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Kinetics of bioreduction of hexavalent chromium by poly vinyl alcohol-alginate immobilized cells of *Ochrobactrum* sp. Cr-B4 and comparison with free cells

Anuradha Hora, Vidya K. Shetty*

Department of Chemical Engineering, National Institute of Technology Karnataka, Surathkal, Mangalore 575 025, India, email: anuradha.hora@gmail.com (A. Hora), Tel. +91 824 2474000, ext. 3606; Fax: +91 824 2474033; email: vidyaks68@yahoo.com (V.K. Shetty)

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ABSTRACT

The cells of *Ochrobactrum* sp. Cr-B4 immobilized in PVA-alginate blended matrix could be successfully used for bioreduction of Cr(VI) from contaminated water. The removal mechanism included adsorption on solid-liquid interface and enzyme catalyzed chromate reduction. At lower concentrations the initial rate of Cr(VI) reduction with immobilized cells was found to be slightly higher than that of free cells owing to adsorption on the immobilization matrix. But after a certain time the rate of Cr(VI) reduction by free and immobilized cells was similar. The estimation of effectiveness factor (η), indicated that there were no diffusional limitations offered by the immobilization of Cr-B4 as the value of η was fond to be near "one" at different concentrations of Cr(VI). The kinetic analysis showed that both free and immobilized cells followed Michaelis–Menten kinetics with K_m and V_{max} of 456.1 mg/L and 14.67 mg/L/h for free cells respectively; 499.4 mg/L and 15.32 mg/L/h for immobilized cells respectively. The kinetic characteristics of Cr(VI) reduction were not altered by immobilization. This study reveals the potential applications of immobilized Cr-B4 in development of industrially feasible and economically viable bioremediation strategy for discharging Cr(VI) free effluent into the environment.

Keywords: Cr(VI); *Ochrobactrum* sp. Cr-B4; Immobilization; Effectiveness factor; Michaelis–Menten kinetics

1. Introduction

Chromium (Cr) is one of the most frequently used heavy metal contaminant found in industrial effluents from leather tanning, metal processing, electroplating, steel and automobile manufacturing, mining, cement, textile, wood preservation, production of paint pigments and dyes. Cr can exist in several oxidation states, ranging from Cr(II) to Cr(VI), but the most stable and common forms are trivalent, Cr(III) and hexavalent, Cr(VI) species [1]. Cr(VI) is highly toxic, mobile and soluble, which generally exists as an oxyanion (CrO_4^{2-}) in aqueous systems. The US Environmental Protection Agency has placed Cr(VI) as a priority pollutant, and classified as a class "A" human carcinogen [2] due to its mutagenic and carcinogenic properties. Cr(III), on the other hand, is insoluble and less toxic [3]. Hence for reduction or

*Corresponding author.

removal of Cr(VI), industrial wastes are treated by various physio-chemical methods such as reduction, precipitation, adsorption, ion exchange, reverse osmosis and electro-dialysis, or disposed through landfill. Recently Barrera-Díaz et al. [4] reviewed the chemical, electrochemical and biological methods for aqueous Cr(VI) reduction. Most of the physic-chemical approaches are economically expensive and have disadvantages like incomplete metal removal, high reagent consumption and energy requirements, while contaminating the ground water because of generation or disposal of toxic secondary wastes. An alternative to overcome these shortcomings and tide over the problem of low concentration of heavy metal, bioremediation appears to have wider implications in Cr(VI) detoxification. Interestingly, certain microbes isolated from the industrial effluents possess the capability to reduce Cr(VI) to relatively less toxic Cr(III), that gives immense opportunities for the development of technologies to detoxify Cr(VI)-contaminated sites.

