SOILS, SEC 5 • SOIL AND LANDSCAPE ECOLOGY • RESEARCH ARTICLE

Biostimulation enhanced the biotic degradation of hexabromocyclododecane in sediments

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Abstract

Purpose The aim of this study was to investigate biodegradation of γ-hexabromocyclododecane (γ-HBCDD) under conditions mimicking three bioremediation strategies: (i) biostimulation: addition of sodium formate and ethanol to stimulate biodegradation as the carbon source and electron donor, respectively; (ii) bioaugmentation: addition of an enrichment culture of *Dehalobium chlorocoercia* strain DF-1; and (iii) natural attenuation: no amendments. To differentiate between biotic and abiotic mechanisms affecting γ-HBCDD degradation, four control microcosms were set up as sterile, negative, abiotic, and contaminant control.

Materials and methods Sediment microcosms were prepared in 20-mL bottles and operated as duplicate sacrificial reactors with a sediment-to-liquid ratio of 3 g wet solid:3.5 mL liquid. Total incubation time was 36 days with sampling every 4 days, except the last day. γ-HBCDD contents of sediments were extracted using ultrasonication and analyzed using GC-MS. Four control microcosms were used to observe the effect of (i) microbial activity (sterilization with mercuric chloride and autoclaving), i.e., sterile; (ii) microbial culture without DF-1 cells, i.e., negative control; (iii) sediments, where kaolinite is used instead of sediments, i.e., abiotic control; and (iv) γ-HBCDD, where no analyte is added, i.e., contaminant control.

Results and discussion Biostimulation showed the highest γ-HBCDD biodegradation rate ($k = 0.0542$ day^{-1}) and enhanced biodegradation compared to natural attenuation ($k = 0.0155$ day^{-1}). Bioaugmentation ($k = 0.0123$ day^{-1}) with DF-1 strain showed a sharp decrease at the beginning, but could not maintain this trend afterwards. Paired comparison of microcosms yielded no statistically significant difference between bioaugmentation and natural attenuation; hence, DF-1 strain did not improve degradation when compared to natural attenuation. This was also substantiated by observations from the negative control set. Sterile and abiotic control sets showed no significant concentration change in time. Consequently, adsorption was not considered as a significant mechanism acting on γ-HBCDD concentration change in our sediment microcosms. Thus, γ-HBCDD decrease observed in bioremediation microcosms was attributed to microbial activity.

Conclusions We reported effective analyte degradation with biostimulation. This was the first study to test bioaugmentation for HBCDD degradation, but we observed no enhancement of degradation with the DF-1 strain tested. Previous studies observed HBCDD reduction in their sterilized controls, hence reported total biotic and abiotic degradation rate. In this study, comparative evaluation of three test and four control microcosms enabled identification of only anaerobic biodegradation rates for γ-HBCDD, providing useful information for bioremediation of contaminated sites.

Keywords Biodegradation · Bioremediation · Flame retardant · Hexabromocyclododecane · Sediment

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1 Introduction

Hexabromocyclododecane (HBCDD) was widely used as a flame retardant in extruded, expanded, and high-impact polystyrene foams for thermal insulation in buildings, and in upholstery furniture, automobile interior textiles, car cushions, and electrical equipment (Covaci et al. 2006; Marvin et al. 2011). Its wide usage resulted in its occurrence in environmental matrices, i.e., soils, sediments, sewage sludge, dust, and atmosphere, and in biota, such as fish, birds, and aquatic species including mammals (Covaci et al. 2006). Since it is persistent in the environment and can have toxic effects, HBCDD production and usage are now regulated (Stockholm Convention 2018). However, its presence in consumer products leads to its ongoing existence in waste streams and eventually in water bodies.

When HBCDD enters the water bodies, it accumulates in aquatic sediments due to its low aqueous solubility (0.0656 mg L^{-1}) and high log Kow (5.62) (other properties are given in Table A1, Electronic Supplementary Material (ESM); European Commission 2008; Marvin et al. 2011). Hence, its fate in the environment is of concern. Few studies so far have investigated the biodegradation of HBCDD in soil, sediment, and sludge (Davis et al. 2005, 2006; Gerecke et al. 2006; Stiborova et al. 2015; Le et al. 2017; Peng et al. 2018). These studies used mineral salt medium (Davis et al. 2006; Le et al. 2017; Peng et al. 2018), nutrients (Gerecke et al. 2006; Stiborova et al. 2015), and primers (Gerecke et al. 2006) to enhance biodegradation. For anaerobic degradation in aquatic sediments, on the other hand, no amendments were made (Davis et al. 2005, 2006). These studies revealed HBCDD half-lives in the range of 0.66 to 115.5 days under anaerobic conditions and 11 to 63 days under aerobic conditions (Davis et al. 2005, 2006; Gerecke et al. 2006; Peng et al. 2018). Furthermore, bacterial culture isolates were used to monitor HBCDD degradation under aerobic (Yamada et al. 2009) and anaerobic conditions (Peng et al. 2015) in the absence of a solid phase.

A number of previous studies on HBCDD biodegradation in the literature showed a decrease in total HBCDD concentration in sterile reactors. Studies of Davis et al. (2005, 2006) and Gerecke et al. (2006) showed a considerable total HBCDD concentration decrease (e.g., 48% decrease in 14 days in sediments, reaching not detected values in 61 days) in sterile control sets. Therefore, rates identified in those studies were stated to reflect degradation rates for the total of biotic and abiotic mechanisms, as differentiation could not be made.

The aim of this study was to investigate biodegradation of γ-HBCDD under various conditions mimicking bioremediation strategies: natural attenuation, bioaugmentation, and biostimulation. For the purpose of differentiating biotic and abiotic mechanisms for HBCDD degradation, control microcosms were set up as sterile, negative control, and abiotic control, as well as a contaminant control set with no HBCDD. Bioaugmentation and biostimulation are the two techniques frequently used for in situ bioremediation of sediments contaminated with halogenated organics (Bedard 2003; Payne et al. 2011). To the best of our knowledge, introducing a microorganism culture in sediments, i.e., bioaugmentation, for the degradation of γ-HBCDD was not investigated previously.

