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Simultaneous biodegradation of carbon tetrachloride and trichloroethylene in a coupled anaerobic/aerobic biobarrier

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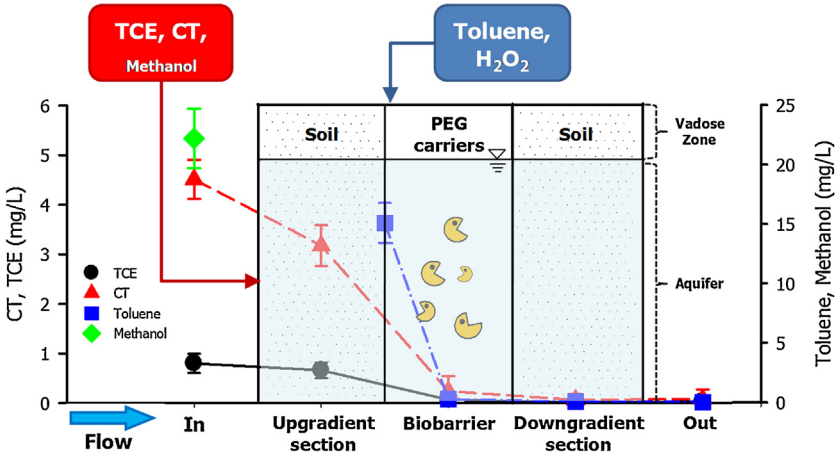
HIGHLIGHTS

- Coupled biodegradation of carbon tetrachloride (CT) and trichloroethylene (TCE) in biobarrier with polyethylene glycol carriers.
- TCE aerobically cometabolized and CT anaerobically dechlorinated.
- Removal efficiencies of over 98%, leaving residuals below or near the regulatory standards.
- Coupled aerobic/anaerobic environments established by H_2O_2 injected at 50% of electron donor.
- Longer retention time (from 3.6 to 7.2 days) achieved satisfactory removal at lower temperature (18 °C).

GRAPHICAL ABSTRACT



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ABSTRACT

Simultaneous biodegradation of carbon tetrachloride (CT) and trichloroethylene (TCE) in a biobarrier with polyethylene glycol (PEG) carriers was studied. Toluene/methanol and hydrogen peroxide (H_2O_2) were used as electron donors and an electron acceptor source, respectively, in order to develop a biologically active zone. The average removal efficiencies for TCE and toluene were over 99.3%, leaving the respective residual concentrations of ~12 and ~57 μg/L, which are below or close to the groundwater quality standards. The removal efficiency for CT was ~98.1%, with its residual concentration (65.8 μg/L) slightly over the standards. TCE was aerobically cometabolized with toluene as substrate while CT was anaerobically dechlorinated in the presence of electron donors, with the respective stoichiometric amount of chloride released. The oxygen supply at equivalent to 50% chemical oxygen demand of the injected electron donors supported successful toluene oxidation and also allowed local anaerobic environments for CT reduction. The originally augmented (immobilized in PEG carriers) aerobic microbes were gradually outcompeted in obtaining substrate and oxygen. Instead, newly developed biofilms originated from indigenous microbes in soil adapted to the coupled anaerobic/aerobic environment in the carrier for the simultaneous and

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almost complete removal of CT, TCE, and toluene. The declined removal rates when temperature fell from 28 to 18 °C were recovered by doubling the retention time (7.2 days).

1. Introduction

Trichloroethylene (TCE) and carbon tetrachloride (CT) are organic solvents frequently found in groundwater [1,2]. According to the International Agency for Research in Cancer (IARC), TCE is considered carcinogenic (Group 1) while CT possibly carcinogenic (Group 2B) to humans [3]. Aerobic cometabolism of TCE in the presence of a primary substrate is well known [4–6] and has been reported in field scales [7–9]. In comparison, a fully chlorinated compound CT is not biodegradable under aerobic conditions due to its carbon oxidation state [10]. However, as a polychlorinated hydrocarbon, CT easily undergoes abiotic and microbial reductive dechlorination under methanogenic, denitrifying, fermenting, and sulfate- or iron-reducing conditions [11].

