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# Cr(VI) Remediation in Groundwater Aquifer Media Using Natural Organic Matter as Carbon Source

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Cr(VI) is toxic to biological systems due to its strong oxidizing potential. However, some microorganisms possess mechanisms that enable them to reduce the toxic of chromium [Cr(VI)] to the less toxic trivalent state [Cr(III)] either as a survival mechanism aimed at reducing toxicity around the cell or as a means of deriving metabolic energy for cell growth. Although Cr(VI) can be reduced by algae, fungi and phyto- and zooplanktons, bacteria have been demonstrated to be the most efficient Cr(VI) reducing agents. Bacteria can reduce Cr(VI) to Cr(III) either aerobically or anaerobically through a variety of biochemical pathways.
In this study, a culture comprising of mostly *Bacilli sp.* and *Enterococcus sp.* achieved high levels of Cr(VI) removal in a continuous flow process under near anaerobic conditions. Up to 93 % removal was obtained under a continuous feed of 40 to 60 mg/L after operation for 45 days. No additional nutrients and carbon sources were added to the feed water as it was assumed that organic compounds produced by decaying roots and organic matter in the soil could serve as carbon sources.
Organics in the soil were characterized using the HPLC, TOC Analyser and GC-MS and were determined to be mostly comprised of breakdown compounds of humic acid. The study demonstrated the feasibility of in situ bioremediation of Cr(VI) contaminated sites using selected cultures of Cr(VI) reducing bacteria while avoiding secondary pollution from additional nutrients.

## 1. Introduction

Chromium, in its hexavalent state, is recognised as one of the most prevalent toxic anthropogenic pollutants on earth. The carcinogenic effects of Cr(VI) have been reported by several organisations and researchers since late 19th century (Newman, 1890). Remediation of Cr(VI) pollution requires the reduction of the most mobile hexavalent state [Cr(VI)] to the less mobile trivalent state [Cr(III)] which readily forms the hydroxide precipitate [$Cr(OH)_3(s)$] under natural pH and redox conditions (Buerge and Hug, 1997). The tendency of Cr(III) to precipitate makes it less mobile in the environment and therefore much easier to manage its ecological impacts than Cr(VI) (Kaimbi and Chirwa, 2015).
Cr(VI) contamination in groundwater systems is conventionally treated using the pump-and-treat methods which involve the extraction of contaminated water from the aquifer, treatment above ground followed by injection of the treated water back into the aquifer (Milkey, 2010).
In situ biological barrier systems (BBS) have been used mainly for removal of toxic organic compounds by introducing organisms or by enhancing the activity of the portion of the indigenous community possessing inherent capability to degrade recalcitrant organic compounds (Ramsburg et al., 2004). Specific application of a biological barrier systems for the removal of Cr(VI) in groundwater has only been attempted lately at pilot level by Jeyasingh at al. (2011). The slow progress towards full implementation of biological barriers for remediation of Cr(VI) pollution has been both due to the unavailability of microorganisms capable of growing under nutrient deficient conditions.
In this study, Cr(VI) removal was evaluated in detail using intermediate sampling ports installed in soil columns. The simulation of internal Cr(VI) concentration profiles enabled us to evaluate the fundamental biological reduction and physical separation processes within the soil columns.

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## 2. Materials and Methods

### 2.1 Microbial Culture Source

The mixed culture of Cr(VI) reducing microorganisms was obtained from dried sludge collected from sand drying beds at the Brits Wastewater Treatment Works (North West Province, South Africa). The treatment plant received periodic flows from a nearby abandoned sodium dichromate processing facility reported to discharge high levels of Cr(VI) in the sewerage works from 1996 to 2005 (SA-IDC, 2014), thus the bacteria at the treatment plant was expected to be acclimatised to Cr(VI) toxicity.

