factors affecting chromium(VI) reduction

Bacterial source. Industrial applications of a bacterial route for Cr(VI) reduction would require that the kinetics of reduction is favourable and/or comparable to other chemical routes. In order to determine the role of isolated bacteria on the reduction profile of Cr(VI), the bacterial consortia A and B, grown in a glycerol-cetrimide broth, were taken and their cell density monitored with time. Under aerobic conditions, the growth profile of bacteria consortium B was significantly higher than that of consortium A. When these consortia, after adjusting to approximately equal cell density ($O.D_{\lambda=610\text{ nm}} = 1.3$), were treated with a Cr(VI) concentration of 50 and 100 mg/L each, the reduction profile for Cr(VI) was found to be better with consortium A

Table 1. Salient observations on the bacterial consortia.

Sl. No.	Test	Consortium A	Consortium B
1.	Gram staining	Gram negative rods	Gram negative rods
2.	Motility	Motile	Motile
3.	Colony morphology	Non-pigmented translucent colonies	Pigmented translucent colonies
4.	Indole test	Negative	Negative
5.	Methyl red test	Negative	Negative
6.	Voges Proskauer test	Negative	Negative
7.	Citrate test	Positive	Positive
8.	Oxidase test	Positive	Positive
9.	Catalase test	Positive	Positive
10.	Confirmatory test on cetrimide agar	Non-pigmented translucent colonies	Pigmented translucent colonies

Fig. 1. Influence of source of bacterial strain on the reduction of Cr(VI) under aerobic conditions.

than with B (Fig. 1). For an initial Cr(VI) concentration of 50 mg/L, a complete reduction of Cr(VI) was possible within 45 min for consortium A while at the same time, consortium B showed a reduction of only 60%. This variation could be explained by tracing back the conditions from which the bacteria were sourced. It is possible that the consortium B, sourced from a tannery soil did not contain specific *Pseudomonas* species like *Pseudomonas fluorescens* LB 300 or *Pseudomonas ambigua* G-1, which are reported to reduce Cr(VI) (Wang and Shen, 1997). It is further possible that the external environment from which the bacteria was isolated had several other compounds or ions, which inhibited the growth of Cr(VI)-reducing bacteria.

Initial concentration of chromium(VI). The effect of the initial concentration of Cr(VI) on the rate of Cr(VI) reduction was investigated over a concentration range of about 2.5–300 mg/L. Figure 2 shows that Cr(VI) reduction occurred even under the highest Cr(VI) concentration. When a complete reduction of Cr(VI) is required within say 48 h, an initial concentration of less than 100 mg/L was ideal. However, the rate of Cr(VI) reduction was higher when initial Cr(VI) concentrations were higher. A complete reduction of Cr(VI) was observed within 6 h for Cr(VI) concentrations up to 15 mg/L, 24 h for 25–50 mg/L and above 48 h for concentrations above 100 mg/L. The results obtained in this study are at variance with that observed earlier with individual strains of *Bacillus* and *Streptomyces* (Wang and Shen, 1995, 1997). In the case of *Streptomyces griseus*, an initial Cr(VI) concentration of 50 mg/L required over 50 h for complete reduction while with the

Fig. 2. Rate of reduction of Cr(VI) with time for varying initial concentrations of Cr(VI).

bacterial consortium used in this study, the same was complete within 24 h (Wang and Shen, 1997). This is possibly due to the fact that mixed consortium would provide adequate tolerance to metal ions than individual strains, similar to that observed in the case of sulphate-reducing bacteria (Cheung and Gu, 2003).

Electron donors. Although Cr(VI)-reducing bacteria may utilize a variety of organic compounds as electron donors for Cr(VI) reduction, the organic compounds are generally limited to natural aliphatics, mainly low-molecular weight carbohydrates, amino acids and fatty acids (Cheung and Gu, 2003). Three different energy sources were analyzed to determine their efficacy in bringing about bacterial growth and subsequently Cr(VI) reduction, while maintaining other ingredients in the growth media constant. At similar mole ratios, the growth of bacterial consortium A was higher with glucose media, followed by glycerol and phenol as observed from the turbidity of the media. At the same time period (5 days), cell densities were much higher for glucose media ($O.D_{\lambda=610\text{ nm}}=1.7$), as against glycerol ($O.D_{\lambda=610\text{ nm}}=1.3$) or phenol ($O.D_{\lambda=610\text{ nm}}=0.7$) media. As bacterial strain A could not effectively use phenol as a source of energy, further studies on Cr(VI) reduction were carried out using glycerol and glucose media only.

