SAND MEDIA TYPE AND CHARGE EFFECTS ON TCE COMETABOLISM IN A FLUIDIZED-BED BIOREACTOR

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ABSTRACT

Fluidized-bed biological reactors (FBBRs) may be used to remove chloroethenes such as trichloroethene (TCE) from groundwater. Proper selection of FBBR aqueous growth medium, biofilm solid support media type and size, and bed charge (bed depth to column length fraction) are critical for establishing a biomass of sufficient quantity and activity to transform TCE via aerobic cometabolism, which requires a supplemental growth substrate. Thus, proper media selection involves facilitating efficient conversion of growth substrate to cometabolic activity for TCE degradation. In this study, five types of sand media (quartz, garnet, ilmenite, hematite, and magnetite) were evaluated in small-scale FBBRs under different conditions of media charge, size, and type. Also, biofilm growth and TCE degradation rates were measured for three aqueous growth media with phenol as the growth substrate: mineral salt waters (MSW), groundwater with excess nutrients (NGW), and amended groundwater with 10 times excess nutrients (AGW). MSW produced the highest level of biomass and highest TCE degradation rates, but the other media were selected for use in the FBBRs due to practical considerations. Differences in biofilm cometabolic properties were related to carbonate versus phosphate buffering of the growth media. Biofilms grown in the small-scale FBBRs on the different sand media were harvested and assayed for their maximum specific TCE degradation rate (k_c) and transformation capacity (T_c). Quartz sand with 30/35 mesh (500 to 594 mm) particle size and 20% column sand charge was identified as a near-optimal media with a k_c of 181 mg-TCE/g-VSS/d and a T_c of 0.23 mg-TCE/g-VSS. Hematite sand was also identified as a media that enhanced cometabolism. When implemented in a continuously operated laboratory pilot-scale FBBR at a flow of 1 L/min and a detention time of 2.4 minutes, TCE removal up to 81% was obtained for an inlet TCE concentration of 123 mg/L using the quartz sand.

Key words: *trichloroethene, fluidized-bed bioreactor, sand media, cometabolism, phenol, nutrients*

INTRODUCTION

Trichloroethene (TCE) has been widely used in the past and its uncontrolled disposal has led to the contamination of many aquifers (USEPA, 1981; Westrick et al., 1984) and soils (Malachowsky et al., 1984). TCE is a suspected human carcinogen (Miller et al., 1983) and is regulated to a level of less than 5 µg/L in drinking water. Over the past decade, many researchers have studied the *in situ* and *ex situ* remediation of TCE-contaminated aquifers. Bioremediation technology has been widely favored because it potentially cost less than other technologies (Smith and McCarty, 1997; Johnston et al., 1996; and Winkler et al., 1995).

The fluidized-bed biological reactor (FBBR) is an *ex situ* bioremediation technology that has drawn interest as a step towards implementing the TCE cometabolism process in the field. A key design aspect for FBBR optimization is the selection of fluidized-bed granular material because of its influence on bioparticle hydrodynamics and microorganism density; thus, media selection strongly affects treatment performance (Karapinar and Fikret, 1996). Various types of mineral sands and carbon can be used as the biofilm attachment media, resulting in the formation of bioparticles with different hydrodynamic properties and catalytic attributes. Some researchers have used gel beads

as the attachment media in an FBBR to treat TCE-contaminated waters and obtained 80 to 90 % removal with influent of TCE at 0.9 to 1.6 mg/L after a residence time of 2.6 hours (Shimomura et al., 1997). Actually, several bed-related factors potentially affect TCE cometabolic activity in FBBRs including media type, settled-bed depth, media diameter, porosity, angularity, specific gravity, composition, and charge (the ratio of clean bed volume to reactor volume). The media affects the thickness and density of biofilm that develops on its surface and may be a population selection factor. Biofilm thickness can impact the biomass activity by limiting substrate availability within the biofilm causing the accumulation of inert material, which is usually inactive biomass. For a particular application, it is desirable to understand the effect of these factors so that better operating conditions can be achieved without excessive trial and error. It is very costly to conduct pilot-scale studies for every type of media condition or to replace poorly performing media.

To study media factors, bioparticles were grown with various types of sand media in small-scale FBBRs and their properties were evaluated. Phenol was chosen as the growth substrate because it supports the growth of biofilm-forming microorganisms that cometabolize TCE (Segar et al., 1995). Furthermore, a laboratory pilot-scale FBBR was operated at a media condition identified as potentially optimal for a typical inlet condition (Foeller, 1998).

EXPERIMENTAL CONDITIONS

Chemicals

Loose crystal phenol with a purity of > 99 %, TCE with a purity of 99.9 %, and chloroform with a purity of 99.9 % were used. HPLC-grade hexane was used as the liquid-liquid extractant. The inorganic compounds used in growth-medium preparation were all reagent grade.

Filter sands were donated by commercial media suppliers. The approximate chemical compositions of five filter sand types (quartz, garnet, ilmenite, hematite, and magnetite) used in the column studies are listed in Table 1. These compositions were reported by the media suppliers and corroborated with Robert et al. (1990). The media were washed and graded by sieving into size classes with ASTM US 20 (840.7 mm), 25 (706 mm), 30 (594 mm), 35 (500 mm), 40 (419 mm), and 50 (297 mm) meshes. Densities of sand media were measured by the water displacement technique.

