TREATMENT OF TRICHLOROETHENE (TCE) WITH A FLUIDIZED-BED BIOREACTOR

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ABSTRACT

Fluidized-bed bioreactors (FBBR's) offer a promising alternative to existing treatment technologies for the treatment of water contaminated with chlorinated solvents. The objective of this research was to test a laboratory-scale FBBR for removal of trichloroethene (TCE) from groundwater and to study the FBBR kinetic behavior so that field-scale treatment systems could be designed. Phenol was selected as the growth substrate for biofilm-forming microorganisms enriched from activated-sludge because phenol induces enzymes capable of cometabolizing TCE and lesser chlorinated ethenes. The biofilm forming microorganisms were identified as *Pseudomonas putida*, a common soil bacterium.

Experiments with a conventional, single-pass FBBR addressed TCE removal as effected by changes in TCE loading, phenol loading, and media type. In this study, TCE removal using quartz filter sand (25/30 mesh) and garnet filter sand (30/35 mesh) as the biofilm attachment media was measured. Removal ranged from 20 to 60% and was not affected by the media type. Also, removal was not affected by inlet TCE concentration over the range of 100 to 500 µg/L provided the phenol loading was decreased with increasing TCE loading. The FBBR was capable of complete phenol removal at an inlet concentration of 20 to 25 mg/L and an empty-bed contact time of 2.7 minutes. However, the empty-bed contact time was insufficient to sustain greater than 40 to 50% removal of TCE in a nutrient-amended groundwater.

Keywords: *fluidized-bed, trichloroethene, phenol, biofilm, groundwater remediation*

INTRODUCTION

Biological treatment techniques for remediation of hazardous organic contaminants are gaining acceptance as an increasing number of effective bioremediation processes have been developed. Trichloroethene (TCE), a common groundwater contaminant, was once considered to be non-biodegradable. However, it has been shown that TCE and other chlorinated organics can be biologically destroyed through cometabolism, which is an enzymatic transformation process that requires a supplemental growth substrate for the microorganisms involved. Simple aromatics (toluene or phenol) and short-chain hydrocarbons (methane, propane, butane) have been used as growth substrates because of their ability to stimulate the production of oxygenase enzymes in indigenous microorganisms (Chang and Alvarez-Cohen, 1995). Non-specific oxygenase enzymes are responsible for cometabolic TCE degradation. Phenol has been used exclusively as a growth substrate in our research with biofilm reactors because it selects

for rapidly growing, biofilm-forming microorganisms that reliably yield an acceptable rate of aerobic TCE transformation (Segar and Speitel, 1995).

Due to their highly efficient performance, fluidized-bed bioreactors (FBBRs) have received increased attention in the treatment of water contaminated with chlorinated solvents. In the FBBR, the biomass grows on an attachment media, such as sand, and the water to be treated flows upward through the bed at velocities sufficient to fluidize the biofilm-covered particles (bioparticles). FBBRs are an attractive alternative to packed-bed biofilm reactors because plugging problems are avoided. Other advantageous features of FBBRs include high surface area for biomass attachment and growth, good contacting of biomass and substrate, and low pressure drop across the bed (Rovatti, et al., 1997).

THEORY

Aerobic cometabolism of TCE requires a growth and energy source that induces oxygen-fixing enzymes (oxygenases). Phenol is a readily-utilized inducer of phenol monooxygenase. The effect of phenol concentration on utilization can be described by Haldane kinetics with an inhibition coefficient in the range of 100 mg/L. Phenol monooxygenase transforms phenol (C_6H_5OH) into catechol ($C_6H_4(OH)_2$, which is rapidly utilized by the organism. Equation 1 shows the unbalanced oxygenation reaction that initiates phenol utilization.

$$C_6H_5OH + O_2 + NADH \xrightarrow{\text{Monooxygenase}} C_6H_4(OH)_2 + NAD^+ \quad (1)$$

NADH is an internal electron (energy) carrier. The non-specific monooxygenase can also epoxidize TCE (C_2HCl_3) to form an unstable epoxide (C_2HOCl_3), as seen in Equation 2.

$$C_2HCl_3 + O_2 + NADH \xrightarrow{\text{Monooxygenase}} C_2HOCl_3 + NAD^+ \quad (2)$$

The primary difference between reactions subsequent to Equations 1 and 2 is that TCE epoxide non-enzymatically decomposes and does not serve to regenerate NADH; thus, the TCE cometabolism reaction cannot be sustained without a co-substrate.

