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Full Length Research Paper

Predictive approach for simultaneous biosorption of hexavalent chromium and pentachlorophenol degradation by *Bacillus cereus* RMLAU1

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Chromium and pentachlorophenol are the major environmental pollutants emanating from tannery effluent. Indigenous *Bacillus cereus* isolate was employed for biosorption and PCP degradation studies under varied environmental conditions such as pH, temperature, contact time, presence of other heavy metals, initial biosorbent and Cr^{6+} concentrations. Best results for Cr^{6+} biosorption (% removal) by living and dead biomass at 2.0 g l^{-1} were found to be 35.2 mg Cr g^{-1} dry wt (63%) at pH 5.0, and 42.5 mg Cr g^{-1} dry wt (70.5%) at pH 4.0, respectively at 35ºC (150 rpm) during 120 min at an initial concentration of 200 mg Cr^{6+} l^{-1} and 500 mg PCP l^{-1}. Among various factors, pH had profound effect on Cr^{6+} biosorption and PCP degradation. Maximum 7.5 % (w/v) PCP degradation ensued in 2 h only by live cells in the presence of 0.4 % (w/v) cometabolite glucose. Presumably, this is the first report on simultaneous biosorption of chromium and pentachlorophenol remediation by native *Bacillus cereus* isolate from tannery effluent. Statistical regressional analysis suitably validated the experimental findings. This strain would be helpful in eco-friendly simultaneous bioremediation allied with a predictive computational approach.

Key words: *Bacillus cereus*, Biosorption, Chromium, Heavy metals, Pentachlorophenol.

INTRODUCTION

In India, over the last few decades, chromium is one of the major environmental pollutants emanating from tannery effluent. Chromium is widely used in tanning of hides/skins, stainless steel manufacturing, textile dyeing and as a biocide in the cooling waters of nuclear power plants, invariably resulting in chromium discharge causing environmental concerns. Two forms of chromium predominantly present in the environment are Cr^{6+} and Cr^{3+}, in which Cr^{6+} is soluble, toxic and carcinogenic (Ackerley et al., 2004). The effluent containing toxic chromium must be treated before discharge to natural environment.

Biosorption is widely used for metal removal from industrial effluents and subsequent recovery (Viera and Volesky, 2000). Biosorption process employs a solid phase (biosorbent, a biological material) and a liquid phase (solvent, normally water) containing a dissolved species of metal to be sorbed (sorbate, metal ions). Microorganisms such as algae, bacteria, fungi and yeasts might serve as potential biosorbents (Volesky, 1987). Several researchers have demonstrated biosorption of Cr^{6+} using different bacterial isolates such as *Bacillus megaterium* (Srinath et al., 2002), *Bacillus coagulans* (Srinath et al., 2003; Quintelas et al., 2008), *Acinetobacter* sp. (Srivastava et al., 2007) and *Pseudomonas* sp. (Srivastava et al., 2008). Since microorganisms have high surface area-to-volume ratio due to their smallness, they can provide a large contact interface, which would interact with metals from the surrounding environment (Zouboulis et al., 2004). During biosorption, metal uptake occurs by physico-chemical interaction between metal and the functional group(s) such as $-NH_2$, -COOH, -SH and –OH, present on the microbial surface thereby serving as sites for interaction with metal ions (Kuyucak and Volesky, 1998).

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The advantages of biosorption over conventional methods are many fold; it can be highly selective, more efficient, easy to operate, short operation time, reusability of biomass and hence, cost effective for treatment of large volumes of wastewaters.

In leather tanning process, pentachlorophenol (PCP) is used as a biocide (Thakur et al., 2001) which is highly toxic and recalcitrant, thereby often occurring in the industrial discharge. Owing to its toxicity, PCP is on the list of priority pollutants defined by the United States Environmental Protection Agency (US EPA). Despite widespread pollution caused by PCP, only a few indigenous bacterial strains capable of degrading PCP have been isolated. However, several non-indigenous bacterial strains such as *Pseudomonas, Serratia marcescens, Sphingomonas chlorophenolicum* capable of PCP degradation have been reported (Yang et al., 2006; Singh et al., 2007; Sharma and Thakur, 2008). Chromium may be present together with aromatic compounds and other metal ions in wastewater from treatment plants, ground water and surface water (US EPA). Thus, microbial remediation of Cr^{6+} with simultaneous degradation of aromatic pollutants may represent a beneficial biological process.

Only a limited research work has been done towards simultaneous bioremediation of Cr^{6+} and phenolics in the tannery effluent (Chirwa and Wang, 2005; Srivastava et al., 2007; Tziotzios et al., 2008), particularly by native microbes. Some researchers have employed either coculture (Chirwa and Wang, 2005) or microbial consortium (Srivastava et al., 2007) for simultaneous bioremediation of PCP and Cr^{6+}. If a single potent native microbial strain is searched, its nutritional requirement, growth and maintenance are likely to be more conveniently managed than a coculture or a consortium. In our earlier study, we have isolated a bacterial strain identified by morphological, biochemical and molecular (16S rDNA and FAME) analyses as *Bacillus cereus* RMLAU1, which could tolerate 500 mg PCP l^{-1} and 200 mg Cr^{6+} l^{-1}, and effectively degrade PCP (56.5%) and bioaccumulate chromium (74.5%) in 48 h (Tripathi et al., 2011). In the present study, attempt have been made to assess the biosorption potential of Cr^{6+} by living and dead cells and simultaneous remediation of PCP by live cells of *B. cereus* RMLAU1.

Lately, statistical analysis of predictive microbiology has attracted the attention (Baranyi et al., 1996; Tarangini et al., 2009) in which the study of microbial responses by mathematical and statistical techniques serve purely as an empirical science. Regression technique is an integral part of predictive microbiology and hence, its application forms the rigorous basis for the development of microbiology and its related paradigm-domains. Regression analysis gives best fit curve which predicts functional and workable relation for interactive situation of prediction; because errors in estimation of predicted variables are minimized by applying the Legendre's least square principle (this process makes the study scientific, valid and convenient for prediction of biological parameters). In other words, an average effect on the dependent or predicted variable is numerically measured by amounting the change to independent or predictor variable. The computational form of microbial indicators charters the new dimension of future application and deeper insight in this domain of knowledge. Therefore, regression model and test of goodness of fit were developed for the estimation of predicted values and for evaluation of statistical significance of the experimental observations.