Analytical Methods

Aqueous TCE concentration was analyzed by gas chromatography (GC) preceded by liquid-liquid extraction. Extractant was prepared by adding 0.5 mg/L chloroform to hexane as an internal standard to improve measurement accuracy (Richard and Junk, 1977). Extracts were analyzed on a Perkin Elmer 8500 gas chromatograph equipped with a DB-624 30-m ´ 0.53-mm capillary column (J&W Scientific) and an electron capture detector (ECD). The carrier gas was > 99.997 % pure helium at 15 mL/min and the auxiliary gas was > 99.998 % pure nitrogen at 45 mL/min. The operating temperatures were injector, 200 °C; detector, 300 °C; oven, isothermal at 40°C for two minutes, then increased at 10 °C/min to 65 °C.

Phenol concentration was measured to monitor growth substrate utilization using an ultraviolet spectrophotometer set at 270 nm. Each sample was centrifuged at 2,000 g for five minutes before measurement.

Total suspended solids (TSS) and volatile suspended solids (VSS) were determined with glass-fiber-filter weight differences according to Sections 2540-D and 2540-E of APHA Standard Methods (1994). Each biomass sample was analyzed in triplicate.

Microorganisms

A mixed-culture of aerobic phenol-utilizing microorganisms was enriched from activated sludge collected at the Columbia, Missouri, Municipal Wastewater Treatment Plant. The enriched culture was fed 100 mg/L phenol and fresh mineral salts water (MSW) every three days under aerated conditions in sequencing (draw/fill) batch reactors.

Growth Medium

Three types of aqueous growth media were prepared from groundwater (GW) and deionized distilled water (DDW) in this study: mineral salts water (MSW), groundwater with nutrients (NGW), and excess-nutrients amended groundwater (AGW) with 10 times higher level of nutrients than NGW. AGW was used only in the small-scale FBBR growth stages to supply nutrients under the recirculation condition. Table 2 gives the composition of the various growth media.

Apparatus

Small-Scale FBBRs

Five small-scale FBBRs made of glass tubing, 50 cm length and 1 cm diameter, were mounted vertically in a rack as shown in Figure 1. The working volume of each column was 39 mL. A threaded reservoir at the outlet accepted an accessory funnel that was useful for filling and retaining the media. A variable speed, multichannel peristaltic pumping system was used to recirculate column effluent. Interconnections among the reactors, the pump, and the feeding lines were 1/8" tubing. The first 1.0 to 1.5 cm of bed at the bottom of each column was filled with 2-mm diameter stainless steel beads as media support. Sand media of the desired volume, size, and type were added according to the matrix presented in Table 3. Feedwater entered the columns from the bottom in an up-flow mode.

Laboratory Pilot-Scale FBBR

A single-stage FBBR was constructed as shown in Figure 2 with three distinct sections: the inlet, the growth region, and the TCE cometabolism region. The total volume was 2.4 L and the FBBR flow rate was 1.0 L/min to yield an empty bed contact time of 2.4 minutes. The FBBR was operated at a constant flow rate yielding 20% initial bed expansion. The inlet concentrations of TCE and phenol were about 100 mg/L and 10 mg/L, respectively. NGW was used as aqueous growth medium in this reactor. Detailed design and operational information is found in Foeller and

Segar (1997) and Foeller (1998).

Growth in Batch Experiments

The difference in biomass activity attributed to MSW and NGW media was evaluated in batch experiments. Two liters of MSW and NGW were prepared separately in 4-L flasks and inoculated with 100 mL of effluent taken from the laboratory pilot-scale FBBR. The flasks were mixed continuously with magnetic stirrers and aerated with an air pump. One-half of the supernatant was discarded after every 12 hours of growth. The flasks were refilled with fresh MSW or NGW and 100 mg/L phenol. After four feeding cycles, the cultures were centrifuged at 2,000 g for five minutes to obtain the biomass used in the TCE degradation assay.

Growth in Small-Scale FBBRs

Flow rates to the sand beds were initially set to provide 20% clean bed expansion. Inoculation was achieved by adding 100 mL supernatant taken from the enriched phenol-utilizing batch culture and 2 L of NGW with 20 mg/L phenol in a 2-L beaker**.** The inoculation water was replaced every day for three days; then the FBBR feed was switched to five-gallon plastic containers of AGW containing 100 mg/L phenol. The effluent was collected in the container and recirculated. Reaeration was obtained by air diffusers and an aquarium pump. Before being used and before each growth medium replacement, the containers were sterilized with 100 mL of 5.25 % sodium hypochlorite (regular bleach) followed by several rinses of DDW. Thus, most of the suspended biomass released from the columns was removed from the system and wall growth did not occur in the feed containers.

The flow rate and the bed height in the reactors were measured daily and flow rate was held constant at the initial values. Complete bed growth required 10 to 15 days after inoculation. To retain sand media in the columns as bioparticles enlarged, biofilm in rapidly growing columns was sheared from the media by agitating the beds with a steel wire. When all beds reached a steady level, in some cases the top of the column, the bioparticles were removed from columns and collected in beakers for analysis. After the biomass was dislodged by shaking, the volumes of the clean sand and the settled biomass were measured in a graduated cylinder.

Three separate experiments were conducted to compare different media charges, diameter, and types of sand media as listed in Table 3. Each growth experiment was completed by harvesting biomass from each column and conducting assays.

TCE Degradation Assay

To avoid competitive effects, phenol was not present in the endogenous TCE cometabolic activity assay. Immediately upon harvesting, settled biofilm was dispersed to flasks containing 200 to 250 mL MSW or NGW aerated by pure oxygen. TSS and VSS were measured. 30 mL biofilm suspension aliquots were transferred into a series of 43-mL vials, then filled with MSW or

NGW. Saturated aqueous TCE stock (~ 1100 mg/L) was added by syringe to each vial, which was sealed headspace-free with a teflon septum and incubated on a shaker. Incubations were ended by injection of hexane extractant. Three control vials filled with culture media without biomass were always used to obtain the initial TCE concentration, which ranged from 97 to 105 mg/L. Experiments were conducted at a room temperature of 23 ± 1 °C.