When TCE and phenol are both present, competitive inhibition occurs between substrates because the monooxygenase affinity for phenol is greater than for TCE. In a FBBR with near plug-flow hydrodynamics, competitive inhibition theoretically would lead to phenol utilization in the lower part of the reactor and inhibition of TCE cometabolism until phenol concentration is sufficiently low. This phenomenon is illustrated in Figure 1 based on inlet TCE and phenol concentrations of 0.1 mg/L and 30 mg/L, respectively. The predicted removal of TCE assumes a uniformly active biomass throughout the reactor. However, phenol is absent in the region of TCE degradation and the biomass would presumably be in a state of endogenous decay. Under starvation conditions, the cometabolism rate declines over time as the microbial viability and enzyme levels decline (Segar, et al., 1992).

Microorganisms have a limited capacity for cometabolic transformation in the absence of growth substrate due to energy availability and toxicity (Alvarez-Cohen and McCarty, 1991a). Thus, regeneration of biomass activity in the region of cometabolism is essential to sustain the degradation process. To overcome the difficulties imposed by enzyme competition and exhaustion of transformation capacity, sequencing techniques using either periodic feeding strategies (Speitel and Leonard, 1992; Segar, et al., 1994) or reactor staging (Alvarez-Cohen and McCarty, 1991b) have been developed. When sequencing is used, microbial growth and cometabolism are separated either temporally or spatially to obtain greater rates of reaction. Sequencing is a well-accepted technique used to enhance the performance of numerous types of biological processes (Zitomer and Speece, 1993) and is becoming commonplace in cometabolism-based treatment processes.

Sequencing of biomass between growth and cometabolism probably occurs to a limited extent in conventional FBBR's due to the internal circulation of bioparticles within the non-uniform flow field of fluidized beds. Such circulation is frequently observed during the backwashing of filter media, where convection cells (boils) form and the media travels up and down in the expanded filter bed (Cleasby, 1990). Experimentation with different types of FBBR media and flow distribution configurations is needed to determine the extent of bioparticle sequencing that occurs and the resulting level of cometabolic TCE degradation obtained.

Spatial separation of TCE cometabolism and phenol utilization may also occur within the biofilms of bioparticles suspended in bulk liquid conditions that would normally inhibit cometabolism (Anderson and McCarty, 1994). This occurs when substrate concentration profiles within the biofilms create regions favorable to cometabolism. It is presently unknown to what extent "intra-biofilm" separation contributes to cometabolic removal in biofilm reactors.

EXPERIMENTAL METHODS

Laboratory-scale FBBR

A laboratory-scale FBBR was constructed to obtain operational data for different designs. The FBBR was constructed of Kimax® glass pressure-piping with three distinct sections as shown in Figure 2. The piping was connected with Teflon®-lined, stainless-steel (SS) clamps. The inlet section was 11.0 in. (27.9 cm) tall by 0.5 in. (1.3 cm) inside diameter (ID) with an inlet port, sample port, and a return port from the bioparticle shear pump. The inlet section was layered with 30 mL of 4.8 mm (3/16 in.) diameter SS beads and 10 mL of 1.6 mm (1/16 in.) diameter SS beads to provide uniform inlet flow distribution and to support the media. The growth section consisted of 1.5 in. (3.8 cm) ID by 29.0 in. (73.7 cm) tall glass piping. The removal section consisted of 2.0 in. (5.1 cm) ID by 48.0 in. (121.9 cm) tall glass piping with a bioparticle shear outlet port located 19.0 in. (48.3 cm) from the FBBR outlet port and an outlet sample port located 7.5 in. (19.1 cm) from the FBBR outlet port.

