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Effects of Slight Variations in Nutrient Loadings on Pore Plugging in Soil Columns

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ABSTRACT

The high nutrient concentrations that would exist near the nutrient injection well during the application of cometabolic *in situ* bioremediation may lead to the development of significant quantities of biomass at this point in the subsurface. This biomass can decrease the porosity of the soil to such an extent that nutrient injection is no longer possible. In this work, experiments were conducted using a porous media biofilm reactor, operated under constant substrate loading conditions, such that the pressure drop across the reactor was allowed to increase to maintain a constant volumetric flow rate through the reactor. Results suggest that biomass production, and hence biofilm thickness, near the injection feed port is highly sensitive to substrate loading. In addition, these variations in biofilm thickness produce dramatic differences in the pressure drop that is attained across the reactor. Use of the Kozeny-Carman equation can be used to predict that once a critical depth has been exceeded, the pressure drop across the bed will increase exponentially within biofilm depth. This result means that pressure is not a reliable indicator of the onset of pore plugging.

Index Entries: *In situ* bioremediation; biofilm; simulation; soil column; nutrient loading.

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INTRODUCTION

During World War II, the Hanford site was selected for production of nuclear materials in support of the United States war effort. As a result of subsequent activities on the site, the ground water has become contaminated by a variety of chemical compounds, including carbon tetrachloride (CCl_4) and nitrate (*1*). *In situ* bioremediation is one technology currently being developed to remediate not only the Hanford site, but also other locations. This technology can make use of indigenous microbial populations to destroy the hazardous compounds contained in the ground water. Previous research has shown that under denitrifying conditions, microbial cultures isolated from the Hanford subsurface will cometabolically degrade CCl_4 with any of several possible electron donors (*2*). The high nutrient concentrations that would exist near the nutrient injection well during the application of cometabolic *in situ* bioremediation may lead to the development of significant quantities of biomass at this point in the subsurface. This biomass can decrease the porosity of the soil to such an extent that nutrient injection is no longer possible. If this reduction in permeability occurs early in the life of the well, the zone of influence can be quite small, significantly increasing the cost of site remediation.

Cunningham et al. (*3*) have addressed the problem of biofouling in porous media. Their experiments, which used a porous media biofilm reactor, showed that a steady-state biofilm can develop and not completely plug the media. However, these tests were performed in a constant pressure system, so that as permeability decreased, volumetric flow rate also decreased. Thus, substrate loading rate decreased throughout the tests. These authors also demonstrated that effects of biofilm accumulation on packed bed permeability can be described by a modified version of the Kozeny-Carman equation. This equation was originally developed to describe the pressure drop during fluid flow through a bed of solid particles without biofilm. The work presented here extends the approach used by Cunningham et al. to a system where the substrate loading rate is held constant and the pressure drop across the porous reactor is allowed to increase to maintain a constant volumetric flow rate. Both experimental and theoretical results demonstrate the extreme sensitivity of pressure drop to biofilm accumulation. The implications of these finding on the field application of *in situ* bioremediation are discussed.

MATERIALS AND METHODS

Bacterial Culture

The bacterial consortium used for these experiments was grown from a soil sample that was aseptically removed from a test well located within