Cr(VI) can be reduced to less toxic Cr(III) by bacteria such as *Desulfovibrio vulgaris* [5], *Pseudomonas aeruginosa* [6] and *Escherichia coli* [7], *Pseudomonas* sp. [8], *Bacillus* sp. [9,10], *P. phragmitetus* LSSE-09 [11]. The extraordinary potential of these bacteria has been exploited for removal of Cr(VI) fortified into the culture flasks. However, the toxic effects of Cr(VI) limits the growth of free cells at very high concentration of Cr(VI). Bacterial cells entrapped in suitable matrix have been shown to have improved tolerance to a variety of toxic and recalcitrant compounds. Moreover, in industrial wastewater treatment systems, immobilized cells prove to be beneficial, as it eliminates the need for cell separation units. Therefore, immobilization of Cr(VI) reducing bacteria could be a better alternative in bioremediation of Cr(VI). The immobilized cells may give the advantage of higher volumetric reaction rates due to higher local cell concentration or altered cell permeability. Moreover, immobilization can also increase the tolerance of Cr (VI) reducing bacteria to the toxic effects of Cr(VI). For instance, Konovalova et al. [12] observed that *Pseudomonas* sp. immobilized in agar–agar films on the surface of synthetic membrane exhibited higher tolerance towards Cr(VI) and higher Cr(VI) reduction activity than free cells, and Camargo et al. [13] demonstrated the feasibility of large scale Cr(VI) detoxification using Ca-alginate immobilized *Bacillus* sp. in a bioreactor. Immobilization of bacteria for Cr(VI) reduction has been reported for *Microbacterium liquefaciens* MP30 [14] and *Serratia marcescens* [15,16], *Acinetobacter* sp. [17].

The isolation of *Ochrobactrum* sp. Cr-B4 capable of reducing Cr(VI) at high concentrations has been reported previously [18]. In the present study, we report immobilization of *Ochrobactrum* sp. Cr-B4 cells in PVA-alginate blended matrix. Studies reported in the present investigation compares the immobilized cells with the free cells. In the present work, PVA-alginate matrix was used for immobilization based on the review by Gentry et al. [19] of several applications of immobilized cells using various types of immobilization materials. Based on this review, polyvinyl alcohol (PVA), a synthetic organic polymer, has been widely used because it provides the strength and high crosslinking capacity to the matrix whereas alginate reduces the agglomeration and increases the surface properties [20]. Natural immobilization matrices have the disadvantage of abrasion and are biodegradable. Synthetic matrices can conquer this disadvantage with an appropriate combination [21]. PVA is a promising type of synthetic polymer and is not toxic to microorganisms, therefore it is very suitable for entrapment of microbial cells in its polymeric matrixes. In the present study, a combination of natural and synthetic material was used for immobilization and the rates of Cr(VI) reduction by free and PVA-alginate immobilized cells at various Cr(VI) concentrations were compared. Despite the fact that immobilized cells present many advantages when compared to the free cells in Cr(VI) reduction, rate of Cr(VI) reduction by immobilized cells may be limited by intra-particle diffusion mass transfer rate within immobilization matrix. Through the examination of intra-particle diffusional mass transfer limitations a better appraisal of the advantages or disadvantages of using immobilized cells for Cr(VI) removal can be obtained. Thereby, Cr(VI) reduction process with both free and immobilized cells were compared through the investigation of the limitation created by diffusional mass transfer into the gel beads on Cr(VI) reduction rates in terms of effectiveness factor. Effectiveness factor (η) is the ratio of actual rate of Cr(VI) reduction by immobilized cells to the rate if not slowed down by diffusion(rate with free cells) [22]. Effectiveness factor of less than "1" indicates that immobilized cell system offers diffusional mass transfer limitations. Lower the value of effectiveness factor below "1", higher is the diffusional mass transfer resistance offered by immobilization matrix. The value of effectiveness factor equal to 1 indicates the absence of diffusional mass transfer limitations. It is important to evaluate the kinetics of Cr(VI) reduction by the bacteria, as design of bioreactors for wastewater treatment often are based on the kinetics of the process. Residence time to be provided for wastewater stream in the bioreactor depends on the reaction kinetics and hence the bioreactor size is determined by the kinetics. Therefore the Cr(VI) reduction

process with both free and immobilized cells were also compared through the analysis of Cr(VI) reduction kinetics.

2. Material and methods

2.1. Chemicals

Polyvinyl alcohol (hot water soluble) was obtained from Himedia, India. Sodium alginate was procured from Sigma Aldrich, USA. Stock solution of Cr(VI) (10,000 mg/L) was prepared by dissolving 2.829 g of $K_2Cr_2O_7$ (Merck (India) Ltd) in 100 mL of distilled water and stored at room temperature. Suitable volume of the stock solution was used while preparation of the Cr(VI) reduction media in aqueous solution containing the optimized media components. All other chemicals were of analytical grade and were procured from Himedia, India.