MATERIALS AND METHODS

Bacterial culture

B. cereus strain RMLAU1 (MTCC 9777, GenBank accession no. FJ959366) capable of simultaneous bioremediation of Cr^{6+} and PCP was isolated from the treated tannery effluent of common effluent treatment plant (CETP), Unnao, India and was employed in the present study.

Biosorbent preparation

The bacterial strain was inoculated in 100 ml sterilized peptone water [1% w/v peptone and 0.5% w/v NaCl in distilled water] in 500 ml Erlenmeyer flasks, and incubated at 35±1ºC for 24 h in an incubator shaker (150 rpm). The cells were grown to late exponential phase, harvested by centrifugation at 10 000 rpm (4ºC) for 30 min and washed thrice with deionized water. Biosorption potential by live bacteria for Cr^{6+} was done by resuspending the cell pellet in deionized water. Biosorbent concentration in cell suspension was determined by drying known volume of an aliquot in a preweighed aluminium foil to constant weight in hot air oven at 80ºC (Puranik and Paknikar, 1999).

Cr^{6+} biosorption potential of dead cells

The harvested washed cell pellet of bacterium was conditioned to a desired pH (3 to 8) by repeated washing with 0.1 N H_2SO_4 or 0.1 N NaOH until the pH of washed water showed no change. The pH-conditioned pellet was then dried in an oven at 80°C till constant weight and finally crushed in pestle and mortar.

Preparation of working solution

The stock solutions 10 000 mg l^{-1} each of Cr^{6+} and PCP were prepared in deionized water using potassium dichromate ($K_2Cr_2O_7$) and sodium pentachlorophenate. All working concentrations were obtained by diluting the stock solution with deionized water. The pH was adjusted to desire values according to the following experimental design with 0.1 N H_2SO_4 or 0.1 N NaOH.

Metal biosorption (% removal) and PCP degradation studies

A sterilized working solution was prepared in distilled water containing 200 mg Cr^{6+} l^{-1} and 500 mg PCP l^{-1} supplemented with glucose 0.4% (w/v) as an additional carbon and energy source. Biosorption of Cr^{6+} by the culture was determined by batch equilibrium method and PCP degradation as per the method of Bergmann and Sanik (1957). The living or dead cells were added (at 2 g l^{-1}) to 25 ml (in duplicate) of above working solution in 150 ml

Erlenmeyer flasks. Pentachlorophenol and Cr^{6+}-free and biosorbent-free solutions were kept as controls. The flasks were incubated at 150 rpm on an incubator shaker as per the varied experimental conditions.

Samples were withdrawn periodically at 20 min intervals till equilibrium was attained. The cells were harvested by centrifugation at 10 000 rpm (4°C) for 10 min and washed twice with deionized water. The biosorbent was then digested with acid mixture 6:1 (perchloric acid and HNO_3). Chromium biosorbed in the digested sample was then analysed using atomic absorption spectrophotometer (AAS) at 357.9 nm. The amount of chromium biosorbed was calculated as mg g^{-1} dry weight. The supernatant was used for analysis of the residual Cr^{6+} and PCP. Cr^{6+} in supernatant samples was determined by 1,5-diphenyl carbazide method (APHA, 1998), extrapolated against the standard curve of $K_2Cr_2O_7$ and reported as percentage (w/v) Cr^{6+} removal. The percentage (w/v) PCP degradation was determined by the estimation of chloride ions released in the supernatant and was extrapolated against the sodium chloride standard curve (Bergmann and Sanik, 1957).

Optimization of various parameters

pH

In order to study the effect of pH on biosorption (% removal) of Cr^{6+} and degradation of PCP, the biosorbent (only dead cells) was conditioned at different pH 3.0-8.0 (live cells were not pH conditioned), prior to inoculation (at 2 g l^{-1}) into working solution (as described above) adjusted to experimental pH range 3.0-8.0 and was taken in duplicate 25 ml volumes. The pH of metal solution was also adjusted to desired corresponding value by addition of aqueous solution of 0.1 N NaOH or 0.1 N H_2SO_4. The flasks were incubated in an incubator shaker at 150 rpm and 35±1ºC for 120 min. Samples were processed and analyzed for biosorbed Cr^{6+} (% removal) and PCP degradation as described above.

Biosorbent concentration

The effect of biosorbent dose (at 1-5 g l^{-1}) was assessed by using the bacterial biomass pellet (25-125 mg) in duplicate 25 ml working solution at optimized pH 4.0 (for pH conditioned dead cells) and pH 5.0 (for live cells). The flasks were incubated in an incubator shaker at 35±1ºC (150 rpm) till equilibrium (120 min) was attained. Samples were processed and analyzed for biosorbed Cr^{6+} (% removal) and PCP degradation as per standard methods.

Initial metal ion concentration

Effect of initial Cr^{6+} concentration on metal biosorption (% removal) and PCP degradation was studied. The Cr^{6+} of varying concentrations (mg l^{-1}: 50, 100, 150, 200, 250) was added to working solution and inoculated with optimized biosorbent (at 2 g l^{-1}) in an incubator shaker (150 rpm) at 35±1ºC for 120 min. The biosorbed Cr^{6+} (% removal) and PCP degradation were analyzed as per standard protocol.

Contact time

To evaluate Cr^{6+} biosorption (% removal) and PCP degradation with respect to contact time (20-140 min), an optimized 2 g biosorbent dose l^{-1} was inoculated in duplicate 25 ml of working solution (pH 5.0 for live cells and pH 4.0 for dead cells) in 150 ml capacity Erlenmeyer flasks. The samples were withdrawn periodically each at 20 min interval up to 140 min, and processed for analyses of biosorbed Cr^{6+} (% removal) and chloride ions released as a measure of PCP degradation.