TCE Kinetic Model

The substrate component of the Monod equation with a biomass activity term addressing transformation toxicity (Alvarez-Cohen, 1991) was used to fit the experimental data. The best-fit values of maximum specific utilization rate, k_c, and transformation capacity, T_c, in equations 1 and 2 were determined by non-linear regression of experimental data using Microsoft Excel Solver 5.0, assuming a half-saturation constant, K_c, of 0.5 mg/L (Segar, 1994). A fourth-order Runge-Kutta technique was used to integrate equation 1.

$$\frac{dC}{dt} = -\frac{k_c * X_a * C}{K_c + C} \qquad \text{Equation 1}$$

$$X_a = X_{oa} - \frac{C_o - C}{T_c} \qquad \text{Equation 2}$$

where, C = TCE concentration at time t, mg/L;
C_o = initial TCE concentration, mg/L;
K_c = TCE half-saturation constant of TCE, mg/L;
k_c = maximum specific TCE degradation rate, mg-TCE/g-VSS/d;
T_c = transformation capacity, mg-TCE/g-VSS;
X_a = active biomass concentration, g-VSS/L; and,
X_{oa} = initial biomass concentration, g-VSS/L.

Transformation capacity is the maximum mass of TCE transformed per unit mass of active biomass prior to complete cell inactivation. The initial active biomass concentration was assumed to be the measured VSS of the biomass suspension. The active biomass concentration, which declines over time, accounts for the detrimental effects of TCE cometabolism. Differences in specific biomass activity as a result of growth conditions in the small-scale FBBR were reflected in the values of k_c obtained from curve-fitting of TCE degradation data.

RESULTS AND DISCUSSION

Proper selection of nutrient conditions is a critical component of applied environmental research with microbial treatments. Routinely, nutrient conditions are selected to be "non-limiting," resulting in solutions with high dissolved solids and strong buffering, so that growth substrate kinetics

may be determined and the full capacity of the microorganisms determined under controlled conditions. However, it is unlikely that such growth medium conditions will be practical under field conditions when contaminated groundwater comprises the growth medium. Natural buffering will be based on the carbonate system, rather than the phosphates used in most laboratory culture media. Addition of excess nutrients and dissolved solids to the groundwater may cause a worse water quality problem that the problem being remediated.

The biomass activity and levels associated with growth and assay in MSW and NGW culture media enous energy storage of the microorganisms. Assay media type did not affect the kinetic parameter values and the small differences were attributed to experiment variance, suggesting that either medium could be used in TCE endogenous activity assays. Although MSW appeared to be a better growth medium because it yielded higher degradation rates, it was an impractical medium for operation of the pilot-scale reactor due to the large quantity of nutrients required (~ 1 kg per day). NGW was chosen as the culture medium to test the laboratory pilot-scale FBBR to obtain an acceptable level of nutrient consumption. Amended groundwater (AGW) was formulated to supply the small-scale FBBRs with excess nutrients for a rapid rate of growth. Recirculation reduced the volume required for the growth experiment to 20 L of AGW every three days, as compared to the pilot-scale FBBR daily NGW requirement of 1440 L.

Sand media charges ranging from 5% to 40% were evaluated with 30/35 quartz sand. Table 5 shows that biomass concentration decreased with increasing media charge, ranging from 469 mg-VSS/L at 5% charge to 284 mg-VSS/L at 40% charge in assay dilutions. These measurements translated to "in-column" biomass concentrations of 12,050 and 5,450 mg-VSS/L, respectively. The biomass levels exceeded the 2,000 to 4,000 mg-TSS/L typical of suspended growth systems, an expected finding that is characteristic of the FBBR. Since biomass concentration decreased with the increase of sand charge, some minimal sand charge will provide for a maximal level of biomass in the reactor; presumably, increased sand volume displaces biofilm or increases interparticle abrasion that dislodges the biofilm.

Biomass activity was expected to vary with the media condition, with differences primarily attributed to biofilm thickness and density. However, data in Table 5 shows that the maximum specific TCE degradation rate did not follow a linear trend with regard to media charge or biomass concentration. Whereas the lowest media charge visibly had the largest bioparticles and highest biomass concentration, it also had the lowest TCE degradation rate. This may be attributed to the excessively thick biofilm that limited the diffusion of phenol and oxygen to the interior of bioparticles, resulting in a portion of the biomass becoming inactive. The highest value of k_c was 167 mg-TCE/g-VSS/d at a 20 % media charge. The biomass under this condition was more active than at lower charges due to higher shear forces that controlled bioparticle size and biofilm thickness. The increase in specific activity at 20 % charge more than offset the slightly lower amount of biomass

compared to 5 % charge; thus, the 20 % charge bed had a greater capacity for TCE degradation than other bed charge conditions.

Transformation capacity, T_c, varied in a manner opposite to k_c as shown in Figure 3. A similar inverse relationship between k_c and T_c was observed in subsequent experiments, although the range of T_c values were lower. Generally, the higher the value of k_c, the lower the value of T_c. Obviously, T_c and k_c were either correlated in the mathematical model or linked biochemically.

With a 30 % and 40 % media charge, the beds enlarged very rapidly during growth and daily agitation was required to keeping the media from flushing out. In a large-scale operation, a shear device would be required to maintain the bed condition, resulting in thin biofilms with a young age. Based on these results, a 20 % media charge was selected as the constant reference condition for subsequent experiments and as potentially optimal for scale-up.