At similar cell densities ($O.D_{\lambda=610\text{ nm}}=1.3$), the reduction profile for Cr(VI) (Fig. 3) indicated a significant change between glucose and glycerol. For an initial Cr(VI) concentration of 100 mg/L a glycerol medium took 24 h while the same was only 3 h for complete reduction in the case of glucose media.

Fig. 3. Rate of reduction of Cr(VI) with varying energy sources.

These results are not in agreement with the earlier observations of Desjardin et al., (2002) who found that pure cultures of *S. thermocaboxydus* NH50 showed more rapid Cr(VI) reduction in glycerol than with glucose, while the same was not observed with soil micro-organisms. This contrasting behavior between soil micro-organisms and pure cultures could be because of an enzymatic activity or metabolic production being repressed by glucose in *S. thermocaboxydus* NH50 while the same may not be the case for a mixed culture. This observation further strengthens the choice of mixed cultures from natural sources over individual strains, as the pure cultures are more prone to repression of activity by varying environmental conditions.

pH. In order to study the growth profile and rate of reduction of Cr(VI) in alkaline and acidic conditions, the cultures were grown at different pH levels of 4.0, 5.8, 7.0 and 8.0. A denser growth was observed in the cultures grown at a pH of 7.0, than in the cultures grown at a pH of 5.8. The percentage reduction of Cr(VI) was also higher at a pH of 7.0 than at a pH of 5.8 (Fig. 4). It was observed from earlier reports that the maximum conversion of Cr(VI) to trivalent form was at a pH range of 6–7, which was observed in the present study as well. Thus in our studies, the pH of the medium was adjusted to around 6.0 ± 0.5 before starting the experiments, as it was the optimal pH for the bacteria to grow.

Temperature. The optimal temperature required for the growth of *Pseudomonas* is $37 \pm 2^\circ\text{C}$ (Cappuccino and Sherman, 1996). The Cr(VI) reduction profile was monitored at different temperatures ranging from

Fig. 4. Influence of pH on the reduction profile of Cr(VI).

Fig. 5. Effect of temperature on the rate of reduction of Cr(VI).

pH: 6.02. Initial Cr(VI) concentration: 50 ppm.

35–75°C. During these experiments the pH was maintained at 6.02. The reduction profile is presented in Fig. 5. As can be seen from the figure, as the temperature of the media is increased, the reduction also increased. For an initial Cr(VI) concentration of 50 mg/L, a complete reduction of Cr(VI) was possible within 6 h at 75°C as against 24 h at 35°C. This is possibly because of an increased enzymatic activity with increasing temperature. Further it needs to be noted that the *Pseudomonas* cultures from source A were resistant to higher temperatures. The Cr(VI) reduction capacity of *Pseudomonas* species fell beyond 75°C. This could be due to loss of viability or metabolic activity of cells on prolonged incubation at higher temperatures.

Additives. Inhibitors: Chromium(VI) in wastewaters

Fig. 6. Effect of varying concentrations of SO_4^{2-} and tannic acid on the reduction of Cr(VI).

pH: 5.96. Time: 3 h. Initial Cr(VI) concentration: 25 ppm.

subjected to microbial reduction is often contaminated with sulfate ions and polyphenols like tannins. The effect of inhibitors on the reduction of Cr(VI) by *Pseudomonas* species was studied in the presence of such contaminants. The inhibitors were added to the culture medium after it had attained a cell density of ($\text{O.D.}_{\lambda=610\text{nm}}$) 1.33, along with Cr(VI) (25 mg/L). In the concentration range studied (0.7–4.2 mM), the inhibitory effect followed the order: tannic acid $>$ SO_4^{2-} . While for control cultures (without inhibitors) a 90% reduction of Cr(VI) was observed in 3 h, the same was only 76, 72, 68 and 64% for 0.7, 1.4, 2.8 and 4.2 mM SO_4^{2-} respectively (Fig. 6). In the case of tannic acid a near complete inhibition of Cr(VI) reduction was observed over the concentration ranges investigated. In correlation with the earlier observation for a predominant Cr(VI) reduction with the microbial consortium from source A over that of source B, it can be seen that the bacterial consortium from source B was obtained from a tannery cluster, where the use of tannic acid or related compounds is predominant. The study indicates that the source of microbe is important in the treatment process; for instance, while treating Cr(VI) wastewaters contaminated with tannins, it is important that the bacteria is sourced from areas where the microbes are not subjected to such compounds. The presence of such compounds perhaps inhibits the enzymatic or metabolic pathways which are responsible for the reduction of Cr(VI).

