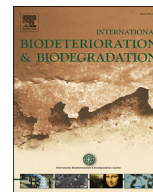




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## Simulation of combined anaerobic/aerobic bioremediation of tetrachloroethylene in groundwater by a column system

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## ABSTRACT

Chlorinated aliphatic hydrocarbons generally cannot be used as the carbon source or energy source for microorganism growth; these compounds are more difficult to be biodegraded by microorganisms in groundwater. The objective of this study was to explore the growth and degradation capability of aerobic mixed culture in a sand column simulating a PCE and DEC-contaminated aquifer. Results of the bio-stimulation study indicated that DCE removal rate was 60% after the operation of 56 days. With the bioaugmentation of aerobic mixed culture, DCE removal rate could be enhanced by 100% in the upstream of a simulated aerobic aquifer after the operation of 105 days. Bioremediation was conducted by addition of mixed aerobic bacteria that can degrade dichloroethylene to investigate the biodegradation of dichloroethylene in a simulated anaerobic and aerobic groundwater system. The results of column tests show that the pollutants in the downstream of the simulated anaerobic aquifer mainly comprised dichloroethylene, vinyl chloride, and methane. Fourteen days after mixed aerobic bacteria were added to the simulated aerobic aquifer, the removal efficiency of most intermediates generated from the anaerobic biodegradation process reached approximately 99%, indicating that the combined anaerobic-aerobic system accelerated the degradation of tetrachloroethylene and dichloroethylene.

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## 1. Introduction

Chlorinated aliphatic hydrocarbons (CAHs) possess the properties of low flammability, low boiling point, high vapor pressure, and high chemical stability. Therefore, CAHs have been widely used in processes such as dry cleaning, pharmaceutical manufacturing, pesticide manufacturing, metal degreasing, and electronic component cleaning (Bradley, 2003; Schmidt et al., 2010). However, most CAHs are potentially carcinogenic and tumorigenic; inappropriate use or leakage of CAHs already threatened the environment and public health (Clewett et al., 2001).

In situ bioremediation is considered as the most promising method for the remediation of organic-contaminated sites because of its several advantages such as low processing cost, limited

environmental disturbance, low wastes production, and the possibility of its integration with other physical-chemical treatment processes (Sponza, 2003; Tiehm and Schmidt, 2011). In an anaerobic environment, CAHs serve as the electron acceptor in reductive dechlorination processes. Reduction of chlorine atoms from CAHs increased their redox potential, resulting in the lower potential to further reductive dechlorination. Through dechlorination, tetrachloroethylene can be converted to trichloroethylene, dichloroethylene, vinyl chloride, and ethylene. As the number of chlorine atoms decreases, the dechlorination rate slows, leading to the accumulation of incompletely decomposed intermediates, such as dichloroethylene and vinyl chloride, which form a dichloroethylene plume that dissolves slowly in groundwater bodies, causing long-term sustained pollution. Therefore, in most anaerobic systems, incomplete dechlorination often causes the accumulation of dichloroethylene or vinyl chloride. Conversely, in an aerobic environment, trichloroethylene can be decomposed through aerobic co-metabolism, whereas dichloroethylene and vinyl chloride can be aerobic biodegraded using dichloroethylene or vinyl chloride as the primary substrate. Furthermore, dichloroethylene and vinyl chloride also can be co-metabolic biodegraded through co-

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metabolism processes with the addition of carbon source. When microorganisms decompose the carbon source, enzymes are produced, which further decompose CAHs (Schmidt and Tiehm, 2008; Abe et al., 2009).

In previous studies, the biodegradation of CAHs in anaerobic and aerobic environments was operated in mutually independent systems (Sponza, 2003; Révész et al., 2006). Therefore, obtaining a microorganism community that combines anaerobic and aerobic degradation abilities is difficult (Beunink and Rehm, 1990). Accordingly, the present study adopted column tests to simulate a saturated groundwater aquifer that was contaminated by dichloroethylene generated from incomplete anaerobic decomposition of tetrachloroethylene from the up-gradient groundwater. Subsequently, a technology using bioaugmentation for in-situ bioremediation was adopted and combined with molecular biotechnology to enable observation of the degradation of CAHs and effects caused by bacterial distribution changes in the simulated aquifer.