2.2. Bacterial strain, growth condition and harvesting of cells

In the present work, a strain of *Ochrobactrum* sp. Cr-B4 with gene bank accession number JF824998, which was earlier isolated [18] from the aerator liquid of wastewater treatment facility of a dye and pigment based specialty chemical industry, was used. This strain had shown resistance up to 1,000 mg/L of Cr (VI) in solid media and is highly capable of reducing Cr(VI) to Cr(III) as reported by Narayani and Shetty [18]. The bacterial biomass for immobilization of cells was prepared by inoculating Cr-B4 cells in 150 mL of LB media at 37˚C, 150 rpm for 24 h. The fully grown culture was then centrifuged at 10,000 rpm for 10 min to obtain the biomass pellet. The wet weight of thus obtained biomass was determined and 1.5 g wet weight of this biomass was suspended in 5 mL of distilled water. This cell suspension was then used for immobilization.

2.3. Immobilization of Ochrobactrum sp. Cr-B4

PVA-alginate beads were prepared by entrapping bacterial cells into a PVA and sodium alginate blend gel. Five grams of PVA and 1 g of sodium alginate were dissolved in 50 ml of distilled water. This mixture was stirred and heated to boiling to obtain a homogeneous mixture. After storage at room temperature for 24 h to cool and remove air bubbles, approximately 1.5 g wet weight of cells suspended in distilled water was added into it. This suspension was filled in a 10 mL disposable plastic syringe and extruded drop-wise into a beaker containing 100 mL solution of 2% calcium chloride ($CaCl_2$) and 1 M boric acid in order to form spherical beads. The beads were allowed to cure in this $CaCl_2$-boric acid solution for 24 h under stirring condition and then rinsed with distilled water to remove excess Ca^{2+} and boric acid. The spherical gel beads were formed without agglomeration, which exhibited rubber like elastic properties. The formed hydrogel beads were then soaked in 1.0 M sodium orthophosphate solution at pH 7.0 for 60 min for hardening. The gel beads were washed thrice in sterile distilled water, and the dilatability of the beads was examined by immersing them in distilled water for 24 h. Diameter of the spherical beads was measured by using vernier calipers. The average size of beads was found to be 4 ± 1 mm. PVA contributed strength and durability to the beads, whereas calcium alginate improved the surface properties, reducing the tendency to agglomerate [14].

2.4. Bioreduction of hexavalent chromium by free and immobilized cells of Cr-B4

Ochrobactrum sp. Cr-B4 cells immobilized in PVA-alginate matrix (having 1.5 g cells in approximately 500 beads) were added into 100 mL of the optimized medium [23] of composition: Na_2HPO_4 (6 g/L), KH_2PO_4 (3 g/L), $MgSO_4{\cdot}7H_2O$ (0.1 g/L), $CaCl_2$ (0.1 g/L), NaCl (0.5 g/L), casein hydrolysate (1.61 g/L) and sucrose (10.05 g/L) with different concentrations of Cr(VI) ranging from 100 to 1,000 mg/L. The pH of the medium was adjusted to 8.4 using either NaOH or HCl solution. These cultures were incubated at 37˚C and 2 mL liquid sample was withdrawn for every 2 h up to 20 h and after 20 h, sample was withdrawn at every 4 h to estimate the amount of Cr(VI) reduced. The optimal medium containing PVA-alginate beads (without cells) served as control to investigate the removal of Cr(VI) by adsorption on to the immobilized beads. Batch Cr (VI) reduction experiments were also performed with free cells under the same conditions as that with immobilized cells, and the inoculum being 1.5 g of bacterial biomass (wet basis). The concentration of Cr (VI) in the cell free sample was determined spectrophotometrically at 540 nm using 1,5diphenyl-carbazide (DPC) reagent in acid solution as the complexing agent for Cr(VI) [24]. Absorbance was measured using UV–vis spectrophotometer (Labomed Inc, USA). To confirm the reproducibility of the results each run of the experiment was done in duplicate and the mean values are reported.

