Formation of Lower Brominated Congeners by Anaerobic Degradation of Decabromodiphenyl Ether in Sewage Sludge

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Introduction

Decabromodiphenyl ether (^{deca}BDE-209[a]) is widely used as a flame retardant in products such as electronic equipment and upholstery furniture (annual global market demand: 56,100 tons in 2001[1]). Its environmental safety has been discussed controversially during the last several years. For example, a European Union risk assessment on ^{deca}BDE-209 recently concluded that there is a need for further information in the area of secondary poisoning, including studies on the degradation of ^{deca}BDE-209 into more toxic and bioaccumulative compounds (e.g. debromination to lower brominated congeners).

Debromination and dehalogenation in general has been described for a variety of halogenated compounds in anaerobic systems[2,3] and anaerobic debromination was also proposed for ^{deca}BDE-209[4]. To our knowledge, degradation of ^{deca}BDE-209 in anaerobic systems has not yet been reported,

In this paper, the transformation of ^{deca}BDE-209 into nona- and octabromodiphenyl ethers was studied in incubation experiments with sewage sludge as inoculum and appropriate controls over a period of 114 days. Alpha-hexachlorocyclohexane (α-HCH) was included to verify microbial degradation of halogenated compounds in our experimental setup. The results obtained enable an experimental test of the hypothesis that ^{deca}BDE-209 is degradable under anaerobic conditions.

Materials and Methods

Incubation of ^{deca}BDE-209 with sewage sludge: Several 300-ml glass serum bottles with a 1-cm layer of glass beads were spiked with stock solutions of technical DecaBDE (Aldrich, 98%), α-HCH as degradation reference (Dr. Ehrensdorfer) and five primers[5,6] (4-bromo benzoic acid (Fluka), 2,6-dibromobiphenyl (Accustandard), tetrabromo-bisphenol A (Aldrich. 97%), hexabromocyclododecane (Fluka, 95%), decabromo-biphenyl (Elf Atochem); 9 - 11 nmol each) and solvents were evaporated over night.

[a] Superscripts (deca, nona, octa etc.) in combination with the Ballschmiter number are used throughout this manuscript to facilitate the recognition of the number of bromine atoms present in a molecule.

Starch (20 mg) and yeast (50 mg) were added immediately before the bottle was filled with 20 mL freshly collected digested sewage sludge (mesophilic stabilizer, ARA Dübendorf, Switzerland, serving 45,000 inhabitants). The bottles were tightly capped and incubated at 37 ± 1 °C for up to 114 days in the dark. Control experiments with heat sterilized sludge (autoclavation at 120°C for 60 minutes, twice 24 hours apart) were performed simultaneously. After predetermined time periods, bottles were opened and internal standards were added ($^{13}C_6$-γ-HCH, $^{13}C_{12}$-^{hepta}BDE-183 and $^{13}C_{12}$-^{deca}BDE-209). Thereafter, the degradation was stopped by addition of conc. sulfuric acid (0.25 mL) and the sample was frozen until analysis.

Analysis: Samples were thawed and transferred into soxhlet thimbles. After air drying, extraction was performed with a 1:1 mixture of hexane and acetone for 6 hours in a soxhlet apparatus. Sulfur was removed by gel permeation chromatography and extracts were purified on a silica gel column. GC analysis was carried out using a DB-1 equivalent stationary phase (10 m × 0.28 mm, film 0.1 μm). Chiral analysis of α-HCH was performed on a column coated with 33% 2,6,-di-O-methyl-3-O-pentyl-β-cyclodextrin in OV-1701-OH (20 m × 0.25 mm, film 0.15 μm). Samples were injected on-column (1 μl). Positive ion EI-MS spectra were acquired on a MAT 95 high resolution mass spectrometer (Thermo Finnigan MAT) in single ion monitoring mode at an ionization energy of 60 eV and a mass resolution of 8,000.

Response factors for ^{nona}BDE-206 and ^{nona}BDE-207 versus $^{13}C_{12}$-^{deca}BDE-209 were determined and used for quantification. ^{nona}BDE-208 was quantified using the average response factors of ^{nona}BDE-206 and ^{nona}BDE -207. The mean of the response factors of ^{octa}BDE-198, -203, -204 and -205 versus $^{13}C_{12}$-^{hepta}BDE-183 was used for quantification of the total (partially co-eluting) octabromodiphenyl ethers.

During incubation and analysis, exposure to light was kept as low as possible, e.g. windows and fume hoods were covered with a UV filter foil. Anaerobic degradation and sterile control experiments were processed at the same time under identical conditions.

