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Biodegradation of decabromodiphenyl ether (BDE-209) by bacterial mixed cultures in a soil/water system

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ABSTRACT

Decabromodiphenyl ether (DBDE) is a brominated flame retardant that is commonly used in many commercial products. Sorption of DBDE within a soil/water system can result in serious bioaccumulation within the ecological system and be a threat to human health. Little is known about aerobic DBDE biodegradation, and the influence of the UV light radiation on DBDE biodegradation has not been considered. This study, for the first time, isolates DBDE biodegrading aerobic mixed bacterial cultures from DBDE-contaminated soil/water systems in Taiwan. The aerobic biodegradation of DBDE as a sole carbon source in the presence of 365 nm UVA irradiation over 10 months was investigated using a clay/water system. The rate constants for DBDE degradation gave values ranging from 0.0121 to 0.0134 day^{-1} in the presence of UV irradiation, which were significantly higher than the 0.0092–0.0102 day^{-1} values obtained in complete darkness. The aerobic metabolites: 2′,3′-dihydroxy-4-bromodiphenyl ether and 2′,3′-dihydroxy-diphenyl ether were identified by GC–MS. Debromination was ascribed to UV irradiation and biodegradation by facultative aerobic bacteria in the micro-anaerobic environment of the clay/water system. The products of debromination included 12 PBDE congeners (tri- to hexa-BDEs) and their concentrations ranged from 34.28 to 83.80 mg l^{-1}. Specific bacteria capable of degrading PBDEs and carrying out nitrification/denitrification were identified. The present findings suggest that systems using a novel combination of photolysis and biodegradation could be developed to carry out DBDE remediation in the future.

1. Introduction

Polybrominated diphenyl ether (PBDE) additives are most commonly used as brominated flame-retardants (BFRs). The chemicals are used in many commercial products including electronic circuit boards and cases, furniture, building materials, textiles, carpets, plastics, airplanes and vehicles (WHO, 1994; Polo et al., 2004). A large amount of solid waste containing PBDE is generated and disposed of at treatment facilities such as MSW landfills and incinerators. PBDE pollutants, such as leachate and ash, are discharged into the environment and have emerged as a major source of pollution across the world. The concentration of PBDE congeners is increasing rapidly worldwide although many developed countries (USA, Canada and Japan) and international organizations (OECD, EEC, etc.) have enforced strict regulations on their use and disposal. Nevertheless, developing countries still continue to use PBDE congeners as a BFR for many purposes.

Deca-BDE, which is called DBDE or BDE-209 in this study, makes up 75% of all PBDE congeners used as BFRs. PBDE congener products with lower brominated numbers (LBNs) are formed by photolytic reactions and by biotransformation when commercial DBDE mixtures are exposed to the natural environment (de Wit, 2002). Mai et al. (2005) indicated that debromination of BDE-209 produces LBN PBDE congeners (tri- to hexa-BDE) such as 2,2′,4,4′-tetrabrominated diphenyl ether (BDE-47), 2,2′,4,4′,5-pentabrominated diphenyl ether (BDE-99) and 2,2′,4,4′,6-pentabrominated diphenyl ether (BDE-100); these have been found to commonly exist in the natural environment. PBDEs have physiochemical characteristics that include low water solubility (<0.1 mg l^{-1}) and high lipophilicity (Log K_{ow} = 6.62–9.97). As a result, their sorption onto many environmental matrixes is almost complete and lasts for a long time (Fangstrom et al., 2005). The widespread distribution of PBDE congeners and their high resistance to degradation means that they are frequently detected in soil/water systems, including surface sediments (Toms et al., 2008; Chen et al., 2010), WWTP sludges (Cincinelli et al., 2012), soils (Wang et al., 2011; Gevao et al., 2011) and similar environments. In Taiwan, 24 PBDE congeners were found at concentrations ranging

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from 103 to 244 μg g^{-1} dry wt. sediment in selected rivers from 2004 to 2010 (Taiwan EPA, 2012). DBDE is the major pollutant making up a large proportion of all PBDE congeners in all samples. An aggressive technology that can bring about DBDE remediation is needed and this approach needs to be applicable to soil/water systems.

PBDE congeners have a high potential in terms of bio-accumulation in humans and wildlife, including fish and mammals, due to movement of the chemicals through food chain trophic levels within ecosystems (Rotander et al., 2012; Yogui et al., 2011; Sjödin et al., 2003). A high risk to human health is generated because PBDEs accumulate in blood, breast milk, and fatty tissue. For example, epidemiological studies have reported an association between PBDE congeners exposure and decreased thyroid function (Herbstman et al., 2008) and impaired spermatogenesis (Akutsu et al., 2008). The diseases of nerve system have been attributed to PBDE exposure due to neurodevelopmental toxicity (Darnerud et al., 2001). PBDEs have been identified as endocrine disruptors and as the cause of carcinogenic diseases (Meeker et al., 2009; Darnerud, 2008; Charboneau and Koger, 2008). Specific PBDEs have been classified as possible human carcinogens by the US EPA (CDC, 2004).