2 Experimental

2.1 Chemicals

Solvents (n-hexane (HEX), dichloromethane (DCM), acetone (ACE)) used for analysis, anhydrous Na$_2$SO$_4$ (granular), copper fine powder (< 63 μm), and aluminum oxide (0.063–0.200 mm) were purchased from Merck KGaA (Darmstadt, Germany). Individual γ-HBCDD isomer was purchased from AccuStandard (New Haven, USA). Internal standard PCB-209 was supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and surrogate standard BDE-208 was purchased from Wellington Laboratories (Canada).

2.2 Sediment microcosms

Surface sediments under 70 cm water depth were collected from five different points in a pond in Camkoru Natural Park (a specially protected forest area) in Ankara, Turkey, and were wet-sieved (2 mm) on-site to remove large particles. Moisture content of the sediment was determined by drying at 105 °C in an oven overnight and found as 36.5 ± 1.53%. Total organic content was then determined by igniting this dried sample at a 550 °C furnace for 4 h (Heiri et al. 2001) and found as 1.43 ± 0.16%. The sediment pH was 8.57, analyzed right after sediment collection. Sediments had a particle size distribution from 0.02 to 2000 μm (Table A2 and Fig. A1 (ESM)). Elemental analysis of sediments was performed using X-ray florescence spectrometry, and the results are given in Table A3 (ESM).

Sediments had no previous HBCDD contamination, as confirmed by GC-MS analysis. The target compound of this study was γ-HBCDD, since it was the dominant isomer in the commercially used technical mixture and in most environmental matrices (Covaci et al. 2006). The target initial γ-HBCDD concentration was 1000 ng g^{-1} dry weight (dw). We selected this initial concentration because it was in the range of previously reported sediment HBCDD levels (0.1–6740 ng g^{-1} as reported by Guerra et al. 2008; Klosterhaus et al. 2012; Li et al. 2012; He et al. 2013; Zhang et al. 2018), and constituted an acceptable concentration considering the instrument sensitivity. Sediment contamination with γ-HBCDD was performed in laboratory. γ-HBCDD standard in toluene was spiked onto air-dried

sediments and mixed until the solvent evaporated. Then, wet sediments were added in this mixture and mixed again to obtain a homogeneous mixture (Tokarz III et al. 2008). Approximately 3 g of this mixture was added in microcosm bottles. Contamination control set was prepared similarly, but spiking the sediments with only toluene.

Microcosms were prepared as duplicate sacrificial reactors: each set was prepared as 16 bottles to be sampled at eight predetermined times. At each sampling time, the full sediment content of two randomly selected bottles was analyzed. Microcosms were operated for 36 days, sampling performed every 4 days except for the last sample. In all reactors, sediment-to-liquid ratio was kept constant as 3 g wet solid:3.5 mL liquid.

Three test microcosms were operated: (i) the biostimulation set contained an organic medium as the liquid portion on top of the sediments. Organic medium was prepared by dissolving several vitamins and minerals in water under $N_2:CO_2$ atmosphere at pH = 6.8 as described by Berkaw et al. (1996). In order to stimulate biodegradation, 10 mM of sodium formate and ethanol was added into the medium as the carbon source and electron donor, respectively. (ii) The bioaugmentation set included *Dehalobium chlorocoercia* strain DF-1 culture. DF-1 was grown in Prof. Dr. Kevin Sowers' Laboratory of Institute of Marine & Environmental Technology, University of Maryland, Baltimore, MD, USA. This strain was enriched from Charleston Harbor sediments and was shown to be successful in anaerobic dehalogenation of PCBs (Payne et al. 2011), chlorobenzenes (Wu et al. 2002; May and Sowers 2016), and PBDEs (Demirtepe 2017; Demirtepe and Imamoglu 2019). It was grown anaerobically in the same organic medium used in biostimulation, using sodium formate as electron donor and PCB-61 as electron acceptor (Berkaw et al. 1996; Payne et al. 2011). To observe the activity of DF-1 cells in sediments, we added 0.5 mL DF-1 culture medium, which corresponded to approximately 6×10^5 cells g^{-1} sediment, as suggested by Payne et al. (2011). (iii) The natural attenuation set had no amendments to sediments, containing only distilled water and contaminated sediment.

Apart from the three test microcosms, four sets of control microcosms were prepared: (i) negative control set was prepared as a control for bioaugmentation, to observe the effects of the culture medium only. For this purpose, a spent growth medium was used, after passing it through 0.22-μm filter to eliminate DF-1 cells. (ii) Sterile control set was established with contaminated sediments and distilled water, with addition of mercury chloride (0.5 mg $HgCl_2$/g sediment) and autoclaving at 120 °C at 1.1 atm pressure for 20 min on three consecutive days to hinder any microbial activity in sediments. (iii) Contamination control set was established with clean sediments (no HBCDD spike) and distilled water, to check for any contamination resulting from incubation conditions in sediments or during analytical procedures. (iv)

Abiotic control set was prepared to differentiate the effect of sediments on γ-HBCDD degradation. For this purpose, kaolinite ($Al_2Si_2O_5(OH)_4$) was used as the abiotic solid medium. It was washed with distilled water and dried in an oven at 105 °C overnight on three consecutive days. Similar to sediments, kaolinite was spiked with γ-HBCDD, mixed vigorously, and distributed to microcosm bottles. Distilled water was then added on top of the kaolinite.

All sets were purged with high-purity nitrogen stream after closing with Teflon-lined septa crimp caps. They were incubated in the dark at 25 °C. During sampling, liquid part was discarded using Pasteur pipettes, and wet sediments were freeze-dried prior to the analysis for γ-HBCDD content. We did not monitor biodegradation products, or system parameters (i.e., nutrients, volatile fatty acids, headspace gas) other than pH.

2.3 Extraction and analysis

The freeze-dried sediment was weighted, was mixed with equal amounts of anhydrous Na_2SO_4, and was extracted twice in 30 mL HEX:DCM:ACE mixture (7:7:1 v/v) by ultrasonication for 30 min, after being soaked into the solvent mixture overnight. Copper powder was added into the extraction solvents for sulfur removal. The two extracts were combined and concentrated to 2–5 mL via a rotary evaporator. To remove any possible interfering organic compounds, the colored extract after the concentration step was treated with concentrated sulfuric acid (U.S. EPA Method 3665A; USEPA 1996). The top clear extract was purified with 0.5 g of alumina (deactivated to 3%) topped with anhydrous Na_2SO_4, and eluted with 5 mL of HEX, followed by 2 mL of HEX:DCM mixture (1:1). The collected extract was concentrated to 2 mL via a rotary evaporator.