In comparison to batch growth conditions in Table 4, AGW yielded TCE degradation rates that were less than those obtained with MSW and much greater than those obtained with NGW. Transformation capacities were similar with AGW and NGW, but AGW generally yielded higher values of T_c than did MSW. The biomass produced in the small-scale FBBR's had maximum specific rates that ranged from 20% to 61% of rates obtained with MSW in batch growth. The lower activity may be due to the accumulation of inert (older age) biomass in the biofilm system.

Media size effects were evaluated at 20 % quartz media charge for sand sizes from 20 to 50 mesh (841 to 297 mm). Flow rates were adjusted for each column to provide an initial (clean bed) expansion of 20 % and held at a constant rate throughout the experiment. It was visually observed that bioparticles grew more rapidly in the columns with smaller media. Hydraulic shearing of biomass decreases with fluid velocity and the required fluidization flow rate decreases with media diameter. The larger diameter quartz bioparticles did not completely fill the column, whereas the smaller diameter bioparticles occupied the entire column volume. As seen in Table 6, the least amount of biomass was accumulated with 30/35 sand and more than 50 % higher biomass levels were obtained with the smallest (40/50) and the largest (20/25) sands.

The kinetic parameters from the media-size experiment are plotted in Figure 4. The maximum TCE utilization rate was 197 mg-TCE/g-VSS/d obtained with 30/35 mesh sand. Other sand sizes yielded significantly lower rates. Transformation capacity did not vary to any significant extent with sand size. The 30/35 sand size was selected for the subsequent mineral type experiment because it yielded the highest rate.

Quartz, garnet, ilmenite, hematite, and magnetite sands were evaluated at 20 % media charge and 30/35 mesh size. All non-quartz sands had densities at least 50 % greater than that of quartz; thus, a given size required higher flow rates for fluidization. Flow rates were adjusted for each column to provide an initial (clean bed) expansion of 20 % and held at a constant rate throughout the experiment. Table 7 presents biomass levels and kinetic parameters. Hematite media yielded

the highest level of biomass and the highest degradation rate. Quartz and ilmenite produced similar results with lower biomass and degradation rates than hematite. Magnetite and garnet also produced similar results, but resulted in the lowest biomass levels and degradation rates of the five media types. The kinetic parameters for biofilm growth on the various media are plotted in Figure 5, which clearly shows the inverse relationship between k_c and T_c. Because media of similar density produced different results, it can be concluded that the mineral chemical composition interacted with the biofilm and was a factor in the FBBR catalytic properties.

Uncertainty existed regarding the significance of the different maximum specific utilization rates and transformation capacities measured in these experiments. For each of the three sets of experiments, a reference condition was identified as 30/35 quartz sand at 20% bed charge. The corresponding TCE-degradation curves from each of the three experiments were analyzed for deviation from their mean and are plotted in Figure 6. The curves were quite similar with initial differences in concentration attributed to different initial levels of TCE dosing (due to variations in stock concentration). Parameter values from kinetic modeling were also analyzed resulting in a mean and standard deviation for k_c of 181 ± 15.1 mg-TCE/g-VSS/d and for T_c of 0.31 ± 0.07 mg-TCE/g-VSS. The relatively small variation in parameter values show that the results are repeatable from one growth experiment to another. The standard deviations represent 8.4 % and 22.6 % of the mean parameter values, respectively; thus, the reported differences in the maximum specific utilization rates, which ranged from 73 to 376 mg-TCE/g-VSS/d, are significant Also, within a set of data from one experiment, differences in kinetic parameters for the various conditions are expected to be significant when the differences in parameters are greater than two standard deviations.

The reference media (quartz with 20% media charge and 30/35 mesh particle size) was used in the laboratory pilot-scale FBBR tests. Table 8 summarizes the operation conditions and measured kinetic parameters for TCE removal in a 48-day test. The detention time was 2.4 minutes and flow rate was 1 L/min. The inlet TCE concentration was about 120 mg/L and the outlet typically ranged between 20 and 30 mg/L. The highest TCE removal was over 80%. Biofilm activity in the reactor was measured by removing some bioparticles and assaying the recovered biofilm suspension. The k_c was determined as 66 mg-TCE/g-VSS/d and T_c was 0.57 mg-TCE/g-VSS. The maximum specific utilization rate was somewhat lower than the rates obtained from the small-scale FBBR tests, which may be attributed to several differences between the pilot and small-scale FBBRs including the use of NGW instead of AGW as growth medium, a greater age of biomass, less efficient hydrodynamics, and continuous exposure of the biomass to TCE. All the aforementioned factors would tend to increase the accumulation of inert biomass in the pilot-scale reactor and reduce the biomass activity.

CONCLUSIONS

The results of this study show that the liquid-growth medium, the attachment-media type,

media particle size, and media charge are important parameters affecting cometabolic TCE removal in FBBRs. The primary effect of liquid-growth medium is on the biofilm catalytic properties and the attachment-media selection affects the hydrodynamic properties and biomass accumulation in the reactor. In relating the attachment-media effects to catalytic properties, the small-scale FBBR study was effective and indispensable for identifying bed conditions to increase FBBR performance.

Among the five types of sand media evaluated in this study, 30/35 mesh quartz at 20% charge was identified as a near-optimal media for TCE degradation in FBBRs. Three separate assays for this media condition yielded a mean maximum specific utilization rate of 181 mg-TCE/g-VSS/d (standard deviation of 15). When tested under simulated field conditions, the laboratory pilot-scale FBBR achieved over 80% TCE removal. Hematite was identified as a media that enhanced the catalytic properties of the biofilm, but has yet to be tested in the pilot-scale reactor.

