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Conceptual Application of Biological Removal of Toxic Metals: In Situ Cr(VI) Reduction

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This study addresses the problem of Cr(VI) pollution at chrome processing foundries in South Africa. A novel culture was isolated from a local treatment works in the North West Province. The isolated culture performed better than cultures previously isolated in the United States. Up to 200 mg Cr(VI)/L was completely removed after incubation in batch studies for about 64.5 hours. This high performing Cr(VI) reducing micro-organism is used to develop an environmentally friendly *in situ* bioremediation process involving the design of a biological permeable reactive barrier (BPRB) for a Cr(VI) contaminated site at Brits, (South Africa).

1. Introduction

Hexavalent chromium is one of the toxic heavy metals with high mobility in soil and groundwater which can produce harmful effects on organisms including humans. It is carcinogenic in mammals and microbes, teratogenic in mammals, toxic to aquatic plants and mobile in aquifers and surface water (*Federal Register*, 1985). Environments and wastewaters containing chromium (VI) are generated by many industries such as chromite ore processing, electroplating, and leather-tanning processes, among others (Chuan and Liu, 1996; Lawson, 1997).

1.1 Background

Hexavalent chromium Cr (VI) compounds are being used in a wide variety of commercial processes and unregulated disposal of the chromium containing effluent has led to the contamination of soil, sediment, surface and ground waters. In South Africa, the generation of toxic divalent species of chromium is on the increase due to the high demand of products that involve the generation of Cr (VI) during processing such as platinum processing, iron smelting, leather tanning, and the manufacture of chrome alloys. South Africa is the leading exporter of chromium ore with 72% of the world's chromium reserves located in the North-eastern region of South Africa (U.S.EPA, 2001). Accidental spills and continued wash off of Cr (VI) into the ground from neglected production sites also contributes to pollution (DWAF, 2005).

The International Agency for Research on Cancer (IARC) has categorized "chromium and certain chromium compounds" in Group 1: sufficient evidence for carcinogenicity in humans and animals. According to the survey conducted by the Blacksmith Institute involving 35 contaminated sites, chromium is among the top three most difficult-to-

remediate pollutants on earth, others include lead and recalcitrant organics (Blacksmith Institute,2006).

1.2 Treatment Options

Cr(VI) may be treated chemically by reduction to Cr(III) at a high pH followed by precipitation as indicated in Equations 1 & 2. The chemical process often generates other harmful by-products that require further treatment.

$$HCrO_4^- + 3Fe^{2+} + 7H^+ \rightarrow Cr^{3+} + 3Fe^{3+} + 4H_2O \quad (1)$$

$$2HCrO_4^- + 3H_2SO_3 + 2H^+ \rightarrow 2Cr^{3+} + 3SO_4^{2-} + 5H_2O \quad (2)$$

Alternatively Cr(VI) may be treated biologically using specialized species of bacteria which offers a cleaner cost effective alternative that can be operated under a natural pH range (6.8-7.2) (Equations 3 & 4) (Brock and Madigan 1991).

$$CrO_4^{2-} + 8H^+ \xrightarrow{CRB} Cr^{3+} + 4H_2O \xrightarrow{neutralpH} Cr(OH)_3(s) + 3H + H_2O \quad (3)$$

$$3CH_3COO^- + 4HCrO_4^- + 4CrO_4^{2-} + 33H^+ \rightarrow 8Cr^{3+} + 6HCO_3^- + 20H_2O \quad (4)$$

Several biological processes have been documented for toxic metal removal including methylation, uptake, bioaccumulation, and reduction by sulfate-reducing bacteria (Haas and Polprasert, 1993; Hao *et al*., 1994).

1.3 Application

In this study, Cr(VI) reducing cultures are isolated for use in the development of biological reactive barriers around Cr(VI) contaminated sites in South Africa. The study involves the evaluation of performance of isolated cultures and the prospect of pollution containment in groundwater aquifers using laboratory-scale microcosms.

2. Materials and Methods

2.1 Culture and Media

Bacteria cultures were obtained from dried sludge samples from sand-drying beds at the Brits Wastewater Treatment Works (North West Province, South Africa). The cultures were cultivated in 100ml sterile LB broth at 30±1^{o}C over days. Colony isolation was carried out using pour plate method with plate count agar.

2.2 Cr(VI) Reduction Experiments

Cr(VI) reduction performance studies were conducted in batch. Experiments were carried out at various initial Cr(VI) concentrations (50, 100, 150, 200, 300, and 400 mg/l) to determine the maximum Cr(VI) reduction rate and Cr(VI) reduction capacity of the cells. Experiments were conducted under micro-aerobic conditions at a constant temperature of 30^{o}C in 250 mL Erlenmeyer flasks covered with cotton plugs. The Cr(VI) reduction capacity of the cells is determined as:

$$R_c = \frac{C_o - C}{X_o - X} \quad (5)$$

where R_c = Cr(VI) reduction capacity (mg Cr(VI) removed /mg cells inactivated), C_o = initial Cr(VI) concentration (mg/L), C = Cr(VI) concentration at any time of incubation t, X_o = initial viable cell concentration (mg/L), and X = viable cell concentration (mg/L) at any time t.

