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Use of specific gene analysis to assess the effectiveness of surfactant-enhanced trichloroethylene cometabolism

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ABSTRACT

The objective of this study was to evaluate the effectiveness of in situ bioremediation of trichloroethylene (TCE)-contaminated groundwater using specific gene analyses under the following conditions: (1) pretreatment with biodegradable surfactants [Simple Green™ (SG) and soya lecithin (SL)] to enhance TCE desorption and dissolution, and (2) supplementation with SG, SL, and cane molasses as primary substrates to enhance the aerobic cometabolism of TCE. Polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), and nucleotide sequence analysis were applied to monitor the variations in specific activity-dependent enzymes and dominant microorganisms. Results show that TCE-degrading enzymes, including toluene monooxygenase, toluene dioxygenase, and phenol monooxygenase, were identified from sediment samples collected from a TCE-spill site. Results from the microcosm study show that addition of SG, SL, or cane molasses can enhance the aerobic cometabolism of TCE. The TCE degradation rates were highest in microcosms with added SL, the second highest in microcosms containing SG, and lowest in microcosms containing cane molasses. This indicates that SG and SL can serve as TCE dissolution agents and act as primary substrates for indigenous microorganisms. Four dominant microorganisms (*Rhodobacter* sp., *Methyloversatilis* sp., *Beta proteobacterium* sp., and *Hydrogenophaga pseudoflava*) observed in microcosms might be able to produce TCE-degrading enzymes for TCE cometabolic processes.

1. Introduction

Groundwater at many existing and former industrial sites and disposal areas is contaminated by halogenated organic compounds that were released into the environment. Chloroethenes are ubiquitous pollutants found at many priority cleanup sites, and they are known or suspected carcinogens [1–3]. Among the various chloroethenes, trichloroethylene (TCE) is among the most ubiquitous chlorinated compounds found in groundwater contamination. One cost-effective approach for the remediation of contaminated aquifers that is attracting increased attention is the application of enhanced bioremediation for contaminant degradation. Because the biodegradation of TCE and its daughter compounds [e.g., dichloroethenes (DCEs) and vinyl chloride (VC)] is generally more efficient under aerobic conditions, introduction of dissolved oxygen (DO) into the TCE plume will increase the TCE biodegradation (cometabolism) rate and significantly reduce the TCE mass flux if sufficient bioavailable primary substrates are present [3–6]. Although microbial cultures are not able to utilize TCE as a growth substrate under aerobic conditions, some aerobic bacterial cultures containing oxygenase enzymes (e.g., methane, toluene, phenol, propane and ammonia oxidizers) could degrade TCE cometabolically [7–10]. The induced enzymes are able to create enzyme active sites to catalyze the degradation of non-growth substrates such as TCE [3]. Several aerobic microorganisms or microbial communities have the ability to synthesize oxygenase enzymes [e.g., toluene oxygenase, phenol oxygenase, particulate methane monooxygenase (pMMO), toluene dioxygenase] that catalyze the initial step in the oxidation of their respective primary or growth substrates, and these enzymes have the potential to initiate the oxidation of TCE and other chlorinated aliphatic hydrocarbons [11–14]. These groups of aerobic bacteria include oxidizers of the following compounds: methane, propane, ethylene, toluene, phenol, acetic acid, propionic acid, cresol, ammonia, and isoprene [7,10]. Among these bacteria, methane oxidizers, phenol degraders, and toluene degraders are the main bacteria that are able to perform the aerobic cometabolic process of TCE [6,7].

Based on the above discussion, in situ aerobic bioremediation is a feasible technology to clean up TCE-contaminated sites if oxygen and biodegradable primary substrates can be provided efficiently to the subsurface. Cane molasses is waste from the sugar industry. It is a good candidate for use as a primary substrate because it is

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relatively inexpensive and rich in bioavailable carbon [12]. Surfactants can increase the solubility of TCE by partitioning it into the hydrophobic cores of surfactant micelles to levels above the critical micelle concentration (CMC). Thus, surfactants are able to improve the mass-transfer of TCE from the solid to aqueous phases and, consequently, the microbial remediation of TCE in groundwater [15–18]. Surfactants are useful for the biodegradation of contaminates because they are able to enhance desorption and increase the solubility of hydrophobic compounds. Some studies have been conducted to enhance the biodegradation of TCE by employing surfactants in contaminated soil and groundwater [19,20]. However, little information is available concerning the effects of surfactants on the enhancement of aerobic TCE cometabolism and the impacts of surfactant application on the subsurface environment. Soya lecithin (SL), a rhizosphere phytogenic and nonionic surfactant that consists of a mixture of phospholipids and quillaya saponin, has been studied in the aerobic biodegradation of phenanthrene and fluoranthene in shake batch cultures of three polycyclic aromatic hydrocarbon (PAH)-degrading bacteria [21,22]. SL was found to have higher PAH and polychlorinated biphenyl (PCB)-solubilizing activity and a lower bacterial toxicity [23,24]. Simple Green (SG), a nonionic surfactant, can be used as a soil washing agent and applied in non-aqueous phase liquid (NAPL) flushing [25,26]. In the current study, the feasibility of using SL and SG for the enhancement of aerobic TCE cometabolism was evaluated.

