Cr(VI) Reduction in Continuous Flow Soil Columns

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Abstract: Bioremediation of Cr(VI) contaminated groundwater using a native bacterial consortium is being investigated as a novel remediation alternative. Laboratory scale soil column experiments are being performed to test our understanding of Cr(VI) reduction in an anaerobic environment. The column contains coarse sand inoculated with a subsurface bacterial consortium. The feed solution consists of a simulated groundwater media (SGM) amended with sucrose and yeast extract as a carbon source. The column influent and effluent are monitored for Cr(VI), nitrate, nitrite, acetate, and carbohydrates. The mean residence time of the column is approximately two hours, based on chloride tracer tests. Column analysis indicated that the majority of the biomass is contained in the first 2 cm of the column inlet. This provided a relatively small "reaction zone" for chromate reduction. In addition, biological markers are being used to monitor community dynamics within the column. The column data will be used to assess the applicability of batch reactor kinetics to continuous flow systems and to expand our understanding of population dynamics in subsurface systems.

Introduction: Chromium(III) is found naturally in air, soil, and water in small quantities. However, chromium(VI) is usually associated with anthropogenic contamination, primarily from industrial operations (1). Of these two valence states, Cr(VI) is the most toxic (2-4). The oxyanions of Cr(VI) (chromate, CrO_4^{2-}, and dichromate, $Cr2O_7^{2-}$) are relatively soluble and mobile in groundwater (5). Conversely, Cr(III) is both less toxic and less mobile (6). Consequently, research has been focused on the reduction of Cr(VI) to Cr(III). The existing treatment processes for treatment of Cr(VI) in soil or groundwater involve physical and chemical methods, e.g., excavation or pumping and off-site disposal, and chemical reduction (7). However, these methods are usually expensive and sometimes generate secondary wastes that require subsequent disposal. Alternatively, *in situ* bioremediation technology can be applied to circumvent the limitations of physical and/or chemical methods. Direct metabolic reduction of Cr(VI) by bacteria has been documented by various researchers (8-15). However, these studies were based on pure cultures, which cannot be readily applied to *in situ* bioremediation. Chromium reduction using natural environmental consortia was reported recently (16) but these studies have been performed in suspended growth batch reactors.

Here, we develop a continuous flow porous media column to show Cr(VI) reduction with a natural environmental consortium, isolated from subsurface soil collected near a chromium plume located on the Hanford Reservation (Richland, Washington). This consortium was shown

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previously to reduce Cr(VI) to Cr(III) in batch experiments (17). The extent of reduction with two different substrates (sucrose and molasses) is also under investigation.

Materials and Methods: The stainless steel soil column had a 2.1 cm ID and 15 cm length (Alltech Associates Inc., Deerfield, IL). The sterile feed was sparged and pressurized with filtered helium gas at 2 psi to maintain anoxic conditions. Flow was afforded by a LC pump (Alltech Associates Inc., Deerfield, IL). A three-way valve was used to take influent samples at a location just prior to the column entrance. The feed solution contained Simulated Ground Water (SGW) (18), 100-200 mg/L nitrate, 150-300 mg/L sucrose, and 15-30 mg/L yeast extract. A coarse sand (average particle size 840 μm) was used as column packing material. The sand was inoculated with the consortia that was initially grown in a 165 ml serum bottle containing SGW amended with 1000 mg/L sucrose, 100 mg/L yeast extract, and 100 mg/L nitrate (16). The inoculated sand was poured into the sterile column aseptically in a laminar flow hood (19).

Cr(VI) concentrations were determined by the diphenylcarbazide method using Hach ChromaVer 3 reagent (Hach Company, Loveland, CO) (17). Cell protein analysis was performed by a total protein assay (Coomassie® Plus Protein Assay, Pierce Chemical Company, Rockford, IL) (16). The nitrate, nitrite, and acetate concentrations were measured by ion chromatography (DX 500 ion chromatograph, Dionex Corporation, Sunnyvale, CA).

Results and Discussion: The result of a non-reactive tracer test to determine the flow characteristics in the column is shown in Figure 1. These data indicate that the column closely approximated an ideal plug flow condition. Attached biomass distribution in the porous media is shown in Figure 2. As can be seen, the first 2 cm contain the largest fraction of biomass, making the effective contact time too small for significant Cr(VI) reduction. Batch kinetics data confirm this, indicating that about 12 hours is needed to achieve complete reduction of 2 mg/L Cr(VI) using molasses as the sole carbon source (16). The data in Figure 3 represent two column experiments that were performed under different feed conditions.

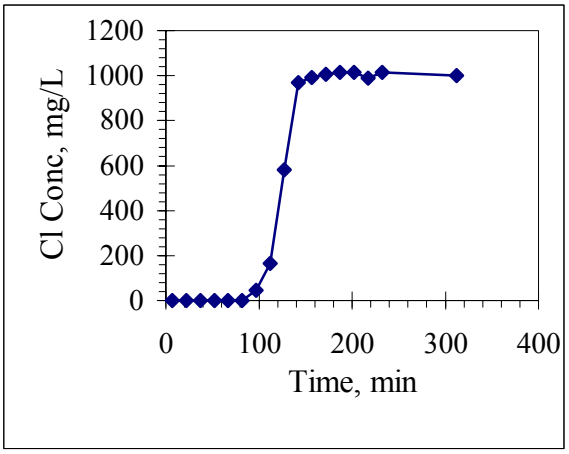

Figure 1. Column tracer test with NaCl.