Effectiveness factor (η) was determined at different concentrations of Cr(VI) during bioreduction. Concentration vs. time data obtained during batch Cr(VI) reduction experiments with immobilized cell system (with 100 mg/L initial Cr(VI) concentration) was

plotted and the slopes of tangents drawn on the plot of Cr(VI) concentrations vs. time data gives the rate of Cr(VI) reduction at that concentration of Cr(VI) in the bulk liquid. Similarly the rates of Cr(VI) reduction by free cells were obtained at the same concentrations using the batch experimental data with free cells. The effectiveness factor (η) was calculated using Eq. (1):

$$\eta = \frac{\text{Rate of Cr(VI) reduction with immobilized cells}}{\text{Rate of Cr(VI) reduction with free cells}} \quad (1)$$

Cr(VI) reduction kinetics for reduction of Cr(VI) by both free and immobilized cells of Cr-B4 were studied in optimized media. Rates of Cr(VI) reduction by both free and immobilized cells of Cr-B4 at different concentrations were obtained by drawing tangents on the plot of Cr(VI) concentrations vs. time data obtained by batch experiments with a 1,000 mg/L initial Cr(VI) concentration. Nonlinear regression analysis on the rate vs. concentration data were performed using Curve Fitting Toolbox of MATLAB 7.14.0 software, to test for the validity of different kinetic models.

3. Results and discussion

The ability of Cr-B4 to reduce Cr(VI) as free cells suspended in optimal medium at aerobic conditions were compared with that of the immobilized cells under same conditions at different concentrations of Cr(VI). For studies on Cr(VI) reduction by immobilized cells, cells of Cr-B4 were successfully embedded in the PVA-alginate beads. The beads had stable structures.

3.1. *Bioreduction of Cr(VI) by free cells of* Ochrobactrum *sp. Cr-B4*

Ochrobactrum sp. Cr-B4 cells have been shown to efficiently reduce Cr(VI). Initially the free cells were grown in optimal medium supplemented with different concentrations of Cr(VI) ranging from 100 to 1,000 mg/L. The Cr(VI) reduction potential of free cells at different initial concentrations of Cr(VI) was determined with respect to time. The representative plots for concentration vs. time data are shown in Figs. 1–3. The free cells have successfully reduced Cr(VI) within 40 h of incubation, when the initial concentration of Cr (VI) was 100 mg/L (Fig. 1). The time for complete Cr (VI) reduction increased with increasing concentration of Cr(VI). When the concentration was increased to 200 mg/L (Fig. 2), free cells of Cr-B4 could reduce approximately 100% of Cr(VI) in 52 h while at the highest concentration of 1,000 mg/L (Fig. 3), Cr-B4 could reduce around 80% of Cr(VI) in 126 h.

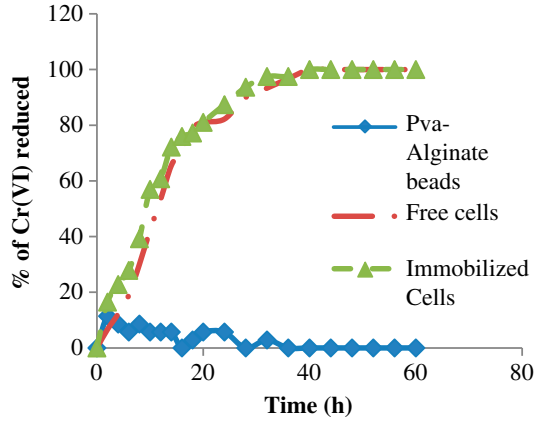

Fig. 1. Comparison of Cr(VI) reduction by free and immobilized cells of Cr-B4. Initial Cr(VI) concentration = 100 mg/L.

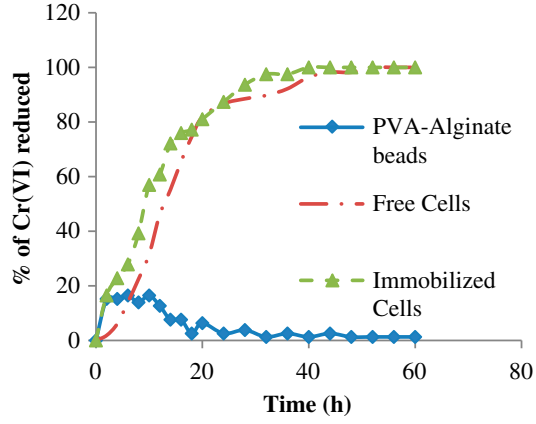

Fig. 2. Comparison of Cr(VI) reduction by free and immobilized cells of Cr-B4. Initial Cr(VI) concentration = 200 mg/L.