In activity assays, the TCE concentration versus time data were fitted well by the mathematical model of cometabolism kinetics, but the governing parameters of maximum specific utilization rate and transformation capacity appear to be correlated. Biofilm kinetic properties were affected in various ways by media factors resulting in different biofilm thickness and substrate availability. For all 15 media conditions evaluated in the small-scale FBBR's, k_c ranged from 73 to 218 g-TCE/mg-VSS/d (mean of 131 and standard deviation of 46) and T_c ranged from 0.19 to 0.81 mg-TCE/mg-VSS (mean of 0.35 and standard deviation of 0.14). The variance of k_c was much lower for three separate tests at the reference-media condition, suggesting that differences in k_c obtained for the various media conditions were significant.

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REFERENCES

Alvarez-Cohen, L., and P.L. McCarty, 1991. A Cometabolic Biotransformation Model for Halogenated Aliphatic Compounds Exhibiting Product Toxicity. *Environ. Sci. Technol.*, 25:1381-1387.

American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1992. Standard Methods for the Examination of Water and Wastewater, 18th edition. Washington D.C..

Chen, S-D., and C-Y Chen, 1996. Treatment of High-Strength Nitrate Wastewater by Biological Methods Operational Characteristics Study. *Water Sci. & Technol.* 34:269-276.

EPA Council on Environmental Quality, 1981. Contamination of Groundwater by Toxic Organic Chemicals, U.S. Government Printing Office, Washington, D.C.

Foeller, J.R., 1998. Cometabolism of Trichloroethene (TCE) in a Fluidized-Bed Bioreactor, Master's Thesis, University of Missouri-Columbia.

Foeller, J.R., and R.L. Segar, Jr., 1997. Treatment of Trichloroethene (TCE) with a Fluidized-Bed Bioreactor, Proceedings of the 12th Annual Conference on Hazardous Waste Research, Kansas City, Missouri, USA. 126-139.

Johnston, J.J., R.C. Borden, and M.A. Barlaz, 1996. Anaerobic Biodegradation of Alkylbenzenes and Trichloroethylene in Aquifer Sediment Down-Gradient of a Sanitary Landfill. *J. Contam. Hydrol.* 23: 263-283.

Karapinar, I., and F. Kaigi, 1996. Effect of Particle Number Density on Wastewater Treatment Performance of a Fluidized-Bed Bioreactor. *Enzyme Microbial Technol.*, 19(2): 140-144.

Malachowsky, K.J., T.J. Phelps, A.B. Teboli, D.E. Minnikin, and D.C. White, 1994. Aerobic Mineralization of Trichloroethylene, Vinyl Chloride, and Aromatic Compounds by *Rhodococcus* Species. *Appl. Environ. Microbiol.* 60: 542-548.

Miller, R.E., and F.P. Guengerich, 1983. Metabolism of Trichloroethylene in Isolated Hepatocytes, Microsomes, and Reconstituted Enzyme Systems Containing Cytochrome P-450. *Cancer Res.*, 43: 1145-1152.

Richard, J.J., and G.A. Junk, 1979. Liquid Extraction for the Rapid Determination of Halomethanes in Water. *J. Am. Water Works Assoc.*, 69(1): 62-64.

Robert, W.L., T.J. Campbell, and Q.R. Rapp, Jr., 1990. *Encyclopedia of Minerals*, Second Edition, Van Nostrand Reinhold , New York.

Segar Jr., R.L., S.L. De Wys, and G.E., Speitel Jr., 1995a. Sustained Trichloroethylene Cometabolism by Phenol-Degrading Bacteria in Sequencing Biofilm Reactors. *Water Environ. Res.*, 67: 764-774.

Segar Jr., R.L., 1994. Endogenous Cometabolism of Chlorinated Solvents by Biofilms Grown on Phenol, Ph. D. Dissertation Thesis, University of Texas at Austin.

Shimomura, T.S., F. Uchiyama, and H.O. Yagi, 1997. Biodegradation of Trichloroethylene by *Methylocystis* sp. Strain M Immobilized in Gel Beads in a Fluidized-Bed Bioreactor. *Water Res.*, 31:2383-2386.

Smith, L.H., and P.L. McCarty, 1997. Laboratory Evaluation of a Two-Stage Treatment System for TCE Cometabolism by a Methane-Oxidizing Mixed Culture. *Biotechnol. Bioeng.*, 54: 650-659.

Westrick, J.J., J.W. Mello, and R.F Thomas, 1984. The Groundwater Supply Survey. *J. Am. Water Works Assoc., 76*: 52-59.

Winkler, J., K.N. Timmis, and R.A. Snyder, 1996. Tracking the Response of *Burkholderia cepacia* G4 5223-PR1 in Aquifer Microcosms, *Appl. Environ. Microbiol.*, 61: 448-455.

Table 1. Characteristics of mineral sands.

Sand Type	Color	Specific Gravity	Composition
Quartz	Brown/clear	2.6	SiO_2
Garnet	Light violet-brown	4.1	$Ca_3Al_2Si_3O_{12}$
Ilmenite	Grey/black/brown	4.2	$FeTiO_3$
Hematite	Black	4.8	Fe_2O_3
Magnetite	Black	4.5	Fe_3O_4

Table 2. Growth -media composition.