One milliliter extract was spiked with internal standard and analyzed in Agilent 7890A GC 5975C inert mass spectrometry (GC-MSD) in EI mode with DB5-MS column (15 m × 0.25 mm ID × 0.10 μm). Injection temperature was 200 °C, ion source temperature was 230 °C, and quadrupole temperature was 150 °C. Helium was used as the carrier gas at a constant rate of 1.5 mL/min. Oven program was as follows: 60 °C for 1 min, raised to 200 °C at 15 °C/min, to 310 °C at 10 °C/min, and held for 5 min. Analysis in scan mode revealed that primary/secondary ions (m/z) used for confirmation are 79/159.1 for HBCDD, 497.8/427.8 for PCB-209, and 721.6 for BDE-208. These ions were then used to analyze samples in SIM mode.

2.4 QA/QC and data analysis

Laboratory control samples (LCS) prepared with spiked sediments were analyzed to check the extraction efficiency, and results yielded an average recovery of 89.3 ± 7.92% (range, 81.4–97.2% for $n = 3$) for γ-HBCDD. γ-HBCDD recovery for LCS with spiked kaolinite was 90.6 ± 8.14% ($n = 4$).

Method detection limits were 34.6 ppb and 80.3 ppb, and limits of quantitation were 110.1 ppb and 255.5 ppb, for γ-HBCDD and BDE-208, respectively. Blanks were analyzed in every batch of 15 samples, and no peaks were detected during analysis. Average surrogate recovery for the whole data set was $86.5 \pm 14.3\%$ (range, 62.4–122.9%). No surrogate and/or blank correction was performed.

The statistical analysis was performed with SPSS 21. Paired t test was used to evaluate the difference between two microcosm sets, and analysis of variance (ANOVA) within-subject design was used to identify the variance within the data set of a reactor.

The degradation kinetics of γ-HBCDD was explained by pseudo-first-order model (Eq. (1)):

$$C = C_0 e^{-kt} \qquad (1)$$

where C is the concentration at sampling times (ng g^{-1} dw), C_0 is the initial γ-HBCDD concentration (ng g^{-1} dw), k is the pseudo-first-order rate constant (day^{-1}), and t is the incubation time (day). Rates were calculated by plotting $\ln(C/C_0)$ vs t, and goodness-of-fit was checked using coefficient of determination, R^2.

3 Results and discussion

3.1 Degradation of γ-HBCDD in test microcosms

We observed clear reduction trends in γ-HBCDD concentrations in all test reactors; no trend was observable in sterile reactors, while slight decrease was observed in the negative control and to a lesser extent in abiotic reactors. The concentration ranges of duplicate reactors analyzed for each sampling time are shown in Fig. 1. The relative percent difference of γ-HBCDD levels in parallel reactors was in the range of 0.74–51.8% (median = 12.3%). As deduced from QA/QC data, variations less than 15% can be attributed to reproducibility. Results from a number of parallel reactors, on the other hand, showed variations higher than 15%, which might be due to the general nature of biological systems, i.e., experimental variation owing to microbial activity, or experimental reasons pertaining to sacrificial operation of microcosms, i.e., uneven distribution of HBCDD between microcosm bottles. Although not explicitly mentioned in the paper, a similar variation was noticeable in anaerobic sediment microcosms of Davis et al. (2005) in which bottles were also sacrificed at each sampling time. For the rest of our discussions, average of parallel microcosm results was used. Percent remaining HBCDD in each set based on average concentrations is given in Fig. A2 (ESM). Additionally, Fig. A3 (ESM) shows the linear trend estimate for each set, using average of parallel microcosms.

The average initial γ-HBCDD concentration in test microcosms ranged 811–862.5 ng g^{-1} dw sediment, while at the end of incubation, they were between 114.5 and 561.6 ng g^{-1} dw (Fig. 1). Both the initial and the final concentrations were in the range of environmental HBCDD levels reported in sediments so far (Guerra et al. 2008; Klosterhaus et al. 2012; Li et al. 2012; He et al. 2013; Zhang et al. 2018). Environmental
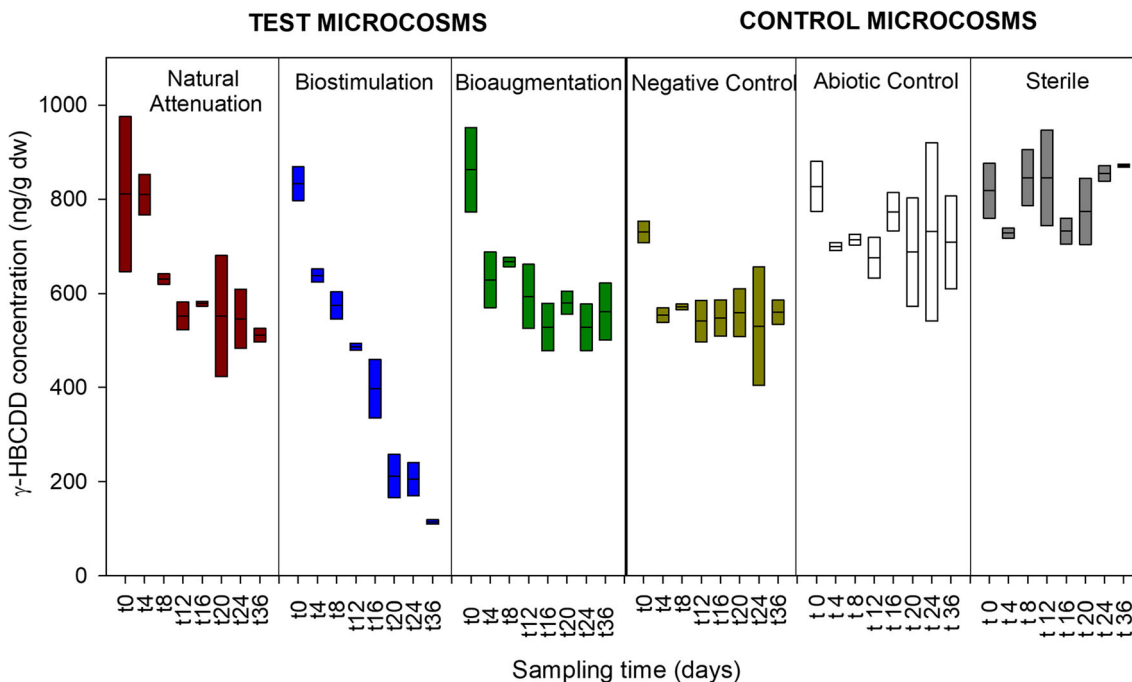

