

Bench-scale column experiments to study the containment of Cr(VI) in confined aquifers by bio-transformation

T. Shashidhar, Ligy Philip*, S. Murty Bhallamudi

Department of Civil Engineering, Indian Institute of Technology Madras, Chennai 600 036, India

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Abstract

Bench-scale soil column experiments were conducted to study the effectiveness of Cr(VI) containment in confined aquifers using in situ bio-transformation. Batch adsorption studies were carried out to estimate the adsorption capacities of two different soils for Cr(VI) and Cr(III). Bio-kinetic parameters were evaluated for the enriched microbial system. The inhibition constant, evaluated using Monod's inhibition model, was found to be 11.46 mg/L of Cr(VI). Transport studies indicated that it would not be possible to contain Cr(VI) by adsorption alone. Transport and bio-transformation studies indicated that the pore velocity and the initial bio-mass concentration significantly affect the containment process. In situ bio-remediation is effective in the case of silty aquifers. Cr(VI) concentration of 25 mg/L was effectively contained within 60 cm of a confined silty aquifer. Cr(VI) containment could be achieved in sandy aquifers when the pore velocity was very low and the initial augmented bio-mass was high. A bio-barrier of approximately one meter width would be able to contain Cr(VI) if the initial Cr(VI) concentration is as much as 25 mg/L. © 2005 Elsevier B.V. All rights reserved.

Keywords: Bio-transformation; Chromium; Contaminant transport; Column experiments

1. Introduction

Many groundwater resources in the world have been contaminated by solid and liquid wastes containing chromium(VI) generated by electroplating, leather tanning and wood preservative industries [1]. Cr(VI) is highly toxic, highly soluble in water, and may be transported over long distances in the subsurface. This transport depends upon geo-hydrological conditions and geo-chemical processes. The contaminated groundwater is usually treated either by chemical reduction followed by precipitation or by biological processes [2]. The first alternative involves large quantities of chemicals and the precipitate formed needs further attention. On the other hand, biological treatment offers an environment friendly and economical alternative. It has been reported in the literature that many micro-organisms, under various environmental conditions, can reduce Cr(VI) to Cr(III) [3–4]. Cr(III) is less toxic and less mobile, hence needs less attention [5–6].

The biological treatment of groundwater can be carried out either ex situ or in situ [2]. Ex situ method involves costly pumping operation. In situ method involves either the enhancement of native chromium reducing bacteria (bio-stimulation) or bio-augmenting the groundwater with enriched Cr(VI) reducing bacteria. A good understanding of the subsurface transport (advection and dispersion) processes along with chemical (adsorption, ion exchange, precipitation, etc.) and bio-chemical reactions is essential for designing the optimal treatment strategy.

Many earlier works on the transport of Cr(VI) concentrated on understanding only the advection, dispersion, adsorption and geo-chemical processes in different soils through batch and continuous column studies [7–12]. There were also several batch studies on bio-transformation of Cr(VI) to Cr(III) under various environmental conditions [4,13–15]. Only recently, combined transport and bio-transformation studies have been reported in literature [16]. In these studies, the focus has been the transport of Cr(VI) through saturated sand column under the influence of adsorption and bio-transformation. So far, no study has truly considered the effectiveness of chromium(VI) containment in confined aquifers under different hydrogeologic conditions. This information is very essential for deciding the treatment option

* Corresponding author. Tel.: +91 44 2257 4274; fax: +91 44 2257 8281.
E-mail address: ligy@iitm.ac.in (L. Philip).

(whether in situ or ex situ) and for designing the optimal in situ bio-remediation. It may be also noted that all the earlier studies were conducted in small laboratory scale columns (10–30 cm), which do not realistically represent the interplay between geo-hydrology and chromium containment.

In this work, bench-scale column experiments were conducted to evaluate the effectiveness of Cr(VI) containment by bio-remediation processes in aquifers. Effects of (i) ground water velocity, (ii) initial microbial concentration and (iii) aquifer soil characteristics on Cr(VI) containment were studied. Experiments were also conducted to study only the transport and adsorption of Cr(VI) in order to assess the role of bio-remediation strategy in Cr(VI) containment.

2. Materials and methods

2.1. Soil

Soils used in this study were collected from the I.I.T. Madras campus, Chennai, India. They were surface soils collected from the unsaturated zone. Soils thus collected were sieved, the portions which passed through 4.75 mm were sterilized, cooled to room temperature and preserved in clean plastic containers for subsequent use. For sand column experiments, river sand, which passed through 0.6 mm and retained in 0.425 mm sieves was washed thoroughly with distilled water and oven dried at 100 °C over night. The soil characteristics were analyzed as per the standard methods [17] and are presented in Table 1. The soils were classified and identified as per ASTM (American Society for Testing and Material) standards, as silty sand (SM, as per ASTM designation D 2487-00), and sand through sieve analysis and Atterberg's limit analysis. The organic matter content and specific gravity of the soil were determined by chromic acid method (IS 2720 (part 22, 1972) and pycnometer method (IS 2720 (part 3/sec.1, 1987).

2.2. Chemicals

All the chemicals used in this study were of analytical reagent (AR) grade and were supplied by Ranbaxy chemicals Ltd., Chennai, India. Glassware used for analysis were equilibrated with Cr(VI) and washed with acid solution followed by distilled water.

Table 1
Soil characteristics

Serial no.	Properties	Value (mass%)	
		Soil A	Soil B
1	Clay content	6.19%	0%
2	Silt content	22.70%	0%
3	Sand content	71.11%	100%
4	Specific gravity	2.543	2.635
5	Organic content	0.92%	ND
6	Bulk density (g/c.c)	1.41	1.60
7	Porosity	0.45	0.375
8	pH	6.59	6.6

2.3. Nutrient media

The nutrient medium (N1) for bacterial growth consisted of Peptone (10 g), beef extract (2 g), yeast extract (1 g), and sodium chloride (5 g) in 1 L of distilled water. The growth medium (M1) for bacteria consisted of peptone (10 g), beef extract (2 g), yeast extract (1 g), in 1 L of distilled water [18]. The medium (M2) for Cr(VI) reduction experiments consisted of K₂HPO₄ (0.03 g/L), KH₂PO₄ (0.05 g/L), MgSO₄·7H₂O (0.01 g/L), NH₄Cl (0.03 g/L), NaCl (0.01 g/L), carbon source (2 g/L) and 1 mL of trace element solution. Trace element solution consists of FeCl₂·4H₂O (12.2 g/L), MnCl₂·4H₂O (4.09 g/L), CoCl₂·6H₂O (0.927 g/L), ZnCl₂ (0.37 g/L), CuCl₂ (0.61 g/L), NaMoO₄·2H₂O (0.579 g/L), H₃BO₃ (0.16 g/L), KI (0.148 g/L), NiCl₂·6H₂O (0.067 g/L), and EDTA Na₂·4H₂O (6.5 g/L) [19]. The pH was maintained at 7 ± 0.2 through the addition of HCl or NaOH. Molasses was used as a carbon source. The media were sterilized by wet autoclaving at 15 kPa and 120 °C for 30 min.