Temperature

Influence of temperature was assessed by inoculating the optimized biosorbent (at 2 g l^{-1}) to duplicate 25 ml working solution (pH 5.0 for live cells and pH 4.0 for dead cells) in flasks and incubated at different temperature range (25-40°C) in an incubator shaker (150 rpm) for optimized 120 min. The biosorption of Cr^{6+} (% removal) and PCP degradation were analyzed.

Other metal ions

The effect of other heavy metals on Cr^{6+} biosorption was studied. Biosorbent was added at 2.0 g l^{-1} to duplicate 25 ml working solution containing binary metals including 200 mg Cr^{6+} l^{-1} with individual heavy metals [mg l^{-1}: Pb (175), As (105), Hg (25), Zn (60), Co (80), Ni (105)], adjusted to optimized pH 5.0 for live cells and pH 4.0 for dead cells, and incubated at 35±1ºC in an incubator shaker at 150 rpm. Biosorption of Cr^{6+} (% removal) and PCP biodegradation were analyzed.

Computing algorithm and statistical analysis

We propose the system of testing the hypothesis that predicted values of parameters are close to the experimental values. We aimed to test the validity of predicted values by applying the test of "goodness of fit".
The main formulae employed are as under:

$$Q_{eq} = V (C_i^* - C_{eq}^*)/M \quad (1)$$

Equation (1) determines the Cr^{6+} uptake value. Where, * values are taken only for Cr^{6+}.

$$\% \text{ removal of PCP/Cr}^{6+} = (C_i - C_{eq}/C_i) \times 100 \quad (2)$$

Equation (2) determines the per cent removal of PCP or Cr^{6+}.
Where, Q_{eq} is biosorption capacity (metal uptake at equilibrium) in mg g^{-1} biomass; V is the volume of solution (l); C_i is the initial concentration of Cr^{6+} or PCP in solution (mg l^{-1}); C_{eq} is the residual concentration of Cr^{6+} or PCP in solution and M is the weight of biomass (g).

We have developed a computing algorithm to compute the various parameters as indicators involved in the equations (1&2), which have been described in the analysis by developing the computing algorithm and implementing it in C^{++} language.

All experiments in present study were performed in duplicate. The regression model of statistical analysis has been used for prediction of experimental data in order to study line of best fit characterizing the functional relationship between them. The correlations between two interactive variables are being depicted in Figs. 1-6.

RESULTS AND DISCUSSION

Biosorption is a metabolism-independent process, and thus, can be performed by both living and dead cells. This process has been attempted in the present study as one of the most promising technologies for simultaneous bioremediation of chromium and PCP from tannery

effluent by living cells only and single remediation of Cr by both living and dead cells of indigenous *Bacillus cereus* RMLAU1 strain isolated in the laboratory from treated tannery effluent. There are more than one variable such as pH, temperature, dose of microbial biomass, initial chromium concentration, presence of other heavy metals and contact time which influence the biosorption process. Some of these factors (pH, metal concentration and contact time) have profound influence on metal removal through this process. Hence, the effect of these factors on simultaneous bioremediation of Cr and PCP has been attempted in the present study.

Effect of pH

pH is one of the most important physical parameters that influence the biosorption process (Paknikar et al., 1999). The pH dependence of metal adsorption can largely be related to type and ionic state of the functional group (s) present on the biosorbent and the type of metal species present in the solution. Chromium^{6+} is present as dichromate ($Cr_2O_7^{2-}$) in acidic environment (pH range 3 to 6) and as chromate ($Cr_2O_4^{-}$) in alkaline environment at above pH 8.0.

In the present investigation, biosorption (removal) of Cr^{6+} and PCP degradation was studied in solutions with pH ranging from 3 to 8. The initial Cr^{6+} concentration at 200 mg l^{-1} and PCP at 500 mg l^{-1} were selected in this experiment based on maximum tolerance limit of the solate (Tripathi et al., 2010). Figure 1 reveals that, maximum Cr^{6+} biosorption (% removal) was 35.2±1.89 (62.5%) and 42.5±1.28 (69.5%) mg Cr g^{-1} dry weight of live and dead cells at pH 5.0 and 4.0, respectively. Further deviation in initial pH, resulted in reduced Cr^{6+} biosorption by live cells of bacteria. When the biomass of living cells was employed for Cr^{6+} biosorption in unbuffered condition, the redox reaction between the cells and liquid caused decrease in the final pH at all the initial pH values under study. In contrast, the equilibrium pH was relatively unaltered in case of dead biomass (not shown). This was due to prior pH conditioning of the biomass to a desired pH of 4.0 based on availability of maximum binding sites for Cr^{6+} biosorption. High adsorption of Cr^{6+} at low pH can be explained by species of Cr and adsorbent surface characteristics. Bacterial cell walls are negatively charged under acidic pH conditions and the cell wall chemical functional groups display a high affinity for metal ions in solution (Collins and Stotzky, 1992). The surface of adsorbent becomes highly protonated and favours the uptake of Cr^{6+} in anionic form under acidic conditions. The degree of protonation and adsorption decreases with increase in pH. Furthermore, there is competition between hydroxyl and chromate ions for binding, as the former being dominant species at higher pH values. Moreover, as the pH increases, the net positive charge on the surface of sorbent decreases due to reduced protonation, which ultimately leads to reduced sorption capacity.

In this study, the biosorption performance of dead cells was 15.8% higher than their living counterparts. Silver (1992) reported that, internalization of Cr^{6+} by living cells could have dominated, thereby affecting the uptake. We may expect an enhancement in Cr^{6+} biosorption by dead cells as heat treatment of cells might have disintegrated the cell wall, thereby yielding more binding sites. Further, during pH conditioning of cells, breakage of cells might have occurred leading to change in Cr^{6+} binding characteristics. Such a dead bacterial biomass is more or less a particulate matter and thus, can be stored and handled easily. Better performance of biosorption by dead cells over the living ones has been demonstrated in *Myxococcus xanthus* and *Saccharomyces cerevisiae* strains for numerous heavy metals including chromium (Omar et al., 1997).