Medium Type Source Water	MSW DDW	NGW GW[a]	AGW GW
		Constituent (mg/L)	
Alkalinity[b]	-	290	290
Na^+	-	50.6	50.6
K^+	-	6.6	6.6
Ca^{2+}	-	60	60
Mg^{2+}	-	29.2	29.2
Sr^{2+}	-	0.9	0.9
PO_4^{3-}	-	0.3	0.3
SO_4^{2-}	-	39	39
BO_3^{3-}	-	0.5	0.5
Cl^-	-	31.4	31.4
H_3PO_4		5.5	55
KNO_3	101	75.5	755
$Na_2HPO_4 \times 7H_2O$	67		
KH_2PO_4	136		
$CaCl_2 \times 2H_2O$	147		
$MgSO_4$	120		
$FeSO_4 \times 7H_2O$	5.6	1.4	14
$ZnSO_4 \times 7H_2O$	0.3	0.3	3
$MnCl_2 \times 4H_2O$	0.2	0.2	2
H_3BO_3	0.6	0.6	6
$CoCl_2 \times 6H_2O$	0.2	0.2	2
$NiCl_2 \times 6H_2O$	0.2	0.2	2
$CuSO_4 \times 5H_2O$	0.3	0.3	3
$(NH_4)_6Mo_7O_{24} \times 4H_2O$	0.2	0.2	2
H_2SO_4[c]	49 mL	49 mL	49 mL
NaOH[c]	as needed	as needed	as needed

[a] Groundwater at the University of Missouri-Columbia on Dec.13, 1995.
[b] mg/L as $CaCO_3$
[c] 6N acid in micronutrient stock solution to prevent precipitation
[d] To obtain pH = 7.2

Table 3. Experimental matrix for small-scale FBBR study.

Experiment	Media Parameter	1	2	3	4	5
#1 Charge Effect	Type	Quartz	Quartz	Quartz	Quartz	Quartz
	Size (mesh)	30/35	30/35	30/35	30/35	30/35
	Charge[a] (%)	5	10	20	30	40
	Volume (cm^3)	1.9	3.8	7.6	11.4	15.1
#2 Size Effect	Type	Quartz	Quartz	Quartz	Quartz	Quartz
	Size (mesh)	20/25	25/30	30/35	35/40	40/50
	Charge (%)	20	20	20	20	20
	Volume (cm^3)	7.6	7.6	7.6	7.6	7.6
#3 Type Effect	Type	Quartz	Garnet	Ilmenite	Hematite	Magnetite
	Size (mesh)	30/35	30/35	30/35	30/35	30/35
	Charge (%)	20	20	20	20	20
	Volume (cm^3)	7.6	7.6	7.6	7.6	7.6

[a] Settled sand bed depth as a fraction of length (48 cm).

Table 4. Biomass levels and kinetic parameters from growth medium studies.

Growth Medium	Assay Medium	X^o mg-VSS/L	k_c[a] mg-TCE/g-VSS/d	T_c mg-TCE/g-VSS
NGW	NGW	177	26	0.36
NGW	MSW	177	12	0.46
MSW	NGW	474	376	0.22
MSW	MSW	474	341	0.23

[a] assuming K_c = 0.5 mg-TCE/L in the curve fitting

Table 5. Biomass levels and kinetic parameters for various sand media charges.

Charge %	Media Depth cm	SBV[a] cm^3	X_o mg-VSS/L	k_c mg-TCE/g-VSS/d	T_c mg-TCE/g-VSS
5	2.4	14.5	469	73	0.40
10	4.8	14.5	413	100	0.34
20	9.6	14.5	356	167	0.31
30	14.4	16.0	376	96	0.43
40	19.2	19.5	284	85	0.81

[a] Settled Bioparticle Volume

Table 6. Biomass levels and kinetic parameters for various sand media sizes.

Sand Size Mesh	SBV cm^3	X_o mg-VSS/L	k_c mg-TCE/g-VSS/d	T_c mg-TCE/g-VSS
20/25	15.5	736	130	0.26
25/30	14.9	560	83	0.35
30/35	11.0	390	197	0.37
35/40	16.4	549	125	0.31
40/50	15.9	633	95	0.32

Table 7. Biomass levels and kinetic parameters for various mineral sand types.

Sand Type	SBV cm^3	X_o mg-VSS/L	k_c mg-TCE/g-VSS/d	T_c mg-TCE/g-VSS
Hematite	16.5	622	218	0.19
Quartz	15.3	463	179	0.23
Ilmenite	16.2	471	178	0.24
Magnetite	14.2	307	130	0.32
Garnet	16.8	411	103	0.30

Table 8. Operating conditions and kinetic parameters for biofilm obtained from the laboratory pilot-scale FBBR.

Parameter	Value	Units
Quartz Media		
Size	30/35	mesh
Charge	20	%
Flow Rate	1.0	L/min
EBCT	2.4	min
Oxygen Concentration		
Inlet of FBBR	25	mg/L
Outlet of FBBR	5 - 10	mg/L
Inlet Phenol Concentration	10.5	mg/L
Inlet TCE Concentration	123	µg/L
Outlet TCE Concentration	24-30	µg/L
Maximum TCE Removal	81	%
Operation Period	48	days
k_c	66	mg-TCE/g-VSS/d
T_c	0.57	mg-TCE/g-VSS