Fig. 1 Change in γ-HBCDD concentrations in microcosms (ng g^{-1} dw). Values for parallel reactors are given as a range, and average of parallel reactors is shown by horizontal lines in the boxes. Contamination control did not yield any γ-HBCDD and not plotted

levels, on the other hand, would be expected to vary depending on whether the site was contaminated due to a past accidental large-scale discharge or has been receiving low levels of continuous discharges. In any case, levels observed in environmental sediments may be regarded as impacted from natural attenuation processes. Due to degradation in sediments, lower HBCDD concentrations than the final concentrations observed in our study might be expected considering that our sediments were incubated for only 1 month. On the other hand, higher concentrations than ours might also be observed since environmental conditions may not be as favorable for microbial activity when compared to our laboratory conditions. Nevertheless, our results indicate that there is a good chance environmental risk associated with high concentrations of γ-HBCDD-contaminated sediments could be alleviated via stimulating microbial degradation.

As can be seen from Fig. 1, the highest reduction in γ-HBCDD was achieved in the biostimulation set, with an overall 86% decrease in initial concentration and a continuous sharp decrease throughout the incubation time (see also Fig. A2 (ESM)). Supplying the sediments with necessary nutrients resulted in a clear enhancement of biodegradation. In natural attenuation, HBCDD started to decrease after the fourth day, achieving a total of 37% reduction at the end of 36 days. Sediments were not historically contaminated with γ-HBCDD; hence, indigenous microbial species might be expected to degrade the analyte at a slower pace, without the help of any amendments. Bioaugmentation with DF-1 strain did not exhibit a sharp decrease as was observed for biostimulation. At the end of incubation period, 35% reduction in γ-HBCDD was achieved, and a plateau seemed to be reached after approximately 12 days. Overall, it can be deduced from the results that bioaugmentation with DF-1 strain did not enhance γ-HBCDD degradation when compared to natural attenuation.

Biodegradation of organic compounds is affected by many environmental conditions, such as pH, organic matter content of the matrix, and presence of substrates, among others (Ghattas et al. 2017). The system pH in our microcosms also showed how microbial degradation proceeded (Fig. 2). In biostimulation microcosms, the system pH was above 6.5 at all times (range, 6.5–8), which falls in the optimum pH range for anaerobic microbial degradation (stated as 6.5–8.2 for successful anaerobic processing in reactors, according to Speece (1996)). On the other hand, in natural attenuation, prepared with distilled water, pH was between 4.5 and 5.5 during incubation. The pH observed in this microcosm is lower than the optimum pH range for anaerobic microbial activity. Previous studies demonstrated lower anaerobic degradation rates of organic compounds in soil at pH around 5 compared to that at higher pH values (Chang et al. 2002). Similarly, HBCDD concentration reduction in our natural attenuation set was much lower than that of the biostimulation set. Lastly, the

pH of bioaugmentation set fluctuated from 5.75 to 7.5. Overall, the pH records also support the favorable abundance of anaerobic microbial activity in biostimulation microcosms.

The sediments used in this study had an average of 1.43% total organic matter, which would correspond to approximately 0.83% organic carbon (Nelson and Sommers 1996). Considering the low solubility and high Kow of HBCDD (Marvin et al. 2011), it would be expected to partition strongly onto sediments, even with low organic content. This can decrease bioavailability of HBCDD and might affect microbial degradation (National Research Council 2003).

The presence of carbon and energy sources in sediments for microbial activity and growth may vary depending on the site conditions, and availability of electron donor can be directly related to degradation of organic compounds (Himmelheber and Hughes 2014). Amendments made in the biostimulation set are therefore believed to promote microbial activity in sediments, when compared to natural attenuation where the only available nutrients were the ones present in the sediments. Similarly, in a previous study of our group, biostimulation of the same sediments provided a greater BDE-209 degradation than natural attenuation (Demirtepe and Imamoglu 2019). These findings are quite promising because they enable a competitive advantage for bioremediation of γ-HBCDD-contaminated sediments.

System parameters such as nutrients, volatile fatty acids, headspace gas, or biodegradation products of γ-HBCDD were not investigated within the scope of this study. The only other study that reports a relevant system parameter was Davis et al. (2006) where methane levels measured in the headspace gas of test microcosms were used as an indication for the presence of microbial activity. Bromide ions were also measured in a couple of studies to monitor debromination (Peng et al. 2015, 2018). Biodegradation products measured previously indicated dihaloelimination mechanism (Davis et al. 2006; Peng et al. 2015). However, Peng et al. (2018) proposed the possibility of transformation mechanisms other than reductive debromination due to the absence of bromide ions in biodegradation microcosms.

3.2 γ-HBCDD concentration change in control microcosms

The concentration ranges of duplicate control reactors analyzed for each sampling time are shown in Fig. 1. We report four important findings from control microcosms: (1) γ-HBCDD concentration showed variations in the sterile reactors over time; however, we found no statistically significant change in time for this set (ANOVA within-subject design, $F(1,7) = 0.05$, $p > 0.05$). (2) The negative control set showed a sharp decline at the beginning and remained unchanged afterwards. The initial decline, as was also observed in bioaugmentation, may be attributed to the triggering effect of

