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Reduction of $Cr(6^+)$ to $Cr(3^+)$ in a Packed-Bed Bioreactor

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ABSTRACT

Hexavalent chromium, $Cr(6^+)$, is a common and toxic pollutant in soils and waters. Reduction of the mobile $Cr(6^+)$ to the less mobile and less toxic trivalent chromium, $Cr(3^+)$, can be achieved with conventional chemical reduction technologies. Alternatively, $Cr(6^+)$ can be biochemically reduced to $Cr(3^+)$ by anaerobic microbial consortia which appear to use $Cr(6^+)$ as a terminal electron acceptor. A bioprocess for $Cr(6^+)$ reduction has been demonstrated using a packed-bed bioreactor containing ceramic packing, and then compared to a similar bioreactor containing DuPont Bio-Sep beads. An increase in volumetric productivity (from 4 mg $Cr(6^+)$/L/h to 260 mg $Cr(6^+)$/L/h, probably due to an increase in biomass density, was obtained using Bio-Sep beads. The beads contain internal macropores which were shown by scanning electron microscopy to house dense concentrations of bacteria. Comparisons to conventional $Cr(6^+)$ treatment technologies indicate that a bioprocess has several economic and operational advantages.

Index Entries: Hexavalent chromium; bacterial reduction; bioprocess; chromate; bioreduction.

INTRODUCTION

Hexavalent chromium ($Cr[6^+]$) has been used extensively in the industrial and government sectors throughout this century *(1,2)*. Consequently, $Cr(6^+)$ is present in soils and ground waters, and presents a considerable health risk as a toxic, mutagenic, and carcinogenic pollutant

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(3). Without remedial activities, $Cr(6^+)$ has been projected to persist in the environment for 1000 years *(4)*.

Health risks significantly diminish on the reduction of $Cr(6^+)$ to trivalent chromium ($Cr[3^+]$) owing to decreased solubility and bioavailability. Previous work indicates that anaerobic bacteria capable of $Cr(6^+)$ reduction may be ubiquitous in soils *(5)*. These findings suggest that a bioprocess operating in conjunction with soil washing and pump and treat technologies could be developed to reduce $Cr(6^+)$ to the more benign $Cr(3^+)$. Turick and Apel *(6)* demonstrated a packed-bed, anaerobic bioprocess, incorporating a mixed culture of $Cr(6^+)$ reducers from soil. This bioprocess has been shown to operate with a continuous stream of $Cr(6^+)$ ranging from 140–750 mg/L at a dilution rate of 0.5 d^{-1}. $Cr(6^+)$ reduction occurred at a rate of 4 mg/L/h. Although these $Cr(6^+)$ reduction rates were low, the results demonstrated the possibility of developing a bioprocess using a mixed culture of soil isolates to reduce $Cr(6^+)$ continuously.

In the present study, an attempt was made to increase bacterial density in the bioreactor and subsequent volumetric productivity of the bioprocess. Solid supports (porcelain saddles) from the previous study were replaced with Bio-Sep beads. The increased porosity of the beads allows for potentially higher densities of bacteria owing to entrapment and immobilization.

An estimation of economics relative to operational costs and size of a scaled-up $Cr(6^+)$ reducing bioreactor is calculated based on data presented here.

MATERIALS AND METHODS

Bioreactor Description

The packed-bed bioreactor consisted of a 1.9-L glass cylinder fitted with a water jacket and sealed with Teflon stoppers at either end. Liquid volume was 0.750 L with the solid supports making up the remaining volume. The operational temperature in the reactor was maintained at 30°C. $Cr(6^+)$ concentration was maintained at 200 mg/L using a syringe pump. $Cr(6^+)$ contacted the Tryptic Soy broth (TSB) via an in-line mixer prior to both the TSB and $Cr(6^+)$ entering the bioreactor. Oxygen-free TSB containing $Cr(6^+)$ was circulated through the reactor with a peristaltic pump positioned upstream of the reactor (Fig.1). The bioreactor was operated anaerobically at a dilution rate of 2 h^{-1}.