Media

Two different attachment media were used in this study: 30/35 mesh (726-847 µm diameter) garnet filtration sand and 25/30 mesh (847-1016 µm diameter) quartz filtration sand. The garnet was selected because it is denser than quartz, but due to its smaller diameter, garnet sand had nearly the same hydrodynamic buoyancy as quartz sand. The smaller size of the garnet sand yielded a larger specific surface attachment area than quartz sand.

Feedwater

A nutrient-amended groundwater spiked with TCE and phenol was fed to the reactor system. The groundwater supply was University of Missouri-Columbia tap water pumped from a deep aquifer and treated with chlorine. MU groundwater composition is

shown in Table 1. The tap water was dechlorinated in the laboratory by adding 2 mg/L of sodium thiosulfate and passing through a granular activated carbon (GAC) bed. Then the following inorganic compounds were added per liter: KNO_3, 75.5 mg; H_3PO_4, 5.5 mg; $FeSO_4 \cdot 7H_2O$, 1.4 mg; $ZnSO_4 \cdot 7H_2O$, 0.3 mg; $MnCl_2 \cdot 4H_2O$, 0.2 mg; H_3BO_3, 0.1 mg; $CoCl_2 \cdot 6H_2O$, 0.2 mg; $NiCl_2 \cdot 6H_2O$, 0.2 mg; $CuSO_4 \cdot 5H_2O$, 0.3 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.2 mg; H_2SO_4, 49 mg. The resulting pH was between 7.0 and 8.0 for the amended groundwater. TCE and phenol solutions were added after aeration and prior to the FBBR inlet resulting in concentrations of 0.1 mg/L for TCE and between 5 to 25 mg/L for phenol as needed to optimize TCE removal. Experiments were conducted at a room and water temperature of 22 to 26°C.

Aeration

The two-stage reactor system consisted of the FBBR and a downflow bubble-contacting aerator (DBCA) as shown in Figure 3. The separate stage aerator was selected for several reasons including the avoidance of TCE stripping within the FBBR, increased oxygen transfer efficiency, and elimination of biofouling problems encountered with fine pore diffusers. The DBCA uses a circulation pump, inlet oxygen injection, and a series of orifice plates to generate small bubbles. A bubble swarm forms in the neck of the DBCA and the water is oxygenated as it flows downward through the swarm of dissolving micro-bubbles. An increase in dissolved oxygen (DO) from near ambient saturation to about 35 mg/L can be obtained with a 3.2 minute water contact time.

Microorganisms

To initiate bioparticle growth, the FBBR was seeded with a mixed-culture of phenol-utilizing microorganisms enriched from activated sludge collected at the Columbia, Missouri municipal wastewater treatment plant. The initial phenol concentration in the FBBR was 100 mg/L and low velocity recirculation (closed-system) expanded the media bed during colonization. When visible growth was observed on the media, the phenol and feedwater pumps were started and the reactor system was switched to overflow (open-system) operation. The flow rate for open-system operation was 1.0 L/min with an empty bed contact time (EBCT) of 2.4 minutes.

The phenol feeding rate was increased as bioparticle growth occurred to maintain an effluent phenol condition of at least 5 mg/L. The aerator was adjusted to obtain an

FBBR outlet DO of 5 to 10 mg/L. Phenol and oxygen levels were monitored at the FBBR inlet and outlet throughout the experiments. After the bed level reached the maximum allowed height, the shear pump was operated at a rate of 100 to 200 mL/min to maintain the bed level and bioparticle condition. Dislodged biomass was continuously flushed out of the FBBR by entrainment in the discharge.

Sampling and Analytical Techniques

Reactor water samples were withdrawn from two side-port locations: the FBBR inlet and above the top of the bed. Samples for bed phenol and TCE profiles were siphoned with a screened tubing from different bed levels and collected in a manner to prevent TCE volatilization losses. Phenol, TCE, and DO were analyzed to determine the removal of each component across the reactor and their input loading. Daily analyses were crucial to maintaining stable (constant concentration) reactor operations.

