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Bioreduction of Cr(VI) using live and immobilized *Phanerochaete chrysosporium*

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ABSTRACT

The aim of the study was to investigate the bioreduction capacity of the live and immobilized *P. chrysosporium*. The basidiospores were immobilized in different matrices viz., Ca alginate, acryl amide and agar. Of the various dosages of inoculums studied for each matrix 10% (v/v) Ca alginate, acryl amide, agar was found to be optimum for the growth and reduction of Cr(VI). The effects of the physical parameters like glucose concentration, pH, and temperature on bioreduction were investigated. A maximum of 98.3% of bioreduction of Cr(VI) was obtained with an initial Cr(VI) concentration of 10 mg L^{-1} at pH 5 and temperature of 25°C. The optimum concentration of glucose for the bioreduction of Cr(VI) was 20 g L^{-1}. The best suitable matrix was optimized to be Ca alginate. An enzyme-based model was also studied.

Keywords: Bioreduction; Cr(VI); White rot fungi; Immobilization; Ca alginate; Acryl amide; Agar

1. Introduction

Chromium is widely used in industrial applications such as electroplating, tanning and textile dyeing. Chromium exists in hexavalent and trivalent forms in wastewaters. Cr(VI) is toxic and highly soluble in water. Cr(III) is less toxic and less soluble in water. Cr(VI) is more toxic and mutagenic in both humans and animals. Chromate poisoning causes severe skin disorders such as allergic dermatitis and liver and kidney damage. It is mandatory to reduce the toxicity of Cr(VI) to the permissible limit before disposal into water bodies. The conventional methods for treatment of chromium-bearing wastewater are electrochemical treatment, oxidation, reduction, filtration, ion exchange and reverse osmosis. Most of these methods require high capital cost and recurring expenses like chemicals, which are not suitable for small scale industries [1]. This prompts the need to develop cheaper and safer methods for the treatment of Cr(VI)-rich wastewater such as biological processes that are more economical, safer and sustainable. The potential use of microorganisms in the detoxification/removal of heavy metals from wastewater is gaining importance. The uptake of heavy metals by microbes is a biphasic process. It involves initial surface binding followed by energy-dependent intracellular reduction.

The application of bacteria for the removal of chromium from wastewater is widely studied. Fungal biomass has certain advantage over bacterial biomass with respect to processing and handling [2]. Many fun-

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gal species have been extensively used for heavy metal biosorption and relatively few studies have been done with *Phanerocahete chrysosporium* [3]. *P. chrysosporium* is a well known white rot fungus and a strong degrader of various xenobiotics. It could also be used for heavy metal removal from wastewater [4]. In industrial or technical operations the immobilized microbial cells provide additional advantages over free cells. Immobilized cells are easy to separate and have less clogging effect during continuous operation [5]. Nancharaiah et al. [6] reported complete removal of uranium and Cr (VI) by immobilized microbial granules.

Natural polymers such as alginate, chitosan, chitin, and cellulose are mostly used as the matrix for the immobilization of microbial cells via entrapment technique. These polymers are also known to bind metal ions strongly [7]. Immobilized fungal cells are found to be far more stable than fungal free cells during metal removal from wastewater [5]. The use of non-living and inactive biomass for large scale utilization is not practicable. The native forms of fungal cells suffer from low mechanical strength and smaller particle size and difficulty in separation from liquid stream [8].

The choice of immobilization matrix is an important factor in the application of immobilized cells in wastewater studies. The polymer matrix determines the mechanical strength and the chemical resistance of the microbial cells. The most extensively investigated biopolymer in the bioremediation studies is Ca alginate [9]. In this work the basidiospores of the white rot fungus *P. chrysosporium* were entrapped using Na-alginate and three other matrices. After growth the entrapped spores were studied for bioreduction of Cr(VI) from aqueous solution in a batch system. The mechanically stable matrix for immobilization of fungi was investigated. Various physical parameters like the temperature and pH for the bioreduction were studied. The chromium uptake capacity of the entrapped spores was studied.

2. Experimental

2.1. Microorganism and media

White rot basidiomycete, *P. chrysosporium* (MTCC 787), a white rot fungus was obtained from the Institute of Microbial Technology, Chandigarh, India. The culture was maintained by subculturing on malt dextrose agar slants. Spore suspensions for immobilization were freshly prepared from 7-day-old culture, grown on malt dextrose agar slants at 25˚C.