Solid Supports

The remaining volume of the bioreactor contained porous, adsorptive, spherical supports (2–3 mm diameter) (Fig. 2) (total dry wt of beads: 1.250 kg) consisting of a polyarimid fibrous matrix and powdered activated carbon (Bio-Sep beads). Inoculation of supports was accomplished in batch by

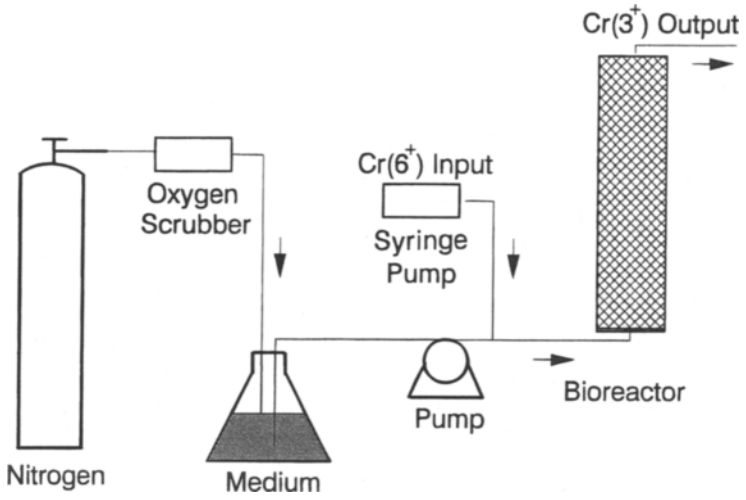

Fig. 1. Schematic of $Cr(6^+)$-reducing bioprocess. Oxygen-free liquid nutrients (TSB) were pumped into a packed-bed bioreactor after contacting a $Cr(6^+)$ stream in an in-line mixer. The process was operated at 30°C with a dilution rate of 2 h^{-1}.

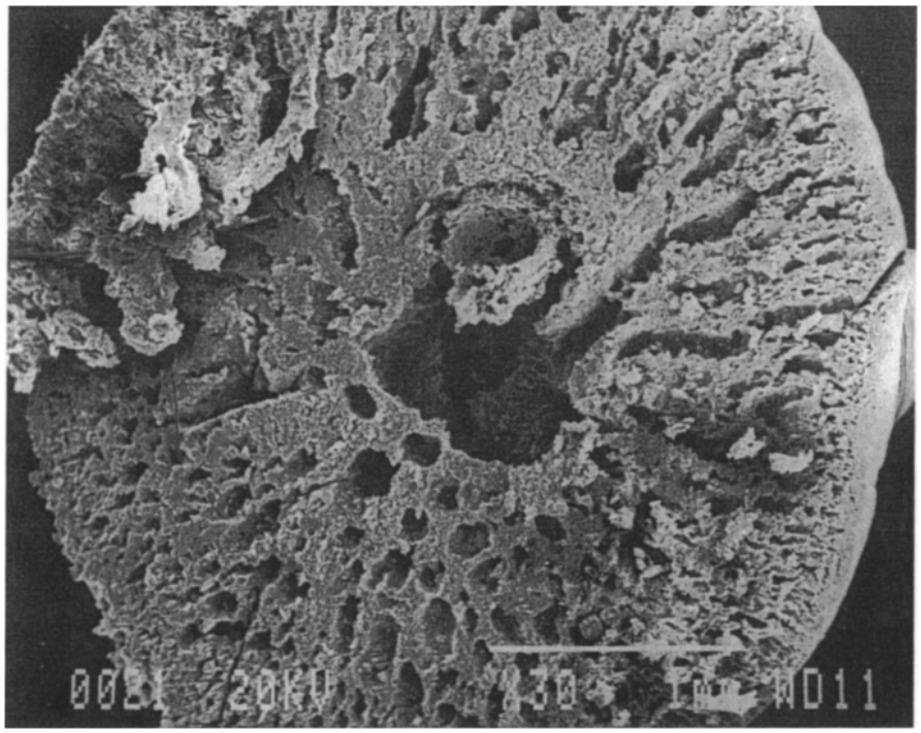

Fig. 2. Cross-section of Bio-Sep bead with scanning electron microscopy (SEM).

contacting the supports with effluent (5 L) from a $Cr(6^+)$-reducing bioreactor (7) containing a mixed culture of $Cr(6^+)$ reducing bacteria and 200–500 mg/L $Cr(6^+)$. Inoculation occurred over 10 d at 20°C with a total of 2175 mg $Cr(6^+)$.