2.4. Enrichment of Cr(VI) reducing bacterial strains

Bacterial strains were isolated from the soil samples collected from the chromium contaminated site located at Ranipet, Tamilnadu, India. About 1 g of soil sample was added to 100 mL of growth medium (N1) and incubated for 24 h in facultative condition. The shake flask cultures were closed using Teflon stoppers. Then, 1 mL of the above was transferred to 100 mL nutrient broth (N1) containing 100 mg/L Cr(VI). This procedure was repeated by progressively increasing Cr(VI) concentration in the nutrient medium up to 500 mg/L. A loopful from the above mixture was streaked on agar slants, incubated for 24 h and stored in the freezer at 4 °C for further use.

2.5. Bacterial cultivation and harvesting

The previously isolated culture from the soil was inoculated to the sterile growth medium (M1), kept in an environmental shaker at 35 ± 2 °C for 24 h and was agitated at 140 rpm. The system was in aerobic condition. The harvested cells were centrifuged at 5000 × g for 10 min. These cells were washed thrice in physiological saline solution and were used for the experiments.

2.6. Batch adsorption study

Sorption equilibrium studies for Cr(VI) were used to estimate the adsorption coefficients for soils A and B. Adsorption kinetic study was conducted using 1 g of sterilized soil and 100 mL of synthetic chromium contaminated water with 50 mg/L of Cr(VI) concentration in various reaction bottles. The reaction bottles were kept in a shaker at 140 rpm. The samples were withdrawn at time intervals of 5, 10, 15, 30, 60, 120, 180, 360, 420 and 480 min on self sacrificing mode. The samples were withdrawn from the reaction bottles, centrifuged and analyzed for residual Cr(VI) concentration. The equilibrium time obtained from the kinetic study was used for isotherm studies. For the isotherm studies, 1 g of soil with 100 ml solution containing Cr(VI) was taken in

several reaction bottles, and kept in a shaker for 6 h (pseudo-equilibrium time) at 140 rpm. The initial Cr(VI) concentrations employed were 2, 5, 10, 20, 30, 50, 100, 200, 300, 400, 500 mg/L. At the end of 6 h, the supernatant was separated and analyzed for Cr(VI) and total chromium. Similarly adsorption isotherm studies were conducted for Cr(III) and lithium. Lithium was used in continuous column studies as a tracer. Adsorption equilibrium studies were also conducted for Cr(VI) in presence of molasses and lithium.

2.7. Studies to estimate bio-kinetic parameters

Bio-kinetic studies were conducted as per standard procedure to estimate the kinetic parameters of Cr(VI) reducing microbial culture [20]. Experiments were conducted with initial chromium concentrations of 0, 1, 5, 10, 20, 50, 100, 200, 300 and 500 mg/L. Bacterial concentration of 36 mg/L was added to the media and hexavalent chromium concentration, COD and bacterial concentrations were measured at 2 h time intervals.

In presence of Cr(VI), specific growth rate of bacteria was assumed to follow the Monod's equation with inhibition [21] as given below

$$\mu = \left(\frac{\mu_{\max} S}{K_s + S} \right) \left(\frac{K_i}{K_i + Cr} \right) \quad (1)$$

where μ_{\max} is the maximum specific growth rate (h^{-1}); S the molasses concentration (COD mg/L); Cr the chromium(VI) concentration (mg/L); K_s the half-saturation constant (COD mg/L); and K_i is the chromium inhibition constant (mg Cr(VI)/L).

2.8. Continuous column study

2.8.1. Experimental setup

The schematic of the bench-scale experimental setup used in this study is shown in Fig. 1a. It was one m long and had a square cross section ($10 \text{ cm} \times 10 \text{ cm}$). An overhead tank with provision for an adjustable head served as the inlet to the column. A porous plate was provided at the inlet in order to achieve a uniform entry of water into the soil. A collection reservoir was provided at the outlet. Water level in the outlet reservoir was maintained above the top of the soil column in order to maintain saturated conditions in the column. Sampling ports were provided on the sides at four different cross sections at distances 20, 40, 60 and 80 cm from the inlet, respectively. Four ports were provided at each cross-section as shown in Fig. 1b. Liquid samples were collected from these ports at regular time intervals using a syringe.

2.8.2. Transport studies without bio-transformation

The prepared sterilized soil was filled in the column in 33 layers of approximately 3 cm thickness. The column was compacted in vertical direction. Each layer was compacted with 25 blows of a 1.2 kg hammer falling from a 30 cm height, in order to get a more or less uniform compaction. The dry weight of the soil was measured before adding the required moisture and filling the column. This information was used to determine the bulk density of the soil. The porosity was determined using the formula relating the bulk density, and dry weight

$$\Phi = \frac{1 - \rho_d}{(G\rho_w)} \quad (2)$$

where Φ is the porosity; ρ_d the dry density of soil (ML^{-3}); ρ_w = the density of water (ML^{-3}); and G is the specific gravity.