The extent of simultaneous PCP degradation only by live cells of *B. cereus* at the initial pH range of 3 to 8 is depicted in Figure 1. As the pH increased from 3 to 5, there was a concomitant increase in PCP degradation from 2.5% (w/v) to 7.5% (w/v) followed by gradual decrease approaching 5.5% (w/v) at pH 8.0. Maximum PCP degradation of 7.5% (w/v) was noted at pH 5.0 which corresponded with maximum biosorption of Cr^{6+}. The degradation of PCP occurred only in the presence of 0.4% (w/v) glucose. In the absence of glucose, no PCP degradation was observed indicating the role of glucose as a co-metabolite. During the course of glucose catabolism as energy source, the chlorinated xenobiotic PCP molecule becomes accessible and therefore, its degradation is the resultant process of co-metabolism (Premlatha and Rajkumar, 1994). The results suggest that, a stringent control on pH makes it an ideal bioremediation technology. Here, it is very interesting that regression model exhibited a positive correlation between most of the experimental and predicted values under consideration. Validity of prediction has been scientifically tested by designing the hypotheses for experimental and predicted values which has been further tested by applying the goodness of fit. Model used and testing performed showed that, predicted values are in good agreement with experimental values at $p < 0.01$, whereas it is significant at $p < 0.05$.

Effect of biosorbent and initial metal ion concentration

The initial Cr^{6+} and biosorbent concentrations are important parameters considered for effective biosorption process. In order to work out the optimum biosorbent dose, varying biosorbent concentrations in the range of 1 to 5 g l^{-1} were taken in the presence of initial 200 mg Cr^{6+} l^{-1} and 500 mg PCP l^{-1} solution supplemented with 0.4% glucose as a cometabolite and the results are presented

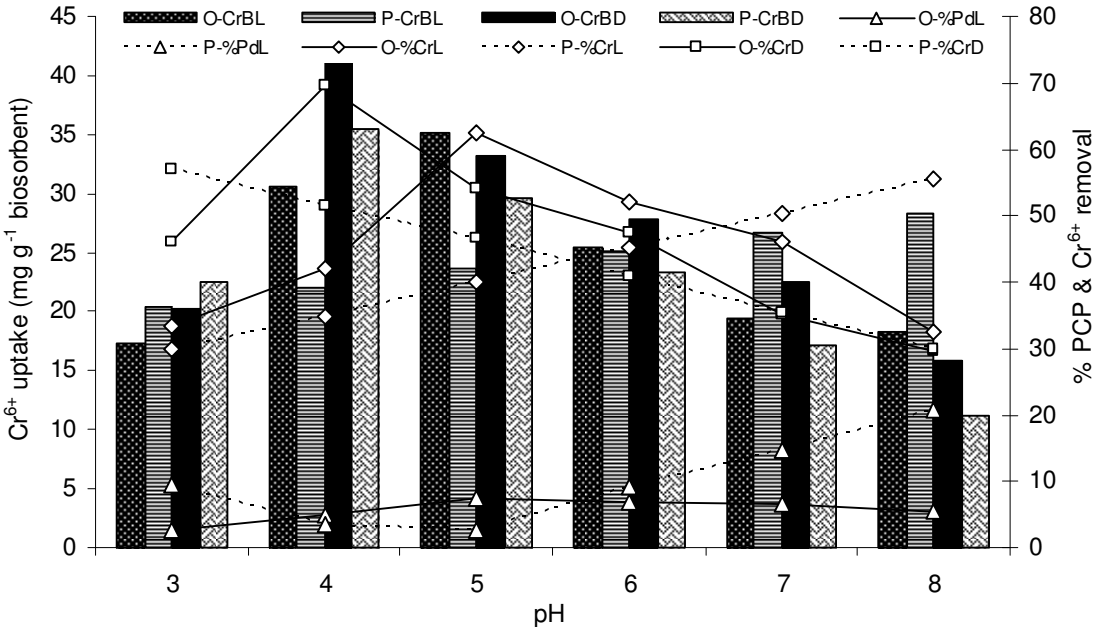

Figure 1. Effect of pH (3 to 8) on simultaneous Cr^{6+} biosorption (CrB)/% removal (% Cr) and PCP degradation (%Pd) by live (L) and dead (D) cells of *Bacillus cereus* RMLAU1. The experiment was performed with 2 g biosorbent l^{-1}, initial Cr^{6+} 200 mg l^{-1}, PCP 500 mg l^{-1} at 35°±1°C and 150 rpm up to equilibrium. Dotted lines (------) and bars (▤, ▨) show the best fit; O-observed value; P-predicted value.

in Figure 2. The optimum biosorbent dose was 2 g l^{-1} for maximum Cr^{6+} adsorption of 34.9 mg g^{-1} (126 mg l^{-1} that is 63%) and 41.7 mg g^{-1} (138 mg l^{-1}, 69%) of live and dead cells, respectively. At optimum biosorbent level, sufficient large number of binding sites were available on the biomass for electrostatic interaction. Whereas, at higher biosorbent concentration, the metal uptake value marginally decreased, as excessive extra free binding sites were still available on biosorbent which might have decreased the electrostatic interaction between metal and binding sites of biosorbent (Fourest and Roux, 1992). At higher biosorbent concentrations, lower uptake of metal ions could be attributed to decreased metal-to-biosorbent ratio (Puranik and Paknikar, 1999). Contrary to Cr^{6+} biosorption, simultaneous PCP degradation was not significantly affected by the concentration of biomass, and maximum remediation (7.5%, w/v) of initial 500 mg PCP l^{-1} was recorded by 2 g live biomass l^{-1} during the contact time of 120 min. With increase in biosorbent concentration from 2 to 5 g l^{-1}, the PCP degradation remained more or less constant (Figure 2).