3.2. *Cr(VI) reduction by PVA-alginate immobilized Cr-B4 cells and comparison with free cells*

Immobilization of *Ochrobactrum* sp. Cr-B4 was very effective. Cr(VI) reduction efficiency of the immobilized cells of Cr-B4 as a function of time with different initial concentration of Cr(VI) ranging from 100 to 1,000 mg/L was studied. The representative plots for concentration vs. time data are shown in Figs. 1–3. As observed in these figures, significant Cr(VI) removal was achieved when PVA-alginate beads with immobilized cells were used, but Cr(VI) removal with bare PVA-alginate beads (without entrapped cells) was negligible. Thus it is

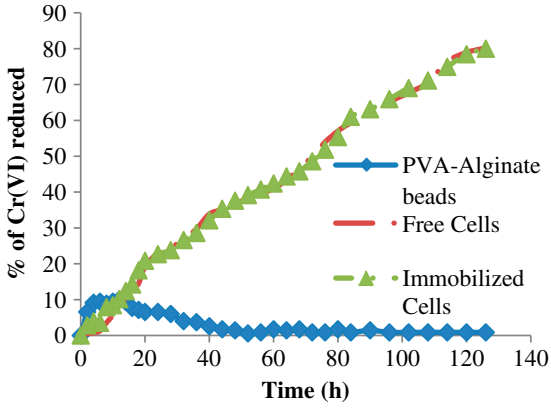

Fig. 3. Comparison of Cr(VI) reduction by free and immobilized cells of Cr-B4. Initial Cr(VI) concentration = 1,000 mg/L.

