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Enricher reactor – Permeable reactive biobarrier approach for removing a mixture of contaminants with substrate interactions

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HIGHLIGHTS

- First study to examine effect of absence of BTEX on the performance of biobarrier.
- Biobarrier lost removal ability for benzene and toluene when they reappeared.
- Benzene biodegradation was inhibited by toluene after an absence period.
- Bioaugmentation from enricher reactor sped recovery of biobarrier.
- Toluene was an effective alternative inducer to BTEX in enricher reactor.

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ABSTRACT

A laboratory-scale enricher reactor (ER) – permeable reactive biobarrier (PRBB) system was studied to address performance loss of a PRBB due to substrate interactions among a mixture of benzene, toluene, ethylbenzene, and xylene (BTEX) in groundwater, when the mixture reappeared after a 10-day absence period. Toluene and BTEX as an inducer in ER were compared to investigate toluene as a potential single inducer in ER. PRBBs without ER augmentation experienced performance losses ranging from 11% to 35% for PRBBs initially inoculated with toluene degraders and 22% to 33% for PRBBs initially inoculated with BTEX degraders. Bacterial communities changed substantially in these PRBBs after the absence period, which could contribute to the performance losses. PRBBs augmented with toluene degraders overcame the inhibition interaction between benzene and toluene, and showed a superior removal performance for toluene degradation suggesting that toluene can be used as a single inducer in an ER.

1. Introduction

Permeable reactive biobarrier (PRBB) is one of the commonly used in situ treatment processes for contaminated groundwater, where microorganisms convert the contaminants into innocuous products. PRBBs are either augmented with bacterial cultures or biostimulated by providing necessary nutrients and environment. Successful PRBB applications for a mixture of contaminants are often influenced by substrate interactions among individual contaminants in the mixture. These interactions can alter degradation rates of individual contaminants either synergistically or antagonistically (Arvin et al., 1989; Barbaro et al., 1992; Wang and Deshusses, 2007; Dou et al., 2008).

Synergistic interactions promote the degradation rates of individual contaminants while the antagonistic interactions reduce the degradation rates through various inhibition processes. Antagonistic interactions such as preferential degradation or diauxie, which is sequential utilization of preferred substrates (Alvarez and Vogel, 1991), can lead to lag phases before other substrates are consumed. PRBBs are often augmented with mixed bacterial cultures that are adapted to target contaminants to address the substrate interactions. Mixed cultures are often found to be more effective than pure cultures in PRBBs because interspecies interactions may be necessary for the complete biodegradation of contaminant mixtures (Deeb and Alvarez-Cohen, 2000).

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The transport of a contaminant or a mixture of contaminants in groundwater is unpredictable and can come in batches or discontinuous plumes as a result from trapped residual non-aqueous phase liquids (Sahloul et al., 2002). Random disappearance of the target contaminants could cause a possible loss of some of the contaminant degraders and/or their biodegradation activity (Qi and Moe, 2006; Mathur and Majumder, 2008; Lee et al., 2009; Kasi et al., 2011). Partial loss of degraders could lead to increased substrate interactions. Increased diauxie (or preferential inhibition) is an example for the increased substrate interaction, where the remaining degraders after the disappearance period prefer an easily degradable (requires less energy) compound within the contaminant mixture. In case of a mixture of structurally similar contaminants such as benzene, toluene, ethylbenzene, and xylene (BTEX), increased diauxie effects after an absence period can be observed leading to unacceptable lag phases in degrading some of the contaminants in the mixture.

In addition to substrate interactions, contaminant mixture degradation can be suppressed by the presence of structurally dissimilar compounds as well. The presence of ethanol was found to repress the production of the inducible enzymes that are needed for starting BTEX degradation, leading to longer lag phases (Corseuil et al., 1998). Catabolic repression due to the presence of more favorable substrates has been extensively described (Duetz et al., 1994; Muller et al., 1996; Chen et al., 2007). Ethanol can be degraded using constitutive enzymes and long term exposure to ethanol in sufficient quantities can reduce the need for the production of enzymes by the bacteria for BTEX degradation. BTEX are typically found along with ethanol in groundwater and ethanol is more soluble than BTEX and hence moves faster and reaches the PRBB sites sooner than BTEX.

At a laboratory scale, an enricher reactor (ER) – PRBB (ER–PRBB) concept has been successfully applied to treat groundwater contaminated with a single contaminant (benzene) that appears in batches (Kasi et al., 2011). ER is an offline reactor where bacterial culture is acclimated to target compound(s) and is used to augment a main treatment system, such as PRBB. Appropriate growth conditions for culture enrichment, such as availability of nutrients and target compounds, and suitable environmental conditions (pH and temperature) are provided in the ER to induce the desired degradation capability. Supplying the enriched bacterial culture from ER was found to maintain the performance of the PRBB when a single contaminant reappeared after a period of absence.