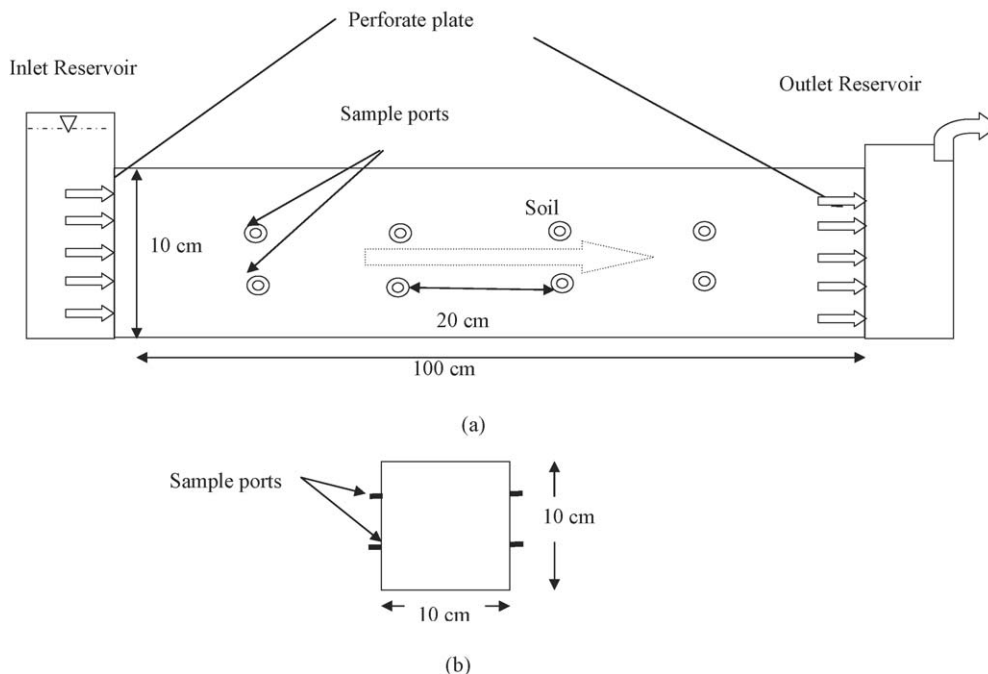


Fig. 1. Schematic of experimental setup.

Constant heads were maintained in the head and tail tanks for 6–24 h (6 h for soil B and 24 h for soil A) to obtain a steady flow rate through the soil column. The flow rate was monitored with respect to time by collecting the water at the outlet. The pore velocity was calculated from the above flow rate (pore velocity = flow rate/(area of cross section \times porosity)). Once steady state was attained, Cr(VI) contaminated water was introduced into the system through the head tank. Liquid samples were taken from the head tank and all the sampling ports at regular time intervals, and were analyzed for Cr(VI) concentration. Experiment was continued till the break-through occurred at the last sampling cross section. These experiments were conducted for soil A with a porosity of 0.36, for three different pore velocities of 22.4, 11.2 and 5.6 cm/h, respectively.

2.8.3. Transport studies with bio-transformation

Transport studies with bio-transformation were conducted in a similar manner, except that the soil was mixed with bacterial cells and mineral medium. The sterilized soil was mixed with highly concentrated cell suspension (the cells were already grown in the prescribed nutrient broth, centrifuged, washed and resuspended in mineral medium) in mineral medium M2 without carbon source. Random samples were taken and checked for the uniformity in mixing. Once uniform mixing was achieved, the columns were packed with the soil as explained earlier. The column was fed with mineral medium (M2 without carbon source) until a steady state velocity was attained. The microbial concentration in column effluent was monitored continuously. After the attainment of constant velocity, the column was fed with medium containing 25 mg/L of Cr(VI), molasses (approximately 2000 mg/L of COD) and lithium (50 mg/L) along with minerals. Samples were withdrawn from various sampling ports at regular intervals using syringes and were analyzed for Cr(VI), total chromium, COD and lithium. After the completion of column run, soil samples were taken from different ports and were analyzed for adsorbed Cr(VI), total chromium, COD, lithium and microbial concentration. These studies were conducted with two different soils namely soils A and B as given in Table 2. For soil B, the studies were conducted with two different velocities (Table 2).

2.9. Analytical procedures

2.9.1. Liquid phase chromium analysis

Cr(VI) concentration was determined by the diphenyl carbazide method (Reference Number: 3500-Cr B colorimetric method) by measuring the absorbance at 540 nm using a UV–vis spectrophotometer (Techcom UK) [22]. Total chromium con-

centration was analyzed using atomic absorption spectrometer (Perkin-Elmer, USA), after acid digestion of the aqueous solution.

2.9.2. Extraction and analysis of Cr(VI) and total chromium in soil

For the extraction of Cr(VI) and total chromium from soil, alkaline digestion method and nitric acid/sulfuric acid digestion method as per standard methods (Reference Number: 3030 G nitric acid–sulfuric acid digestion) [22] were used, respectively. The hexavalent chromium was measured colorimetrically at 540 nm by reaction with diphenyl carbazide in acidic conditions. In the case of Cr(III), potassium permanganate was used to oxidize Cr(III) to Cr(VI).

2.9.3. Measurement of cell density in liquid phase

Overnight cultures were centrifuged, and cell pellets were washed with physiological saline water thrice. This was followed by resuspension in saline water and homogenization. The homogenized solution was used as stock solution. Different dilutions were made from the stock solution. A known volume of these solutions was filtered through 0.45 μ m filter paper (Millipore, USA) to find out the dry weight of cells. Corresponding absorbance was measured at 440 nm using a spectrophotometer. This information was used to prepare a calibration curve between dry weight and absorbance. For unknown samples, the absorbance was measured at 440 nm and was converted to dry weight using absorbance versus dry weight calibration curve [23].

2.9.4. Microbial quantification

2.9.4.1. Protein estimation. The total protein of intact cells was determined according to the method of Herbert et al. [24]. The cell suspension (0.5 mL) was mixed with 1 mL of 1 N NaOH and was kept in boiling water bath for 5 min. The contents were then cooled in cold water. To this, 5 mL of freshly prepared alkaline copper reagent was added and allowed to stand for 30 min for the colour development. Reagent blank containing 0.5 mL distilled water instead of bacterial suspension was treated in the same way. The optical density was measured at 750 nm using a spectrophotometer against the reagent blank. Known bacterial concentrations were used for preparing the calibration curve.

2.9.4.2. Bacterial cell count. The bacterial cell count was carried out as per standard procedure [25]. It involves serial dilution in physiological saline water followed by plate count. Spread plate technique was adopted in the present study.

2.9.5. Chemical oxygen demand

COD of liquid and soil samples were estimated as per standard methods (Reference Number 5220 chemical oxygen demand) [22]. Closed reflex method was followed.

2.9.6. Lithium

Lithium was analysed using flame photometer (Elico, India) method as described in standard methods (Reference Number 3500-Li B flame emission photometric method) [22].