Fig. 2 pH of microcosms during incubation period (average of parallel microcosms)
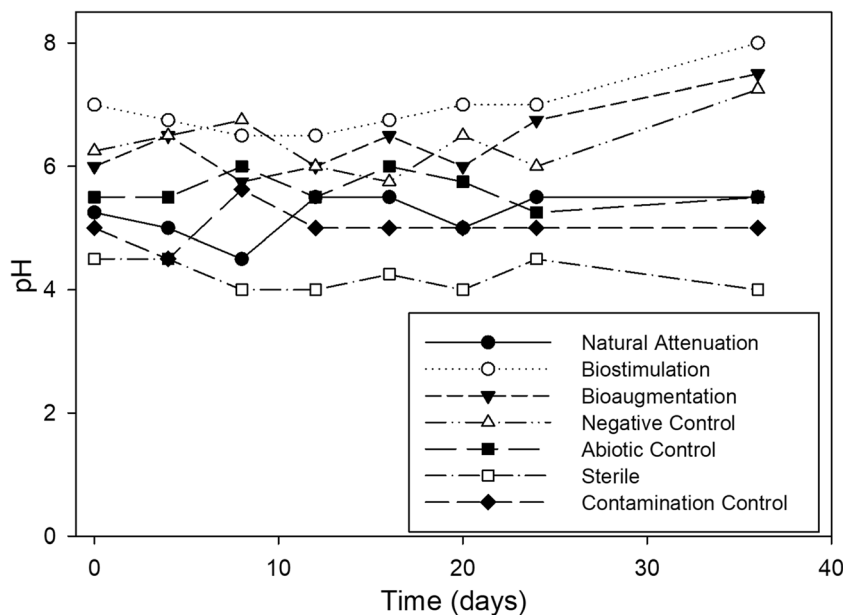
culture media added. γ-HBCDD degradation nearly stopped after the fourth day, possibly due to consumption of nutrients. Hence, we may deduce that initial decrease in bioaugmentation set was due to the effect of culture media, and not the DF-1 cells themselves. The overall reduction in γ-HBCDD in negative control set was 23% at the end of 36 days. (3) The abiotic control set, i.e., kaolinite microcosms, demonstrated a variation between sampling times, but our analysis yielded no statistically significant change in time (ANOVA within-subject design, $F(1,7) = 1.99$, $p > 0.05$). (4) No γ-HBCDD was detected at any time in the contamination control set.

An interesting observation from the literature is that previous studies on HBCDD degradation all showed decrease in HBCDD levels to some extent in their sterile/control reactors (Davis et al. 2005, 2006; Gerecke et al. 2006; Le et al. 2017; Peng et al. 2018). For instance, in Davis et al.'s (2005) study, total HBCDD reached "not detect" levels in 60 days in sterile sediments, while 33% reduction was observed in 113 days in the Davis et al. (2006) study. They observed remarkable decrease in concentration also in sterile soil and sludge microcosms (Davis et al. 2005, 2006). The control reactors showed no viable cells in cell count; therefore, authors concluded that abiotic degradation mechanisms (e.g., presence of coenzymes such as vitamin B_{12}) were acting on HBCDD reduction in these reactors (Davis et al. 2006). Nevertheless, our sterile set did not demonstrate any significant change in time. Hence, our study showed that addition of mercuric chloride and autoclaving the sediments concurrently successfully inhibited any microbial activity and the reactivity of enzymes.

Le et al. (2017) observed a 9% decrease in total HBCDD in control reactors after 21 days under anaerobic conditions and related this phenomenon to sorption of HBCDD on soil. Similarly, Peng et al. (2018) concluded that sorption

occurred in autoclaved sediment microcosms, which showed 49% reduction in 3 days. We set up a control reactor using kaolinite for the purpose of investigating any possible abiotic mechanism affecting γ-HBCDD. It can be considered similar to our sediments in terms of elemental content. SiO_2 and Al_2O_3 contents of our sediment were 52.5% and 16.3% (Table A3 (ESM)), while those of kaolinite are 46.5% and 39.5% (theoretical), respectively. Although the variation observed in the abiotic control set was not statistically significant, the γ-HBCDD concentrations (for parallel microcosms) measured in the first day are higher than all other measurements, except the 24th day. Variations in concentration ranges are observable with no clear decreasing trend in time. Figure A3 (ESM) also shows the linear trend estimate for this set has a milder slope when compared to that of the other sets. This could indicate initial adsorption of HBCDD with no further change in time, afterwards. However, if adsorption was acting on sediments, then a similar trend would have been observed for the sterile reactors, which was not the case. Previous studies on sorption of organic compounds on sediments and kaolinite revealed lower log K_d and log K_f^0 values for kaolinite when compared to sediments (Site 2001). For instance, log K_d of kaolinite was 3.22 for Aroclor 1254, while that of bay sediments was 4.05 (Site 2001). Therefore, sorption of halogenated organic compounds is expected to be higher on sediments than kaolinite, which would mean the effect of adsorption would have been more prominent in the sterile set when compared to the abiotic control set. Since this is not the case, we believe adsorption is not a significant mechanism acting on γ-HBCDD concentration change in our sediment microcosms, and hence, the observed decrease in γ-HBCDD levels in our test microcosms was due to anaerobic biodegradation of γ-HBCDD.

Table 1 Paired sample *t* test scores between microcosm pairs (*NA*, natural attenuation; *BS*, biostimulation; *BA*, bioaugmentation; *NC*, negative control; *AC*, abiotic control; *St*, sterile). Paired *t* test statistic *t* is presented with *p* scores given in parentheses ($p < 0.05$ indicates a significant difference between pairs)

	NA	BS	BA	NC	AC
NA					
BS	3.51 (0.01)				
BA	0.18 (0.86)	− 3.11 (0.02)			
NC	1.52 (0.17)	− 1.91 (0.10)	2.35 (0.05)		
AC	− 2.75 (0.03)	− 3.66 (0.01)	− 3.44 (0.01)	− 10.53 (< 0.001)	
St	− 3.48 (0.01)	− 3.94 (0.01)	− 4.58 (0.003)	− 8.14 (< 0.001)	− 3.03 (0.02)

3.3 Paired comparison of microcosms and comparison of results with literature

To elucidate the differences between reactors, we conducted paired sample *t* test. Table 1 presents *t* scores for paired *t* test statistic and *p* scores (given in parenthesis) representing the statistical significance of difference between samples, corresponding to the given test statistic *t*. We observed significant differences between abiotic control set and others, and between sterile set and others ($p < 0.05$). Hence, these control reactors simulated the absence of microbial activity as they were planned to be. Additionally, the difference between these two control reactors was significant ($t(7) = -3.03$, $p < 0.05$). Natural attenuation showed no significant difference with the bioaugmentation set ($t(7) = 0.18$, $p > 0.05$). Therefore, we concluded that introducing DF-1 strain in sediments had no effect on HBCDD degradation and that DF-1 was not able to degrade HBCDD in sediment microcosms. Negative control set showed no significant difference with any of the test reactors ($p > 0.05$). Addition of a small amount of culture media in this set could not supply the necessary nutrients for enhanced biodegradation; hence, this can explain the similarity with natural attenuation and bioaugmentation. However, a clear reason could not be found for no significant difference between biostimulation and negative control.