ensured that the phenomena of Cr(VI) removal by PVA-alginate beads with entrapped cells is bacterial bioreduction and not adsorption onto the beads. When Cr(VI) contaminated water with 100 mg/L was subjected to Cr(VI) reduction by immobilized cells of Cr-B4, the initial rate of Cr(VI) reduction was found to be slightly higher than that of free cells as observed from the trend of percentage reduction vs. time plots (by visual observation and analysis of the slopes of the plots), but after a certain time the rates of Cr(VI) reduction by free and immobilized cells were similar and complete reduction of Cr(VI) took place in 40 h by both free and immobilized cells of Cr-B4 (Fig. 1). Similar observations were made with Cr(VI) reduction in Cr (VI) contaminated water at higher concentrations. During the initial period of around 2 to 4 h, the rates of Cr (VI) removal with cell immobilized PVA-alginate beads were found to be similar to that with bare PVA-alginate beads. The initial rates of removal were very high as compared to those at later times. This may be due to initial adsorption of Cr(VI) on to the beads. During this initial period biological Cr(VI) reduction rate may be very minimal owing to very low concentration of cells in the beads. Adsorption being the physical phenomena, takes place at a higher rate than the biological Cr (VI) reduction process. Lowering of the rate after initial adsorption period may be owing to the saturation of bead surface with Cr(VI) and the matrix can no longer adsorb Cr(VI). Meanwhile the cells in the bead matrix grow by consuming the nutrients that diffuse into the beads from the media. At later times, the adsorbed Cr (VI) may have been reduced by the cells in the bead matrix. This can further lead to mass transfer from the bulk liquid to the bead surface followed by diffusion to the inner pores and reduction to Cr(III) by the cells. Biological Cr(VI) reduction process being the slower process than adsorption, it governs the rate of Cr(VI) removal from the bulk liquid. As Cr(VI) concentration in the bulk liquid reduces the rate of mass transfer from the bulk liquid to the bead reduces and the overall rate falls to a greater extent. Owing to absence of adsorption phenomena with free cells, the initial rate is lower with free cells up to around 2 to 4 h. But at later times the rates of Cr(VI) reduction by free cells were almost similar as the immobilized cells with all the initial Cr(VI) concentrations. The reason for free cells exhibiting similar rate of reduction as the immobilized cells at later times, may be due to biological process governing the rate of Cr(VI) removal from the bulk even with immobilized cells rather than adsorption at later times. Studies on chromate reduction reported by Konovalova et al. [12] also support the observation in the current study. Slightly higher rates of Cr(VI) reduction with immobilized cells as observed at lower concentrations of 100 and 200 mg/L, may be due to adsorption playing a major role in Cr(VI) removal for a longer duration of time. This may be the result of larger time being taken for the beads to achieve saturation owing to lower rate of mass transfer at lower bulk concentrations which is due to smaller concentration gradients as compared to that at higher concentrations. It may also be due to localized concentration of reductase enzymes in the microenvironment of the gel matrix and hence increasing the probability of binding of Cr(VI) on to the active sites of enzymes and leading to higher rate of Cr(VI) reduction with immobilized cells. The time for complete Cr(VI) reduction increased with increase in initial Cr (VI) concentration and as the concentration increased the rate of Cr(VI) reduction also decreased. At a concentration of 200 mg/L of Cr(VI) (Fig. 2), complete reduction by immobilized cells of Cr-B4 was observed in 48 h while with free cells complete reduction was observed in 52 h. With 300 mg/L (Figure not shown) of Cr(VI) in reduction media, the complete reduction of Cr(VI) was observed in 60 and 56 h with free and immobilized cells of Cr-B4 respectively. When the concentration was increased to 400, 500, 600 and 700 mg/L of Cr(VI) (Figures not shown), complete reduction with both free and immobilized cells of Cr-B4 was observed after 64, 72, 76 and 108 h respectively. At 800 and 900 mg/L of Cr(VI), both free and immobilized cells were able to reduce around 90% of Cr(VI) in 108 h (Figures not shown). With highest concentration of 1,000 mg/L of Cr(VI) (Fig. 3), though complete reduction was not observed, Cr-B4 was able to reduce 80% of Cr(VI) in 126 h. The lower reduction rates at higher concentration of Cr(VI) may be due to the inhibition of bacterial growth inside the gel matrix at high Cr(VI) concentration. This corroborated the findings of Ganguli and Tripathi [25] who demonstrated that low levels of chromate

reduction by *P. aeruginosa* A2Chr was due to the inhibitory effects of high initial Cr(VI) concentration on cellular metabolism. It can be observed from Figs. 1–3 that the immobilized cells of Cr-B4 show almost a similar or slightly higher Cr(VI) reduction rate as that of free cells. This indicates that the immobilization matrix of PVA-alginate does not offer diffusional mass transfer limitations and hence the rate of removal of Cr(VI) from contaminated water is governed by rate of Cr(VI) reduction by the bacteria through enzymatic process, but not by the diffusional mass transfer rate in immobilized cell systems. Table 1 presents the values of effectiveness factor at different concentrations of Cr(VI). These values of effectiveness factor (η), also indicate that there are no diffusional limitations offered by the immobilization of the Cr(VI) reducing bacteria, Cr-B4 in PVA-alginate beads, as the value of η was fond to be nearly "one", as estimated at different concentrations of Cr(VI) using the concentration vs. time data obtained with 100 mg/L initial Cr(VI) concentration. The value of η is little higher than "one" at initial concentration of 100 mg/L, which may be owing to adsorption of Cr(VI) on the gel beads during the initial time of the reaction. The adsorption rate is higher initially as the bulk liquid concentrations are higher at initial times and the concentration of Cr(VI) in the gel matrix is smaller, causing large driving force for mass transfer followed by adsorption.

3.3. Kinetics of Cr(VI) reduction by free and immobilized cells

The kinetics of Cr(VI) reduction process by free and immobilized cells were evaluated using the concentration vs. time data with 1,000 mg/L initial Cr (VI). The rates of Cr(VI) reduction were determined only after the initial adsorption period. The rate data for initial fast adsorption period were not considered, as the rate during this time does not represent the enzymatic process. The kinetics analysis of both free and immobilized cells showed that Cr(VI) reduction by both free and immobilized cells of Cr-B4 followed Michaelis–Menten kinetics given by Eq. (2).