Adsorption

Solid supports were added to 9-mL glass tubes and contacted continuously at 20 and 30°C with distilled water containing 200 mg/L $Cr(6^+)$, at a dilution rate of 0.5 h^{-1}. $Cr(6^+)$ concentrations were analyzed to determine the degree of adsorption. Rate constants for $Cr(6^+)$ adsorption (k) (h^{-1}) and total $Cr(6^+)$ adsorbed (a_1) (mg/g) were calculated using least-squares fit of the data (Statgraphics version 5 STSC, Inc.) to a first-order kinetic model described by the following equation:

$$a = a_1(1 - e^{-kt}) \quad (1)$$

Cr and pH Analysis

Influent samples were taken periodically and analyzed for pH and $Cr(6^+)$. $Cr(6^+)$, total Cr, and pH of the effluent were monitored in the reactor effluent. $Cr(6^+)$ concentrations in the samples were measured by clarifying via centrifugation, diluting the clarified solution 1:100 or 1:1000, adding 0.09 g of ChromaVer 3 Chromium Reagent Powder (Hach Chemical, Loveland, CO), and measuring the absorbance of the mixed solution at 542 nm on a Shimadzu 160U UV VIS spectrophotometer. Total chromium was analyzed using inductively coupled plasma emission spectroscopy (Model 3410, ARL).

Scanning Electron Micrographs

Supports were removed from the bioreactor after operation, thin-sectioned, and gold-sputter-coated. They were photographed during inspection under a scanning electron microscope.

Carbon Source Study

An equal number of inoculated solid supports from the bioreactor were transferred to 10-mL serum vials and incubated anaerobically with equal volumes of TSB or mineral salts medium containing: 10 g/L sucrose; 10 g/L K_2HPO_4; 3.5 g/L $Na(NH_4)HPO_4 \cdot 4H_2O$; and 0.2 g/L $Mg\ SO_4 \cdot 7H_2O$ at 30°C. $Cr(6^+)$ concentrations were measured over time as described above. Results were used to determine the economic utility of using sucrose as an inexpensive sole carbon source in future scale-up studies.

RESULTS AND DISCUSSION

The $Cr(6^+)$ adsorptive capacity of the supports was estimated (Eq. [1]) to be 0.18 and 0.77 mg/g at 20 and 30°C, respectively. Similarly, $Cr(6^+)$ adsorption rates were determined as 0.18 and 0.25 h^{-1}. Based on these

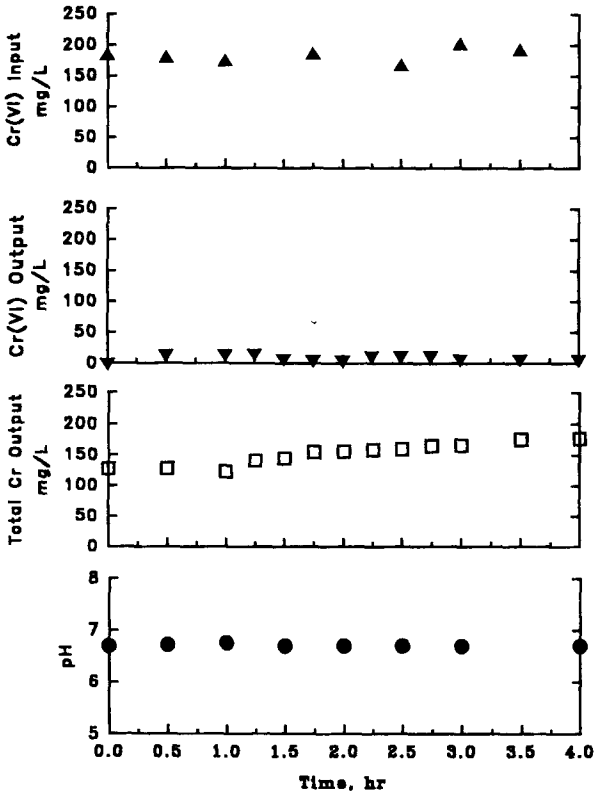

Fig. 3. Results of $Cr(6^+)$-reducing bioreactor study.

values, 90% of the adsorption capacity of the supports was attained within four reactor volumes. These data indicated that $Cr(6^+)$ adsorption onto the beads during experimental operation was not a significant factor, since adsorption occurred during bioreactor inoculation, as described above.

Approximately 95% of the $Cr(6^+)$ entering the bioreactor was reduced to $Cr(3^+)$ (Fig. 3), resulting in a reduction efficiency of 260 mg/L/h at a dilution rate of 2 h^{-1}. Throughout the experiment, the total Cr concentrations in the effluent increased with time and were similar to $Cr(6^+)$ influent concentrations (Fig. 3), indicating that sorption in the reactor was minimal.