Table 2
Transport and bio-transformation studies

Run no.	Soil type	Initial pore velocity (cm/h)	Initial bacterial concentration added (mg/g)
1	Soil A	5.833	0.0205
2	Soil B (run-1)	6.67	0.0205
3	Soil B (run-2)	1.16	0.0405

3. Results and discussion

3.1. Batch adsorption studies

Adsorption studies were conducted to understand the role of adsorption on the transport/containment of Cr(VI) and Cr(III) in contaminated aquifers. Kinetics of adsorption of Cr(VI), Cr(III), Li and COD on the soil matrix were studied using batch experiments. Adsorption studies were conducted at a pH equal to 4.0–5.0 to avoid precipitation. Adsorption was very fast during the initial few minutes and attained a pseudo-equilibrium state at approximately $t = 6$ h. Usually it takes days to attain the true equilibrium. Therefore, $t = 6$ h is used as the equilibrium time for all the isotherm studies. Figs. 2–4 show the adsorption isotherms for Cr(VI), Cr(III) and Li, respectively for soil A. In all these figures, Freundlich isotherm was used for fitting the experimental data because soil is a heterogeneous medium with different functional groups. Table 3 shows the Freundlich coefficient (K_f), exponent ($1/n$) and the corresponding correlation coefficient for all the isotherms.

It can be seen that adsorption of Cr(III) is much higher than Cr(VI) as expected. The residual Cr(VI) concentration and total chromium concentration in liquid phase were almost same in case of Cr(VI) isotherm studies. Adsorption of Li is almost negligible, indicating that it is a conservative pollutant, which serves

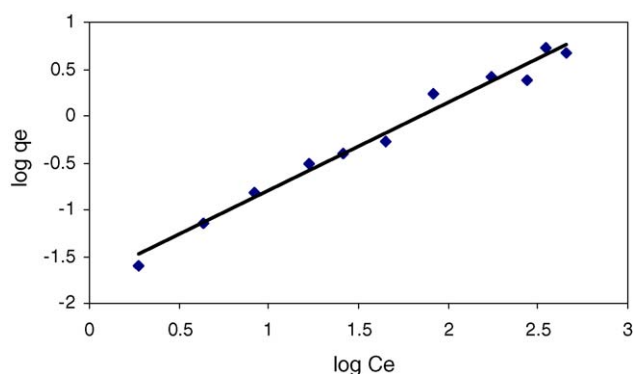


Fig. 2. Freundlich adsorption isotherm for Cr(VI) for soil A (pH 4.2–5.0, $C_0 = 1.0$ –500 mg/L).

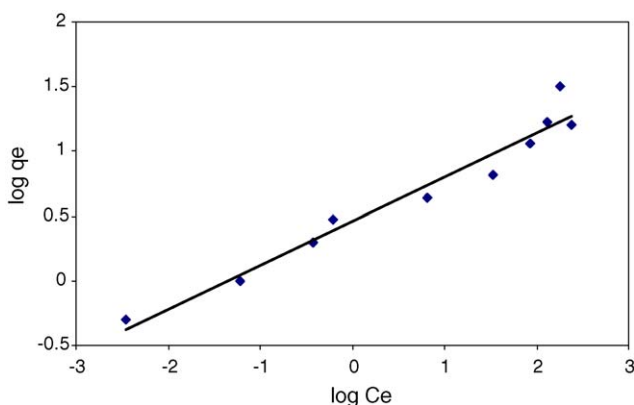


Fig. 3. Freundlich adsorption isotherm for Cr(III) for soil A (pH 4.0–5.0, $C_0 = 1.0$ –500 mg/L).

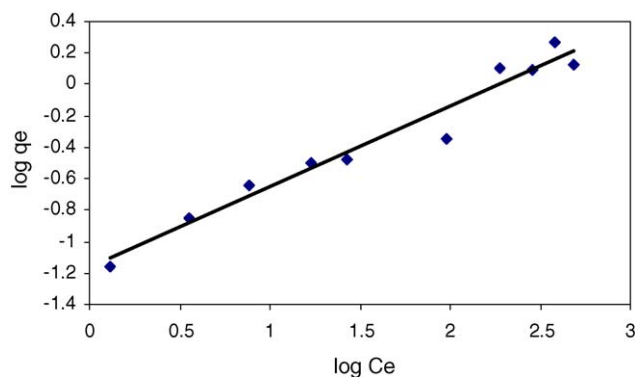


Fig. 4. Freundlich adsorption isotherm for Lithium for soil A (pH 4.0–5.0, $C_0 = 1.0$ –500 mg/L).

as a tracer to determine dispersion characteristics. Adsorption studies were also conducted for Cr(VI) and Cr(III) in presence of COD and Li to understand the interference of these components on adsorption as it is a non-selective process. High adsorption of Cr(III) was observed when the pH was adjusted to 7.0, which was the pH of the mineral medium used for column studies. This high value is due to the precipitation of Cr(III). It is difficult to differentiate between the adsorption, precipitation, ion exchange and polymerization processes. Also, the focus of this work is on containment of chromium. Hence, all these reactions are considered together as “adsorption” in this study.

The results are presented in Table 3. It is clear that COD affected the adsorption of Cr(III) to a certain extent compared to Cr(VI).

3.2. Batch bio-transformation studies

Kinetics of Cr(VI) bio-transformation by the enriched microbial culture isolated from contaminated soils was conducted. These results are presented in Fig. 5. The enriched microbes

Table 3
Isotherm constants for soil adsorption

	Freundlich Isotherm		
	K_f	$1/n$	r^2
Soil A			
Cr(VI)	0.01798	0.9445	0.98
Cr(III)	2.9	0.339	0.96
Li	0.068061	0.5128	0.96
Cr(VI) in presence of molasses and lithium			
Soil A	0.012303	1.9135	0.96
Soil B	0.010325	1.0512	0.94
Molasses in presence of Cr(VI) and lithium			
Soil A	0.055017	0.764	0.95
Soil B	0.050687	0.7575	0.90
Cr(III) in presence of molasses and lithium (pH 7.0)			
Soil A	0.1667	4.628	0.859
Soil B	0.734	5.778	0.933
Cr(III) in presence of molasses and lithium			
Soil A	0.1979	0.704	0.855
Soil B	0.0257	0.8443	0.983