### 2.2 Experimental Setup

Soil media used in soil columns was compacted together with a dried sludge sample in the ratio of 1 g sludge to 1 kg soil. The underlying aquifer at the target contaminated site consists of decomposed nolite rock with a top soil layer no deeper than 200 cm from the surface. Soil samples collected from about 1 m depth at the site were loaded in 100 mm diameter 90 cm long tubes and operated as plug-flow systems with equally spaced sampling ports along the length (Figure 1). Three sets of experiments were installed consisting of (1) a sterile abiotic control, (2) columns inoculated with sludge bacteria installed with no added carbon source, and (3) columns inoculated with sludge bacteria with ground biomass as added carbon source.

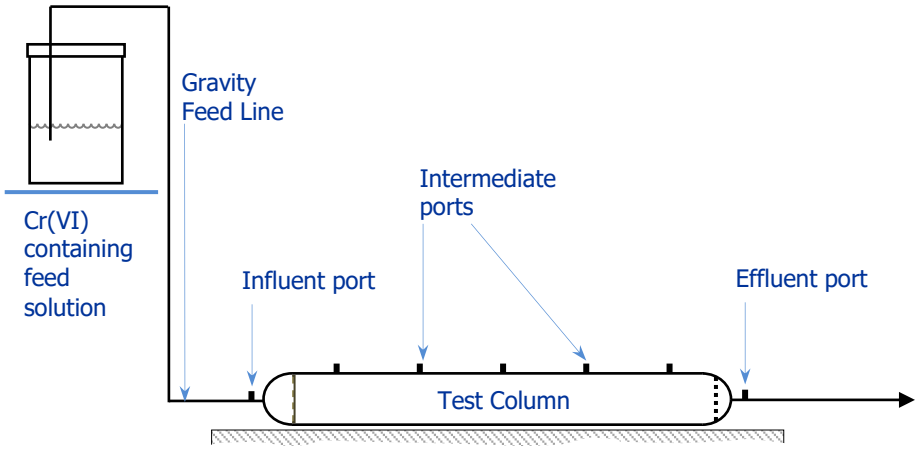


*Figure 1. Test column setup indicating intermediate sampling ports for spatial dynamic modelling.*

### 2.3 Microbial Culture Characterisation

The microbial culture distribution and species succession during operation was monitored using the 16S rRNA genotype fingerprinting technique. In the 16S rRNA characterisation method, DNA was extracted from the pure cultures grown from colonies using a ZR Fungal/Bacterial DNA MiniPrep™ (Zymo Research). The 16S rRNA genes were amplified by PCR primers targeting the common promotor sequence for the 16S rRNA gene. The amplified genome was purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany). The 16S rRNA was then sequenced using the ABI 3500XL Genetic Analyzer (Applied Biosciences, USA). The DNA sequence for each pure colony was then uploaded to the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI). A phylogenetic tree was constructed from the identified 16S rRNA sequences using the neighbor-joining method in the MEGA Version 6 software (Tamura et al., 2013).

### 2.4 Analytical Methods

Cr(VI) was measured in water samples by a UV/Vis spectrophotometer (WPA, Light Wave II, Labotech, South Africa) at a wavelength of 540 nm (10 mm light path) in 2 mL samples acidified by 1M $H_2SO_4$ treated with 0.2 mL of a 15 % solution of 1,5-diphenyl carbazide (APHA, 2005). Total Cr was measured at a wavelength of 359.9 nm using a Varian AA-1275 Atomic Adsorption Spectrophotometer (AAS) (Varian, Palo Alto, California, USA) equipped with a 3 mA chromium hollow cathode lamp.
Total organic carbon (TOC) in the sludge residue was measured from 20 mL water samples using the Model TOC-VWP Shimadzu TOC Analyzer (Shimadzu Corporation, Kyoto, Japan) following a modified protocol for TOC analysis from the Standard Methods for the Examination of Water and Wastewater (APHA, 2005).
Semi-volatile organic compounds in soil and water samples were characterised using the Perkin-Elmer Clarus

500 GC-MS equipped with Clarus 600T headspace autosampler (Perkin Elmer, Connecticut, USA). Separation of compounds in the GC was performed in a Perkin–Elmer Elite—5MS capillary column (30 m × 0.25 mm ID × 0.5 µm fixed phase) with helium as a carrier gas with the GC operating in a split-less mode. The oven temperature was kept initially at 60 °C for 5 min, followed by an increase to 300 °C at a rate of 15 °C/min.