Throughout this study, pH values of the bioreactor effluent remained circumneutral (Fig. 3), varying little from the input values of 7.0. Therefore, additional pH treatment of the effluent is not required.

Inoculated Bio-Sep beads demonstrated an increase of $Cr(6^+)$ reduction rates by nearly 65 times relative to previous work *(6)*. Improved volumetric productivity was probably achieved by increasing bacterial density in the bioreactor through immobilization into beads. High bacterial density is evident in the solid supports used in this study and is exhibited in Fig. 4. This improvement in the rate of $Cr(6^+)$ reduction, relative to previous studies *(6)*, can be presumed to be owing to increased bacterial density

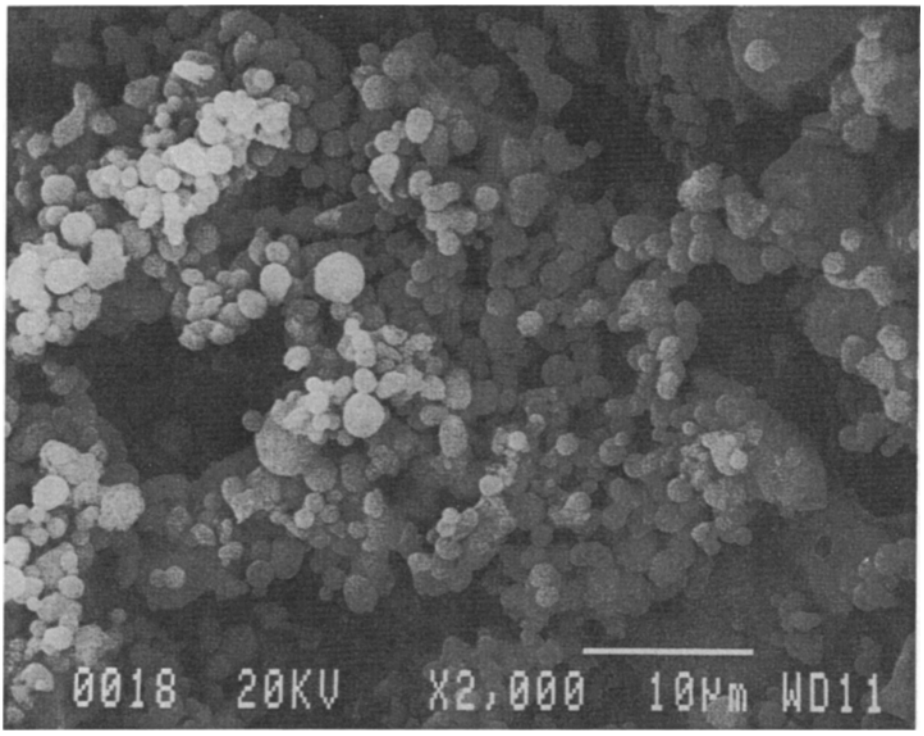

Fig. 4. Cross-section of inoculated Bio-Sep bead with SEM, demonstrating high bacterial density.

obtained during the present work. Analysis of bacterial density in the beads was not performed in this preliminary study owing to the difficulty in obtaining accurate values. The beads were found to interfere with protein analysis, and their robust physical properties did not allow for blending, eliminating the potential of plate counts of immobilized bacteria. The rich nutrient medium reduced the reliability of bacterial activity measurements by analysis of carbon utilization. Future studies are planned to deal with this problem by using a simplified carbon and energy source more amenable to physiological measurements of bacterial activity.

Batch studies amended with sucrose (10 g/L) demonstrated rates of $Cr(6^+)$ reduction similar to batch experiments using TSB (data not shown). These findings support the use of sucrose-rich feedstocks, such as molasses, as suitable, inexpensive sources of carbon and energy for future studies. The above data allow for the calculation of size and operational costs of a scaled-up bioprocess. The resulting estimation, based on 200 mg/L of $Cr(6^+)$ at a flow rate of 37.85 L/min into a bioprocess (10 gal/min), indicates a size range of 2436–2832 L (86–100 cubic feet) with an operating cost range from \$0.20–0.50/3785 L (1000 gal), based on economic evaluations of sucrose-rich feedstocks, such as molasses (*7*). Based on operational costs and size, a $Cr(6^+)$-reducing bioreactor using sucrose as a carbon and

energy source would compare favorably to conventional chemical processes for $Cr(6^+)$ reduction. Advantages of a bioprocess for $Cr(6^+)$ reduction would include low capital and maintenance costs, as well as potential modular design.

ACKNOWLEDGMENT

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