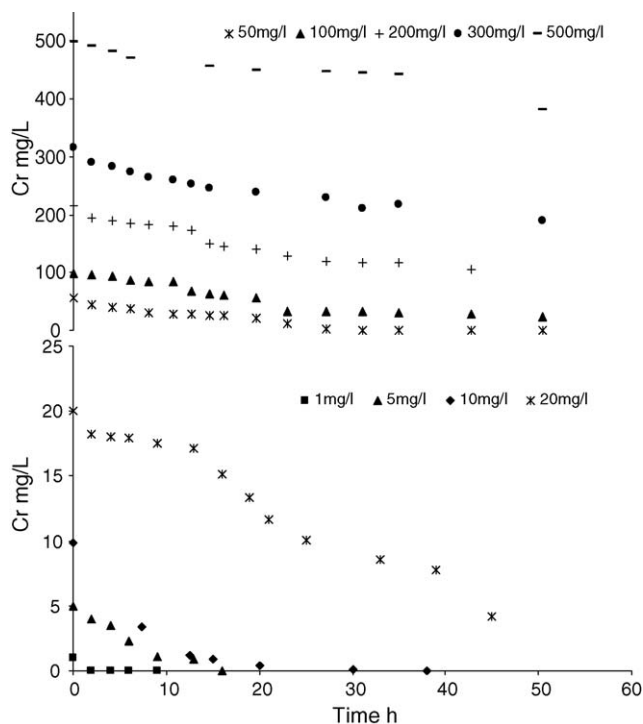


Fig. 5. Kinetics of Cr(VI) reduction for different initial Cr(VI) concentration (pH 7, Cr(VI) concentration 1–500 mg/L).

were able to reduce Cr(VI) even when the initial Cr(VI) concentration was as high as 500 mg/L. The Cr(VI) reduction was faster at low Cr(VI) initial concentrations. At higher concentrations of Cr(VI), microbial growth might have been inhibited. Measured residual COD values (results not shown) indicated that the system was working under substrate unlimiting conditions with an initial COD value of 2000 mg/L. In an earlier study, control experiments with Cr(VI) and molasses without any microbes had shown that the abiotic Cr(VI) reduction was less than 2% [4].

3.3. Studies to estimate bio-kinetic parameters

Data from bio-kinetic studies conducted without chromium were used to determine μ_{\max} and K_s in Eq. (1). Lineweaver–Burk plots [26] were used for this purpose, and $\mu_{\max} = 0.5846 \text{ h}^{-1}$ and $K_s = 3835 \text{ mg/L}$ (as COD) were determined. Experiments for microbial growth rate in the presence of chromium were then used to determine the inhibition constant K_i and it was found to be equal to 11.46 mg/L of Cr(VI). The yield coefficient, Y_T and decay constant K_d were found to be equal to 0.2615 and 0.091 h^{-1} , respectively.

3.4. Transport studies with no bio-transformation

To study the role of bio-transformation in the containment of Cr(VI) in aquifers, it is essential to understand the trans-

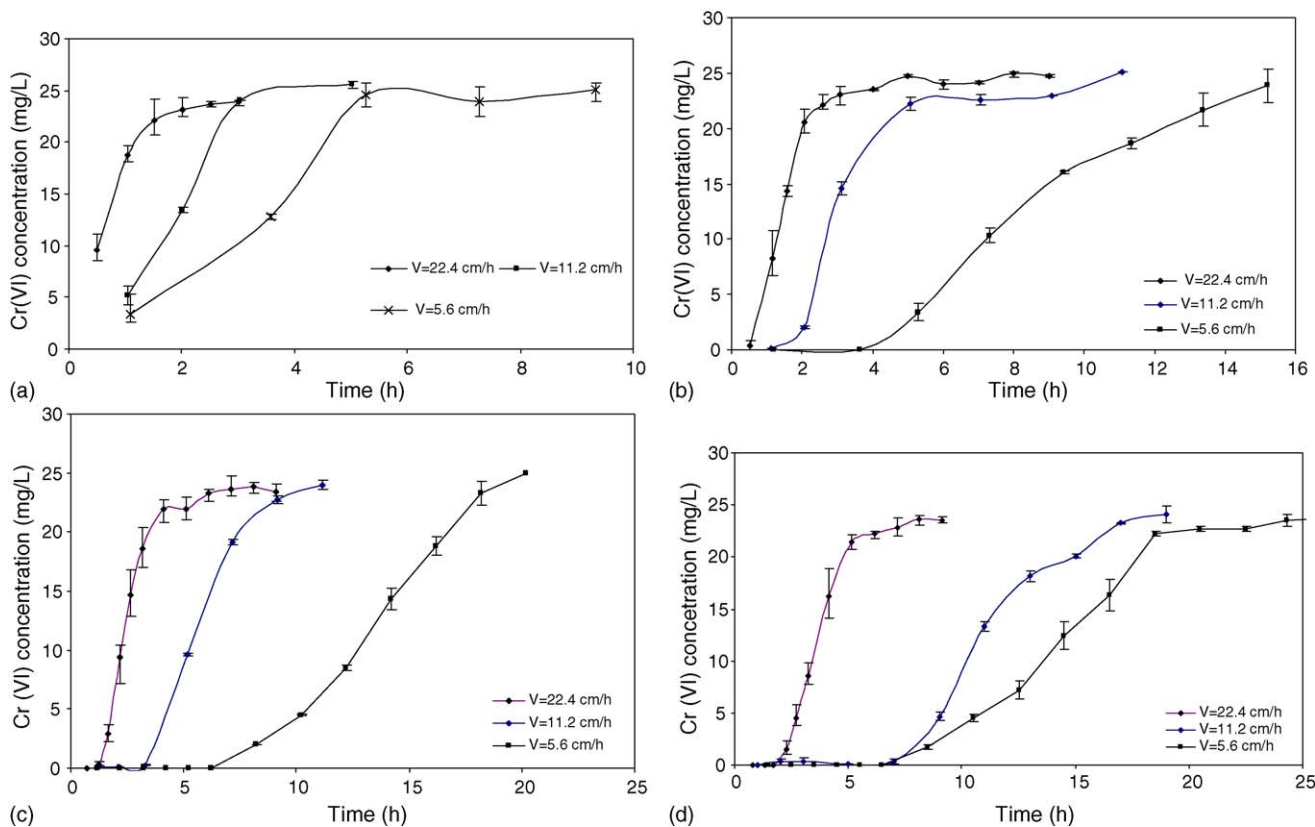


Fig. 6. (a) Break-through curve at 20 cm port for different pore velocities; no bio-transformation (pH 6.7–7.0, inlet Cr(VI) concentration 25 mg/L). (b) Break-through curve at 40 cm port for different pore velocities; no bio-transformation (pH 6.7–7.0, inlet Cr(VI) concentration 25 mg/L). (c) Break-through curve at 60 cm port for different pore velocities; no bio-transformation (pH 6.7–7.0, inlet Cr(VI) concentration 25 mg/L). (d) Break-through curve at 80 cm port for different pore velocities; no bio-transformation (pH 6.7–7.0, inlet Cr(VI) concentration 25 mg/L).