Recently, molecular biology has been used in site remediation studies to confirm the effectiveness of the bioremediation and identify the bacterial species that are critical for the biodegradation of contaminants of concern [27,28]. Results from other studies reveal that polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), and nucleotide sequence analysis techniques provide a direct evaluation of bacteria that actively contribute to contaminant biodegradation. In addition, these techniques can be used to understand the microbial ecology at the site, which can be used to identify trends in the biodegradation process [12,27]. The main objectives of this study were the following: (1) to develop a specific gene analytical method to confirm the existence of TCE-degrading enzymes at a TCE-contaminated site, (2) to evaluate the effectiveness of aerobic cometabolism of TCE-contaminated groundwater using specific gene analyses in the presence of cane molasses and biodegradable surfactants (SG and SL) for the enhancement of TCE desorption and dissolution in a microcosm study, and (3) to determine the dominant microorganisms responsible for aerobic cometabolism of TCE in microcosms using a series of molecular biology techniques, including DNA extraction, PCR amplification, DGGE, and DNA analysis. Total bacterial DNAs and DNA amplification were used to verify the presence of phenol, methane, and toluene-degrading enzymes in microcosms and field soil samples that had been subjected to TCE degradation. PCR, DGGE, and nucleotide sequence analysis were applied to monitor the variations in activity-dependent microbial diversity and dominant native microorganisms.

2. Site description

An industrial park site located in southern Taiwan was chosen as the site at which to perform the present study. At this site, a TCE storage tank had leaked and contaminated the groundwater with TCE. Soil and groundwater samples from monitor wells were collected and analyzed to determine the local hydrogeology and delineate the TCE plume during a previous site investigation study. Site investigation results showed that the components of the site soils are consistent with a sandy loam texture (48% sand, 43% silt, and 9% clay). The water table is generally found at depths ranging from 6 to 7 m below ground surface. The site groundwater flows to the northeast at a velocity of 7.2 cm/day and with a hydraulic conductivity of 0.006 cm/s. Fig. 1 presents the site map that shows the groundwater flow direction, estimated plume boundary, and one groundwater and four soil sampling locations chosen for this study.

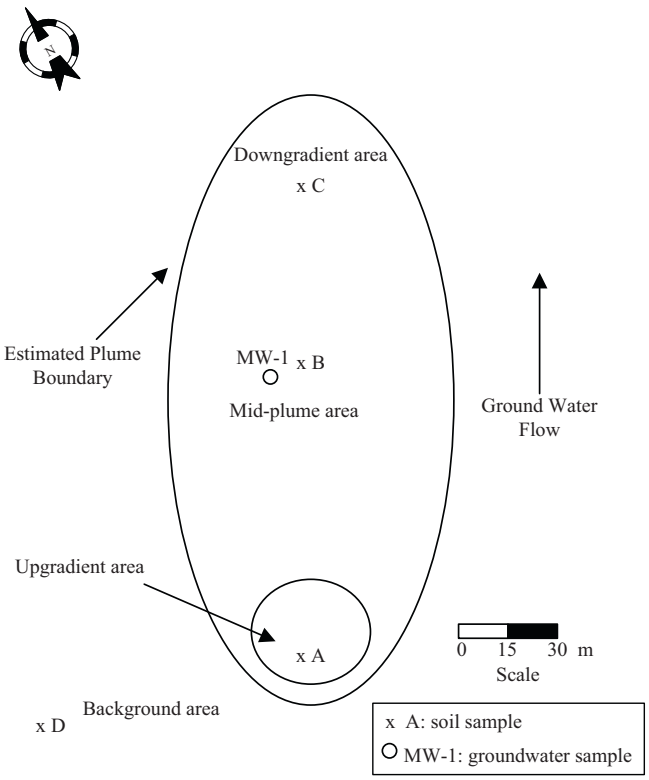

Fig. 1. Site map showing the groundwater flow direction, estimated plume boundary, and one groundwater and four soil sampling locations chosen for this study.

3. Materials and methods

3.1. Chemicals

All chemicals used in this study were analytical grade and purchased from Wako Chemical (Kyoto, Japan). The TCE was purchased from Fisher Chemical (Fair Lawn, NJ) (99.99%) and used as received. This study employed the nonionic surfactants SL and SG, which were supplied by Prodotti Gianni Spa (Italy) and Sunshine Makers, Inc. (USA). Ninety-six percent (w/w) of SL (CENTROLEX-E322) is made up of phospholipids, of which 73% are pure phospholipids (23% phosphatidylcholine, 20% phosphatidylethanolamine, 14% phosphatidylinositol, 8% phosphatide acid, and 8% other phospholipids), 15% glycolipids, 8% carbohydrates, and 3% neutral lipids. SG has an average molecular weight of 106 and a molecular formula of $HOCH_2H_2O$-$(CH_2)_3CH_3$. Cane molasses was purchased from Taiwan Sugar Corp., Ltd. (Taiwan) and contained 78.6 g/L of glucose, 79.7 g/L of fructose and galactose, 170.9 g/L of sucrose, 10.2 g/L of raffinose, and 339.4 g/L of fermentable sugar.

3.2. Collection of sediment and groundwater samples

Aquifer sediments (collected from locations A to D) and groundwater (collected from monitor well MW-1) were collected from the TCE-spill site, and it was analyzed for the presence of specific TCE-degrading genes and for groundwater quality (Fig. 1). Groundwater samples were analyzed for TCE and geochemical indicators including pH, oxidation–reduction potential (ORP), and DO. TCE