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Cr(VI) reduction in packed-column microcosm reactors using chromium reducing microorganisms

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This research was the initial step towards the development of an *in situ* bioremediation process to contain the spread of a Cr(VI) plume in a groundwater aquifer at contaminated site in Brits, South Africa. South Africa holds about 72% percent of the world's chromium resources the majority of which is mined in the North Eastern region of the country formally known as Transvaal. The remediation process was first observed in aquifer microcosms in which up to 50 mg/L was completely removed under a hydraulic loading of 0.304 cm^3/h. The proposed *in situ* bioremediation process is expected to be more cost effective and less environmentally intrusive than the currently employed pump-and-treat method at the site. Microbial culture characterisation results showed a change in culture composition after 45 days of reactor operation, indicating *Bacillus* and *Enterobacter* species as the most dominant species in reactors that reduced Cr(VI) . The predominance of *Bacillus* and *Enterobacter* species was either due to resilience against toxicity or adaptation to the changing conditions in the reactor.

1. Introduction

Chromium is one of the most widely used metals in industry. It is used in activities such as metal finishing, petroleum, power plants and nuclear facilities resulting in large quantities being discharged into the environment (Wang and Shen, 1995). Cr(VI) is toxic, carcinogenic and mutagenic to animals as well as humans and is associated with decreased plant growth and changes in plant morphology. In contrast, trivalent chromium (Cr(III)) is relatively less toxic and less mobile (Rama *et al.*, 2005). Cr(III) is actually essential for carbohydrate metabolism in humans as it contributes to the glucose tolerance factor necessary for insulin-regulated metabolism (Zayed and Terry, 2003).
The discharge of Cr(VI) to surface water is regulated to below 0.05 mg/L by the U.S. EPA (Baral and Engelken, 2002) and the European Union (Journal of the European Communities, 1998), whereas total Cr, including Cr(III), Cr(VI) and its other forms is regulated to below 2 mg/L.
Common treatment technologies for removing chromium from industrial waste include ion-exchange, electrodepositing, and chemical reduction with iron- and sulfur-containing solutions ($FeSO_4$, Na_2SO_3, $NaHSO_3$, and $Na_2S_2O_5$), followed by precipitation at a high pH (Zhao and Duncan, 1997). These methods, although effective, can be quite costly, requiring high energy input or large quantities of chemical reagents, and can

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create other secondary waste with their own unique environmental concerns which could be detrimental to the environment.

The biological reduction of Cr(VI) to Cr(III) provides a less costly approach to soil and aquifer remediation. Although many bacterial strains have been shown to mediate reduction of Cr(VI) to Cr(III), few studies have examined the potential of *in situ* treatment of Cr(VI) using microorganisms.

This research forms part of the project in which an *in situ* bioremediation process is being developed to contain Cr(VI) pollution at a contaminated site in Brits, South Africa. The project addresses the need to develop cleanup technologies for chromium-6 contaminated sites in South Africa. The target of the study is to apply an *in situ* bioremediation process using indigenous bacteria capable of reducing Cr(VI) to remediate or prevent the spread of Cr(VI) into groundwater aquifers in the region..

2. Materials and Methods

2.1 Culture and Media

A consortium of Cr(VI) reducing bacteria was isolated from a local treatment plant in the North West Province (Molokwane *et al.*, 2008). When tested in suspended growth systems, the mixed cultures achieved 100% Cr(VI) removal under initial concentrations up to 300 mg/L, a much higher concentration than the current highest groundwater Cr(VI) concentration at the remediation wells at the study site (≈40 mg/L).

2.2 Microcosm Reactor Studies

Cores from an actual environment were set up in the laboratory as microcosm reactors as shown in Figure 1 below. Contaminant loading was simulated by gravity feeding as is the case in open aquifers at the site. Experimental systems were installed and operated as packed-bed reactors. All microcosm reactors were operated at a feed concentration of 50mg/L, representing a higher Cr (VI) concentration than that observed at the actual site where the concentration is 40mg/L. 1mL samples drawn from the influent and effluent were centrifuged at 6000 rpm for 10 minutes to remove soil particles followed by analysis for Cr(VI) and total Cr as described below.