port of Cr(VI) without any bio-transformation, considering only the adsorption. These transport studies were conducted for soil A, with three different pore velocities. Fig. 6a–d present the break-through curves for Cr(VI) at 20, 40 and 80 cm ports, respectively. Each figure shows the variation of Cr(VI) concentration in liquid phase with respect to time for different pore velocities. It is clear from these figures that the dispersion effect is more predominant than the advection effect when the pore velocity is low, as expected. The maximum Cr(VI) concentration at 80 cm port was almost equal to the inlet concentration when the pore velocity was equal to 11.2 cm/h. Further, the maximum Cr(VI) concentration at 80 cm port was almost equal to the inlet concentration even when the pore velocity was as low as 5.6 cm/h. Break-through curves for both Cr(VI) and Li at 80 cm port for a pore velocity of 22.4 cm/h (not shown here) matched closely, indicating that the adsorption and hence retardation of Cr(VI) is insignificant. This is also evident from the adsorption isotherm constants for Cr(VI) and Li presented in Table 3. Therefore, it can be inferred that adsorption alone cannot significantly retard Cr(VI) transport in the aquifer. Model based parameter estimation using the advection–dispersion equation for Cr(VI) accounting for retardation showed that the dispersivity in these studies was equal to 4.46 cm. The dispersion coefficient varied linearly with pore velocity.

3.5. Transport studies with bio-transformation

Bench-scale experiments were conducted for transport along with bio-transformation in saturated, confined aquifer systems.

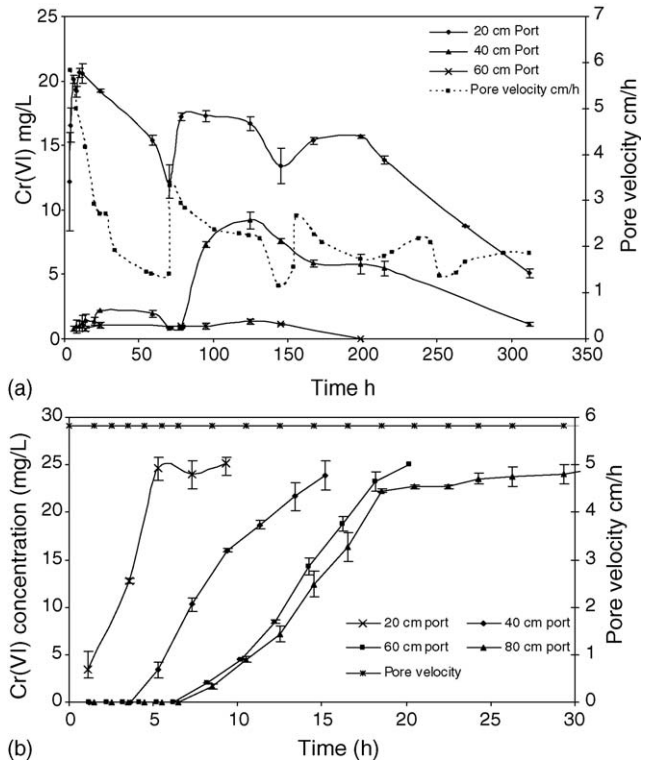


Fig. 7. (a) Cr(VI) break-through curve with bio-transformation in soil A (pH 6.2–7.2, inlet Cr(VI) concentration 25 mg/L). (b) Cr(VI) break-through curve without bio-transformation in soil A (pH 6.7–7.0, inlet Cr(VI) concentration 25 mg/L).