## 3. Results and Discussion

### 3.1 Microbial Culture Composition

Changes to microbial culture composition due to long-term exposure to Cr(VI) was monitored by the 16S rRNA fingerprinting method. The results presented in the Phylogenetic trees (Figure 2a and 2b) confirm that, after long-term operation (seven weeks), the well-known Cr(VI) reducers, Bacillus thuringiensis and Bacillus cereus remained persistent in the reactors. The Gram-negative species Enterococcus faecium and Enterococcus villorum also remained persistent after long-term operation (Figure 2b).

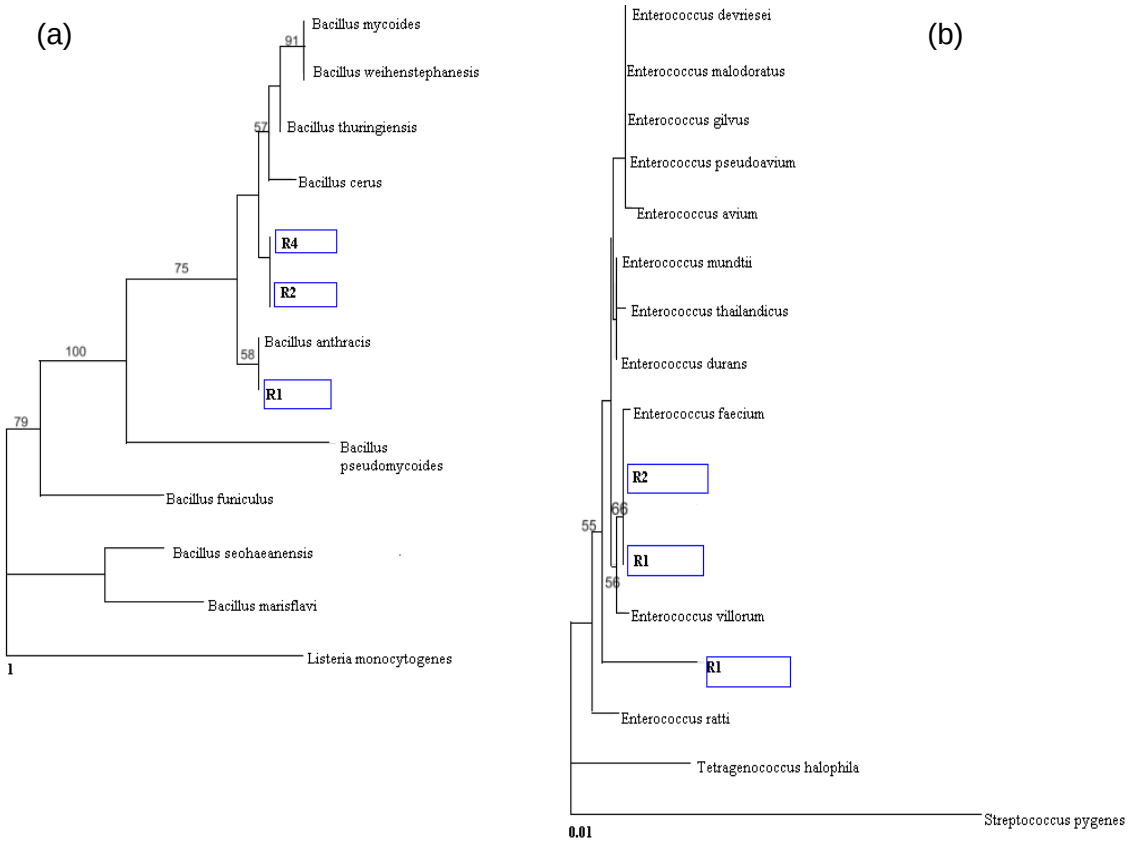


*Figure 2. Phylogenetic analysis of Cr(VI) reducing colonies for (a) Gram-positive colonies, and (b) Gram-negative colonies. The cultures were predominated by Bacillus cereus and Enterococcus villorum species.*