Experiment 1 had a feed solution amended with 150 mg/L sucrose, 15 mg/L yeast extract, 100 mg/L nitrate, and 2 mg/L Cr(VI); whereas experiment 2 had 300, 30, 200, and 1 mg/L of sucrose, yeast extract, nitrate, and Cr(VI) respectively. Observed Cr(VI) reduction averaged 13% and 32% in experiments 1 and 2, respectively. It is interesting to note that the target Cr(VI) feed concentration for experiment 2 was 1 mg/L but the average measured feed concentration was approximately 0.5 mg/L. Currently, we feel that the increase in yeast extract concentration may have resulted in abiotic reduction. This hypothesis is currently being investigated. Experiments in the immediate future will include the use of larger diameter columns to increase contact period and the investigation of alternate substrate (e.g., molasses).

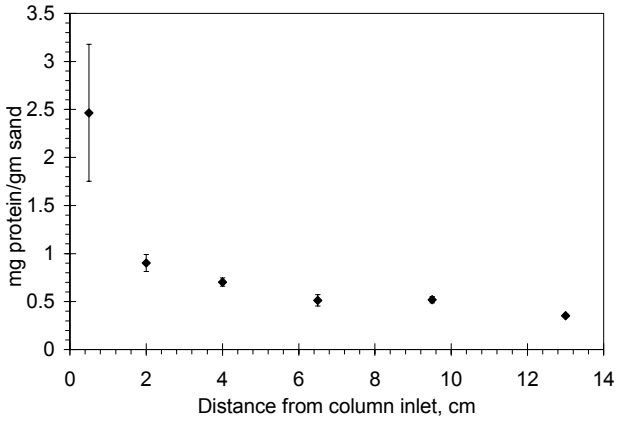

Figure 2. Column biomass distribution.

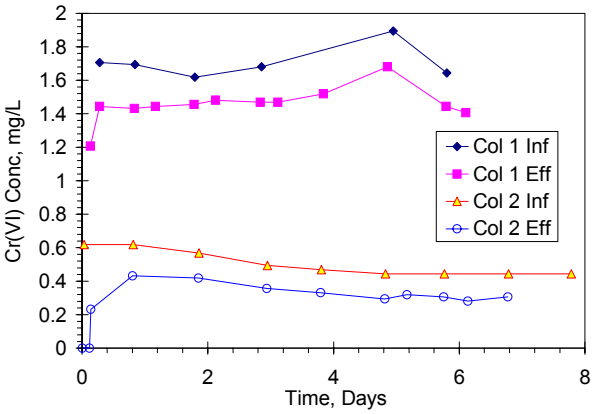

Figure 3. Cr(VI) Reduction at two different feed conditions

References

(1) Langard, S (1980). *Metals in the environment*, H.A. Waldron (ed.), Academy Press Inc., New York, 111-132.

(2) Roe, FJC and Carter, RL (1969). *Brit. J. Cancer*, **23**, 172-176.

(3) Enterline, PE (1974) *J. Occup. Med.*, **16**, 523-526.

(4) Mertz, W (1974). *Trace elements metabolism*, WG Hoekstra, JW Suttie, KE Ganther, and W Mertz, eds., University Park Press, Baltimore, MD, **2**, 185-198.

(5) Dragun, J (1988). The Soil Chemistry of Hazardous materials, Hazardous Materials Control Resources Institute, Greenbelt, MD, 75.

(6) Fendorf, SE, and Sparks, DL (1994). *Environ. Sci. Tech*., **28**, 290-297.

(7) Nyer, EK (1992). *Groudwater Treatment Technology*, Van Nostrand Reinhold, New York, 218

(8) Horitsu, H (1987). *Agric. Biol. Chem*., **51**, 2417-2420.

(9) Romanenko, VI, and Koren'kov, VN (1977). *Mikrobiologiya*, **43**, 414-417.

(10) Bopp, LH, and Erlich, HL (1988). *Arch. Microbiol*., **150**, 426-431.

(11) Wang, P, Mori, T, Komori, K, Sasatsu, M, Toda, K, and Ohtake, H (1989). *Appl. Environ. Microbiol*. **55**, 1665-1669.

(12) Fujie, K, Tsuchida, T, Urano, K, and Ohtake, H (1994). *Wat. Sci. Tech*., **30**, 235-243.

(13) Fujie, K, Hu, HY, Huang, X, Tanaka, Y, Urano, K, and Ohtake, H (1996). *Wat. Sci. Tech*., **34**, 137-182.

(14) Shen, H, and Wang, YT (1995). *Appl. Environ. Microbiol*., **61**, 2754-2758.

(15) Chen, JM, and Hao, OJ (1996). *Wat. Environ. Res*., **68**, 1156-1164.

(16) Schmieman, EA, Yonge, DR, Rege, MA, Petersen, JN, Turick, CE, Johnstone, DL, and Apel, WA (1998). *J. Environ. Eng.,* **124(5)**, 449-455.

(17) Schmieman, EA, Petersen, JN, Yonge, DR, Johnstone, DL, Samuel, YB, Apel, WA, and Turick, CE (1997). *Appl. Biochem. Biotech*, **63-65**, 855-864.

(18) Petersen, JN, Skeen, RS, Amos, KM, Hooker, BS (1994) *Biotech Bioeng.*, **43(6)**, 521-528

(19) Clement, TP, Peyton, BM, Skeen, RS, Jennings, DA, and Petersen, JN (1997). *J. Contam. Hydro*., **24**, 269-285.