Adsorption experiments at initial Cr^{6+} concentrations from 50 to 250 mg l^{-1} were performed with optimized fixed dose (2.0 g l^{-1}) of live and dead bacterial biomass. The results depicted in Figure 3 indicate that, percentage of Cr^{6+} removal from the solution decreased with increase in initial Cr^{6+} concentration. The Cr^{6+} removal ranged from 97.5 to 57% and 98.5 to 59% by live and dead cells, respectively at initial Cr^{6+} concentration of 50 to 250 mg l^{-1}. Our findings are in accordance with that of Quintelas et al. (2006) who reported decreased percent removal of Cr^{6+} at higher initial concentrations. They offered possible explanation that the chromate ($Cr_20_4^-$) with its toxicity and high oxidative potential might have inhibited the biological activity of *Arthobacter viscosus* biofilm. However, if our results are interpreted in terms of biosorption of Cr^{6+} g^{-1} biomass, there was an increase in the rate of metal adsorption with increase in metal ion concentration by both live as well as dead cells (Figure 3).

At lower concentrations (<200 mg Cr^{6+} l^{-1}), metal ions present in the solution possibly interacted with the binding sites, and thus, facilitated more %Cr^{6+} removal. On the other hand, at higher concentrations, more Cr ions were left unabsorbed in solution due to saturation of the binding sites. This appears to be due to increased moles of chromium competing for the available binding sites in the biosorbent and hence, the removal percentage of chromium is dependent on the initial percentage (Puranik and Paknikar, 1999). Furthermore, at higher Cr^{6+} concentrations, the average distance between adsorbing species is reduced, which affects the charge distribution of its neighbors, thereby altering the ability of species to migrate to the biomass surface, resulting in reduced adsorption (Horsfall et al., 2006).

This is amply evident from the foregoing that it is very essential to know the optimum ratio of biomass to metal concentration (Puranik and Paknikar, 1999). The Cr^{6+} biosorption results depicted in Figures 2 and 3 reveal that, metal uptake by biomass were chemically equilibrated and involved saturable mechanism. Thus, there was

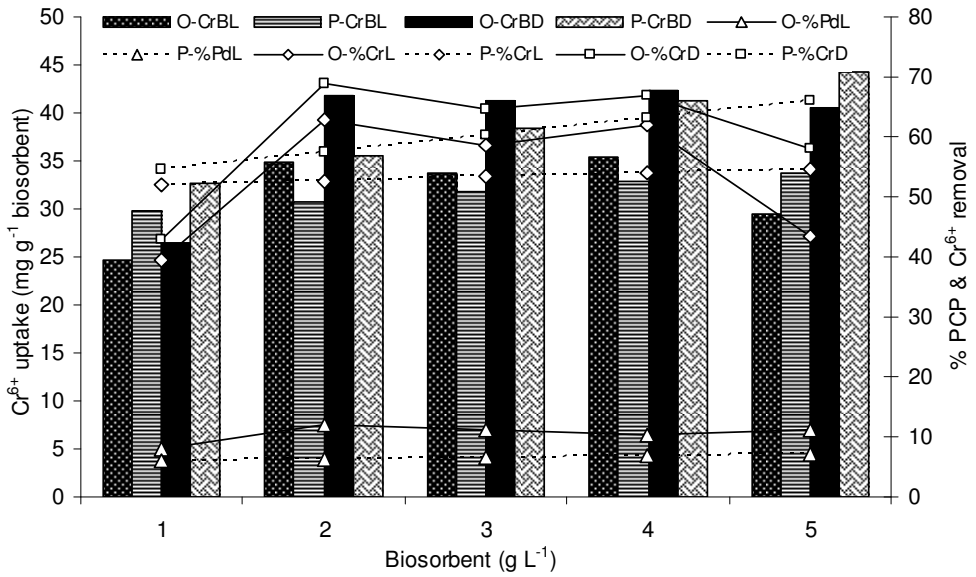

Figure 2. Simultaneous biosorption of Cr^{6+} (CrB)/% removal (% Cr) and PCP degradation (%Pd) at different concentrations (1 to 5 g l^{-1}) of biosorbent (live (L) and dead (D) cells). The experiment was performed with initial Cr^{6+} 200 mg l^{-1}, PCP 500 mg l^{-1} at optimized pH 4.0 for dead cells and 5.0 for live cells at 35°±1°C and 150 rpm up to equilibrium. Dotted lines (------) and bars (▤, ▨) show the best fit; O-observed value; P-predicted value.