### 3.2 Cr(VI) Reduction Kinetics

The kinetic for Cr(VI) reduction was developed from batch studies reported earlier (Mtimunye and Chirwa, 2011). It was observed that Cr(VI) reduction under oxygen-stressed conditions is normally catalysed by a Cr(VI) reductase associated with the cytochrome *a,a*$_3$ enzyme complex of the transmembrane electron transport system (ETS) (Figure 3a) (Chirwa and Molokwane, 2011). The reduction kinetics were limited by the Cr(VI) reduction capacity of the cells and the toxicity of Cr(VI) to the cell such that Cr(VI) reduction could be represented by the modified Michaelis-Menten kinetic

$$-\frac{dC}{dt} = \frac{k_{mc} \cdot C}{K^{1-Cr/C_o} \cdot (K_c + C)} \left( X_o - \frac{C_o - C}{R_c} \right) \quad (1)$$

where $C$ = Cr(VI) concentration ($ML^{-3}$) at any time $t$ ($T$), $k_{mc}$ = maximum specific Cr(VI) reduction rate coefficient ($T^{-1}$), $K_c$ = half velocity constant ($ML^{-3}$), the term ($C_o$-$C$)/$R_c$ represents cells killed due to exposure to Cr(VI), $K$ = dimensionless competitive inhibition coefficient and $C_r$ = Cr(VI) inhibition coefficient ($ML^{-3}$). The observed Cr(VI) reduction under oxygen-stressed conditions agreed with the modified M&M enzyme kinetics as shown in Figure 3a. Best fitting parameters for model fir against batch data are shown in Table 1.

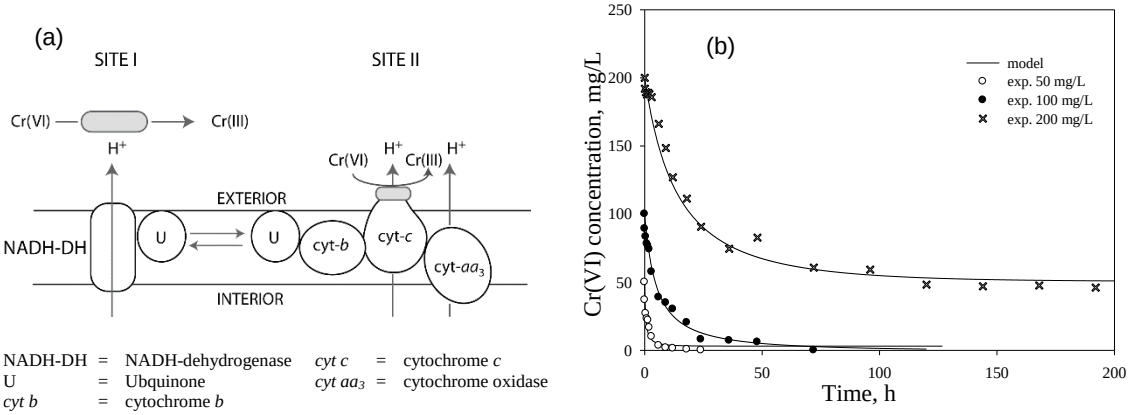


*Figure 3. The model of Cr(VI) reduction in bacteria under oxygen stressed conditions is shown in (a). Part (b) of the figure shows the fit of the kinetic model to experimental data*.

