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COMPARISON OF CHROMIUM ADSORPTION TO STARVED AND FRESH SUBSURFACE BACTERIAL CONSORTIUM

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1. Summary

The adsorption of chromium (Cr^{+6}) to a denitrifying consortium was investigated for starved and fresh cells under three pH values (pH 6.0, 7.5 and 9.0). Cells starved 50 days adsorbed approximately 10-15% more Cr^{+6} than fresh (0 day) cells at those three pH conditions.

2. Introduction

Bacterial/fungal biomass can be effective for removing heavy metals, organics, or radionuclides. In a pump-and-treat situation, it may be more cost effective to use biomass for removing heavy metals than conventional treatment systems (Hutchins *et. al.*, 1986). Additionally, subsurface bacterial populations may be play an active role in the establishment of heavy metal equilibria in groundwater. For example, bacterial populations may concentrate chromium and retard its migration by acting as an adsorption or ion exchange medium. Current information regarding the sorptive nature of subsurface bacteria under *in situ* conditions is limited; however, knowledge of system conditions that impact *in situ* bacterial removal of chromium from groundwater is necessary for the development of appropriate remediation techniques.

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There has been considerable interest in the phenomenon of adsorption/desorption of metals to biomass. Previous investigators have used metabolically active cultures or killed cells to study metal adsorption phenomena under different physicochemical conditions. The primary physicochemical/biological parameters of interest have been temperature, pH, metal concentration, competing ions, and type of biomass. Batch and continuous flow reactors or return sludge from conventional wastewater treatment have been used to produce biomass for these studies (Brown and Lester, 1982; Tobin *et al.*, 1988; Tsezos et al.,1986; Tsezos et al., 1989; Tsezos and Bell, 1989).

The cell age of the biomass used in previous equilibria adsorption isotherm studies ranges from freshly grown and harvested cells to cells obtained from activated sludge treatment with average cell age (solids retention time) up to 60 days (Uloth and Mavinic, 1977; Stoveland and Lester, 1980; Brown and Lester, 1982). The results of previous studies using metabolically active cells are applicable to pump and treat technology. However, for *in situ* applications, the use of metabolically active cells may not accurately describe the applicable sorption phenomena. In the subsurface environment, average cell age may approach hundreds of days with generation times approaching one year (Morita, 1988). This extreme oligotrophic environment induces starvation in existing microorganisms, resulting in unique physiological conditions (Beloin *et al.*, 1988; Fredrickson et al. 1989; Morita, 1988). The physiological state and its impact on contamination sorption cannot be accurately represented using cells that are metabolically more active. The relationship between sorption and physiological state was addressed in this work by developing a series of sorption isotherms at three pH values utilizing both fresh and starved cells.

3. Materials and Methods

Two denitrifying strains of Pseudomonas stutzeri (designated as DN2 and DN5) originally isolated from groundwater at the U.S. Department of Energy's Hanford site were used in this investigation (Brouns *et. al.*, 1990). Simulated groundwater (SG) amended with acetate (SGM) was used for cultivating cells. The SG was formulated to approximate the major ion concentration of the Hanford groundwater. The SGM contained the following constituents in deionized water: $Na_2SiO_3\ 9H_2O$, 455 mg/L; Na_2CO_3, 100 mg/L; Na_2SO_4, 6 mg/L; NaF, 44 mg/L; NaOH, 287.5 mg/L; $NaNO_3$, 548.4 mg/L; KCl, 26.3 mg/L; $CaCl_2$

$2H_2O$, 8.1 mg/L; HCl, 399.2 mg/L; $Mg(H_2PO_4)_2$, 125.7 mg/L; and $C_2H_4O_2$ (acetic acid), 381.8 mg/L.

Cells were cultured using SGM in a completely mixed, fill and draw reactor. The temperature of the reactor was controlled at 30°C, and the pH was maintained at 7.5 by addition of sterile acetic acid using an automatic pH controller. Reactor solution was withdrawn on a daily basis at a volume that resulted in a 4 day hydraulic and solids retention time. Harvested cells were washed with sterile SG twice using centrifugation (10,000xg for 10 min). To reduce nutrient carryover, the washed cells were resuspended in sterile SG and rested at room temperature (22±1°C) for 48 hr after purging with sterile N_2 (Kong, 1988). Following the rest period, cells were washed again and placed in a sterile starvation flask. This flask was then purged with sterile N_2 to achieve denitrifying conditions. Starvation was carried out at 18°C (average groundwater temperature at Hanford site) and 100 rpm.