The general growth media for growth of the fungi *P. chrysosporium* consisted of malt extract 20 g L^{-1}, dextrose 20 g L^{-1}, and peptone 1 g in 1 L of distilled water. The pH of the medium was maintained at 6 using 0.1 N HCl. The media for bioreduction contained malt extract 20 g L^{-1}, dextrose 20 g L^{-1}, and peptone 1 g spiked with different concentrations of chromium in 1 L of distilled water. All media were autoclaved (Indfos 110 PB) at 15 kPa and 120˚C for 20 min.

2.2. Chemicals

All reagents were of AR grade and procured from Merck, India. Cr(VI) stock solution (1000 mg L^{-1}) was prepared by dissolving $K_2Cr_2O_7$ salt in deionized water. For bioreduction experiments, concentrations ranging from 10 mg L^{-1} to 40 mg L^{-1} were prepared. The chemicals used for the biopolymeric matrices are Ca alginate (Loba chemie, India), acryl amide (Merck, India), agar (Merck, India).

2.3. Immobilization of *P. chrysosporium* basidiospores

The entrapment of the basidiospores was done on various polymer matrices viz., Ca alginate, acryl amide and agar.

A 2% slurry of Ca alginate was prepared in hot water. After cooling, different concentrations (2% to 10% v/v) of the fungal spore suspensions (2.8×10^8 spores per mL) were added and stirred. The mixture was introduced into a solution containing 0.1 M of $CaCl_2$ through a burette and stirred. The height of the burette was adjusted to get a uniform bead size of 4 mm every time. The beads were cured in $CaCl_2$ for 2 h and washed thrice with 200 mL of deionized water.

A (10% w/v) polyacryl amide gel was prepared with varying concentrations of biomass (2% to 10% v/v) and the polymerized gel was cut in to beads of equal size (3 mm). The gel beads were preserved at 4˚C.

A 2% agar solution was prepared by boiling agar in hot water. The solution was allowed to cool for polymerization and 2–10% of fungal spore suspension was added to the solution. The resultant polymer was cut in to equal sized beads of (3 mm). The agar beads were stored at 4˚C before use.

2.4. Bioreduction studies

The immobilized beads were transferred to 100 mL of the growth media in a 250 mL flask and incubated in an orbital shaker (Daihan LabTech Co. Ltd., Model LSI 3016-R) at 25˚C for 120 h. The mycelia growth was monitored using a microscope. The metabolically active immobilized fungus was transferred from growth media to the media spiked with (10–40 mg L^{-1}) of chromium. For all the experiments carried out with immobilized fungus, spore-free matrices were used as control to study the effect of the matrices on bioreduc-

tion. The effect of media pH, temperature and initial chromium concentration on the bioreduction was also studied.

2.5. Analytical procedure

After the bioreduction the supernatants were collected by centrifugation (Remi R-24) and analyzed for total chromium concentration using Atomic Absorption Spectrophotometer (Varian AA140). The Cr (VI) concentration was measured using a UV–vis spectrophotometer (Perkin Elmer, Model Lambda 35) at 540 nm by complexation with 1,5 diphenyl carbazide.

3. Kinetics of Cr(VI) reduction

Shen and Wang [10], demonstrated that Cr(VI) reduction by enzymes can be expressed in the form of Monod Eq. (1). The model proposed by Shen and Wang considered the concentration of active cell mass and proposed the Cr(VI) reduction model.

$$-\frac{d\mathrm{Cr}}{dt} = \frac{K_m \mathrm{Cr}}{K_c + \mathrm{Cr}} X \tag{1}$$

where Cr ($\mathrm{mg\,L^{-1}}$) is the concentration of Cr(VI) at time t (h), X is the concentration of biomass ($\mathrm{cells\,L^{-1}}$). K_m and K_c are the specific Cr(VI) reduction rate ($\mathrm{mg\,Cr(VI)\,cell^{-1}\,h^{-1}}$) and half velocity constant ($\mathrm{mg\,Cr(VI)\,L^{-1}}$) respectively.

The concentration of the Cr(VI) was dependent on the concentration of the active biomass, X ($\mathrm{cells\,L^{-1}}$). It was assumed that the active cell mass concentration decreases with time due to the toxicity of the Cr(VI),

$$X = X_o - \frac{\mathrm{Cr}_o - \mathrm{Cr}}{R_c} \tag{2}$$

where R_c is the maximum Cr(VI) reduction capacity of the cells ($\mathrm{mg\,Cr(VI)\,cell^{-1}}$).