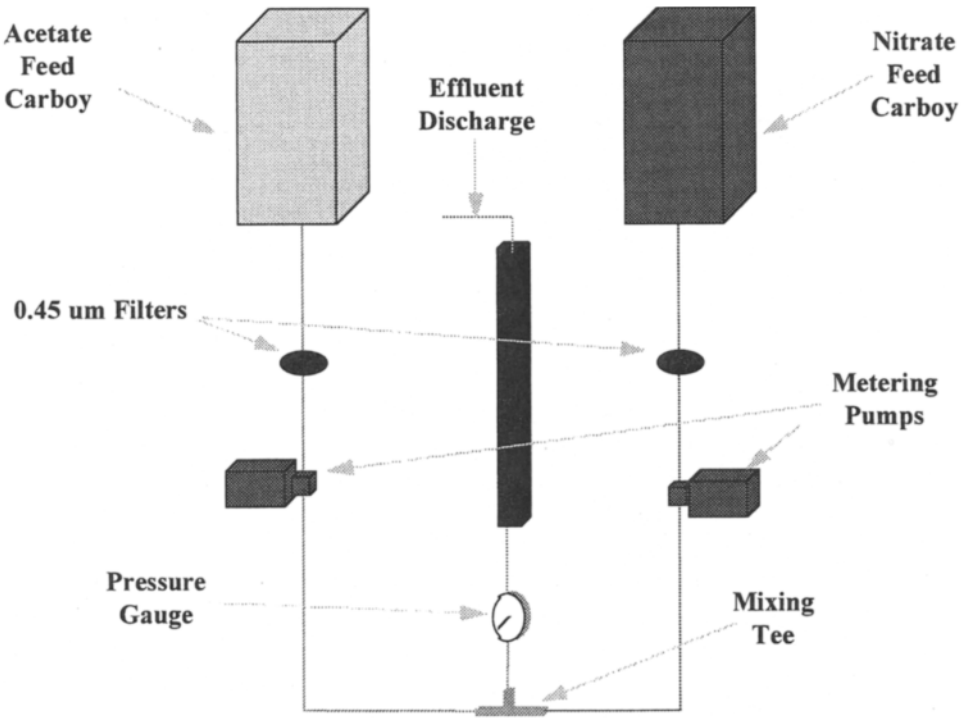

Fig. 1. Schematic representation of the experimental setup.

the CCl_4 plume on the Hanford site (4). Microbial stock solution was prepared using the method reported by Petersen et al. (5). The biomass needed for each experiment was grown by adding 100 μL of the microbial stock to a sterile 250-mL flask containing 200 mL of simulated ground water medium (SGM) amended with 1500 mg/L nitrate and 2000 mg/L acetate. This culture was incubated aerobically at 27°C on a 125-rpm orbital shaker for 3 d. Aerobic growth processes were used to generate biomass quickly for sand column experiments, since it has been shown previously that the microbial species of interest are facultative anaerobes (2).

Sand Column Experiments

All sand column experiments employed 316 stainless-steel HPLC columns (Alltech Associates, Inc., Deerfield, IL) of 50-cm length and 2.25-cm OD, providing a total working volume of 199 mL. Columns were supplied with a flow distributor and a stainless-steel frit at the entrance and exit to minimize channeling.

A schematic diagram of the column flow system is shown in Fig. 1. Acetate and nitrate were fed from separate 10-L carboys using high-pressure precision metering pumps (Model #A-30-S, Eldex Labs, Napa, CA).

Acetate and nitrate discharge tubing was routed from the metering pumps to a mixing tee, through a 0–1000 psi pressure gage, and to the inlet of the packed columns. The tubing diameter and length allowed a residence time of the mixed solutions of <5 s. All of the required connections were made with 0.16-cm pressure fittings.

The columns, tubing, filters, and pressure gages were steam-sterilized before column inoculation. Metering pumps were sterilized by pumping a 75% alcohol solution through the liquid cylinder head for 30 min, followed by a 5-min rinse with sterile deionized water. Silicate sand of 200-μm average particle diameter was prepared for column loading using the procedure outlined by Vandevivere and Kirchman to ensure that no organic compounds were carried over (*6*). The sterile control column was filled with dry sterile sand, sealed with end fittings, and resterilized, whereas the remaining sand was sterilized separately. In all cases, steam sterilization was used to eliminate microbial contamination. To inoculate the columns, 250 mL of culture with approx 10^9 cells/mL were mixed with 600 g of the sterilized sand, and the resulting slurry was aseptically poured into the columns. This resulted in an initial porosity of 0.44 in all columns.

Feed solutions of acetate and nitrate were adjusted to pH 7.0, and sparged with helium gas for approx 30 min prior to use to remove any dissolved oxygen. Feed carboys were then pressurized to 5 psig, and nutrient solutions were introduced to the columns. Each feed pump was set to deliver 1 mL/min of acetate and nitrate stock solutions giving a combined flow rate of 2 mL/min. Effluent flow rates and column temperature and pressure drop were measured daily.

Feed, Effluent, and Core Analysis

Column feed and effluent solutions were sampled, filtered, and analyzed daily using a Dionex 4000i ion chromatograph (Dionex, Sunnyvale, CA), equipped with a conductivity detector and a cation-suppression column. NaOH eluent (100 m*M*) was fed at 1 mL/min to a Dionex AS-11 anion-exchange column. Tests with this column indicate that all reported anion concentrations are accurate to ± 10 mg/L.