The structure of PBDEs is similar to that of polychlorinate biphenyl (PCBs), but with an oxygen atom between the aromatic rings. The microbial degradation of PBDE congeners is extremely slow, which is similar to the situation with PCB congener biodegradation. Few studies are available exploring the biotic and abiotic transformation of PBDE congeners. Mechanisms of PBDEs biodegradation include anaerobic debromination and the cleavage of the aromatic rings. Biodegradation of high bromide number (HBN) PBDE congeners (hepta-nano-BDE) is known to occur under anaerobic conditions. Gerecke et al. (2005) demonstrated the 30% removal of DBDE by biodegradation in anaerobic sludge over 238 days (Gerecke et al., 2005). Mixed bacterial cultures, which are equipped with a wide range of different enzymes, are likely to be more effective at PBDE degradation. When two selected strains; *Sulfurospirillum multivorans* and *Dehalococcoides* sp. were used to degrade DBDE, it was found that they were unable to completely debrominate DBDE in an anaerobic environment. *S. multivorans* was able to break down DBDE to octa-BDE and hepta-BDE, but was unable to continue on to produce lower bromine number PBDEs. The *Dehalococcoides* sp. was unable to debrominate DBDE, but was able to breakdown octa-BDE to di-BDE (He et al., 2006). Aerobic biodegradation of PBDE congeners has focused on LBN PBDE congeners as a sole carbon source. Kim et al. (2007) reported that *Sphingomonas* sp. PH-07 was able to aerobically breakdown several LBN BDE congeners (Mono- and di-BDEs). PCB-degrading bacteria, such as *Rhodococcus jostii* RHA1, *Burkholderia xenovorans* LB400 and various other strains, including *Rhodococcus* sp. RR1 and ether-degrading *Pseudonocardia dioxanivorans* CB1190, have been identified as capable of carrying out the aerobic degradation of LBN PBDEs (Robrock et al., 2009). A new aerobic stain *Pseudomonas stutzeri* is isolated to biodegrade BDE-47 as a sole carbon source (Zhang et al., 2013).

Photolysis by solar or UV light is one important potential approach to the transformation of PBDEs in various environmental matrix (Söderström et al., 2004; Eriksson et al., 2004). UV radiation is able to generate active free radicals that can destroy the complex chemical structure of persistence organic pollutants (POPs). The chemical reduction of PBDEs is called debromination and when UV irradiation is present, debromination occurs resulting in the release of a series of bromide atoms. Previous studies have studied this reaction and shown that reductive debromination by photodegradation in aqueous solution is able to produce five PBDEs (BDE-47, BDE-100, BDE-99, BDE-154 and BDE-153 (Sanchez-Prado et al., 2005)). Shih and Wang (2009) showed that the order of photolytic products during DBDE degradation is BDE-206, then BDE-207 and finally BDE-208. The debromination of BDE-15 by the UV light irradiation in aqueous solution resulted in the conversion into the diphenyl ether. Reductive debromination of BDE-15 to BDE-3 by photolysis was found to occur via homolytic C–Br bond cleavage (Rayne et al., 2003).

Biodegradation of halogen-containing POPs by UV light radiation has great potential as remediation method for contaminated soil. Aerobic biodegradation of organic compounds can be accelerated by photodegradation (Marsolek et al., 2008). Tamer et al. (2006) applied a combination of the UV radiation and biological degradation to a mixture of 4-chlorophenol (CP), 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP). This integrated remediation approach has a number of advantages including a reduction in the economic cost of remediation and the effective removal of halogen-containing POPs. Bacteria have a number of DNA repair pathways if their genetic material is affected by the radicals formed during UV irradiation (Walker, 1984). These DNA repair mechanisms are ubiquitous and a source of bacterial UV tolerance (Miller and Kokjohn, 1990). Stress-induced responses, such as oxidative protection, are known to occur in parallel to the induction of DNA damage repair mechanisms (Hengge-Aronis et al., 1993). Specifically, Suh et al. (2009) demonstrated the potential ability to carry out PBDE biodegradation in the presence of UV irradiation.

The objective of this study is to investigate the aerobic biodegradation of DBDE as a sole carbon source in the presence or absence of UV irradiation. Mixed bacterial cultures, isolated from either PBDE-contaminated river sediments or municipal wastewater treatment plant (WWTP) activated sludge was used to bring about the biodegradation of DBDE as a sole carbon source for the first time. Removal efficiency and the kinetics of DBDE degradation were explored in a soil/water system. All possible byproducts were measured in order to understand the series of reactions that brings about the biological/chemical debromination and biodegradation of DBDE. The changes in the bacterial community during DBDE biodegradation were monitored by the PCR-denaturing gradient gel electrophoresis (DGGE). Specific bacteria and their functions in the soil/water system were identified by PCR and cloning. The findings of this research provide details that help to evaluate the possibility in the future of using a novel DBDE remediation system, namely biodegradation in the presence of UV irradiation.

2. Materials and methods

2.1. Chemicals

2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether (called DBDE or BDE-209; 99% purity), which was used as a sole carbon source for biodegradation, was obtained from Alfa Aesar (Karlsruhe, Germany). In addition, a standard solution of DBDE (50 μg ml^{-1}) dissolved in the isooctane–toluene solution (9:1, v/v) was brought from AccuStandard, Inc. (New haven, CT, USA) and used for the GC analysis. A standard solution of 24 BDE congeners mixture dissolved in isooctane-toluene (8:2, v/v) was purchased from Wellington Labs. (Guelph, Canada) and used for the HSGC/HSMS analysis; the congeners consisted of BDE-17, BDE-28, BDE-47, BDE-49, BDE-66, BDE-71, BDE-77, BDE-85, BDE-99, BDE-100, BDE-119, BDE-126, BDE-138, BDE-153, BDE-154, BDE-156, BDE-183, BDE-184, BDE-191, BDE-196, BDE-197, BDE-206, BDE-207 and BDE-209. All organic solvents used in this study were of HPLC grade. All chemicals used in this study were of reagent grade with a purity above 99%. The Milli-Q water was double-distilled and deionized by a Millipore water purification system. The stock and experimental