We compared the results with limited literature on HBCDD degradation. Previous studies reported degradation of HBCDD mixture with varying reduction percentages in various solid media and conditions. Aquatic sediments with no amendments demonstrated 61.5% reduction in 113 days (Davis et al. 2006). The reduction was approximately 27% at around 30 days (Davis et al. 2006), which was similar with the percent reduction observed in our natural attenuation set. Digester sludge incubated with a mineral salt medium also showed a comparable reduction percentage with our biostimulation set, with 90% reduction in 28 days (Davis et al. 2006). Similar results were also obtained in a very recent sediment microcosm study which showed approximately 90% reduction of HBCDD mixture in sediments with anoxic basal salt medium after 38 days (Peng et al. 2018). On the other hand, Le et al. (2017) found that HBCDD mixture degradation percentage in both rhizosphere and non-rhizosphere soils receiving mineral salt medium was lower than our observation in the
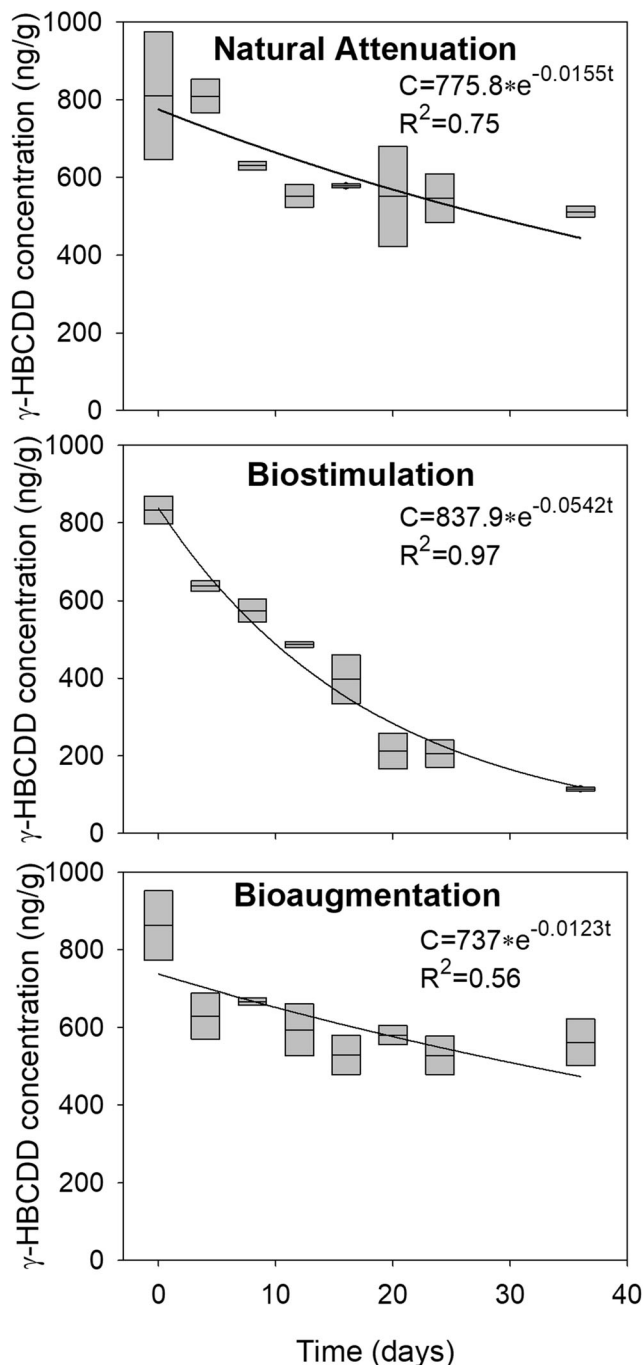

Fig. 3 Pseudo-first-order degradation kinetics for test microcosms with fit equations and corresponding R^2 values

Natural Attenuation
$C = 775.8 * e^{-0.0155t}$
$R^2 = 0.75$

Biostimulation
$C = 837.9 * e^{-0.0542t}$
$R^2 = 0.97$

Bioaugmentation
$C = 737 * e^{-0.0123t}$
$R^2 = 0.56$

biostimulation set. Variations in incubation conditions (including incubation time and concentration), microbial diversity, contents, and characteristics of the media studied are likely to result in variations in anaerobic degradation. Another possible reason for variations in degradation might be the use of HBCDD mixture in literature studies and instead the gamma-isomer in the present study. Variation in degradation of isomers could impact overall degradation rates reported in the literature.

3.4 γ-HBCDD degradation rate

The γ-HBCDD degradation rate in sediment microcosms was explained by pseudo-first-order reaction kinetics as shown in Fig. 3. The calculated degradation rates and half-lives are given in Table 2. The biostimulation set showed the highest degradation rate among all with 0.0542 day^{-1}. Natural attenuation and bioaugmentation sets revealed low R^2 values for pseudo-first-order reaction kinetics, yet did not fit zero-order reaction kinetics, either (data not shown). It was clear that biostimulation enhanced the degradation of HBCDD in sediments with 3.5 times higher rate with respect to natural attenuation. It should however be emphasized that, under environmental conditions, lower degradation rates would be expected due to the presence of other contaminants and less favorable conditions such as low temperature and nutrient availability (Magar et al. 2005).