Reactor water samples were immediately acidified with 1 μL/mL of 6N H_2SO_4. The DO of samples was determined with a YSI 540 meter and probe. Phenol samples were filtered through a 0.3 μm glass fiber filter and the filtrate was analyzed with a Milton Roy Spectronic 601 UV detector at a wavelength of 270 nm.

FBBR water samples analyzed for TCE were sealed immediately after collection in headspace-free vials with Teflon®-faced silicone septa. Acidification occurred immediately after sealing. Extraction of TCE was obtained with hexane containing chloroform as an internal standard. The extracts were analyzed by manually injecting splitless 2 μL into a Perkin Elmer 8500 gas chromatograph (GC) equipped with a DB-624, 30-m x 0.53-mm capillary column and an electron capture detector. Helium at 15 mL/min was the carrier gas with nitrogen as the make-up gas. The GC was operated at the following temperatures: injector, 200°C; detector, 300°C; oven, isothermal at 40°C for 2 minutes, then increased at 10°C/min to 65°C.

Bioparticle samples were siphoned from the fluidized bed and collected in vials. Then, the vial and contents were agitated to remove all biomass from the media. Total (TSS) and volatile (VSS) suspended solids measurements were made according to APHA-2450 D&E. The measurements were used to estimate the biomass concentration in the FBBR.

Water samples were collected from the effluent of the mature bed to determine the types of microorganisms present in the FBBR. Effluent was directed into a Whirl-Pak bag; the bag was sealed and shipped overnight on ice to MiL Laboratories in St. Louis, Missouri. Upon receipt, the sample was diluted and plated onto R2A agar. Colonies were counted at 24 and 48 hours. Then a typical colony was identified by the Biolog® system and by GC-FAME (Fatty Acid Methyl Esterification).

RESULTS AND DISCUSSION

Identification of Microorganisms

Based on the homology of the plated colonies, it was concluded that the biofilm in the FBBR was practically a monoculture. Bacterial numbers were about 10^7 org/mL in the FBBR discharge. Both microbial identification techniques were in agreement that *Pseudomonas putida* was the dominant microorganism. Previous studies with different innoculum sources also resulted in positive identification of *P. putida* in the acclimated phenol-utilizing culture (Segar and Speitel, 1995). *P. putida* is frequently studied by environmental microbiologists and there is no indication that it harbors the potential to cause adverse health effects. Thus, reinjection of the FBBR effluent could be used to promote *in situ* bioremediation coupled with FBBR treatment of extracted groundwater.

Media Type Comparison

The phenol-utilizing bacteria rapidly colonized the FBBR sand beds over a period of two weeks. After the media became conditioned, a high rate of phenol uptake and biological bed growth occurred. The growth period for the garnet was a few days longer than for quartz, but once the biofilm was established, bioparticle size and appearance were similar for the two media. A lower biomass density was obtained for garnet (9 g-VSS/L) than for quartz (13 to 21 g-VSS/L). However, the garnet-supported biomass had a higher phenol uptake (20 to 25 mg/L versus 10 to 20 mg/L for sand), which indicated a more active biomass was present in the garnet bioparticles.

Since TCE degradation is somewhat dependent the amount of active biomass in the FBBR, more TCE removal was expected when garnet was used as the media than when quartz was used. However, TCE removals for both media were similar as seen in Figure 4. The TCE removal averaged between 30 and 40% with a maximum of 60% at

an inlet TCE concentration of 100 to 200 μg/L. The TCE removal in these experiments with nutrient-amended groundwater was low in comparison to the 70 to 80% obtained in previous studies with phosphate-buffered mineral salts water (Segar, et al., 1997). Preliminary indications are that the cometabolizing activity of biomass grown in the groundwater is less than that grown in synthetic medium. This result would suggest that pilot studies using the water to be treated should be conducted before a large-scale implementation is undertaken.