Table 1
Effectiveness factor η at different concentrations of Cr(VI)

Cr(VI) concentration (mg/L)	η
100	1.06
60	1.00
50	1.00
30	1.00
10	1.00

$$V = \frac{V_{\max} S}{K_m + S} \tag{2}$$

where V is the rate of removal of Cr(VI), mg/L/h, S is the concentration of Cr(VI), mg/L, $V_{\max}$ is the maximum rate of Cr(VI) reduction, mg/L/h, and K_m is the Michaelis–Menten constant, mg/L.

The values of kinetic parameters K_m and $V_{\max}$ for Cr (VI) reduction kinetics with free and immobilized cells of Cr-B4, along with the parameters representing the goodness of fit, are presented in Table 2. Figs. 4 and 5 show the plots of rate vs. Cr(VI) concentration obtained from the experimental data and from the kinetic model predicted data with the optimized media for free and immobilized cells respectively. As shown in Figs. 4 and 5, the hyperbolic shape of plots in case of both free and immobilized cells showed dependence of Cr(VI) removal on chromium concentration which is typical of enzyme catalyzed reactions. The kinetics parameters obtained for Cr(VI) reduction with both free and immobilized cells of Cr-B4 were nearly same indicating that the kinetic characteristics of Cr(VI) reduction is not altered by immobilization. These findings show that immobilized cells are suitable for use in bioremediation applications for Cr(VI) removal and do not offer any limitations on its use for industrial scale operations. On the contrary immobilized cell systems show a slightly enhanced performance as compared to free cells. Hence the PVA-alginate matrix with immobilized cells of Cr-B4 has been proven to be beneficial over the free cells for Cr(VI) removal in wastewater treatment facilities.

On comparison with other Cr(VI)-reducing bacteria, immobilized cells of Cr-B4 showed higher Cr(VI) reducing efficiencies. For example, the agar immobilized cells of *D. vulgaris* required 22 h to reduce 26 mg/L Cr(VI) to 5.2 mg/L; agar immobilized cells of *Microbacterium* sp. NCIMB 13776 required 65 h to reduce 26 mg/L Cr(VI) to 13.2 mg/L [26]; while the PVA-alginate immobilized cells of *Streptomyces griseus* removed 25 mg/L Cr(VI) in 24 h [21] and that of *Bacillus spahericus* AND 303 reduced 87.50% of 1 mg/L of Cr(VI) in 24 h [27]. Furthermore, whole cell immobilization has the advantages over free cells in being more stable; eliminate solid–liquid separation and minimal clogging in continuous systems [26,28].

Table 2
Kinetic parameters for Cr(VI) reduction with free and immobilized cells of Cr-B4

Cells	$V_{\max}$ (mg/L/h)	K_m (mg/L)	R^2	Adj R^2
Free Cr-B4	14.67	456.1	0.9905	0.9890
IB Cr-B4	15.32	499.4	0.9542	0.9465