We compared our calculated rate constants with all available relevant degradation rates reported in the literature (Table 2). Generally, our rates were lower, except for the anaerobic sediment microcosms of Davis et al. (2006). Freshwater sediment microcosms without any additional substrates revealed very similar rates with our natural attenuation set (Davis et al. 2006). γ-HBCDD degradation in pure culture revealed higher degradation rate than that in our study (Peng et al. 2015). Relatively higher rates were expected with pure culture isolates, while in the presence of a solid phase, degradation rates would be anticipated to be lower due to the sorption of compound on solid phase, i.e., limited bioavailability of compounds in solid phase (Semple et al. 2004; Payne et al. 2013). However, previous studies showed that anaerobic degradation in soil and sediments (Davis et al. 2005; Peng et al. 2018) and sewage sludge (Gerecke et al. 2006; Davis et al. 2006) was much faster than that in bacterial strains (Peng et al. 2015). Additionally, studies reporting isomer-specific degradation rates were scarce. Nevertheless, the reported γ-HBCDD rates were very similar to the rates of HBCDD mixture, i.e., same up to two significant digits (Davis et al. 2006). The order of degradation rates of isomers varied according to environmental media and conditions and microbial consortia as well (Gerecke et al. 2006; Davis et al. 2006; Peng et al. 2015; Le et al. 2017; Karahan 2018). However, two studies on contaminated aquatic sediments both found the order of degradation rates as β- > γ- > α-HBCDD (Davis et al. 2006;

Table 2 Degradation rate constants for pseudo-first-order reaction kinetics of this study and comparison with literature

Media and conditions	Isomer	k (day^{-1})	$t_{1/2}$ (day)	R^2	Reference
Lake sediment microcosms without any substrates	γ	0.0155	44.7	0.75	This study
Lake sediment microcosms with carbon source + electron donor	γ	0.0542	12.8	0.97	
Lake sediment microcosms with *Dehalobium chlorocoercia* strain DF-1	γ	0.0123	56.3	0.56	
Anaerobic soil microcosms with activated sludge simulating land application of sludge	All	0.10[a]	6.9	–	Davis et al. (2005)
River sediment microcosms without any substrates	All	0.45[a]	1.5	–	
Creek sediment microcosms without any substrates	All	0.61[a]	1.1	–	
Digester sludge microcosms with mineral salt medium	All	0.1278	5.4	–	Davis et al. (2006)
	α	0.1327	5.2		
	β	0.1522	4.6		
	γ	0.1248	5.6		
Freshwater sediment microcosms without any substrates	All	0.0109	63.6	–	
	α	0.0060	115.5		
	β	0.0140	49.5		
	γ	0.0113	61.3		
Creek sediment microcosms with mineral salt medium	All	0.31	2.24	–	Peng et al. (2018)
Digester sludge microcosms with nutrients and primers	All	1.1	0.66	–	Gerecke et al. (2006)
Mixed liquor reactors with inoculums of two individual isolated strains[b]	α	0.1286/0.1217	5.4/5.7	0.94/0.96	Peng et al. (2015)
	β	0.0847/0.0726	8.2/9.6	0.89/0.96	
	γ	0.0789/0.0721	8.8/9.6	0.96/0.98	

[a] Calculated from the half-lives given

[b] Results obtained from two different strains given as separated with "/"

Karahan 2018). The results of our study on γ-HBCDD degradation together with those from literature can then be used to understand the abundance of this isomer in environmental matrices.

The relation between soil/sediment organic carbon and HBCDD degradation cannot be found due to a limited number of studies on HBCDD degradation and even less (only Davis et al. 2005, 2006) that report organic carbon content of their matrices. Higher HBCDD degradation rates were observed in sediments with higher organic carbon content, but sediment microcosms of Davis et al. (2006) report degradation rate that is very similar to our natural attenuation set, although their sediments had three times higher organic carbon than ours. These HBCDD degradation studies had factors other than organic carbon changing in their experimental design, such as initial concentrations and incubation conditions. Since none of these studies, including ours, were designed to investigate the sole effect of organic carbon content, it was not possible to arrive at a conclusion on the relation of organic content and degradation rates.

4 Conclusions

We investigated the anaerobic degradation of γ-HBCDD in sediments under various conditions to assess the efficiency of bioremediation strategies: natural attenuation, biostimulation, and bioaugmentation. Concurrent investigation of these strategies along with four control sets enabled a comparative evaluation of degradation behavior and rates. We found that biostimulation of sediments with a carbon source and electron donor–rich organic medium resulted in more than tripling the degradation rate. Bioaugmentation with *Dehalobium chlorocoercia* strain DF-1 yielded no statistically significant difference with natural attenuation, revealing the limited ability of this strain to degrade a cycloaliphatic compound.

The degradation rates reported previously in the literature represented total biotic and abiotic degradations of HBCDD in environmental matrices due to observed reduction in sterilized controls. In this study, comparative evaluation of three test and four control microcosms enabled the identification of only anaerobic biodegradation rates for γ-HBCDD in sediments. These rates can be used in designing bioremediation of contaminated sites, where anaerobic degradation will be the prevailing mechanism for the removal of HBCDD. Monitoring the contaminants in freshly spiked sediments under controlled laboratory conditions facilitates the assessment of bioremediation strategies. However, environmental conditions would probably result in lower degradation rates and require further inquiry. Overall, under favorable conditions, biostimulation holds potential to result in very low or even below detection γ-HBCDD concentrations in sediments.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

Bedard DL (2003) Polychlorinated biphenyls in aquatic sediments: environmental fate and outlook for biological treatment. In: Haggblom MM, Bossert ID (eds) Dehalogenation: microbial processes and environmental applications. Kluwer Academic Publishers, Boston, pp 443–465

Berkaw M, Sowers KR, May HD (1996) Anaerobic ortho dechlorination of polychlorinated biphenyls by estuarine sediments from Baltimore Harbor. Appl Environ Microbiol 62:2534–2539

Chang BV, Shiung LC, Yuan SY (2002) Anaerobic biodegradation of polycyclic aromatic hydrocarbon in soil. Chemosphere 48:717–724

Covaci A, Gerecke AC, Law RJ, Voorspoels S, Kohler M, Heeb NV, Leslie H, Allchin CR, de Boer J (2006) Hexabromocyclododecanes (HBCDs) in the environment and humans: a review. Environ Sci Technol 40:3679–3688

Davis JW, Gonsior S, Marty G, Ariano J (2005) The transformation of hexabromocyclododecane in aerobic and anaerobic soils and aquatic sediments. Water Res 39:1075–1084

Davis JW, Gonsior SJ, Markham DA, Friederich U, Hunziker RW, Ariano JM (2006) Biodegradation and product identification of [14C]hexabromocyclododecane in wastewater sludge and freshwater aquatic sediment. Environ Sci Technol 40:5395–5401

Demirtepe H (2017) Sustainable remediation of aquatic sediments contaminated with polybrominated diphenyl ethers and hexabromocyclododecane. Ph.D. Dissertation, Middle East Technical University, Ankara, Turkey

