Bioresource Technology 123 (2012) 207–213

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Molecular approach to evaluate biostimulation of 1,2-dibromoethane in contaminated groundwater

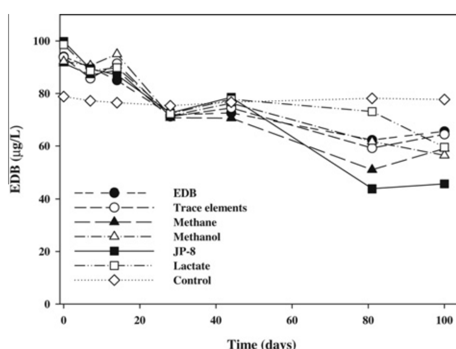
Kyunghwa Baek [a], Robert McKeever [b], Kahlil Rieber [a], Diane Sheppard [b], Chul Park [b], Sarina J. Ergas [b,c], Klaus Nüsslein [a,*]

[a] Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA
[b] Department of Civil & Environmental Engineering, University of Massachusetts, Amherst, MA 01003, USA
[c] Department of Civil & Environmental Engineering, University of South Florida, Tampa, FL 33620, USA

HIGHLIGHTS

- ▶ This study investigated the potential for biostimulation of 1,2-dibromoethane in contaminated groundwater using a molecular approach.
- ▶ Microcosm experiments diligently mimicked in situ conditions.
- ▶ The addition of jet fuel (50 mg/l) yielded the highest biodegradation of EDB.
- ▶ Jet fuel addition led to highest bacterial numbers compared with other amendments.
- ▶ Members of genera associated with monooxygenase dominated all microcosms.
- ▶ Gene abundances for monoxygenase were significantly higher in jet fuel treatments.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 March 2012
Received in revised form 21 May 2012
Accepted 23 May 2012
Available online 29 May 2012

Keywords:
1,2-Dibromoethane
Aerobic biodegradation
Biostimulation
Groundwater
Leaded gasoline

ABSTRACT

This study investigated the effect of co-substrate amendments on EDB biodegradation under aerobic conditions. Microcosms were established using contaminated soil and groundwater samples and maintained under in situ conditions to determine EDB degradation rates, and the diversity and abundance of EDB degrading indigenous bacteria. After 100 days of incubation, between 25% and 56% of the initial EDB was degraded in the microcosms, with added jet fuel providing highest degradation rates ($2.97 \pm 0.49\ yr^{-1}$). In all microcosms, the quantity of dehalogenase genes did not change significantly, while the number of BTEX monooxygenase and phenol hydroxylase genes increased with jet fuel amendments. These results indicate that EDB was not degraded by prior dehalogenation, but rather by cometabolism with adapted indigenous microorganisms. This is also reflected in the history of the plume, which originated from an aviation gasoline pipeline leak. This study suggests that biostimulation of EDB is possible at aerobic groundwater sites.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

The compound 1,2-dibromoethane, also known as ethylene dibromide (EDB), was primarily used as a lead scavenger in anti-knock gasoline mixtures, particularly in aviation fuels

* Corresponding author. Tel.: +1 413 545 1356; fax: +1 413 545 1578.
E-mail address: nusslein@micro.umass.edu (K. Nüsslein).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.05.119

(Aronson and Howard, 2008; Kszos et al., 2003; Wilson et al., 2008). In the past, EDB has also been used in agriculture, as an insecticide, pesticide, and soil fumigant for various crops. EDB is highly toxic and potentially carcinogenic and the US EPA banned its agricultural use in 1983 (US EPA, 1983). The current US EPA drinking water maximum contaminant level (MCL) for EDB is 0.05 μg/l, while the Massachusetts Department of Environmental Protection has a more stringent MCL for EDB of 0.02 μg/l (AFCEE, 2007).

One particular site where EDB has been persistent is Fuel Spill 12 (FS-12) at the Massachusetts Military Reservation (MMR). At this site, a fuel pipeline leak of approximately 265,000 l occurred in the early 1970s, which released ~159 kg of EDB to the subsurface. This plume ranges in depth from 46 to 76 m below the ground surface in an aquifer characterized by low biomass and nutrients. The aerobic zone has a mix of coarse and fine-grained sand, while the anaerobic zone is comprised of dark gray, very fine and densely packed silt. Current groundwater sampling indicates that EDB remains above the Massachusetts MCL of 0.02 μg/l. An extremely low natural attenuation rate of 0.04 yr^{-1}, has been calculated for this site using a simple mass balance approach and assuming first-order kinetics (Falta, 2004). However, prior to this study the level of natural attenuation and the potential for enhanced natural attenuation within the plume had not been validated through in depth studies.