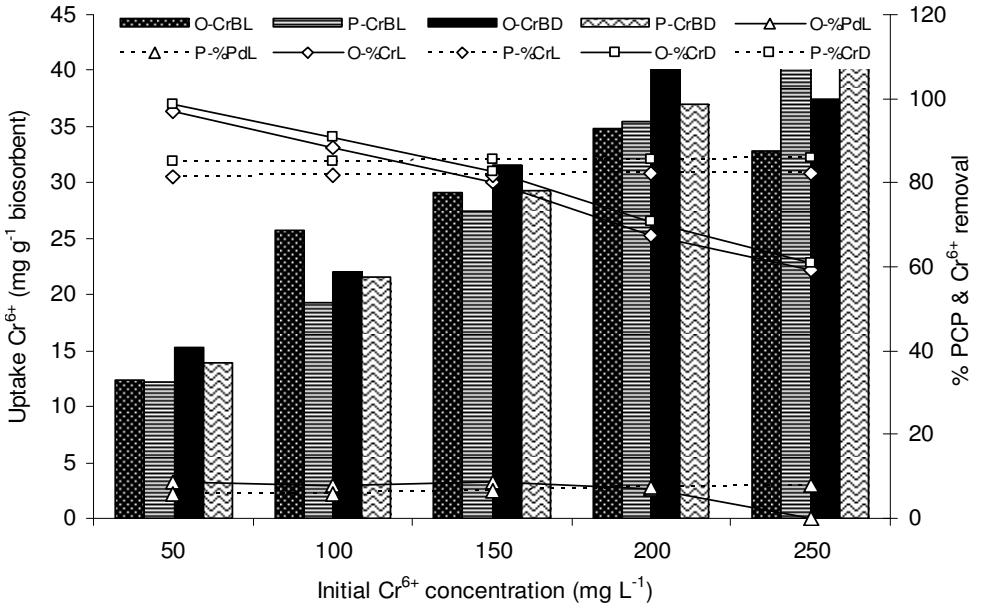

Figure 3. Effect of initial Cr^{6+} concentrations (50 to 250 mg l^{-1}) on simultaneous biosorption of Cr^{6+} (CrB)/% removal (% Cr) and degradation of PCP (%Pd) by live (L) and dead (D) of *Bacillus cereus* RMLAU1. The experiment was performed with 2 g biosorbent l^{-1}, PCP 500 mg l^{-1} at 35°±1°C, pH 4.0 for dead cells and pH 5.0 for live cells at 150 rpm up to equilibrium. Dotted lines (------) and bars (▤, ▨) show the best fit; O-observed value; P-predicted value.

an increase in metal uptake as long as binding sites were free. The extent of simultaneous PCP degradation was maximum (8.5%, w/v) at 50 mg $Cr^{6+}l^{-1}$, which gradually decreased with increase in Cr^{6+} concentration up to 200 mg l^{-1} approaching zero PCP degradation at 250 mg Cr^{6+} l^{-1} indicating that, this level is highly toxic for *B. cereus*

live biomass. The predicted values are compared with the corresponding experimental data, and it could be inferred that the experimental values are in congruence with predicted values at $p < 0.02$, whereas it is significant at $p < 0.05$. Therefore, experimental data were validated with predicted values at $p < 0.02$ (Figures 2 and 3).

Effect of temperature

Biosorption (% removal) of Cr^{6+} and PCP degradation were studied at different temperatures. The results reveal that isolate offers a broad range of temperature from 25° to 40°C (Figure 4). With increase in temperature from 25° to 35°C, there was concomitant marginal increase in Cr^{6+} biosorption (% removal) ranging from 30.3 to 35.0 mg g^{-1} biomass (49.5 to 61.5%) and 39.1-42.3 mg g^{-1} biomass (56.5 to 68.5%), respectively by living and dead cells of *B. cereus* isolate. Further increase in temperature to 40°C caused slight decrease in the extent of biosorption as well as percent removal of Cr^{6+} from the solution. Simultaneous degradation of PCP to the extent of 3.5 to 7.5% (w/v) was noted in the temperature range of 25° to 35°C by live biomass only, which was reduced to more than half (3.0%, w/v) when temperature was further elevated to 40°C. Therefore, maximum Cr^{6+} biosorption (removal) as well as PCP degradation was achieved at 35°±1°C (Figure 4). It may be inferred from the results that fairly good efficiency of Cr^{6+} biosorption (% removal) and PCP degradation was achieved throughout the temperature range under study.

The mechanism of biosorption is metabolism independent and appears to be little affected by changes in certain temperature range; however, this can not be generalized for all the biosorbents and metals. Srinath (2001) has reported that, biosorption of Cr^{6+} by *Bacillus coagulans* was less affected and had a broad optimal temperature range of 20° to 40°C for the purpose. Such a situation is evident when the chemical adsorption plays the dominant role (Sag and Kustal, 1996). The enhancement in metal sorption can be attributed to increase in energy level of the system that facilitates metal attachment to the cell surface. Further increase in temperature from the optimum causes decrease in sorption possibly due to distortion of some chemical sites on the cell surface available for metal adsorption (Al-Asheh and Duvnjak, 1995). It is also suggested that, increase in metal uptake with temperature increase could be due to either higher affinity of sites for metal or an increase in binding sites on the relevant biomass (Marques et al., 1991). Thus, our results reveal that the adsorption sites present on *B. cereus* biosorbent are sufficiently stable and therefore, offer better efficiency for Cr^{6+} interaction. The flexibility in temperature range for Cr^{6+} biosorption makes the process applicable to various geographical locations and broadens the scope of applicability in various industrial sectors which face the problem of safe disposal of Cr^{6+} laden effluent. In this study, PCP degradation by live biomass of *B. cereus* was significantly affected as it is a metabolism dependent event in which supplemented glucose serves as an energy source and PCP is co-metabolically degraded enzymatically. The comparison between the experimental results and those predicted by regression model is shown in Figure 4. The experimental values were very close to predicted ones at $p < 0.05$, thereby fairly validating our experimental results with predicted values at $p < 0.05$.

Effect of other metal ions

Industrial effluent with the presence of single metal ion species can never occur. They are usually a blend of numerous metals and non metals as cations and anions, thus, may impart some hindrance or assistance in binding of concerned metals. In a previous study, the presence of other metal ions in the treated tannery effluent has been reported (Tripathi et al., 2011). The presence of these ions complicates the biosorption process depending on their competitiveness and toxicity (Volesky and Holan, 1995). Therefore, in the present study, the effect of other heavy metals on simultaneous Cr^{6+} biosorption and PCP degradation was attempted. Figure 5 reveals that, other metal ions (mg l^{-1}) such as mercury (25), lead (175), arsenic (105), nickel (100), cobalt (60) and zinc (75) negligibly affected the Cr^{6+} uptake by both live and dead biomass whereas, PCP degradation was adversely affected to a varied extent by live biomass only. The order of percent inhibitory effect of other metal ions on PCP degradation was: mercury (100)>arsenic (66.67)> nickel (46.67)> cobalt (40)> zinc (33.34)> lead (20).

Our results are in agreement with the findings of Srinath (2001) who has reported insignificant effect of cationic and anionic (except sulphate ions) heavy metals on biosorption of Cr^{6+} by both live and dead biomass of *B. coagulans.* The uncompetitveness of these cations may be attributed to the optimum pH in acidic range for Cr^{6+} biosorption. Since the biomass is highly protonated at acidic pH range, repulsion of cations is eminent and has to compete with H^+ and H_3O^+ for adsorption sites (Antuner et al., 2001). Regression model provided sufficient evidence for the existence of positive correlation between observed and predicted value at $p < 0.05$ indicating that, experimental results are very close to predicted values at $p < 0.05$ (Figure 5).