2.3 Microbial Diversity Analysis

Phylogenetic characterization of cells was performed on individual colonies of bacteria from grown aerobically and anaerobically from soil samples extracted from the microcosm medium at the beginning and end of the experiment. LB and Plate Count (PC) agar was used for colony development. In preparation for the 16S rRNA sequence identification, the colonies were first classified based on morphology.

Genomic DNA was extracted from the pure cultures using a DNeasy tissue kit (QIAGEN Ltd, West Sussex, UK) as per manufacturer's instructions. The 16S rRNA genes of isolates were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) using primers pA and pH1 (Primer pA corresponds to position 8-27; Primer pH to position 1541-1522 of the 16S gene) (Coenye *et al.*, 1999; Molokwane *et al.*, 2008). An internal primer pD was used for sequencing (corresponding to position 519-536 of the 16S gene). The resulting sequences were matched to known bacteria in the GenBank using a basic BLAST search of the National Centre for Biotechnology Information (NCBI, Bethesda, MD).

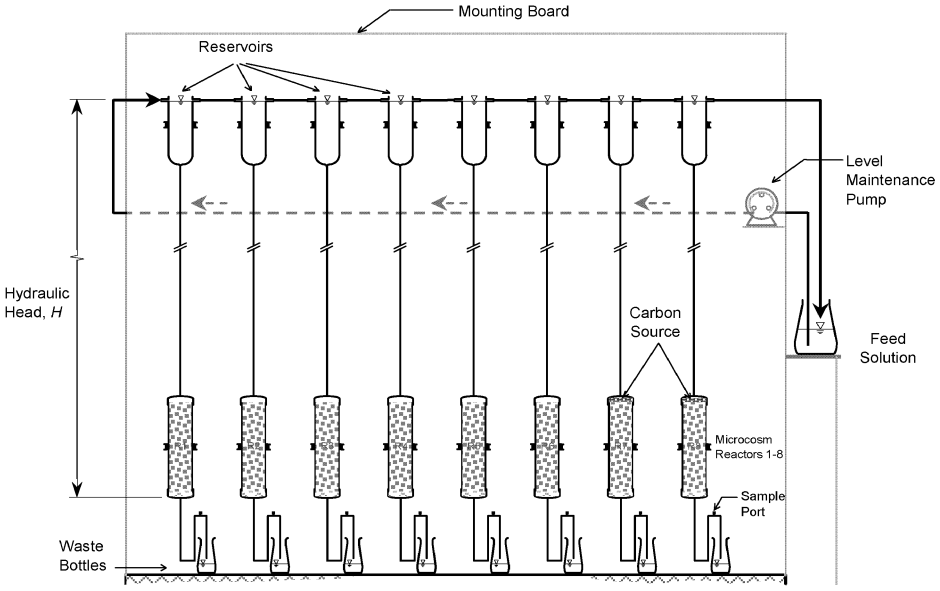

Figure 1: Laboratory microcosm setup

2.4 Analytical methods

Cr(VI) was measured using a UV/VIS spectrophotometer (WPA, Light Wave II, Labotech, South Africa) measured at λ = 540 nm (10 mm light path) after acidification of 0.2 ml samples with 1 N H_2SO_4 and reaction with 1,5-diphenyl carbazide to produce a purple colour (APHA, 2005). Total Cr was measured at a wavelength of 359.9 nm using a Varian AA – 1275 Series Flame Atomic Adsorption Spectrophotometer (AAS) (Varian, Palo Alto, CA (USA)) equipped with a 3 mA chromium hollow cathode lamp. Cr(III) was determined as the difference between total Cr and Cr(VI) concentration..

3. Results and Discussion

3.1 Microcosm Performance

Figure 2 shows that column inoculated with Cr(VI) reducing bacteria(R5) achieved significant removal of Cr(VI), however, the effectiveness of removal increased in reactors with an added carbon source(R6 and R7). Saw dust was used as a carbon source in order to avoid interferences and introduction of unnatural products into the system and that it is a better representation of the natural vegetation system at the contaminated site. Reactor 1 (sterile un-inoculated control) did not show any chromium reduction. Overall performance of different reactors is summarised in Table 1.