Demirtepe H, Imamoglu I (2019) Degradation of decabromodiphenyl ether (BDE-209) in microcosms mimicking sediment environment subjected to comparative bioremediation strategies. J Environ Manag 233:120–130

European Commission (2008) Risk assessment hexabromocyclododecane. European Communities, Luxembourg

Gerecke AC, Giger W, Hartmann PC, Heeb NV, Kohler HPE, Schmid P, Zennegg M, Kohler M (2006) Anaerobic degradation of brominated flame retardants in sewage sludge. Chemosphere 64:311–317

Ghattas AK, Fischer F, Wick A, Ternes TA (2017) Anaerobic biodegradation of (emerging) organic contaminants in the aquatic environment. Water Res 116:268–295

Guerra P, Eljarrat E, Barceló D (2008) Enantiomeric specific determination of hexabromocyclododecane by liquid chromatography-quadrupole linear ion trap mass spectrometry in sediment samples. J Chromatogr A 1203:81–87

He M-J, Luo X-J, Yu L-H, Wu JP, Chen SJ, Mai BX (2013) Diasteroisomer and enantiomer-specific profiles of hexabromocyclododecane and tetrabromobisphenol A in an aquatic environment in a highly industrialized area, South China: vertical profile, phase partition, and bioaccumulation. Environ Pollut 179: 105–110

Heiri O, Lotter AF, Lemcke G (2001) Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. J Paleolimnol 25:101–110

Himmelheber DW, Hughes J (2014) In situ biotransformation of contaminants in sediments. In: Reible DD (ed) Processes, assessment and remediation of contaminated sediments. Springer, New York, USA

Karahan I (2018) Investigation of biotic degradation of hexabromocyclododecane (HBCDD). MS Thesis, Middle East Technical University, Ankara, Turkey

Klosterhaus SL, Stapleton HM, La Guardia MJ, Greig DJ (2012) Brominated and chlorinated flame retardants in San Francisco Bay sediments and wildlife. Environ Int 47:56–65

Le TT, Son M-H, Nam I-H et al (2017) Transformation of hexabromocyclododecane in contaminated soil in association with microbial diversity. J Hazard Mater 325:82–89

Li H, Zhang Q, Wang P, Li Y, Lv J, Chen W, Geng D, Wang Y, Wang T, Jiang G (2012) Levels and distribution of hexabromocyclododecane (HBCD) in environmental samples near manufacturing facilities in Laizhou Bay area, East China. J Environ Monit 14:2591–2597

Magar VS, Brenner RC, Johnson GW, Quensen JF (2005) Long-term recovery of PCB-contaminated sediments at the Lake Hartwell superfund site: PCB dechlorination. 2. Rates and extent. Environ Sci Technol 39:3548–3554

Marvin CH, Tomy GT, Armitage JM, Arnot JA, McCarty L, Covaci A, Palace V (2011) Hexabromocyclododecane: current understanding of chemistry, environmental fate and toxicology and implications for global management. Environ Sci Technol 45:8613–8623

May HD, Sowers KR (2016) "Dehalobium chlorocoercia" DF-1 - from discovery to application. In: Adrian L, Löffler FE (eds) Organohalide-respiring bacteria. Springer, Berlin Heidelberg, Germany, pp 563–586

National Research Council (2003) Bioavailability of contaminants in soils and sediments processes, tools and applications. National Academies Press, Washington DC

Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter. In: Sparks DL, Page AL, Helmke PA et al (eds) Methods of soil analysis. Part 3—chemical methods. Soil Science Society of America, Inc., Madison, pp 961–1010

Payne RB, May HD, Sowers KR (2011) Enhanced reductive dechlorination of polychlorinated biphenyl impacted sediment by bioaugmentation with a dehalorespiring bacterium. Environ Sci Technol 45: 8772–8779

Payne RB, Fagervold SK, May HD, Sowers KR (2013) Remediation of polychlorinated biphenyl impacted sediment by concurrent bioaugmentation with anaerobic halorespiring and aerobic degrading bacteria. Environ Sci Technol 47:3807–3815

Peng X, Huang X, Jing F, Zhang Z, Wei D, Jia X (2015) Study of novel pure culture HBCD-1, effectively degrading hexabromocyclododecane, isolated from an anaerobic reactor. Bioresour Technol 185:218–224

Peng Y-H, Chen Y, Chang M, Shih Y (2018) The effect of zerovalent iron on the microbial degradation of hexabromocyclododecane. Chemosphere 200:419–426. https://doi.org/10.1016/j.chemosphere.2018.02.123

Semple KT, Doick KJ, Jones KC, Burauel P, Craven A, Harms H (2004) Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environ Sci Technol 38:228A–231A

Site AD (2001) Factors affecting sorption of organic compounds in natural sorbent/ water systems and sorption coefficients for selected pollutants. A review. J Phys Chem Ref Data 30:187–439

Speece RE (1996) Anaerobic biotechnology for industrial wastewaters. Archae Press, Nashville

Stiborova H, Vrkoslavova J, Pulkrabova J, Poustka J, Hajslova J, Demnerova K (2015) Dynamics of brominated flame retardants removal in contaminated wastewater sewage sludge under anaerobic conditions. Sci Total Environ 533:439–445

Stockholm Convention (2018) Stockholm Convention Website. http://chm.pops.int/TheConvention/ThePOPs/. Accessed 1 Oct 2018

Tokarz JA III, Ahn M-Y, Leng J et al (2008) Reductive debromination of polybrominated diphenyl ethers in anaerobic sediment and a biomimetic system. Environ Sci Technol 42:1157–1164

USEPA (1996) Method 3665A sulfuric acid/permanganate cleanup, Revision 1, December 1996, Final Update III to the Third Edition of the Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, EPA publication SW-846

Wu Q, Milliken CE, Meier GP, Watts JEM, Sowers KR, May HD (2002) Dechlorination of chlorobenzenes by a culture containing bacterium DF-1, a PCB dechlorinating microorganism. Environ Sci Technol 36:3290–3294

Yamada T, Takahama Y, Yamada Y (2009) Isolation of Pseudomonas sp. strain HB01 which degrades the persistent brominated flame retardant gamma-hexabromocyclododecane. Biosci Biotechnol Biochem 73:1674–1678

Zhang Y, Lu Y, Wang P et al (2018) Transport of hexabromocyclododecane (HBCD) into the soil, water and sediment from a large producer in China. Sci Total Environ 610–611:94–100