2.3 Sample Preparation

1 mL samples were withdrawn from the reactors at predetermined intervals. The samples were then centrifuged at 6000 rpm for 10 minutes in a Hermle 2323 centrifuge (Hermle Laboratories, Wehigen, Germany) to remove or settle cells. 0.2 mL of the supernatant (0.1 mL for concentrations >200 mg/L) was withdrawn for colorimetric analysis of Cr(VI).

2.4 Analytical Methods

Cr(VI) and total chromium was measured with a UV/VIS spectrophotometer (WPA, Light Wave II, Labotech, South Africa) at 540nm wavelength (10mm path length) after acidification of 0.2 mL (0.1 mL for concentrations >200 mg/L) samples with 1N H_2SO_4 and reaction with 1,5-diphenyl carbazide to produce a purple colour. Cr(VI) reduction kinetic parameters were determined by non-linear regression using the Marqaudt-Levenberg Algorithm in SigmaPlot 9 (Systat Software, Inc., San Jose, CA). All statistical analysis was performed using SigmaPlot and SigmaStat.

3. Results and Discussion

3.1 Preliminary Results

Dried sludge cultures from the Wastewater Treatment Works at Brits (NW) reduced Cr(VI) at higher concentrations and at a higher rate than known Cr(VI) reducing cultures obtained from abroad, these include pure cultures of *Bacillus* sp., *Pseudomonas fluorescens* LB300, and *Escherichia coli* ATCC 33456 isolated from soil from Cr(VI) contaminated sites in Newark (New Jersey) and other parts of the United States (Chirwa and Wang, 1997a; Chirwa and Wang, 1997b, Shen and Wang, 1993). Abiotic Cr(VI) reduction in the indigenous sludge cultures was ruled out by the absence of Cr(VI) reduction activity in cell free and heat killed controls (e.g., Figure 1).

Near complete removal of Cr(VI) from solution was observed at concentrations as high as 200 mg/L, with total removal in 50 mg/L after incubating for only 4.5 hours. This rate of removal was observed to be much higher than the removal rate observed in the *Bacillus* culture where only 50% removal was observed after incubation of the the 50 mg/L culture for 100 hours (Wang and Shen, 1997).

So far, Cr(VI) loading did not significantly affect the concentration of viable biomass. But this may be due to unreliability in the method used to determine the viable cell concentration, i.e., the pour plate colony count method (APHA, 1992).

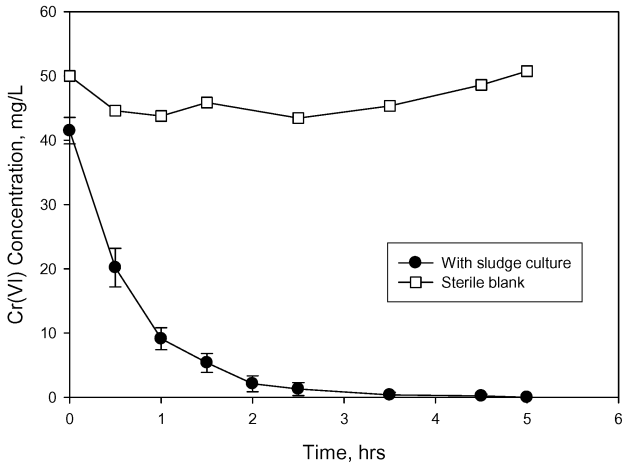

Figure 1: Demonstration of biological Cr(VI) reduction in cultures from dried sludge at 50 mg/L.

3.2 Cr(VI) Reduction Experiments

Figure 2 (A and B) shows that the rate of Cr(VI) reduction decreased significantly as the initial Cr(VI) concentration was increased from 50 to 400 mg/L. The highest rate of removal was observed at 50 mg/L. However, the highest amount of Cr(VI) reduced per gram of cells incubated was observed at 200 mg/L. These data suggest that the optimum Cr(VI) reduction efficiency could be located somewhere between 200 and 300 mg/L initial concentration. These data are consistent with earlier observations by Chirwa and Wang (1997b), in which the optimum Cr(VI) reduction depended on the Cr(VI) reduction capacity of the cells. In all experiments, Cr(VI) reduction in cell free cultures was insignificant. The decreasing Cr(VI) reduction rate at higher initial concentrations was attributed to the toxic effect of Cr(VI) and depletion of the hydrogen ion which resulted in an increase in the pH of the solution (Equations 1-4).

3.3 Application

The research is part of an effort to develop the pollution containment strategy for the site of an abandoned Cr(VI) processing plant at Brits, North West Province (Figure 3, Site Layout). The proposed method involves the installation of an *in situ* permeable reactive barrier around the site. This will be combined with operation of scavenger pumps to reverse the groundwater flow followed by *ex situ* treatment of the extracted Cr(VI). The figure shows the strategic location of the barrier. The above strategy will ensure the savings in energy and pumping costs and protection of the surrounding aquifer which is used as drinking water supply source for local residents in the area.