The breakthrough characteristic of the column is typical of packed-media reactors with moderate dispersion depicting an exponential rise to a maximum followed by reduction in effluent as the Cr(VI) culture becomes more established. The graph indicating the cumulative Cr(VI) reduction over time was used to show the comparative performance

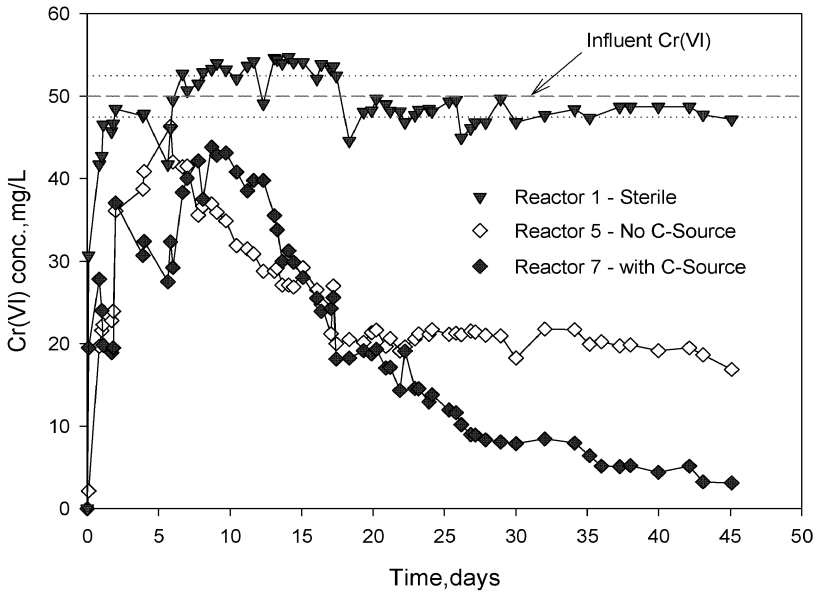

Figure 2: Performance of inoculated microcosm columns with and without carbon source in comparison with sterile control.

Table 1: Capability of mixed cultures in reducing Cr(VI) (microcosm) at 48 ±2mg/l

Reactor No.	Flow rate (Q) cm^3/day	Cr(VI) Concentration after 45 days mg/L	Cr(VI) Removal after operation for 45 days %	Cumulative Cr(VI) Removal Rate mg/d
R1	6.66	47.174	0	0
R2	25.095	45.8371	4.51	0.63
R3	12.714	48.702	0	0.48
R4	29	48.766	0	1.126
R5	0.228	16.8706	66.26	6.045
R6	0.430	13.497	73.01	5.73
R7	0.304	3.1195	93	10.161
R8	0.304	10.886	78.23	6.8716

of the reactors (rate, mg Cr(VI) removed/d) (Table 1, Figures 3 & 4). The results show that the best performing reactor (Reactor 7) did not reach system failure which could have been indicated by an asymptote when no reduction takes place as shown in the poorer performing systems (Reactors 2 and 3).

The data in the best performing reactors (5 and 7) shows that the system was self-sustaining indicating a steady increase in Cr(VI) removed up to the end of the experiment. The data for Reactor 5 without carbon source and Reactor 7 with carbon source shows that the live cultures from the soil played a role in reducing Cr(VI).