Replacing the term biomass with the active biomass concentration in Eq. (1),

$$-\frac{d\mathrm{Cr}}{dt} = \frac{K_m \mathrm{Cr}}{K_c + \mathrm{Cr}} \left(X_o - \frac{\mathrm{Cr}_o - \mathrm{Cr}}{R_c} \right) \tag{3}$$

Eq. (3) was integrated,

$$t = \frac{K_c}{K_m \left(\frac{\mathrm{Cr}_o}{R_c} - X_o \right)} \ln \left[\frac{\mathrm{Cr} X_o}{\mathrm{Cr}_o \left(X_o - \frac{\mathrm{Cr}_o - \mathrm{Cr}}{R_c} \right)} \right] + \frac{R_c}{K_m} \times \ln \left[\frac{X_o}{\left(X_o - \frac{\mathrm{Cr}_o - \mathrm{Cr}}{R_c} \right)} \right] \tag{4}$$

Eq. (4) represents the Cr(VI) reduction by the active biomass at various time intervals.

It was evident from Eq. (4), that the maximum amount of Cr(VI) that can be reduced was dependent on the active biomass concentration.

4. Results and discussion

The effect of the glucose, temperature and pH on the bioreduction of Cr(VI) with immobilized *P. chrysosporium* was studied and the results are discussed in this section. Various matrices for immobilization like Ca alginate, acryl amide and agar were investigated. The best suitable matrix with high mechanical stability and chemical resistance was optimized.

4.1. Glucose consumption during bioreduction of Cr(VI)

Glucose was tested as the sole carbon source for the growth and reduction of Cr(VI) by *P. chrysosporium*. The glucose concentration in the media was varied from 0 to $20\,\mathrm{g\,L^{-1}}$ and a trace concentration of glucose was analyzed periodically using dinitro salicylic acid method. It was observed that the glucose was completely utilized for the initial concentrations of 10 and $20\,\mathrm{mg\,L^{-1}}$ of Cr by the fungus entrapped in Ca alginate (Fig. 1). This was a clear indication that the fungus was metabolically active throughout the study. Glucose provided the essential energy required for the metabolic activity of the fungi. The decrease in glucose consumption for a concentration of Cr(VI) exceeding $20\,\mathrm{mg\,L^{-1}}$ was due to the fact that chromium acts as an inhibitor of growth of the organism and there by affected the metabolic activity of the fungi.

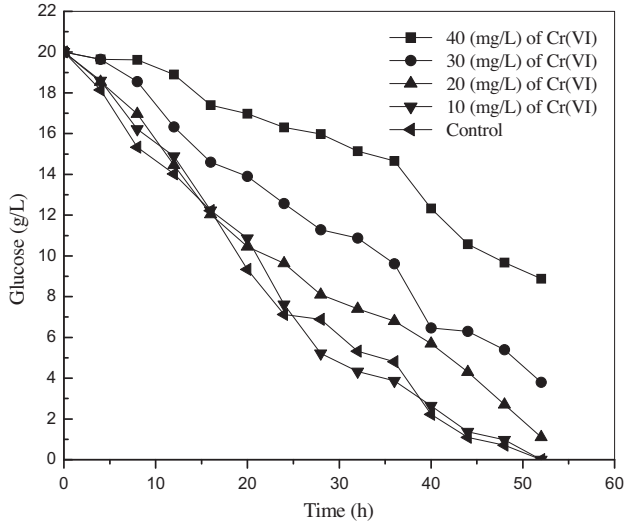

Fig. 1. Glucose consumption profile.

4.2. Effect of pH on bioreduction

The medium's pH affects the solubility of the metal ions and the ionization state of the functional groups. pH of the media affects the activity of the biosorbents [11]. The bioreduction was studied at various pH ranging from 2 to 7. Maximum bioreduction was obtained with pH 5. A maximum of 24, 48, 78, 98.3, 92.58, 90.24% of bioreduction were reported for pH 2, 3, 4, 5, 6, 7 respectively with an initial Cr(VI) concentration of $10\,mg\,L^{-1}$ (Fig. 2). The increase in bioreduction from pH 3 to 5 was due to the physicochemical interaction between the metal ions with the charged groups of the matrix. As the pH was increased further the charge on the cell wall was neutralized and thereby the bioreduction capacity decreased. It was found that pH 5 was the optimum pH for the bioreduction using immobilized beads.