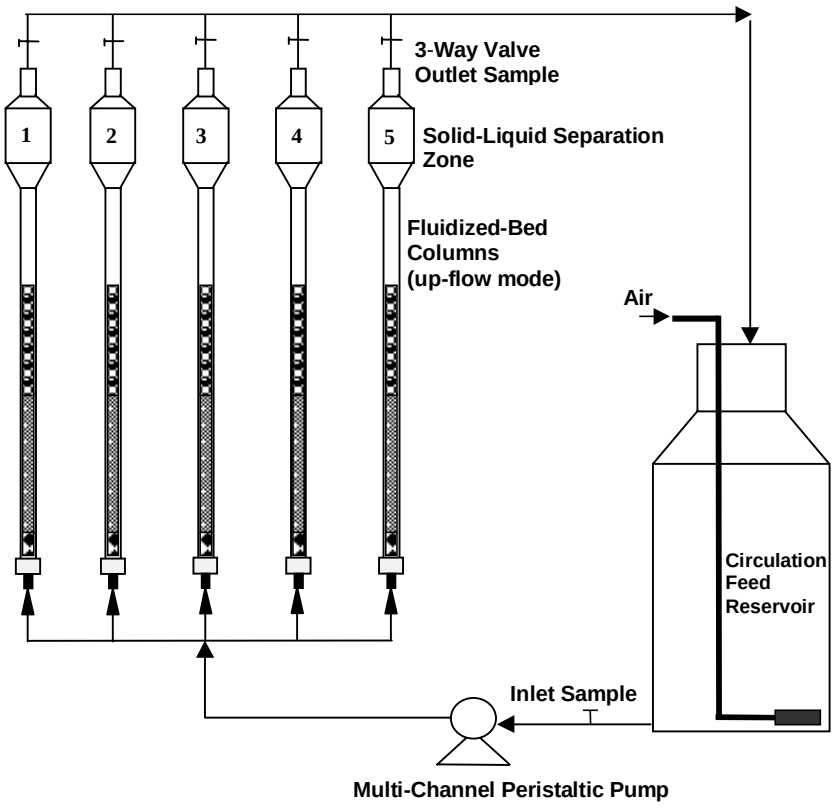

Figure 1. Schematic of the laboratory small-scale FBBRs.

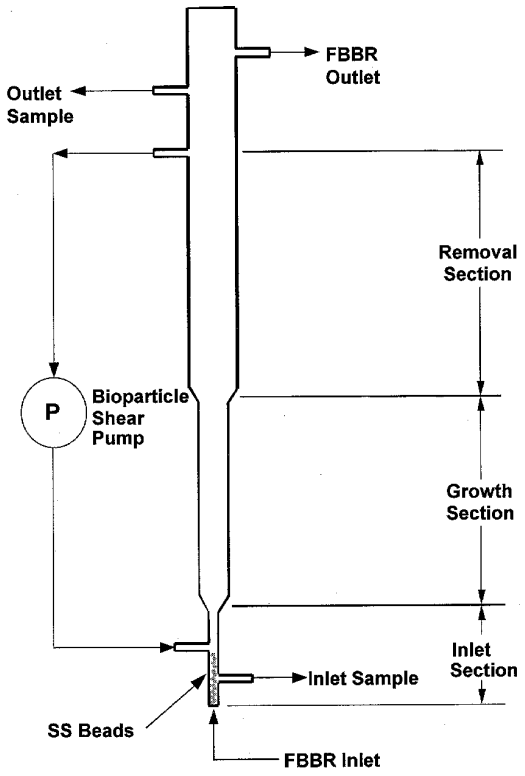

Figure 2. Schematic of the laboratory pilot-scale FBBRs.

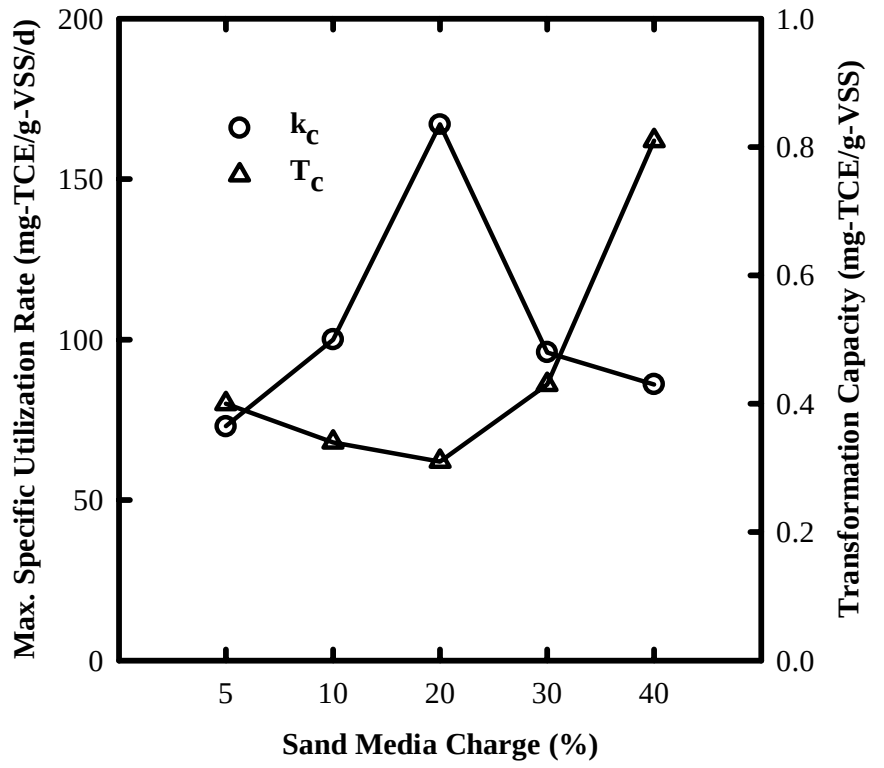

Figure 3. Effect of media charge on kinetic parameters T_c and k_c.