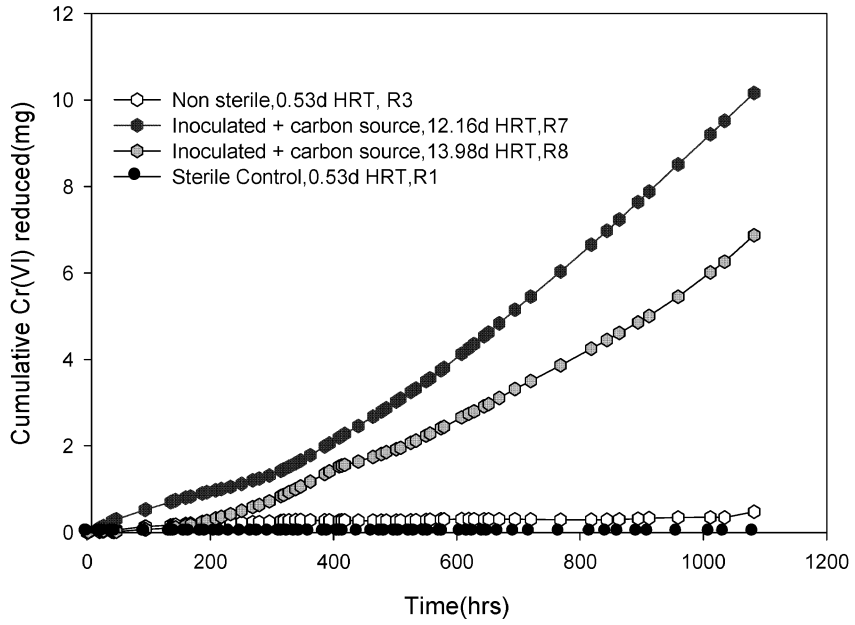

Figure 3: Cumulative Cr (VI) reduction in a microcosm system (Reactor 7, Reactor 8, Reactor 3 and Reactor 1)

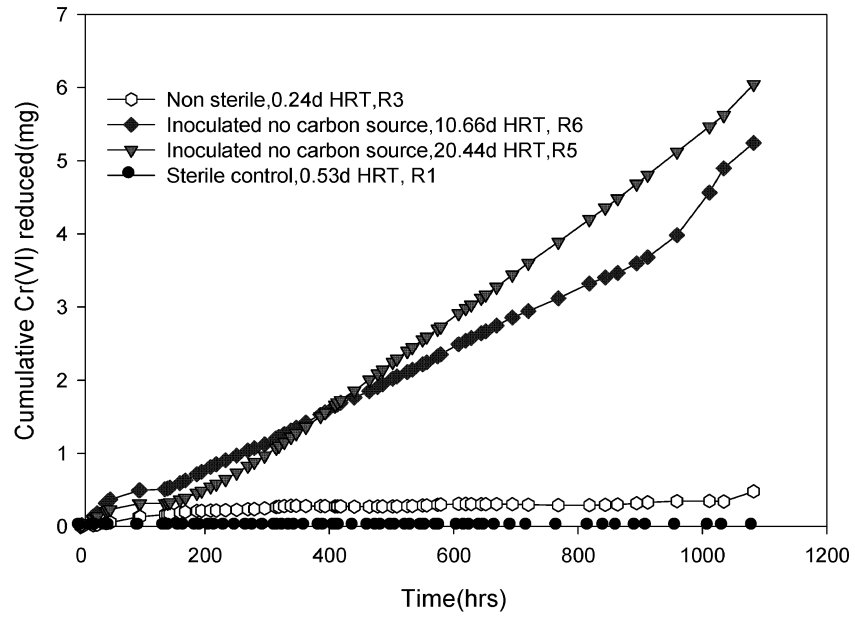

Figure 4: Cumulative Cr (VI) reduction: comparison of R5, R6 (native culture and sludge culture-no carbon source),R3(Native culture) and R1-Control.

Overall, inoculated reactor performed better than the non-inoculated reactors whereas the reactors with carbon source (R7&R8) with a performance of about 93%± 2mg/L in reactor 7. There was no reduction in reactors without any bacteria. The findings of this study indicate that the introduction of Cr(VI) reducing bacteria from activated sludge could attenuate the spread of Cr(VI) pollution in the aquifer material.

Table 2: Characterisation of the remaining bacterial species after operation

Sample	Blast result	ID Index	Alternative Species
R 2	*Bacillus cereus*	99%	
R 2	*Clostridium bifermentans strain* SH-C65	99%	
R 5	*Bacillus cereus*	99%	
R 7	*Uncultured Rhodobacter*	94%	*Alcaligenes faecalis*
R 7	*Klebsiella sp., Enterobacter cloacae*	--	
R 7	*Enterobacter cloacae*	--	*Enterobacter sp., Pantoea sp.*

4. Conclusion

The introduction of Cr(VI) reducing bacteria from activated sludge could could be used in the formulation of biological permeable barriers for protection against the spread of Cr(VI) from hot spots in the area. The microbiology of the soil material favoured the more Cr(VI) adaptive species capable of reducing Cr(VI) with *Bacillus cereus*, *Enterobacter sp.*, and *Alkaligenes sp.* as the predominant species.

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