Results of in-bed phenol sampling are shown in Figure 5. For this operating condition, the TCE removal was about 20% at an EBCT of 2.7 minutes with phenol loading of 20 mg/min. Phenol was depleted within the bed and phenol removal exceeded 99%. The correspondingly low TCE removal indicates that the FBBR is overloaded with phenol and an insufficient region of TCE cometabolism occurs. Essentially, the contact time for uninhibited TCE degradation is too short to obtain much TCE removal. To increase TCE removal, the phenol profile in the bed must be reduced in its penetration depth. The loadings of 14 mg/min and 7 mg/min yielded 30 to 40% TCE removal (TCE profiles were not obtained). For the 7 mg/min loading, phenol was completely consumed by the mid-point of the reactor, yet TCE removal was not high. This result indicates that for the amended groundwater that a detention time of 2.7 minutes is insufficient to obtain high TCE removal regardless of the phenol loading.

TCE Concentration Effects

TCE removal was determined for two inlet TCE concentration ranges as shown in Figure 6. On the fifth day of the experiment, the TCE concentration was increased from 0.12 to 0.40 mg/L. Initially, removal did not decrease as would be expected; rather, it increased from 40% to 50%. By the next day, the removal had declined to 20% and remained there for several days. Eventually, the removal was increased by decreasing the phenol feed rate. Conclusions from this experiment are that short-term increases in TCE concentration do not change the removal in the FBBR, but sustained changes require phenol adjustment. At higher TCE concentration, a greater transformation yield for phenol is obtained.

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REFERENCES

Alvarez-Cohen, L. and P.L. McCarty. 1991a. Effects of Toxicity, Aeration and Reductant Supply on Trichloroethylene Transformation by a Mixed Methanotrophic Culture. *Applied and Environmental Microbiology.* 57(1): 228-235.

Alvarez-Cohen, L. and P.L. McCarty. 1991b. Two-Stage Dispersed-Growth Treatment of Halogenated Aliphatic Compounds by Cometabolism. *Environmental Science and Technology.* 25(8): 1387-1391.

Anderson, J.E. and P.L. McCarty. 1994. Model for Treatment of Trichloroethylene by Methanotrophic Biofilms. *Journal of Environmental Engineering. ASCE.* 120(2): 379-400.

Chang, H.L. and L. Alvarez-Cohen. 1995. Transformation Capacities of Chlorinated Organics by Mixed Cultures Enriched on Methane, Propane, Toluene, or Phenol. *Biotechnology and Bioengineering.* 45(5): 440-449.

Cleasby, J.L. 1990. Filtration. *In* Water Quality and Treatment, 4th ed. American Water Works Association., 455-560.

Rovatti, M., R. Di Felice and C. Nicolella. 1997. Biomass Concentration in Fluidized Bed Biological Reactors. *Water Research.* 31(4): 936-940.

Segar, R.L. Jr. and G.E. Speitel Jr. 1995. Cometabolism of Chloroethene Mixtures by Biofilms Grown on Phenol. *In* Bioremediation of Chlorinated Solvents. R.E. Hinchee, A. Leeson, and L. Semprini, Eds. Vol. 3(4): 245-253. Battelle Press, Columbus, OH.

Segar, R.L. Jr., S.L. DeWys and G.E. Speitel Jr. 1995. Sustained Trichlorothylene Cometabolism by Phenol-Degrading Bacteria in Sequencing Biofilm Reactors. *Water Environment Research.* 67(5): 674-684.

Segar, R.L. Jr., S.-Y. Leung, and S.A. Vivek. 1997. Treatment of Trichloroethene-Contaminated Water with a Fluidized-Bed Bioreactor. *Annals of the New York Academy of Sciences.* 829: 83-96.

Speitel, G.E. Jr. and J.M. Leonard. 1992. A Sequencing Biofilm Reactor for the Treatment of Chlorinated Solvents Using Methanotrophs. *Water Environment Research.*64(5): 712-719.

Zitomer, D.H. and R.E. Speece. 1993. Sequential Environments for Enhanced Biotransformation of Aqueous Contaminants. *Environmental Science and Technology.* 27(2): 227- 244.

Table 1. MU groundwater analysis.