During the course of the experiment, the sand column effluent was collected in order to enumerate both the number of colony forming units (CFU) and total biomass. CFU determination consisted of serial dilutions that were subsequently plated out on sterile tryptic soy agar spread plates (*7*). The number of colonies formed on the agar after 2 d was counted, multiplied by a dilution factor, and recorded. Total cell enumeration (cells/mL) was performed by direct counting using an oil-immersion compound microscope and a Petroff-Hausser Chamber (Hausser Sci., Blue Bell, PA) (*6*).

At the termination of each experiment, sand columns were removed from the housings using a fabricated column extruder. Intact sand columns

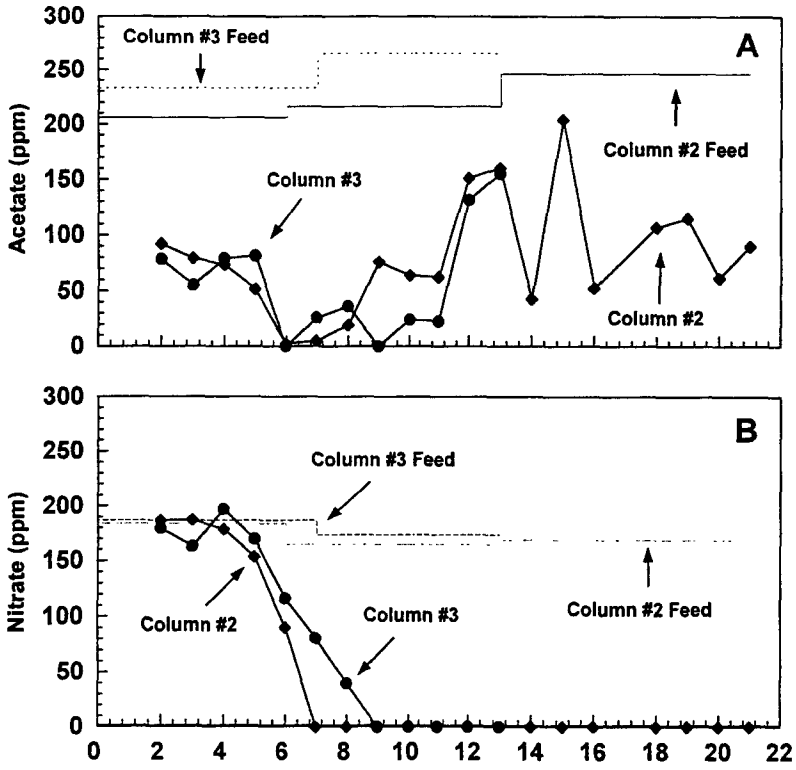

Fig. 2. Acetate and nitrate concentrations in the feed column effluent streams as functions of time.

were then cleaved into axial segments of varying volume, with shorter segments located near the column entrance. Viable and total cell concentration was determined for each column segment by diluting sand samples with 10 mL of 0.1% sodium pyrophosphate (pH 7). The resulting suspension was mixed for 15 min, and enumeration was completed on 1-mL aliquots using the methods described by Gerhardt (*7*). Protein analysis on column segments was completed using Lowry's method (Micro BCA Assay: Pierce Chemical Co., Rockford, IL) (*8*).

RESULTS AND DISCUSSION

Figure 2, on which the nutrient concentrations in both the feed and effluent streams are displayed as functions of time, shows that similar nutrient feed concentrations were used for all the three sand columns. In these columns, the ratio of acetate to nitrate in the feeds ranged from 1.1 to 1.5 mg/mg, resulting in excess acetate. This ratio is consistent with previously reported kinetics that show a range for the acetate to nitrate yield coefficient of approx 0.6–0.8 mg/mg (*9*). Column 1 was maintained as a

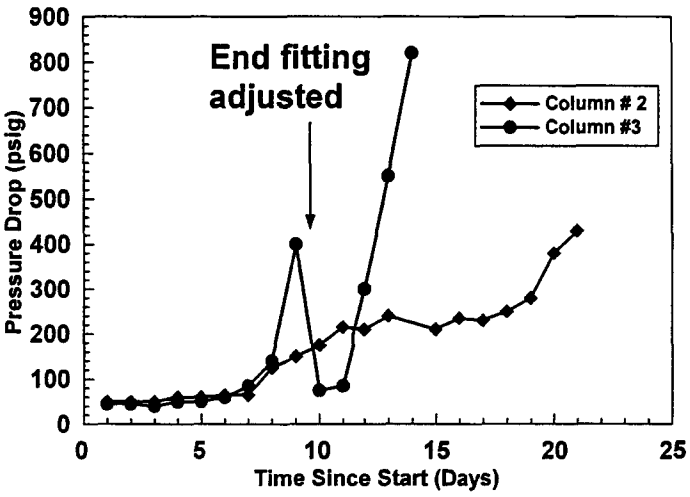

Fig. 3. Pressure drop across the sand columns as a function of time.

sterile control, and no biomass growth was observed. This suggests that changes in the measured properties for the other columns were caused by biological activity.