4. Conclusion

The first step of identifying local cultures to be used in an in situ bioremediation process for the chrome processing industry was achieved. Cultures isolated during this study outperformed earlier isolated and purified cultures from abroad. Using these culture, it

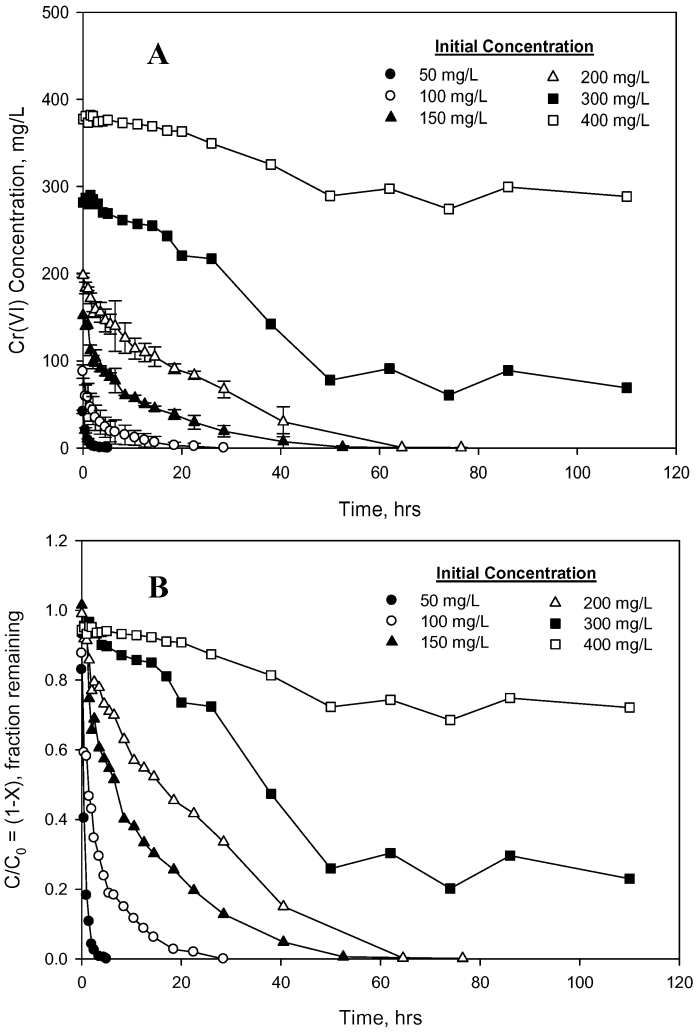

Figure 2. (a) Cr(VI) reduction at different initial concentrations, and (b) representation as a fraction of Cr(VI) remaining in solution.

will be possible to design and test a pilot scale in-situ bioremediation system at Brits in the North West Province. However, the isolated cultures will need to be purified further and possibly optimised for the underground environment. To achieve this, it is necessary to characterise the culture (determine the culture composition) and determine the minimum medium requirement of the bacteria. This process if proven successful will underscore the importance of adopting natural processes for pollution control without the worry of managing harmful byproducts. more efficient (and smaller) reactor systems may be used to biologically treat Cr(VI) using these cultures of bacteria. The results shows the feasibility of *in situ* clean up processes for Cr(VI) contaminated sites using local cultures with minimum environmental consequences.

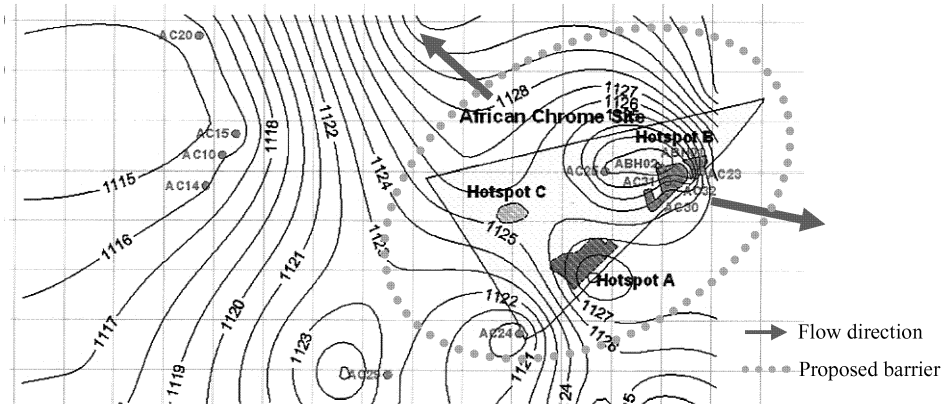

Figure 3. Site layout at the Cr(VI) Contaminated site at Brits, North West Province.

5. ACKNOWLEDGMENTS

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