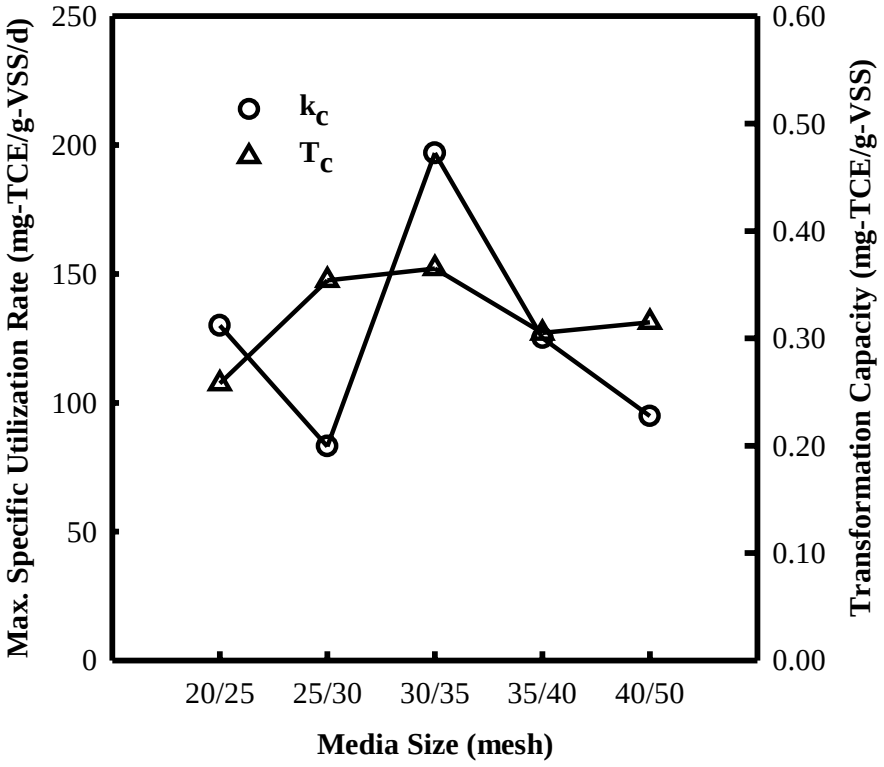

Figure 4. Effect of sand size on kinetic parameters T_c and k_c.

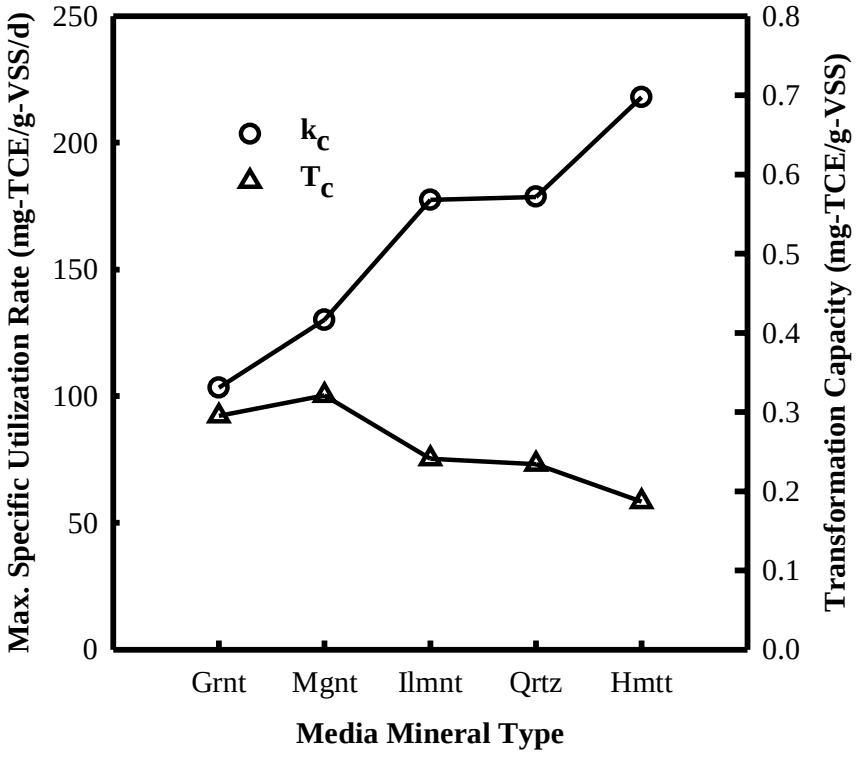

Figure 5. Effect of Mineral Type on Kinetic Parameters T_c and k_c.

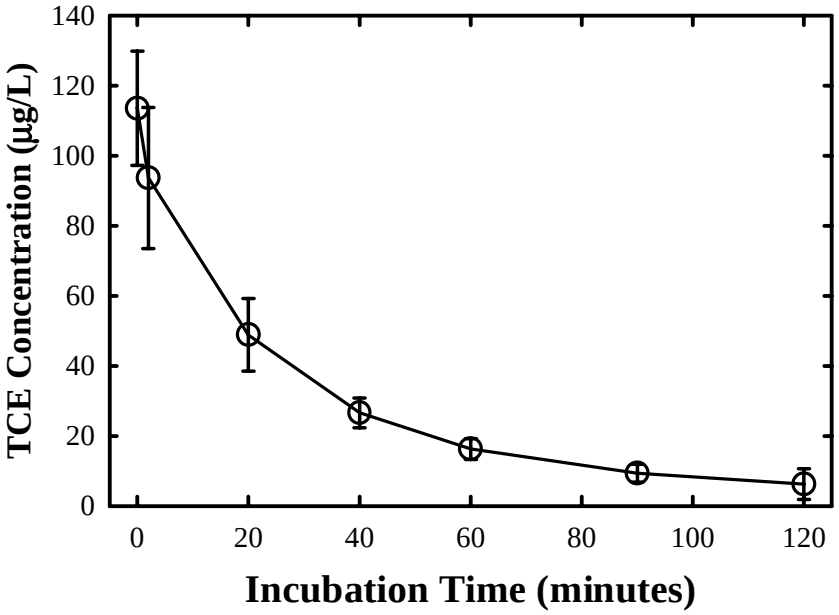

Figure 6. Composite of three TCE activity assays with identical media condition (30/35 Quartz, 20% Charge). Mean and standard deviation are shown.