4.3. Effect of time on bioreduction

The bioreduction of Cr(VI) was studied for an extended period of 52 h. It was observed that the bioreduction of the Cr increased with time. A maximum of 98.3% bioreduction of Cr(VI) was reported at 52 h for an initial concentration of $10\,mg\,L^{-1}$ with fungus entrapped in Ca alginate (Fig. 3). It was also observed that the Ca alginate was the best suitable matrix. The other two matrices reduced 92.6 and 90.3% of Cr(VI) at 52 h.

4.4. Effect of temperature on bioreduction

The bioreduction of Cr(VI) was studied at various temperatures ranging from 10°C to 35°C. The bioreduction capacity of the organism was found to increase when the temperature increased from 10°C to 25°C. When the temperature was further increased there was no significant reduction. It was observed that 25°C was found to be the optimum temperature for the bioreduction. The maximum bioreduction obtained at 25°C was 98.3% (Fig. 4). The decrease in the bioreduction with increase in temperature can be attributed to the fact that the organism was acclimatized to a specific temperature (25°C). The substrate consumption was also dependent on the temperature. Even a small change in the operating temperature affected the metabolic activity of the fungi which in turn affected its bioreduction capacity.

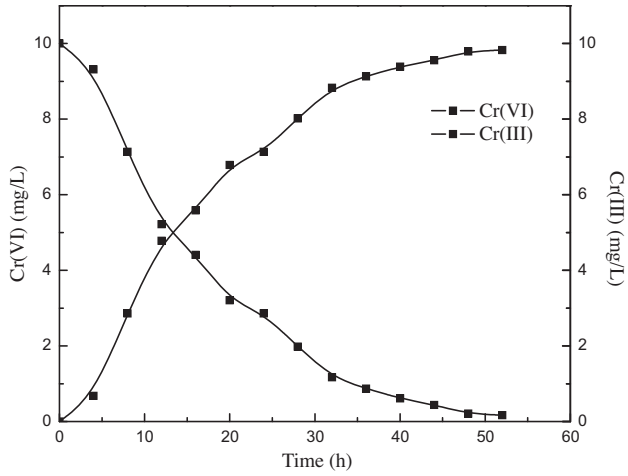

Fig. 3. Effect of time on bioreduction of Cr(VI).

4.5. Effect of inoculum size

The inoculums size was varied from 2% (v/v) to 10% (v/v). It was observed that the bioreduction was

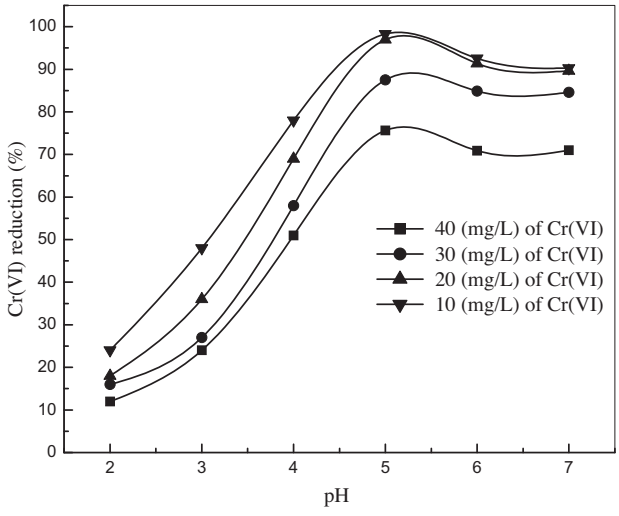

Fig. 2. Effect of pH on bioreduction of Cr(VI).