Experimental data with initial Cr(VI) concentration of 100 mg/L was initially used to estimate the kinetic parameters, $k_m$, $K_c$, $R_c$ and $K$. The validation of this model was performed and Figure 3 confirms that the kinetics parameter values obtained at 100 mg Cr(VI)/L simulated Cr(VI) reduction data very well for a broader range of Cr(VI) concentrations under anaerobic conditions (50 and 200 mg/L). However, the maximum Cr(VI) reduction capacity was not experimentally observed which implies that a unique value of the model kinetic parameter, $R_c$, cannot be obtained. Cr(VI) reduction capacity of cells, $R_c$, in this model was observed to increase with increasing initial Cr(VI) concentration Table 1.

*Table 1. Optimum kinetic parameter in anaerobic batch cultures*

| $C_o$ (mg/L) | $k_m$ ($h^{-1}$) | $K_c$ (mg/L) | $K$ (mg/L) | $R_c$ (mg/mg) | $\chi^2$ |
|---|---|---|---|---|---|
| 50 | 0.131816 | 672.09135 | 10 | 0.089395 | 259.1872 |
| 100 | 0.131816 | 672.09135 | 10 | 0.090764 | 268.6806 |
| 200 | 0.131816 | 672.09135 | 10 | 0.124514 | 1306.9093 |

### 3.3 Steady-State Performance

Cr(VI) reduction across the packed column followed plug flow kinetics for packed media with a porosity $n$ and surface area of adsorption determined by the average particle size in the reactor. The nominal reaction rate is given by the term:

$$-\frac{dC}{dL} = \frac{\rho A_f}{Q_{in}} \cdot \frac{k_{mc} \cdot C}{K^{1-Cr/C_0} \cdot (K_c + C)} \tag{2}$$

where $Q_{in}$ = hydraulic loading rate ($L^3T^{-1}$), $\rho$ = density of aquifer soil particles ($ML^{-3}$), $A_f$ = catalytic reaction interface ($L^2$) which is a function of viable cell density in the column; and $L$ = length of the reactor ($L$). In this case, the reaction area contains the biomass term, $A_f = \sigma X$, where $\sigma$ = the area-biomass proportionality constant ($L^5M^{-1}$), and $X$ at any position is subject to inactivation by the Cr(VI) gradient, i.e., the viable cells, $X_{Z_{i+1}} = X_Z - (C_Z - C_{Z_{i+1}})/R_c$ .

The response of biomass to different Cr(VI) loading conditions is illustrated in Figures 4a-c. The concentration of Cr(VI) at the entrance to the column was generally lower than the concentration at the effluent end of the reactor. This was attributed to cell inactivation due to Cr(VI) concentration at the entrance. The viable biomass in barrier regenerated as some Cr(VI) is reduced as the flow progresses along the reactor. For the reactor with

the high loading (60 mg/L), the regeneration was not quick enough to remove all the Cr(VI) (Figure 4c). Reactor biomass density in the columns operated with no added organic carbon source proceeded towards an equilibrium state after running the reactors for 15 d. By day 15, the biomass in the system stabilised and this was accompanied by the flattening of the effluent Cr(VI) concentration curve. The initial cell concentration $X_{in}$ was approximately 8.5 g/m$^3$ and the maximum value observed was approximately $X_f$ = 45.5 g/m$^3$. The maximum in reactor with added carbon source was, $X_f$ = 65.3 g/m$^3$.