Parameter	Value	Units
pH	7.4	pH units
Alkalinity	290	mg/L $CaCO_3$
Iron	0.04	mg/L
Manganese	<0.02	mg/L
Sodium	52	mg/L
Potassium	7.1	mg/L
Calcium	53.3	mg/L
Magnesium	28.2	mg/L
Nitrate	<0.05	mg-N/L
Sulphate	39	mg/L
Chloride	36	mg/L
Fluoride	1.4	mg/L
Phosphate	0.0	mg/L
Chlorine residual	0.5 to 1.0	mg/L

Figure 1. Ideal phenol and TCE profiles in the FBBR.

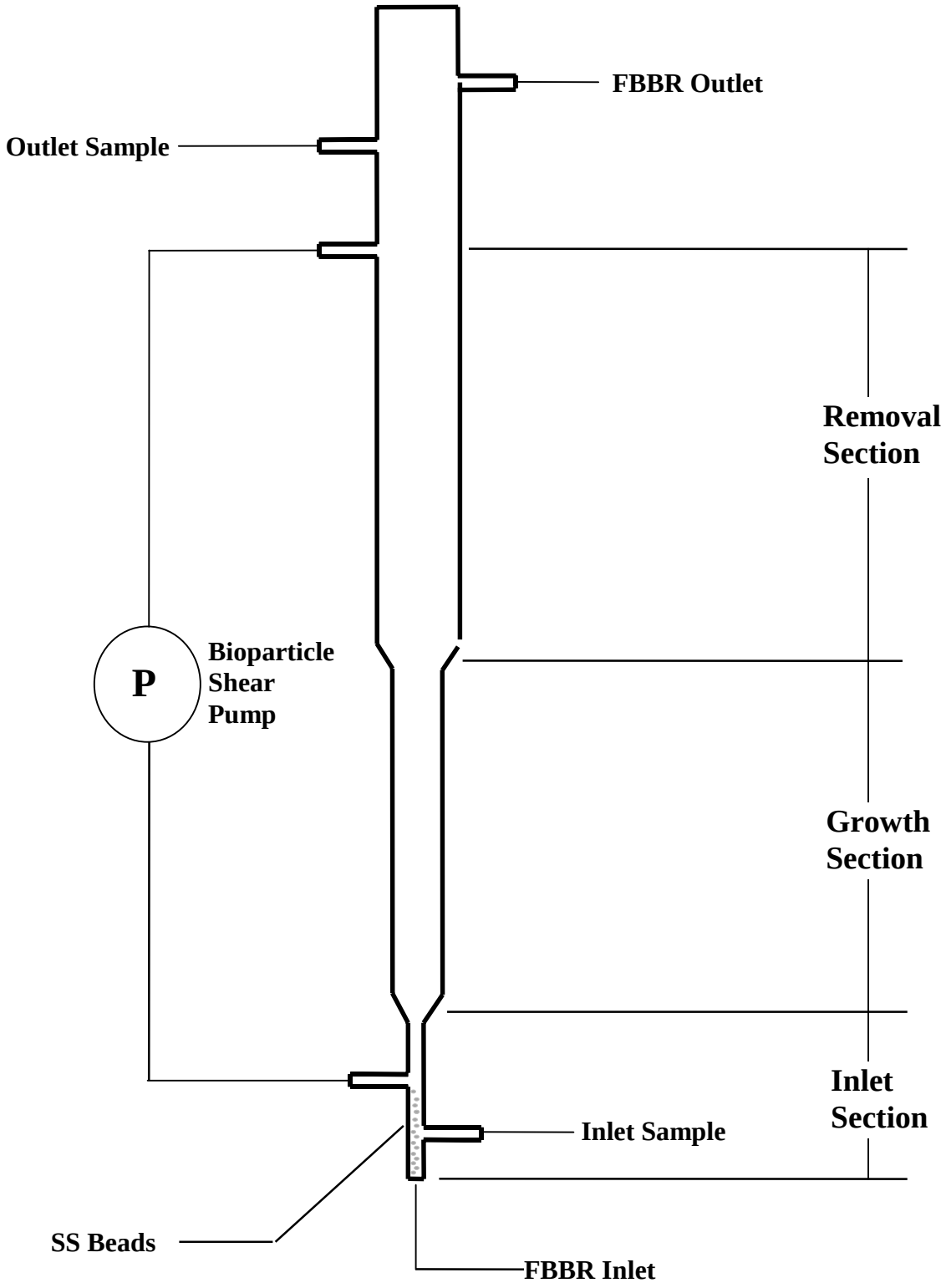

Figure 2. FBBR configuration.