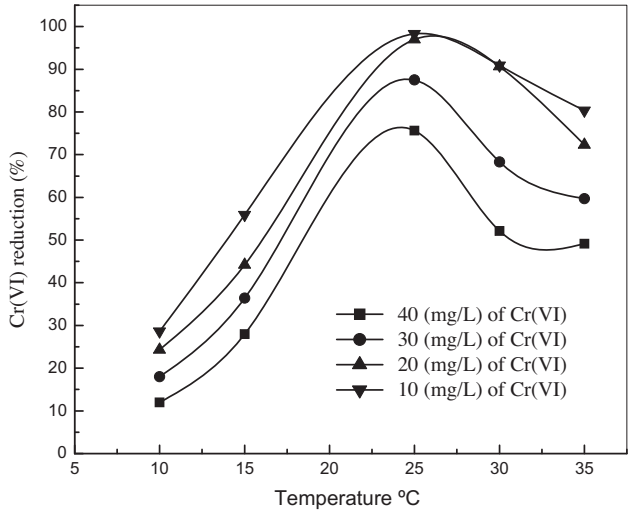

Fig. 4. Effect of temperature on bioreduction of Cr(VI).

Table 1
Percentage reduction with different matrices

Matrix	Cr(VI) reduction (%) Initial Cr(VI) concentration (mg L^{-1})			
	10	20	30	40
Ca alginate	98.3	96.95	87.53	75.65
Acrylamide	90.3	68.4	46.6	29.2
Agar	92.6	89.3	56.13	54.9

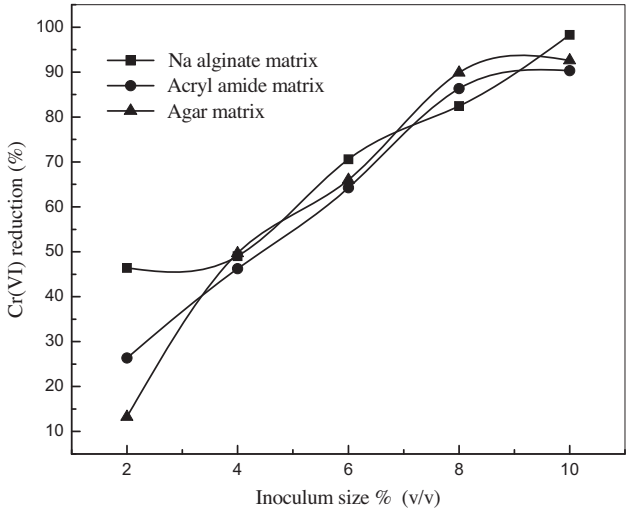

Fig. 5. Effect of inoculum dosage on bioreduction of Cr(VI).

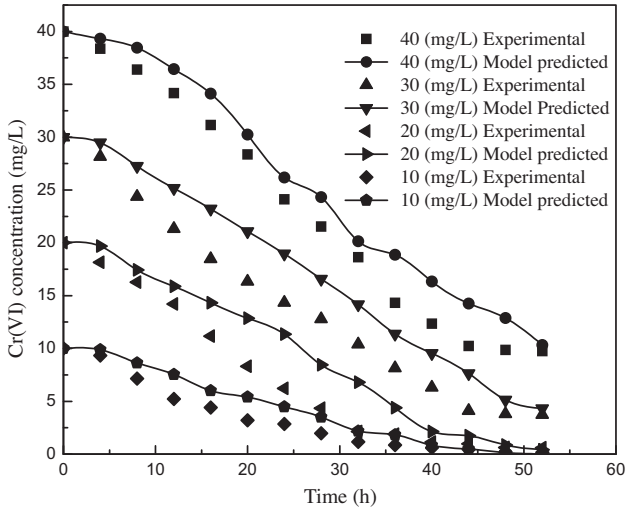

Fig. 6. Kinetic modeling of Cr(VI) bioreduction.

Table 2
Kinetic parameters for Cr(VI) reduction (40 mg L^{-1}) using Ca alginate immobilized *P. chrysosporium*

Matrices	K_m (mg Cr(VI)/cell^{-1} h^{-1})	K_c (mg Cr(VI) L^{-1})	R_c (mg Cr(VI) cell^{-1})	X_o (cells L^{-1})	Maximum reduction capacity (mg Cr (VI) L^{-1}) R_cX_o	
					Model predicted	Experimental
Alginate	3.63×10^{-9}	0.9742	1.452×10^{-7}	2.08×10^{8}	30.201	30.26
Acrylamide	1.56×10^{-9}	1.374	6.245×10^{-8}	1.87×10^{8}	11.678	11.68
Agar	3.03×10^{-9}	1.24	1.215×10^{-7}	1.81×10^{8}	21.99	21.98

Table 3
Comparison of of Cr(VI) reduction using immobilized microbes reported in literature