Effect of contact time

Contact time of biomass with metal ions for attaining equilibrium is relevant for the knowhow of a process as well as it is the driving factor for the economy of a process to be used for industrial purpose. Biosorption is a rapid phenomenon, where equilibrium is attained within

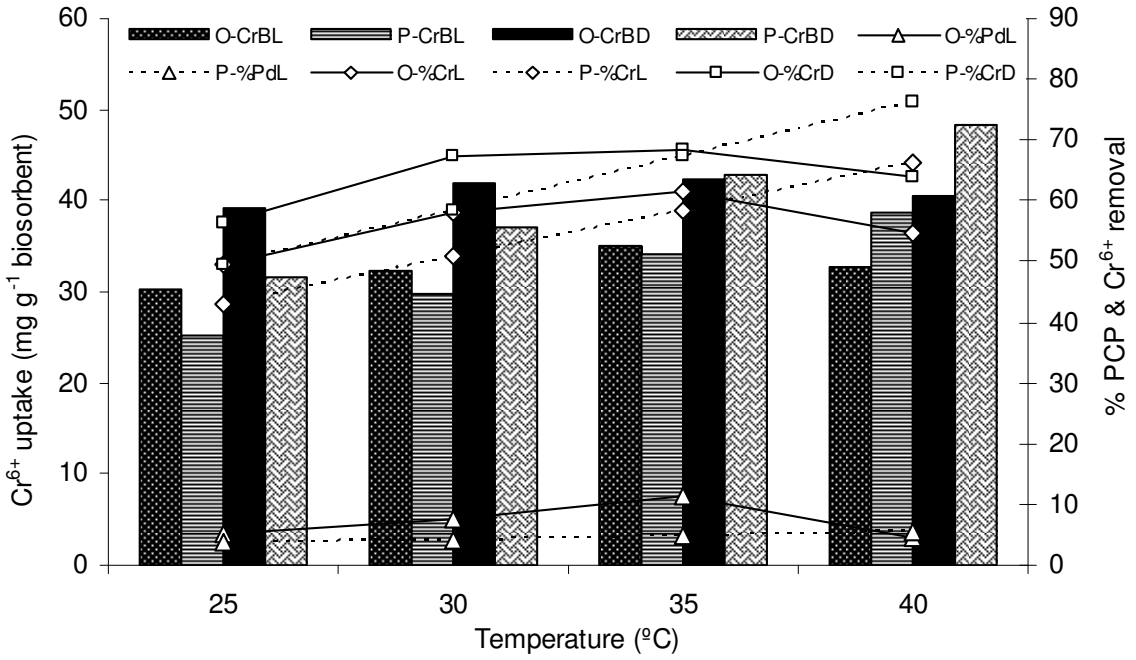

Figure 4. Influence of temperature (25° to 40°C) on simultaneous biosorption of Cr^{6+} (CrB)/% removal (% Cr) and PCP degradation (%Pd) by live (L) and dead (D) cells of *Bacillus cereus* RMLAU1. The experiment was performed with 2 g biosorbent l^{-1}, initial Cr^{6+} 200 mg l^{-1}, PCP 500 mg l^{-1} at pH 4.0 for dead cells, 5.0 for live cells at 150 rpm up to equilibrium. Dotted lines (------) and bars (▤, ▨) show the best fit; O-observed value; P-predicted value.

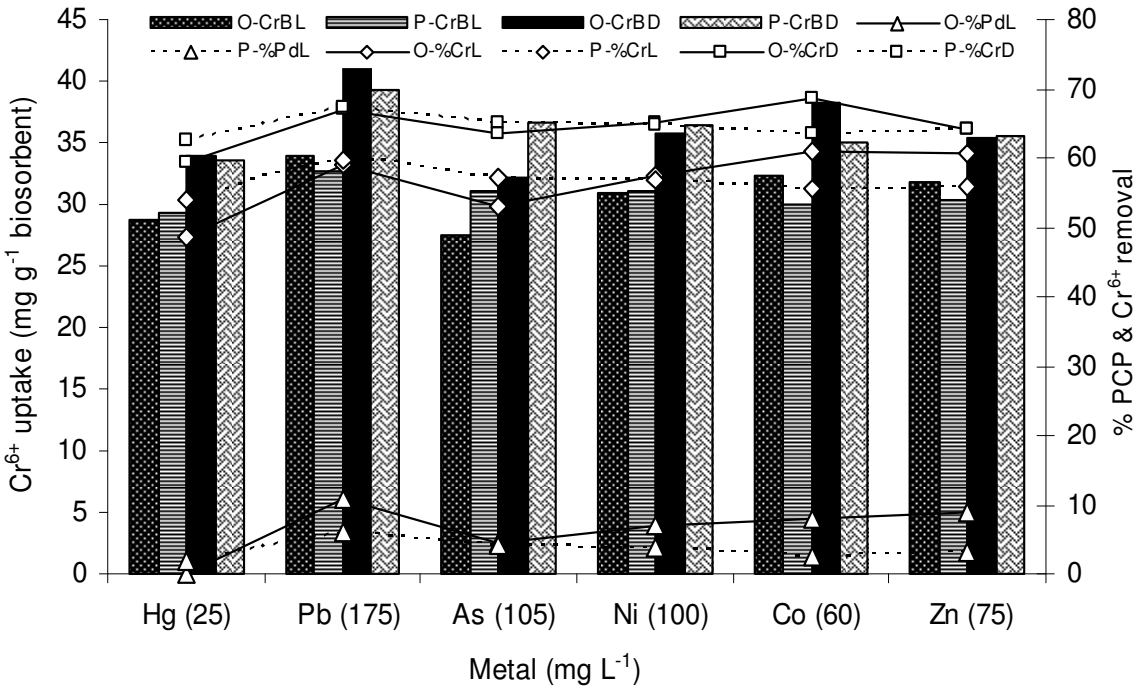

Figure 5. Dual effect of other heavy metals with Cr^{6+} on simultaneous biosorption of Cr^{6+} (CrB)/% removal (% Cr) and PCP degradation (%Pd) by live (L) and dead (D) cells of *Bacillus cereus* RMLAU1. The experiment was performed with 2 g biosorbent/l at 35°±1°C, pH 4.0 for dead cells, 5.0 for live cells at 150 rpm up to equilibrium (120 min) with initial 200 mg Cr^{6+} l^{-1} and PCP 500 mg/l concentration. Dotted lines (------) and bars (▤, ▨) show the best fit; O-observed value; P-predicted value.