Simultaneous dehalogenation of chlorinated ethenes and CT had been demonstrated in the laboratory under anaerobic conditions [12,13]. CT and its dechlorinated intermediate chloroform (CF) are known to inhibit the degradation of chlorinated ethenes [12,14,15]. Thus, the remediation strategies using dehalorespiration under anaerobic conditions to remove mixtures of chlorinated ethenes and CT or CF would be a significant challenge at co-contaminated sites. Furthermore, the potential accumulation of toxic and carcinogenic metabolites formed from the anaerobic treatment, particularly vinyl chloride, is another environmental concern [16]. A sequential treatment such as anaerobic dechlorination followed by aerobic cometabolism is known to be effective and leads to the complete mineralization of the chlorinated pollutants [17], although the complexity of the system may still offset the cost effectiveness of bioremediation. On the other hand, the coupled aerobic/anaerobic system could create both oxidation and reduction conditions in a single biofilm (or bio-granule) reactor [18–20]. By maintaining the granule sludge aerobic outside and anaerobic inside, both reductive dechlorination and aerobic oxidation could be accomplished simultaneously. In particular, methane produced in the anaerobic core where PCE (perchloroethylene) was reduced to TCE could be used as the electron donor for TCE cometabolism by methanotrophs. Burns et al. [21] conducted Bio-Trap® based *in situ* microcosm studies to evaluate EHC-M® stimulated degradation of mono-, di-, and trichlorobenzenes in anaerobic groundwater, and the obtained results supported the view that both aerobic and anaerobic metabolism can be important to the complete degradation of chlorinated benzenes and may coexist in predominantly anaerobic groundwater systems.

Therefore, this study investigated the simultaneous biodegradation of CT and TCE in a pilot-scale biobarrier, using the artificially contaminated water mimicking groundwater. The biobarrier was filled with polyethylene glycol (PEG) pellets that were initially immobilized with *Burkholderia vietnamiensis* G4, a TCE cometabolizer. Toluene was provided as a growth substrate and hydrogen peroxide was used to deliver oxygen to the medium. The main focus of this study was to verify if the system could effectively/stably/simultaneously remove the contaminants to the required concentration levels, which is essential for the field application. To the best of our knowledge, the simultaneous and almost complete removal of CT, TCE, and toluene in a single biobarrier has never been reported.

2. Materials and methods

2.1. Enrichment and cell immobilization

B. vietnamiensis G4 (LMG 22486) was used due to its excellent ability to cometabolize TCE by the synthesis of toluene monooxygenase [22,23]. Cell enrichment was carried out in nutrient broth (NB; Bacto-peptone 2.5 g/L, Bacto-beef extract 1.5 g/L). Fig. S1 illustrates the procedure used to immobilize cells into PEG. Cells were prepared at the optical density (OD_{600}) 1.0 and mixed with chemicals for the PEG polymer synthesis. The synthesis ratio in PEG polymerization was oligomer: monomer: crosslinker: promotor: initiator, 3.67: 0.17: 0.33: 1: 1 (%, w/v). All the chemicals, except the initiator, were mixed well with the cell suspension, followed by the addition of initiator. The mixture was then spread on a plate for gelation. After the PEG gel was set with microorganisms, it was cut into 4 × 4 × 4 mm pellets. Further details on the immobilization are described elsewhere [24].

2.2. Fabrication of biobarrier reactor

Fig. 1 shows a schematic diagram of the stainless steel biobarrier reactor (total volume, 465 L), consisted of three parts with the artificially contaminated water moving along the flow path, mimicking groundwater.

The upgradient and downgradient sections were filled with natural soil (screened through a 5 mm-mesh sieve), whereas the biobarrier section was filled with PEG pellets immobilized with *B. vietnamiensis* G4. Later, the downgradient soil section was replaced by 56 cm in width of sand wall due to clogging. TCE (and later CT) dissolved in water was fed through the inlet placed at the center of the front face. Two injection ports were placed in the biobarrier section for the toluene and hydrogen peroxide supply to develop a biologically active zone (BAZ). Water extracted from the two extraction ports (corresponding to 1Q, same as the influent flow) was re-circulated into the injection ports. The outlet port was placed 190 mm below the top of the soil surface, leaving a vadose zone above. Nine horizontal ports (diameter 35 mm) were set across the reactor for sampling. All the ports were sealed with Teflon-faced septa. The reactor surface was initially open to the atmosphere, but later completely sealed with a stainless steel cover. A control reactor containing PEG pellets but without immobilized cells and electron donor/acceptor injection was operated in parallel to monitor the volatilization of contaminants.

2.3. Artificially contaminated water and mineral medium

The TCE and CT stock solutions dissolved in methanol and diluted in water were stored in a collapsible Tedlar bag and fed into the reactor with tap or chloride-free deionized water. The influent concentrations of TCE, CT, and methanol were 0.8, 4.5, and 25 mg/L, respectively. The superficial flow velocity of the feed water was 9.2 cm/d, giving the approximate (theoretical) travel times, assuming the pore ratio to be 0.4, of 0.7 days, 3.6 days, and 4.5 days to the sampling ports in upgradient section, BAZ, and downgradient section, respectively. A mixture of toluene, mineral medium, and hydrogen peroxide (H_2O_2) was directly injected to the biobarrier through two injection ports. The injection flow rate was one thirti-