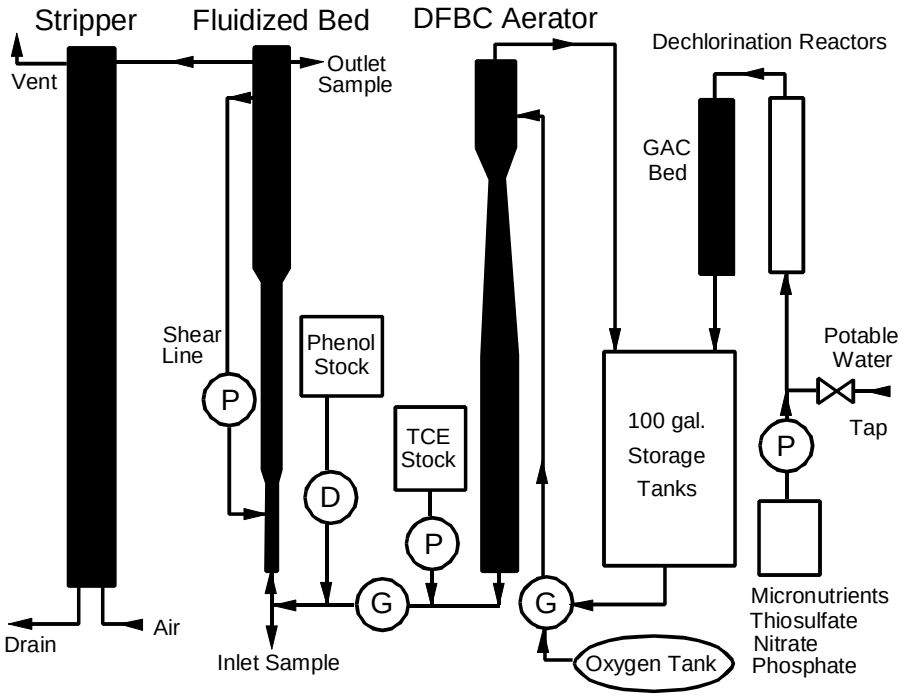

Figure 3. Diagram of the FBBR system.

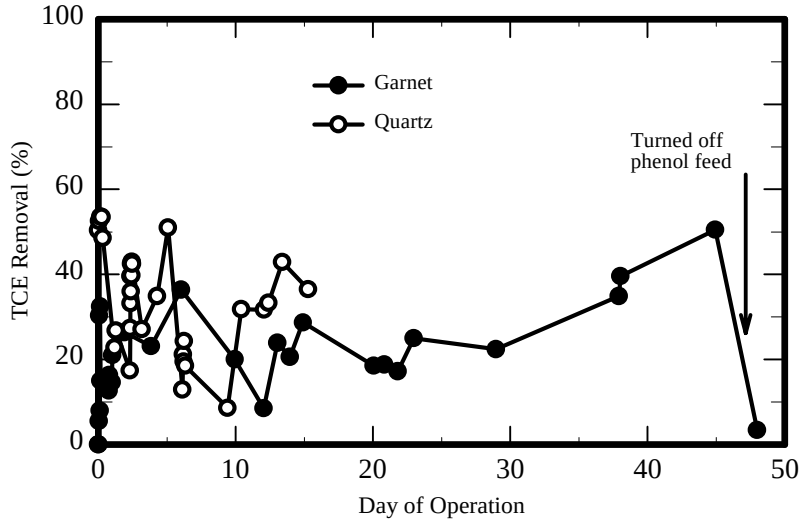

Figure 4. Comparison of TCE removal for quartz and garnet media.