The column effluent results in Fig. 2 show that the rate of nutrient consumption was similar in columns 2 and 3. (The high acetate concentration in the effluent of column 2 on day 15 was the result of a gas bubble in the nitrate feed stream that temporarily affected the amount of acetate that was consumed in the column.) In contrast to the similar nutrient consumption profiles, the data shown in Fig. 3 indicates that the pressure drops that developed across the columns are not the same. On day 8, the pressure drop across column 3 began to increase rapidly, whereas that in column 2 rose much more slowly. This result is indicative of the presence of large amounts of biomass in column 3, and is typical of the plugging phenomenon that can limit the life of *in situ* bioremediation. In fact, the pressure drop was large enough to cause the column end fitting to slip down the column and to deform the column prepacking frit such that the center of the frit was distended approx 3 mm from horizontal. Because the column end fitting was still connected to the column, however, it was resealed under aseptic conditions, and column 3 was restarted. This experiment was terminated only 3 d later because the pressure drop once again increased dramatically.

The extreme difference in the pressure profiles may be caused by slight variations in the nitrate feed concentration. This is suggested in Fig. 4, which shows the pressure drop predicted for different biofilm thicknesses using the Kozeny-Carman equation as modified by Cunningham et al. (3). Once the biofilm thickness is >25 μm, the pressure drop increases exponentially. To verify that the observed differences in pressure

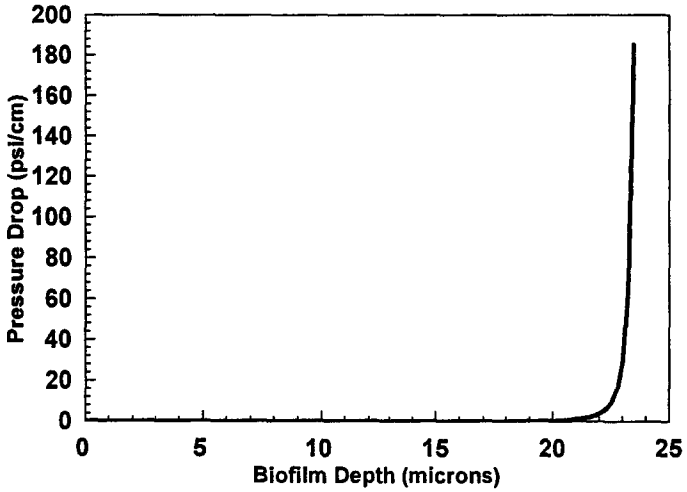

Fig. 4. Predicted pressure drop in a sand column, operated under the conditions used in the experiments reported here, as a function of biofilm depth.

drop are caused by differences in biofilm thickness, the biomass profiles predicted for columns 2 and 3 were calculated using the Kozeny-Carman equation and the measured pressure drops. This evaluation indicates that at day 8, when the pressure rose dramatically in column 3, there was $<1.0\%$ difference between the total biomass in the two columns. Based on biomass-to-nitrate yields demonstrated from the previously reported kinetics (9), this pressure difference could occur if the concentration of nitrate fed to column 3 were 0.3–2 mg/L higher than that fed to column 2. Such a small discrepancy could not be detected using the analytical methods reported here. Further experiments are being conducted to verify these results.

These results will have a dramatic impact on *in situ* bioremediation. The pressure of the injection well is an easily measured operating parameter that might be misused as an indication that nutrient loading should be changed to avoid biofouling. However, these results indicate that pressure is not a reliable indicator for the onset of pore plugging.

CONCLUSIONS

The experimental results presented here suggest that biomass production near the injection feed port is highly sensitive to substrate loading. This fact is compounded by the existence of a critical biofilm depth that, when exceeded, results in a rapid rise in pressure drop. Thus, pressure is not a reliable indicator of the onset of pore plugging.

ACKNOWLEDGMENTS

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