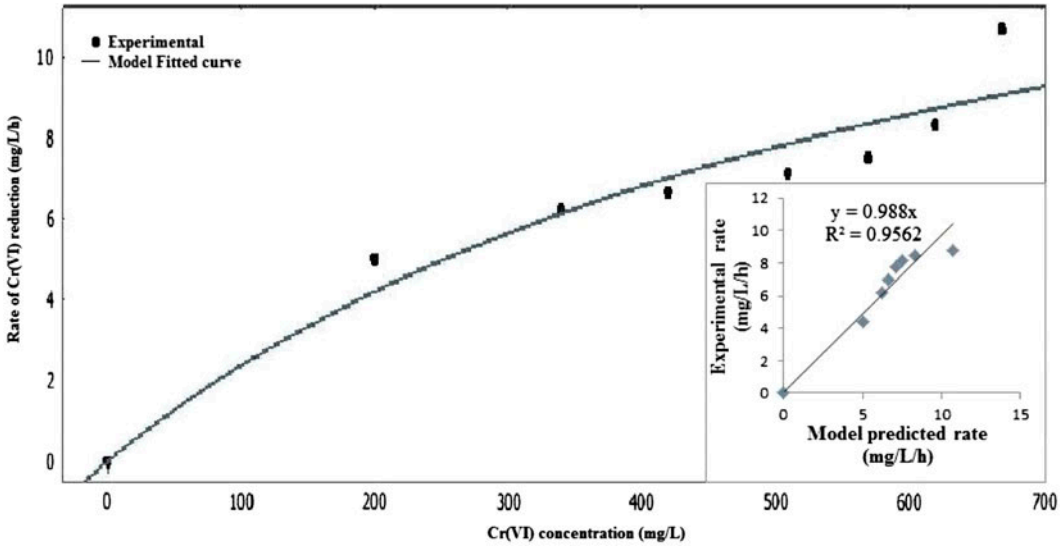

Fig. 4. Rate of Cr(VI) reduction (mg/L/h) vs. Cr(VI) concentration (mg/L) for Cr(VI) reduction with free cells of Cr-B4.

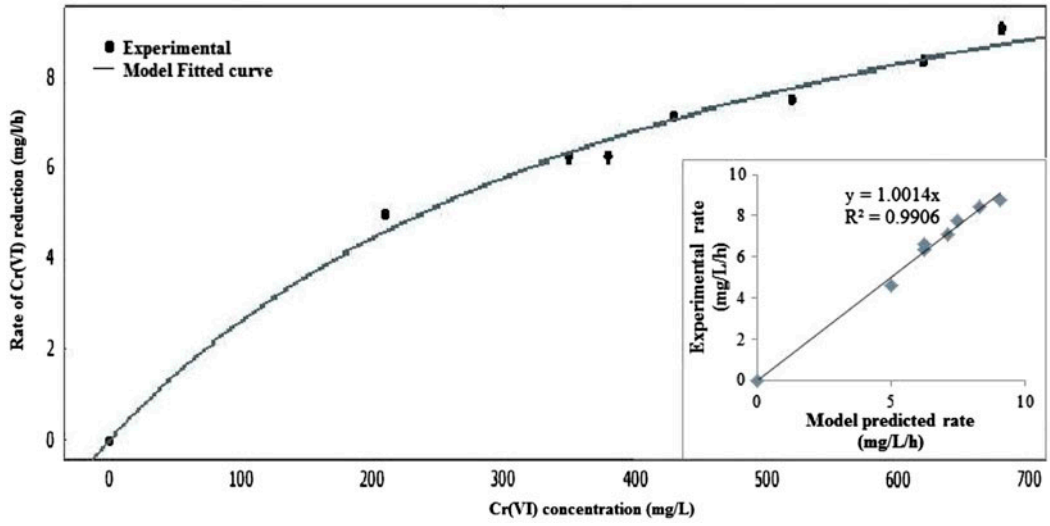

Fig. 5. Rate of Cr(VI) reduction (mg/L/h) vs. Cr(VI) concentration (mg/L) for Cr(VI) reduction with immobilized cells of Cr-B4.

Using the PVA-alginate matrix combination would be a very good choice for immobilization of bacteria to be used in biological water treatment systems. Given the notable properties of Cr(VI) reduction, this *Ochrobactrum* sp. exhibits a great potential for use in removal of Cr(VI) from industrial effluents.

4. Conclusion

The cells of *Ochrobactrum* sp. Cr-B4 immobilized in PVA-alginate blended matrix could be successfully used for bioreduction of Cr(VI) from contaminated water. The removal mechanism included adsorption on solid-liquid interface and enzyme catalyzed chromate reduction. The comparison of rates of Cr(VI) reduction by free and immobilized cells, and resultant value of η to be near "one" at different concentrations of Cr(VI) showed that there were no diffusional limitations offered by the immobilization of Cr-B4. The kinetic analysis showed that both free and immobilized cells followed Michaelis–Menten kinetics. The K_m values for free and immobilized cells were found to be

456.1 mg/L and 499.4 mg/L respectively. The V_{max} values obtained were 14.67 mg/L/h for free cells and 15.32 mg/L/h for immobilized cells. The kinetic characteristics of Cr(VI) reduction were not altered by immobilization. Using the PVA-alginate matrix combination would be a very good choice for immobilization of bacteria to be used in biological water treatment systems. This study reveals the potential applications of immobilized Cr-B4 in development of industrially feasible and economically viable bioremediation strategy for discharging Cr(VI) free effluent into the environment.

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