Supply of bacteria from an ER after an absence period can augment the biobarrier with active bacterial culture to make up for the loss of degraders acclimated to the target contaminant. The activity of bacterial culture in the ER is maintained through the supply of necessary growth materials as well as the target contaminant itself. Application of an actively enriched bacterial culture to address substrate interactions among mixtures of target contaminants, such as BTEX, has not been addressed. The active culture, although may not completely eliminate the antagonistic substrate interactions (inhibition), could minimize the effects of these interactions by maintaining the communities in sufficient numbers necessary to degrade each compound in the contaminant mixture. Hence, when used to augment a PRBB, supply of this actively enriched culture can minimize the performance losses after a period of absence due to increased substrate interactions by providing the communities necessary to degrade all compounds in a contaminant mixture. For structurally similar compounds, the culture can also be enriched in a single ER by supplying all compounds in the same reactor or even supplying a single compound as an inducer in the ER, which eliminates the cumbersomeness of enriching the necessary bacterial cultures on individual compounds in multiple ERs.

The goal of this research is to apply the ER–PRBB approach to address the performance loss of a PRBB due to substrate interactions among a mixture of contaminants in groundwater, when the mixture reappears after a period of absence. A mixture of BTEX was chosen as the model contaminants in a laboratory-scale study. Effect of ethanol during the BTEX absence period on the substrate interactions among BTEX was investigated. Ethanol is a common additive to gasoline in many countries and is commonly found with BTEX in contaminated groundwater. Toluene and BTEX as an inducer in ER were compared since bacterial cultures grown on toluene alone were found to effectively degrade BTEX compounds individually and as a mixture (Kasi et al., 2013). Changes in bacterial community structure in the PRBBs due to BTEX absence period and the supply of ethanol during BTEX absence period were also investigated.

2. Methods

2.1. Cultivation of toluene and BTEX degraders

A mixed bacterial culture was acclimated to toluene (toluene degraders or TD) and BTEX mixture (BTEX degraders or BTEXD) in separate reactors following the acclimation procedure described by Kasi et al. (2011). Mixed liquor suspended solids from the Moorhead Wastewater Treatment Plant, Moorhead, MN, USA were used as the mixed bacterial culture source. Benzene (99% purity), ethylbenzene (99% purity), and xylenes (99% purity) were purchased from Sigma–Aldrich Chemical Co., MO, USA. Toluene (99% purity) was purchased from VWR International Co., PA, USA.

Initially, the mixed bacterial culture was grown in synthetic groundwater with methanol as a carbon source under denitrifying conditions (nitrate as an electron acceptor). The synthetic groundwater, hereafter referred to as mineral salt medium (MSM), was prepared according to the composition described by Kasi et al. (2011). Mineral salts, vitamins, and trace metals used for MSM preparation were purchased from VWR International Co., PA, USA. The cultivation reactor made of polyethylene (PE) with a working volume of 3 L was operated as a sequencing batch reactor (SBR). The SBR operation cycle included 30 min for filling, 70 h and 20 min for reaction, 1 h for settling, and 10 min for drawing. During the filling, the reactors received MSM and were purged with nitrogen gas for 30 min before the addition of methanol. During the reaction period, the reactors were closed with an airtight cap to maintain anoxic conditions and the solution was mixed using a horizontal shaker (DS-500E, VWR International). Methanol was injected into the reactors through valves (made of Teflon®) attached to the cap using a glass syringe.

The denitrifiers were later adapted to toluene in one reactor and BTEX mixture in the other reactor by gradually increasing their concentrations in their respective reactors, while gradually reducing the methanol in the feed at the same time (Kasi et al., 2013). The total concentration of organic carbon (27.7 mg C/L) supplied in the feed was kept constant during the gradual acclimation. The final concentrations in the synthetic groundwater that the culture was exposed to were 30 mg/L of toluene for TD and 8 mg/L of each BTEX compound for BTEXD.

2.2. ER operation and activity test

The acclimated TD and BTEXD cultures were continuously enriched in their respective ERs to maintain their degradation activities. The MSM was constantly supplied with toluene for TD culture and BTEX for BTEXD culture. The ERs made of PE with a working volume of 3 L were operated as SBRs. The SBR operation cycle was similar to that described for the acclimation of the cultures in the preceding section.