Type of microorganism used	Matrix	Initial chromium concentration ($mg\,L^{-1}$)	Percentage reduced (%)	Optimum pH	References
B. coagulants	Acryl amide	26	100	7	Philip et al. [12]
A. haemolyticus	Wood husk	15	97	7	Zakaria et al. [13]
S. griseus	PVA–alginate	25	100	7	Poopal and Laxman [14]
Pseudomonas sp.	Alginate	100	66.5	6–8	Murugesan and Maheswari [15]
Bacillus sp.	Celite, amberlite, and Ca-alginate	2–8	98	7	Camagro et al. [16]
P. chrysosporium	Alginate, acryl amide, agar	10	98.3	5	Present study

found to increase with the increase in the inoculums size. The increase in bioreduction with increase in inoculums dosage was due to the fact that bioreduction was dependent on the growth of the white rot fungi. The number of viable colonies increased with increasing inoculums dosage. The bioreduction reached a constant value for inoculums dosage exceeding 8% (v/v) (Fig. 5). The optimum inoculums dosages for the other matrices, acryl amide and agar are found to be 6% and 10% respectively.

4.6. Effect of different matrices on Cr(VI) bioreduction

A maximum of 98.3% of reduction was reported with alginate matrix for an initial Cr(VI) concentration of $10\,mg\,L^{-1}$. The acryl amide and agar matrices yielded a Cr(VI) reduction percentage of 90.3 and 92% respectively. The acryl amide matrix was found to disintegrate as the study was continued for a period of 52 h. The agar matrix was found to be soluble in the media causing difficulty in analyzing the Cr(VI) concentration. Table 1 showed the percentage reduction of Cr(VI) obtained for different matrices studied with different initial concentrations of Cr(VI) ranging from $10\,mg\,L^{-1}$ to $40\,mg\,L^{-1}$.

4.7. Kinetics of Cr(VI) bioreduction

Fig. 6 represents the experimental and model fit of Cr(VI) reduction by immobilized *P. chrysosporium*. It was observed that the experimental and the model predictions are closer for all the concentrations studied (Fig. 6). The model predicted a maximum reduction capacity of $30.2\,mg\,L^{-1}$ for an initial Cr(VI) concentration of $40\,mg\,L^{-1}$ at initial cell density of 2.08×10^8 cells $mL\,L^{-1}$ with alginate as the immobilization matrix (Table 2). The maximum reduction obtained for other concentrations with Ca alginate is 9.83, 19.37, $26.26\,mg\,L^{-1}$ respectively for an initial Cr(VI) concentration of 10, 20, $30\,mg\,L^{-1}$. The predicted value was closer to the experimental reduction capacity $30.26\,mg\,L^{-1}$. The results indicated that the model studied fitted well for the Cr(VI) reduction by immobilized *P. chrysosporium*.

4.8. Comparison of Cr(VI) reduction using immobilized microbes

Comparison of other immobilized microbes used for Cr(VI) reduction reported in literature is summarized in Table 3. From the present study it was observed that a maximum of 98.3% reduction of Cr(VI) was reported with an initial concentration of $10\,mg\,L^{-1}$ of Cr(VI) at pH 5. Immobilization provides the advantage of separation of biomass. Usage of live microbes proves to be advantageous over other process as no costly chemicals are required for the treatment of Cr(VI). Immobilized beads were able to reduce Cr(VI) under adverse conditions.

5. Conclusion

Bioreduction using the immobilized *P. chrysosporium* was dependent on the pH, temperature and metabolic activity of the organism. Glucose at $20\,g\,L^{-1}$ was an essential nutrient for the bioreduction and growth. A maximum of 98.3% of bioreduction was reported for $10\,mg\,L^{-1}$ of initial Cr(VI) concentration. Results indicated that higher cell concentrations (2×10^8) were necessary for significant reduction of Cr(VI). The Cr(VI)

reduction rate was also affected by the initial Cr(VI) concentration. The optimum reduction was observed at pH 5 and 25°C. The Shen and Wang model fitted well for the experimental data. The model predicted a maximum reduction capacity of 30.2 mg L^{-1} for an initial Cr(VI) concentration of 40 mg L^{-1}. Ca alginate was found to be the best suitable matrix for the immobilization of the fungus. Thus, immobilized cells of *P. chrysosporium* can be effectively used for the reduction Cr(VI) present in wastewater.

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