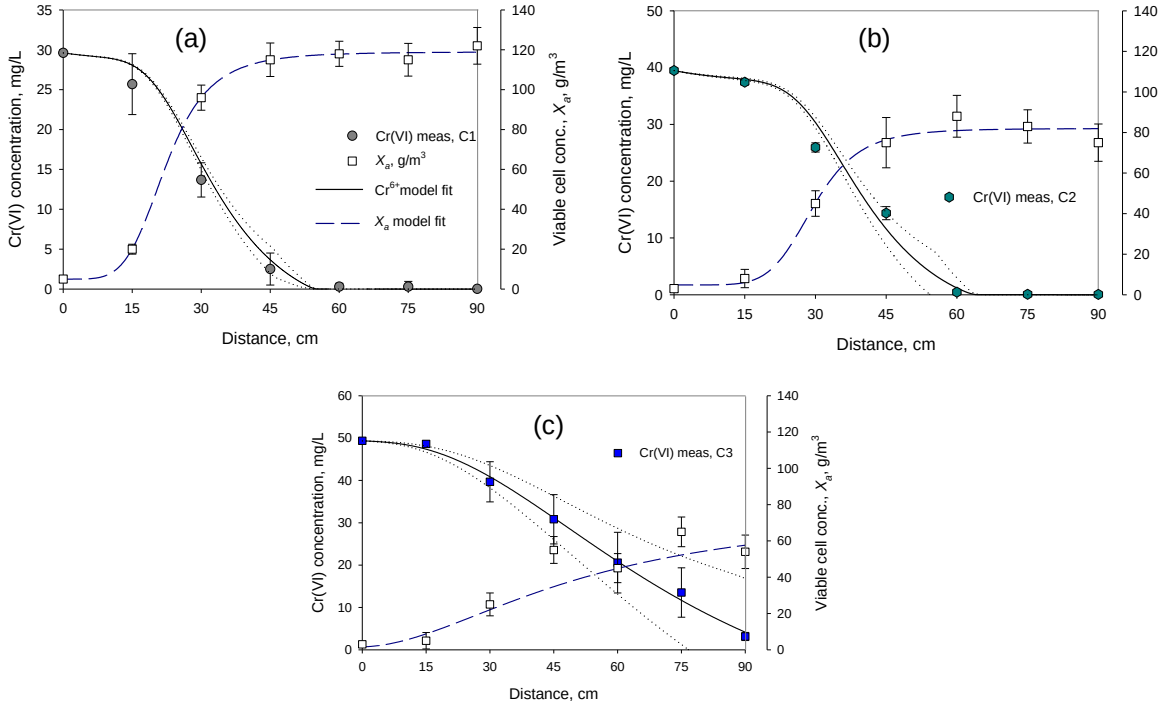


*Figure 4. Spatial response of Cr(VI) and biomass to Cr(VI) loading at (a) 30 mg/L, (b) 40 mg/L and (c) a high loading of 60 mg/L influent Cr(VI).*

### 3.4 Composition and Distribution of Carbon Sources

The organic content of the leachate from milled plant matter that served as the carbon source in the experiments was predominated by breakdown products of humic acid and an array of aromatic compounds originating from the plants (Figure 5).

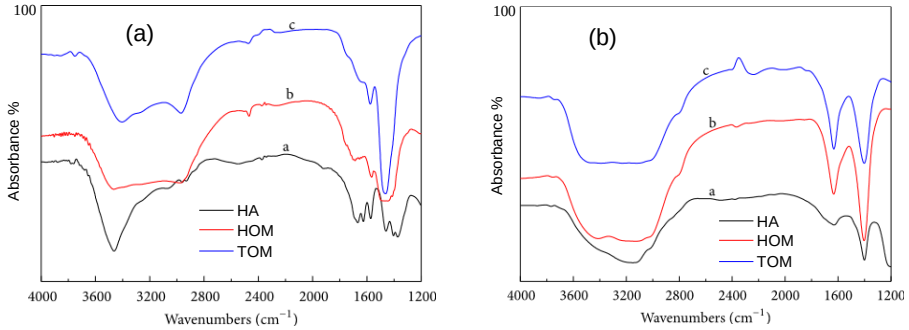


*Figure 5. (a) Humic acid (HA) and (b) hydrophilic organic matter (HOM) and (c) total organic matter (TOM) FTIR spectra from the leachate sample from simulated organic loading using plant material as carbon source.*

## 4. Conclusions

The composition of Cr(VI) reducing bacterial species from the sludge source was consistent with results from earlier studies which showed the predominance of Bacillus cereus and Enterococcus sp. in the soil. The

microbial composition distribution was not lost after operation of the columns for 45 d without re-inoculation of the columns during operation. Over 90 % Cr(VI) removal by bio-reduction and bio-precipitation was possible in the simulated in situ barriers in the presence of natural carbon sources.

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