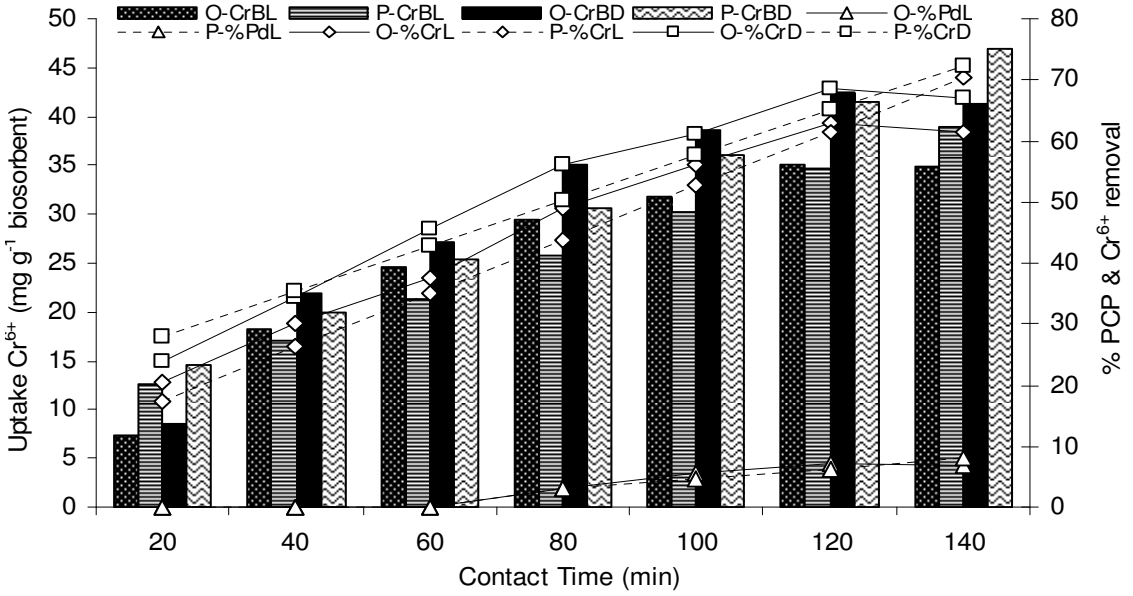

Figure 6. Effect of contact time (20 to 140 min) on simultaneous biosorption of Cr^{6+} (CrB)/% removal (% Cr) and PCP degradation (%Pd) by live (L) and dead (D) cells of *Bacillus cereus* RMLAU1. The experiment was performed with 2 g biosorbent l^{-1} at 35°±1°C, pH 4.0 for dead cells, 5.0 for live cells with 150 rpm up to equilibrium with initial 200 mg Cr^{6+} l^{-1} and PCP 500 mg l^{-1} concentration. Dotted lines (------) and bars (▤, ▨) show the best fit; O-observed value; P-predicted value.

few hours under optimum conditions. In the present study, the effect of contact time on biosorption of Cr^{6+} and PCP degradation was performed under optimum conditions during 140 min incubation and the results are presented in Figure 6. The process attained equilibrium at 120 min and remained almost constant upon further increase in contact time to 140 min. The percentage of Cr^{6+} removal, biosorption g^{-1} biomass and PCP degradation increased with time up to 120 min followed by constancy of all parameters at 140 min contact time. In a previous study, the simultaneous PCP degradation of 56.5% (w/v) and 74.5% (w/v) Cr^{6+} reduction occurred during 48 h incubation period (Tripathi et al., 2011). However, in this study, the maximum 7.5% (w/v) PCP degradation and 70.5% Cr^{6+} removal was achieved within 120 min only, which is time wise more feasible and economical for industrial applications.

Our isolate appears to be more efficient too for simultaneous bioremediation of Cr^{6+} and PCP in a very short operation time than the strains of bacteria reported by other researchers (Quintelas et al., 2006; Srivastava et al., 2007, 2008; Parameswari et al., 2009). Thus, *B. cereus* RMLAU1 can be employed to mitigate the contamination of PCP and Cr^{6+} simultaneously in industrial effluents, particularly tannery wastewater in a shorter operation time. Regression model showed positive correlation between the experimental and the predicted value at $p < 0.05$; consequently, our prediction is validated against the experimental observations at $p < 0.05$ (Figure 6).

Conclusions

The indigenous bacterial isolate of *B. cereus* RMLAU1 can be efficiently employed as an economically feasible and eco-friendly biosorbent owing to its resilience to a wide range of environmental conditions which makes it an alternative to conventional methods for remediation of Cr^{6+} and PCP from tannery effluent. The Cr^{6+} biosorption (% removal) and degradation of PCP predicted by regression model at optimal conditions clearly revealed the positive correlation with most of the experimental data as validated by the statistical technique of testing of the hypothesis. Cr^{6+}concentration in industrial wastewater range from 0.5 to 270 mg l^{-1}. The discharge limit for Cr^{6+} into inland surface waters is 0.1 mg l^{-1} and in potable water is 0.05 mg l^{-1} (Dubey and Gopal, 2007). The isolate has a tolerance limit for Cr^{6+} at 200 mg l^{-1} and PCP at 500 mg l^{-1} and is capable of efficiently bioremediating both of them simultaneously. The future prospect includes the use of this promising isolate as a biosorbent for the treatment of industrial tannery effluent for simultaneous bioremediation of PCP and other heavy metals including chromium.

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