Adsorption isotherms were developed on fresh (0 day) and aged cells (50 day) using a constant cell mass and variable Cr^{+6} ($K_2Cr_2O_7$) concentrations (eight initial Cr^{+6} concentrations). Twentyfive mL of cells (2 g/L as dry weight) and 25 mL of an appropriate Cr^{+6} solution were mixed, and the pH adjusted to either 6.0, 7.5, or 9.0. The adsorption vessels were then shaken at 100 rpm and 18°C. After a 24 hr equilibration period, the cells were separated by membrane filtration (0.45μm, Millipore), and Cr^{+6} concentrations in the aqueous phase and the solid phase (biomass) were analyzed by atomic absorption spectrometry (Varian, model 300). To analyze Cr^{+6} in the solid phase, cells and membrane were dissolved with concentrated nitric acid. Chromium analytical procedures were based on Standard Methods (APHA, 1989)

4. Results and Discussion

The adsorption isotherms indicated that 50 day starved cells adsorbed approximately 10 to 15% more Cr^{+6} than fresh (0 day) cells at pH 7.5 (Fig 1). For other pH values, a similar pattern of differences in adsorption was observed. Adsorption isotherms for starved cells and fresh cells yielded "typical" adsorption isotherm traces. Statistical analysis indicated that both the Freundlich and Langmuir equations yielded acceptable ($r > 0.95$) data descriptions at all three pH values. Chromium sorption was found to be inversely related to solution pH. Although the data in Fig. 2 indicated a

maximum Cr^{+6} uptake at pH 6.0, the isotherm traces for pH 6.0 and pH 7.5 were not significantly different at the 95% level. Statistically significant differences were observed between pH 7.5 and 9.0 and pH 6.0 and 9.0. Similar results were obtained for both fresh and starved cells.

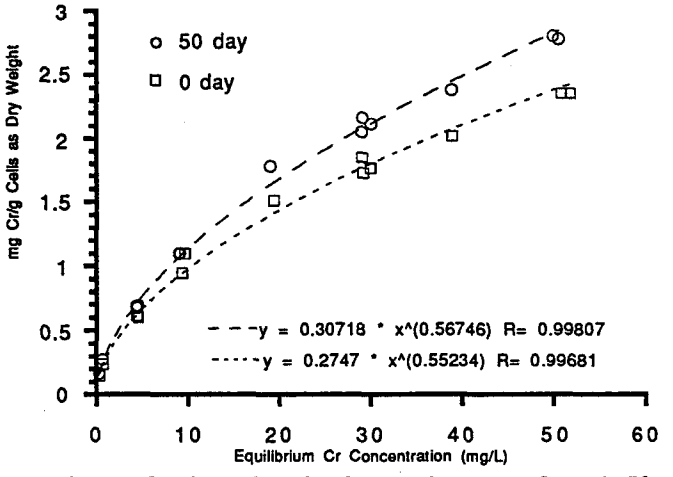

Fig 1. Comparison of adsorption isotherms between 0 and 50 day cells at pH 7.5.

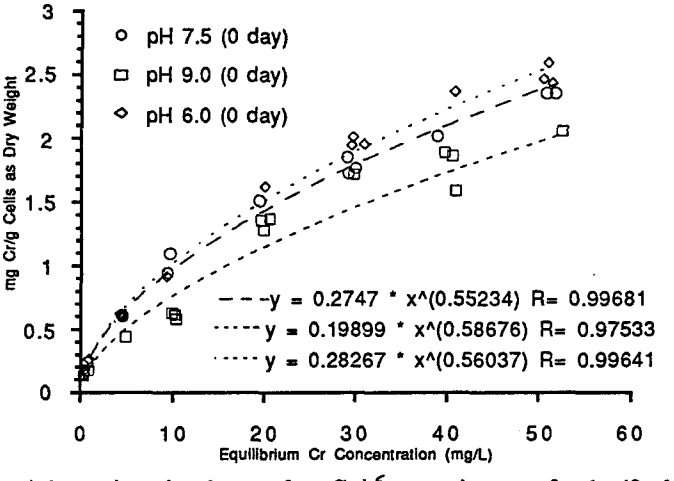

Fig 2. Adsorption isotherm for Cr^{+6} sorption to fresh (0 day) cells at pH 6.0, 7.5, and 9.0.

Previous studies on Cr^{+6} adsorption with killed cells (Zoogloea ramigera) indicated that maximum Cr^{+6} uptake was observed at pH 2 and 25°C (Kutsal

and Sag, 1989a and 1989b). Under these conditions the amount of Cr^{+6} adsorbed per gram of cells was approximately 28 mg/g, substantially higher than the results from our sorption tests (2.5 mg/g at pH 6.0). This difference may be attributable to species variation, different equilibration conditions (pH and temperature), or the different physiological status of the biomass. In our sorption tests with starved and fresh cells, the different physiological status of the testing biomass is likely to have a significant effect on higher Cr^{+6} sorption with starved cells.

Biomass used for our starved cell adsorption isotherms was viable. Cell viability was monitored with spread plate (APHA, 1989), and over 80% of the initial cell population was culturable after the 50 day starvation period. Therefore, metal sorption mechanisms may extend beyond simple extracellular adsorption. To understand these sorption mechanisms, an analysis of metal distribution in the subcellular components is necessary.

5. Conclusions

Initial results show starved cells adsorb more Cr^{+6} than fresh cells. This may be favorable to *in situ* remediation because starved cells approximate the physiological conditions of subsurface indigenous bacterial populations. Apparently, the adsorption phenomena between fresh and starved cells were governed by more than pH effects and may be linked to Cr^{+6} incorporation within the cells. Work is being performed to elucidate the observed differences between starved and fresh cells by fraction and subcellular component analysis.

6. Acknowledgements

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