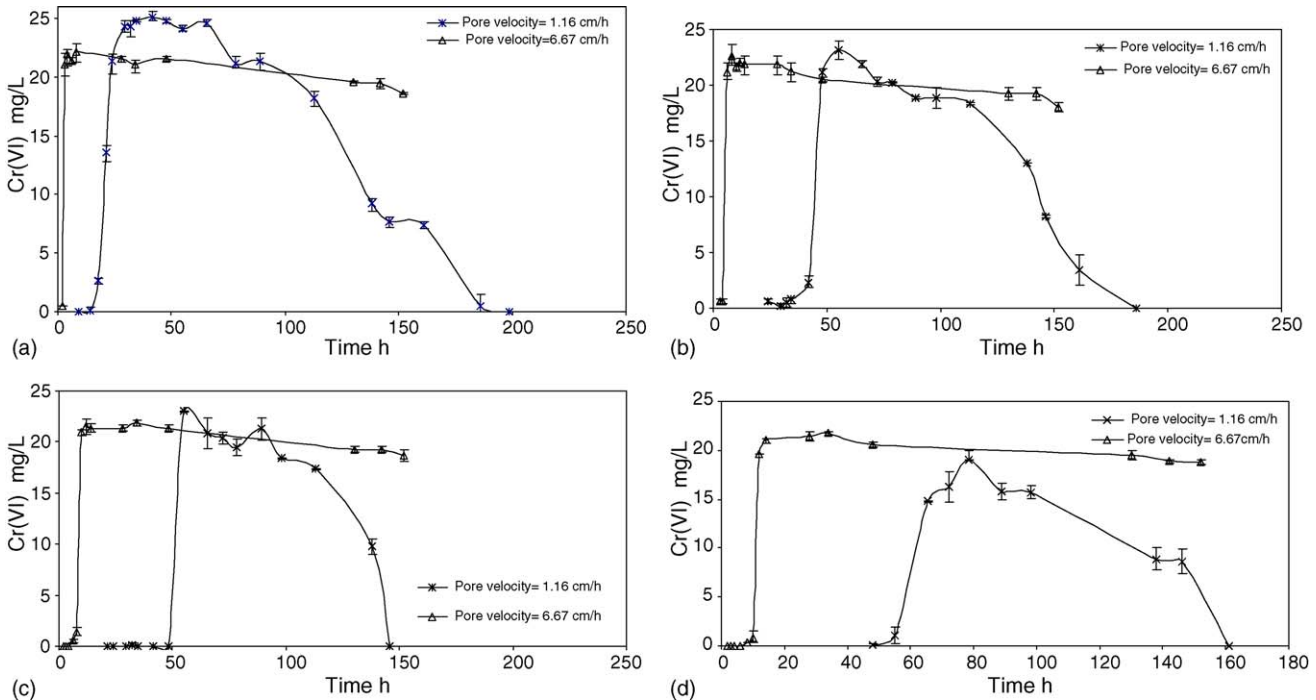


Fig. 8. (a) Cr(VI) break-through curve at 20 cm port in soil B; with bio-transformation (pH 6.2–7.2, inlet Cr(VI) concentration 25 mg/L). (b) Cr(VI) break-through curve at 40 cm port in soil B; with bio-transformation (pH 6.2–7.2, inlet Cr(VI) concentration 25 mg/L). (c) Cr(VI) break-through curve at 60 cm port in soil B; with bio-transformation (pH 6.2–7.2, inlet Cr(VI) concentration 25 mg/L). (d) Cr(VI) break-through curve at 80 cm port in soil B; with bio-transformation (pH 6.2–7.2, inlet Cr(VI) concentration 25 mg/L).

Different soil types and different pore velocities were used as shown in Table 2. The pH of the systems was monitored intermittently. The values were in the range of 6.2–7.2. In an earlier study on bio-remediation of soil by authors [4,18], it was found that bio-transformation of Cr(VI) to Cr(III) was most effective in the pH range of 6–8.

3.5.1. Cr(VI) containment with and without bio-transformation

Fig. 7a and b present Cr(VI) break-throughs in the confined aquifer in soil A, with and without bio-transformation, respectively. These figures present the break-through curves at 20, 40 and 60 cm ports. It may be noted that the initial pore velocity for both the runs was approximately equal to 5.6 cm/h. However, in the case of experiment with bio-transformation, the pore velocity reduced with respect to time. It might have been due to the change in the permeability resulting from gas release and microbial growth. Water level in the upstream head tank was adjusted periodically to obtain approximately an average pore velocity of 2.3 cm/h. The pore velocity variation with time is also shown in Fig. 7a. It can be observed from Fig. 7b that, in the experiment without bio-transformation, the break-through ($\approx 95\%$ of initial concentration) occurred at 9, 15, 20 and 25 h at 20, 40, 60 and 80 cm ports, respectively. In the case of experiment with bio-transformation, even though break-through occurred at 10 h at the 20 cm port, the Cr(VI) concentration reduced drastically in the next 300 h. In the same experiment, the maximum concentration at 40 cm port was only 8 mg/L ($\approx 33\%$ of initial concentration) and it occurred at time $t = 125$ h. The maximum concentration in 60 cm port never exceeded 1 mg/L ($\approx 4\%$ of initial concentration). There was no significant reduction of the Cr(VI) by molasses as discussed in an earlier work [4]. The transport experiments without bio-transformation showed very little containment of Cr(VI) in the soil column. Moreover, adsorption equilibrium studies with soil and molasses showed insignificant adsorption of Cr(VI) though there was some adsorption of Cr(III) (Table 3) This clearly indicates the effectiveness of bio-transformation on hexavalent chromium containment in contaminated aquifers.

In the case of column study with bio-transformation, break-through of Cr(VI) at 20 cm port occurred almost at the same time as that in column without bio-transformation. This might be due to the acclimatization period of microbes present in the system. As the microbes got acclimatized, the rate of bio-transformation and hence the containment of Cr(VI) increased. This is clearly evident from the results for ports 40 and 60 cm. Cr(VI) was contained completely within 80 cm as the bio-barrier distance through which Cr(VI) transport occurred was more.

3.5.2. Effect of velocity and cell concentration on bio-transformation

Fig. 8a–d presents the variation of Cr(VI) concentration at 20, 40, 60 and 80 cm ports, respectively, for the case of transport and bio-transformation experiments in soil B. Results for two different pore velocities of 6.67 and 1.16 cm/h are presented in these figures. It is clear that the effect of bio-transformation

Table 4a
Substrate mass balance

Soil	$TCOD_{baect(ama)}$ (mg)	$TCOD_{baect(amt)}$ (mg)	$TCOD_{baect(ama+amt)}$ (mg)	$TCOD \times V_c$ (mg)	$COD_b \times V_c$ (mg)	$\int_0^t (COD_i - COD_o) Q_{out} dt$ (mg)	$\int_0^t (COD_i - COD_{in}) dt \times V_s$ (mg)	Left hand side of Eq. (3) (mg)	Right hand side of Eq. (3) (mg)
Soil A	5511.7	15445.36	20957.06	36650.88	26853.12	19812	2122.01	48787.13	52096.24
Soil B (run-1)	1588.16	4450.47	6038.63	8842.08	0	13746.84	440.69	14187.53	13292.55
Soil B (run-2)	2801.78	7851.4	10653.18	5967.75	0	13203	643	13647.53	13819.15

Table 4b
Total chromium mass balance

Soil	$\int_0^t (\text{Cr(VI)}_i - \text{Cr}_o) Q_{\text{out}} dt$ (mg)	$\int_0^t (\text{Cr(VI)}_i - \text{Cr}_{\text{av}}) dt \times V_s$ (mg)	Left hand side of Eq. (4) (mg)	Total Cr $\times V_c$ right hand side of Eq. (4) (mg)
Soil A	605.3	34.3	639.6	621.27
Soil B (run-1)	195.52	10.07	205.58	218.63
Soil B (run-2)	400	8.53	408.53	438.5

on Cr(VI) containment is significant in the case of low pore velocity. Here, although break-through did occur, the Cr(VI) concentration reduced to zero subsequently. In case of high pore velocity, break-through of Cr(VI) occurred much earlier and also the maximum concentration was almost equal to the inlet concentration even after 150 h.