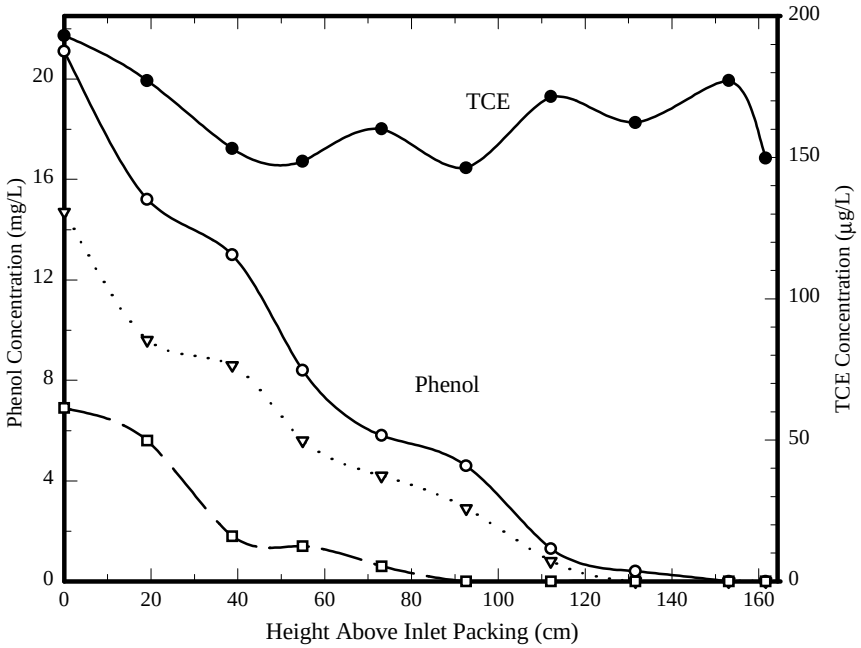

Figure 5. Substrate concentration profiles in the FBBR.

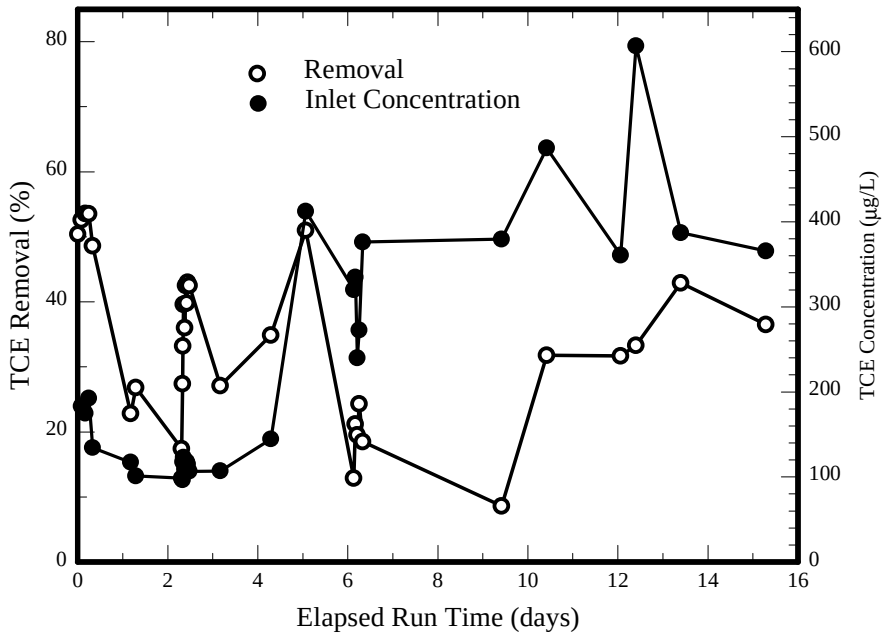

Figure 6. Effect of TCE concentration change on removal.