The use of microorganisms to clean up polluted environments through *in situ* or *ex situ* bioremediation is particularly attractive for both environmental and economic reasons. In biostimulation, the activity of the indigenous microbial populations is increased through the addition of rate limiting nutrients and/or a terminal electron acceptor (Dafale et al., 2008; Olaniran et al., 2011). This type of bioremediation is advantageous, as it is directed to native microorganisms, well-suited to the subsurface environment, and well distributed spatially within the subsurface. Biological degradation of EDB has been studied under both aerobic (Freitas dos Santos et al., 1996; Hartzell et al., 2001; Polelarends et al., 1999) and anaerobic conditions (Henderson et al. 2008; Tandol et al., 1994). Although in some instances moderate to rapid EDB degradation rates have been reported, many of these studies were conducted under strict anaerobic conditions, at relatively high temperatures (>20 °C), with non-environmentally relevant EDB concentrations, or with materials not representative of conditions found within a deep aquifer. In addition, there is limited information on changes in microbial communities or mechanisms that occur during biodegradation of EDB.

For the success of any bioremediation process, it is necessary to understand the behavior of microbial populations responsible for the degradation of the targeted contaminants. It is also important to understand the members of microbial populations within that community that are not directly responsible for the degradation, because they might affect the behavior of the degrading bacteria through microbial interactions. Culture-independent nucleic acid–based techniques have provided helpful insights by determining active microorganisms within complex microbial communities (Malik et al., 2008; Pandey et al., 2009). Nevertheless, there has been no attempt to identify the organisms in the complex microbial community associated with EDB contaminated sites using these methods.

This study investigates the biostimulation of EDB at an aviation fuel spill site (FS-12) at the MMR in eastern Massachusetts. While petroleum hydrocarbons, such as benzene and toluene, from this fuel spill have been remediated, a plume of low concentration EDB remains in the groundwater aquifer at concentrations above current federal and state drinking water standards (AFCEE, 2007). To evaluate natural attenuation potential and the effect of biostimulation on EDB degradation, microcosms were set up with varying substrates with natural groundwater and soil under conditions similar to those at the contaminated site, including comparable EDB concentration, oxygen saturation, and temperature (12 ± 2 °C) as well as static incubation. Furthermore, how the composition of the microbial community shifted during the degradation of EDB was investigated, and the specific functional genes that might be involved in the degradation of EDB were quantified.

2. Methods

2.1. Soil and groundwater

Soil core samples (0.15 m diameter × 3 m length) were obtained by sonic core drilling from the Fuel Spill-12 (FS-12) site (MMR, MA) in early October, 2009. Samples were taken at a depth of 61–64 m below the ground surface. This area represented an aerobic zone with varying concentrations of EDB, based on groundwater monitoring data (AFCEE, 2007). The EDB concentration within the aerobic zone ranged from just above the Massachusetts MCL of 0.02 μg/l to approximately 23 μg/l. The dissolved oxygen concentration was between 4.5 and 5.5 mg/l. Annual groundwater temperature was 12 ± 31 °C and the pH value was 6.5. Upon retrieval of the cores, visual inspection revealed a mix of coarse and fine grained sand with a light to medium brown color, indicating an aerobic zone. Samples were extruded, in a manner as to minimize disturbance, in 30 cm intervals into headspace free sterile Pyrex glass containers (volume 1.8 l) and transported on ice to the laboratory, where they were maintained at 4 °C until analyzed.

2.2. Chemicals & bacterial strains

EDB (>99% purity) was purchased from Sigma–Aldrich. Methane (>99.9%) was purchased from Supelco (Belleonte, PA). All solvents (hexane and methanol) were HPLC grade. All other chemicals were ACS reagent grade.

Mycobacterium sp. GP1 and *Polaromonas* sp. JS666 were obtained from the research groups of D. Janssen (University of Groningen, The Netherlands) and J.M. Gossett (Cornell University, USA), respectively. Both pure culture strains were used as positive controls for the detection of specific functional genes.

2.3. Microcosms

Microcosms were prepared with six parallel treatments: (1) no amendment control, (2) addition of mixed trace elements [2 ml addition of a solution of (per liter), $FeSO_4 \cdot 7H_2O$ 3000 mg, $ZnSO_4 \cdot 7H_2O$ 70 mg, $MnCl_2 \cdot 4H_2O$ 20 mg, H_3BO_3 20 mg, $CaCl_2$ 100 mg, $CuCl_2$ 100 mg, $NiCl_2$ 20 mg, $Na_2MoO_4 \cdot 2H_2O$ 30 mg] and vitamin B_{12} (0.001%), (3) addition of methane (final conc. 52 nmol/bottle), (4) addition of methanol (final conc. 128 mg/l), (5) addition of jet fuel JP-8 (with final conc. 50 mg/l), and (6) addition of lactate (final conc. 224 mg/l). Fresh groundwater (800 ml) and soil (200 g) were added into 1.2 l Pyrex bottles supplied with a Teflon septum under the main lid, and a Mininert® valve (Aldrich, St. Louis, MO) at an additional entry port (Glass Blowing Facility, UMass, Amherst). The killed controls were autoclaved for 20 min, and in addition 0.04% (w/v) sodium azide was added. To maintain conditions similar to sampled environmental conditions, the microcosms were incubated at 12 ± 2 °C without agitation in the dark. All experiments were performed in triplicate.

2.4. Analytical methods

EDB was extracted using EPA method 504.1 and monitored with a 5890 series II Plus Hewlett–Packard chromatograph, equipped

Download English Version:

https://daneshyari.com/en/article/7085600

Download Persian Version:

https://daneshyari.com/article/7085600

Daneshyari.com