## 2. Materials and methods

### 2.1. Column tests

The anaerobic and aerobic columns were operated in series both columns were 100 cm in length with an inner diameter of 3 cm and three sampling ports (the first sampling port was set at 10 cm from the influent side, the second port was set at 50 cm, and the third port was set at 90 cm). The groundwater sampling tubing was prepared by drilling ten fine holes in a 1/8-inch Teflon tube. The sampling tubing was then set into the sand columns to ensure the homogeneity of the groundwater samples. Subsequently, a control valve was attached to each tube to facilitate the sample extraction directly by a syringe. At the connecting pipe between the anaerobic and aerobic columns, another sampling port was prepared using the same method (Fig. 1). The columns were filled with sand with particle sizes ranging from 0.25 to 0.59 mm. After columns had been filled, the properties and operating conditions of the columns were determined, as shown in Table 1.

### 2.2. Anaerobic column

For the acclimation purpose, the microorganisms were collected from a paddy field and the effluent of a petroleum wastewater treatment plant. The mixture of microorganisms collected from the field was then acclimated with a chemostat. The anaerobic chemostat and column were continuously fed with tetrachloroethylene at  $5 \text{ mg l}^{-1}$  ( $30.15 \text{ } \mu\text{mol l}^{-1}$ ), and nutrients are given in Table 2. The flow rate for the anaerobic sand column was  $86.4 \text{ ml day}^{-1}$  resulting a velocity of  $34.6 \text{ cm day}^{-1}$ . The acclimated anaerobic microorganisms (Table 3) were used as the cell source for the

up-gradient sand column.

### 2.3. Aerobic column

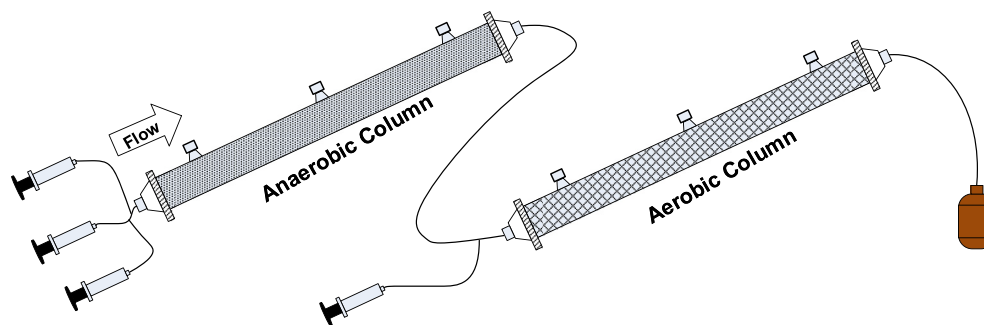
In this experiment, an aerobic sand column was connected to the up-gradient anaerobic sand column to form the integrated anaerobic/aerobic groundwater system (Fig. 1). Between these two different environmental condition columns,  $50 \text{ mg l}^{-1} \text{ H}_2\text{O}_2$  was injected into to convert the down-gradient column to the aerobic environment. The mixture of aerobic microorganisms can degrade naphthalene, pyrene, chlorophenol and nitrophenol by the long-term acclimation of the chemostat. These aerobic mixed bacteria (Table 4) were used as the source of dichloroethylene biodegrade. First, 10 ml each of aerobic mixed bacteria were added to a reaction

**Table 1**  
Column properties and operating conditions.

Item		Unit
Length of sand filling in the columns	200.0	cm
Overall density	1.7	$\text{g cm}^{-3}$
Specific gravity of sand	2.6	
Porosity	34.6	%
Pore volume	460.0	$\text{cm}^3$
Cross-sectional area of pores	2.5	$\text{cm}^2$
Influent flow rate	5.4	$\text{ml hr}^{-1}$
Pore velocity	2.2	$\text{cm hr}^{-1}$
Theoretical hydraulic retention time	3.6	day

**Table 2**  
Tetrachloroethylene and nutrient compositions and concentrations.

Materials	Concentration ( $\text{mg l}^{-1}$ )
$\text{C}_2\text{Cl}_4$ (tetrachloroethylene)	5.0
$\text{CH}_3\text{COONa}$	250
$\text{C}_3\text{H}_6\text{O}_3$	184
$\text{NaHCO}_3$	20.3
$\text{NH}_4\text{Cl}$	56.3
Phosphorous	
$\text{KH}_2\text{PO}_4$	2.5
$\text{K}_2\text{HPO}_4$	3.25
$(\text{NaPO}_3)_6$	1.15
Mineral	
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1.53
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.55
$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	0.525
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.6
Micronutrient	
cysteine	0.1
$\text{NH}_4\text{VO}_3$	0.025
$\text{H}_3\text{BO}_3$	0.025
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.025
$\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$	0.025
$\text{Na}_2\text{SeO}_3 \cdot 2\text{H}_2\text{O}$	0.025



**Fig. 1.** Column system.

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