NADH as an enhancer: NADH was evaluated as an additive for *Pseudomonas* culture at varying con-

Fig. 7. Effect of NADH on the rate of reduction of Cr(VI).

centrations. The concentration of NADH was increased from 0.7–4.2 mM. For an initial concentration of 25 mg/L Cr(VI), the reduction was complete within 15 min even at 0.7 mM NADH. The Cr(VI) reduction was found to increase by 60% with a complete reduction observed within 180 min for 4.2 mM NADH (initial Cr(VI) concentration 100 mg/L) (Fig. 7).

In earlier reports it has been suggested that chromate reduction required either NADH or (NAD(P)H) for maximum activity (Ishibashi et al., 1990); further, the metabolic activity involved with Cr(VI) reduction was specific, involving an NADH pathway. Our studies indicate that NADH acts only as enrichment, while enzymatic or metabolic activities related to Cr(VI) reduction take place even in the absence of NADH.

Kinetics of chromium(VI) reduction

First-order model. This model is based upon the assumption that the change in substrate concentration with respect to time is proportional to the power one. The rate of reaction r , can be expressed as:

$$-r = -\frac{dC}{dt} = k_1 C \quad (1)$$

Separating and integrating equation (1) with respect to limits $C=C_0$ at $t=0$ and $C=C$ at any time t :

$$-\int_{C_0}^C \frac{dC}{C} = k_1 \int_0^t dt \quad (2)$$

or

$$\ln \frac{C}{C_0} = -k_1 t \quad (3)$$

Table 2. First-order kinetic constant with corresponding correlation coefficient for reduction of Cr(VI) at various initial concentrations of chromium.

C_0 (mg/L)	k_1 (min^{-1})	R_1^2	k_2 (L/mg s)	R_2^2
2.5	3.69×10^{-3}	0.824	3.08×10^{-3}	0.839
5.0	7.33×10^{-3}	0.947	4.14×10^{-3}	0.966
7.5	9.40×10^{-3}	0.755	5.47×10^{-3}	0.839
15	5.67×10^{-3}	0.699	1.55×10^{-3}	0.814
25	4.86×10^{-3}	0.953	4.74×10^{-3}	0.927

Thus the rate constant k_1 can be calculated from the slope of plot between $\ln(C/C_0)$ versus time t (figure not shown). The calculated k_1 values and their corresponding linear regression correlation coefficient R_1^2 values are shown in Table 2. From the table it is observed that the data can be represented by the first-order kinetic model at all chromium concentrations with an average higher correlation coefficient of 0.836.

Second-order model. The reduction kinetics was further analyzed using a second-order model. This model is based on the assumption that the following reaction proceeds:

where A is Cr(VI), which is being reduced by the bacterial consortium. Then the rate of reaction r can be written as:

$$-r = -\frac{dC}{dt} = k_2 C^2 \quad (5)$$

Integrating equation (5) with respect to limit $C=C_0$ at time $t=0$ and $C=C$ at any time t , the equation simplifies to:

$$\frac{1}{C} = \frac{1}{C_0} + k_2 t \quad (6)$$

The second-order rate constant k_2 can be calculated from the slope of plot between $1/C$ versus time t (Fig. 6). The determined second-order rate constants k_2 and the corresponding linear regression correlation coefficient R_2^2 values are shown in Table 2. From the table it is observed that the kinetic data are well represented by the second-order model (average $R_2^2=0.877$). As the R_2^2 values are found to be higher than that of R_1^2 values for all chromium concentrations, the reduction of Cr(VI) tends to follow second-order rate kinetics.

Conclusions

Though Cr(VI) reduction using aerobic and anaerobic cultures is known, for the industrial application of the method, aerobic systems are preferable. In this study an aerobic reduction of Cr(VI) to Cr(III) by employing mixed *Pseudomonas* cultures isolated from a marshy land has been reported. The mixed *Pseudomonas* culture was found to resist higher concentrations of Cr(VI), with cells being viable up to 300 mg/L of Cr(VI). Under normal conditions of temperature (37°C), pH (~ 6.0), 15 mg/L Cr(VI) was reduced completely within 360 min. The bacteria were viable at higher temperatures ($\sim 75^\circ\text{C}$). NADH addition reduced the time required for 50% reduction of 100 mg/L Cr(VI) from 360 min to 20 min. The presence of sulphates and polyphenols inhibited the metabolic activity relating to Cr(VI) reduction. This study indicates the feasibility of employing a mixed *Pseudomonas* culture for complete reduction of Cr(VI).

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