Pore velocity had a significant effect on bacterial retention on the soil matrix. Initially, the columns were operated with only mineral medium until steady state flow conditions were attained (6 h). High pore velocity resulted in significant bacterial cell wash out during this initial period. The bacterial concentration reduced from 0.021 to 0.005 mg/g in the case of column with high pore velocity, while it reduced from 0.04 to 0.027 mg/g in the case of column with low pore velocity. Thus column with low pore velocity had an advantage over the column with high pore velocity since the rate of any biological activity depends upon the bio-mass concentration. Cr(VI) containment in the case of column with low pore velocity was delayed to some extent because the bacteria introduced to the system might have taken time to get acclimatized. It can be seen from Fig. 8a–d that the rate of Cr(VI) containment increased with respect to time because of corresponding increase in bio-mass concentration in the system. It is clear from these results that initial bio-mass concentration as well as the pore velocity play a significant role in Cr(VI) containment in contaminated aquifers.

Mass balance was made for the system with respect to chromium, bio-mass and organic matter. These results are summarized in Tables 4a and 4b. Following equations were used for this purpose.

3.5.2.1. *Mass balance equation for substrate.* Total COD entered–total COD left + COD present in the system = COD consumed by bacteria + COD adsorbed by soil

$$\int_0^t (\text{COD}_i - \text{COD}_o) Q_{\text{out}} dt + \int_0^t (\text{COD}_i - \text{COD}_{\text{av}}) dt \times V_s + \text{COD}_b \times V_c = \text{TCOD}_{\text{bact (ana+cata)}} + \text{TCOD}_{\text{soil}} - \text{TCOD}_{\text{bact (ana)}} \quad (3)$$

where COD_i is the inlet COD, COD_o the outlet COD, COD_{av} the average COD of the samples, V_s the volume of the liquid samples, V_c the volume of the column, $\text{TCOD}_{\text{bact (ana+cata)}}$ the total COD consumed by bacteria for metabolism, $\text{TCOD}_{\text{soil}}$ the total COD of soil, $\text{TCOD}_{\text{bact (ana)}}$ is the total COD consumed for bacterial anabolism. Q_{out} the outlet flow rate, and COD_b initial soil COD.

3.5.2.2. *Mass balance equation for total chromium.* Total Cr(VI) entered – total Cr left = total Cr retained in the column

$$\int_0^t (\text{Cr(VI)}_i - \text{Cr}_o) Q_{\text{out}} dt + \int_0^t (\text{Cr(VI)}_i - \text{Cr}_{\text{av}}) dt \times V_s = \text{total Cr} \times V_c \quad (4)$$

where Cr(VI)_i is the inlet Cr(VI) concentration, Cr_o the outlet total Cr concentration, and Cr_{av} is the average total Cr concentration of the samples. It can be seen from these tables that mass balances for Cr and COD are satisfactory ($\pm 7\%$ error).

3.5.3. *Cr(VI) containment in aquifer with different soils*

Fig. 7a and Fig. 9 shows the variation of concentration of Cr(VI) with time for the transport and bio-transformation in soils A and B, respectively. Cr(VI) concentrations at 20, 40 and 60 cm ports are shown in these figures. Though initial bacterial concentrations and initial pore water velocities were approximately the same in both the experiments (bacterial conc ≈ 0.0295 mg/g and pore water velocity ≈ 6 cm/h), Cr(VI) was effectively contained in aquifer with soil A, where as there was only negligible containment in the case of aquifer with soil B. This was because there was more bacterial retention in soil A (concentration ≈ 0.0148 mg/g) compared to that in soil B (concentration ≈ 0.0053 mg/g), at the start of bio-transformation process. It may

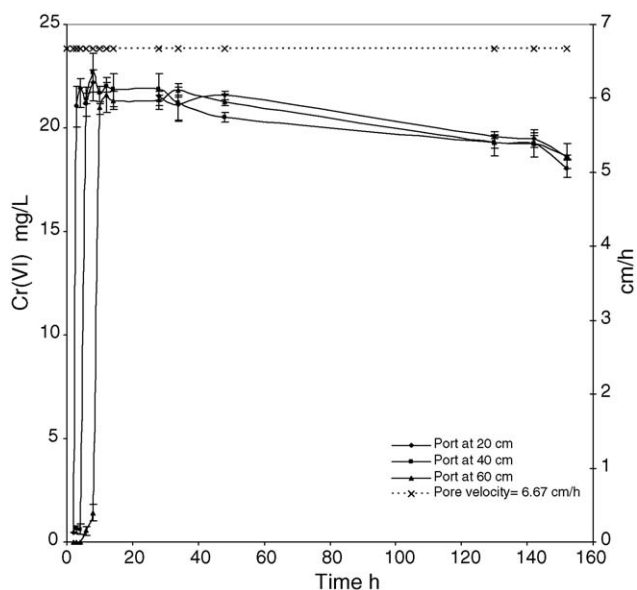


Fig. 9. Cr(VI) break-through curve in soil B for pore velocity = 6.67 cm/h; with bio-transformation (pH 6.2–7.2, inlet Cr(VI) concentration 25 mg/L).

be inferred from these results that it is easier to contain Cr(VI) in silty aquifers as compared to sandy aquifers.

3.6. Strategies for Cr(VI) containment in contaminated aquifers

Results from the present study shed light on the strategies to be adopted for containing Cr(VI) in aquifers. It can be inferred that it is a better choice to utilize pump and treat technology in case of sandy aquifers with high hydraulic gradients in ground water levels. Pore velocities in such cases would be very high and it would be hard to retain the bio-mass on the soil matrix. Subsequently, the advective effect would be more predominant than the bio-transformation effect, potentially leading to ineffective containment in case of in situ strategies.

The in situ bio-transformation strategy can be adopted for silty aquifers. Initial bio-mass concentration is a significant parameter in this case. It can be inferred from this study that it is a better choice to utilize bio-augmentation to have effective faster containment. This bio-augmentation can be implemented using a bio-barrier of a short width transecting the flow path. Based on the results of this study, width of the bio-barrier could be as small as 1 m if the Cr(VI) concentration is in the range of 1–25 mg/L.

4. Conclusions

Batch adsorption and bench-scale column transport studies indicated that it may not be possible to contain Cr(VI) in aquifers using only adsorption. Bio-kinetic parameters were evaluated for the enriched microbial consortium and the inhibitory Cr(VI) concentration was estimated using the Monod's inhibition model. Bench-scale transport and bio-transformation studies showed that bio-transformation is an effective way of chromium containment in contaminated aquifers. Most significant parameters in the containment of Cr(VI) through bio-transformation are pore water velocity and the initial bio-mass concentration. Containment is more effective in aquifers with silty soils. Bio-barriers of relatively small thickness with bio-augmentation can effectively contain the Cr(VI) in saturated confined aquifers.

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