Results and Discussion

Microbial activity: Gas production was detected in the sample bottles, indicating methanogenic conditions during the incubations. No gas formation was observed in the sterile control samples. The degradation rate of α-HCH during the incubations was comparable to the one observed by Buser et al.[2] (Table 1) and indicated that the microbial community in our experiments was capable to degrade halogenated compounds with similar reactivity as reported previously. The change in the enantiomeric fractions during the incubations was a further indication for biological activity.

Table 1 Degradation rates of α-HCH

Species	Ref	Anaerobic degradation $k_{pseudo\ first\ order}$ [d^{-1}]	Sterile control $k_{pseudo\ first\ order}$ [d^{-1}]
α-HCH	this paper	0.40 ± 0.036	4.4×10^{-3} ± 3.4×10^{-4}
(+)-α–HCH	ref [2]	0.47	2.4×10^{-2}
(-)-α–HCH	ref [2]	0.17	2.4×10^{-2}

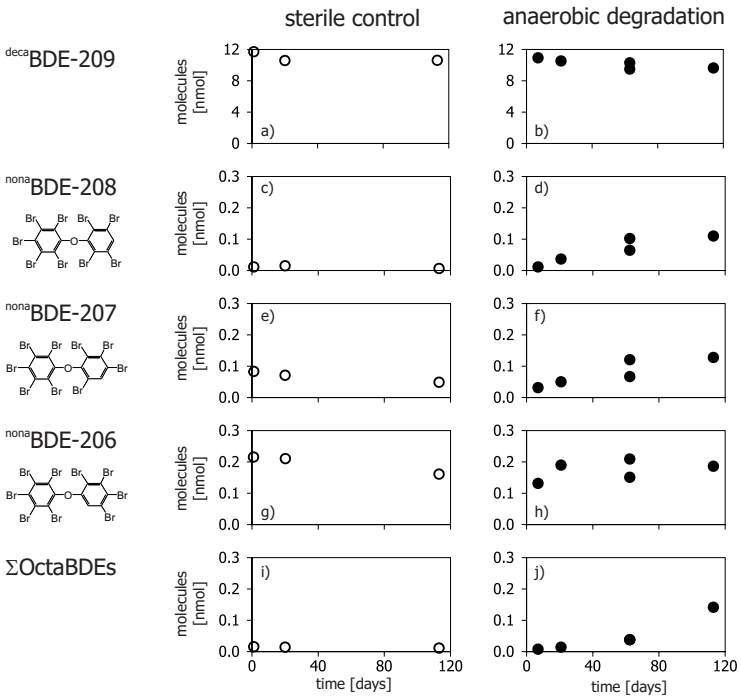

Figure 1 Measured levels of brominated diphenyl ethers in individual batches of sterile control (○) and anaerobic degradation experiments (●). At day zero, 11 nmol of ^{deca}BDE-209 were present. ^{nona}BDE-208 and octa brominated congeners were formed under anaerobic conditions in sewage sludge.

Degradation of ^{deca}BDE-209: Figure 1a and 1b illustrate the levels of ^{deca}BDE-209 over time determined in the sludge and sterile control. All measured values were within the margin of error (15%) of the initial level (11 nmol). Thus, it was not possible to deduce from these data that ^{deca}BDE-209 was degraded. Given the margin of error of 15%, at least 75% of the initial ^{deca}BDE-209 were still present after 114 days and hence the pseudo-first order degradation rate constant was below 3×10^{-3} d^{-1}.

Presence and formation of nona- and octabromodiphenyl ether congeners: As shown in Figure 2, the technical DecaBDE used in this work contained traces of two nona congeners (about 1% of ^{nona}BDE-206 and 0.2% of ^{nona}BDE-207). But, neither ^{nona}BDE-208 nor any octabromo congeners were detected in this technical mixture. In the sterile control experiments, neither ^{nona}BDE-208 nor any octabromo congeners were formed (Fig. 1c, 1i and 2e). In contrast, about 0.1 nmol of ^{nona}BDE-208 and octabromo congeners were measured after 114 days of incubation. Therefore, at least 2% of the spiked ^{deca}BDE-209 were reduced to ^{nona}BDE-208 and octabromo congeners. The data for ^{nona}BDE206 and -207 were not as conclusive. A microbially mediated degradation of ^{deca}BDE-209 into these congeners can not be shown based on our results.

Further, we can conclude that the observed debromination of the *para*-position leads to lower brominated congeners, being distinguishable from lower brominated congeners contained in technical Penta- and Octa-BDE formulations. In these products, all major congeners reported are brominated at both *para*-positions (exception: ^{tri}BDE-17).

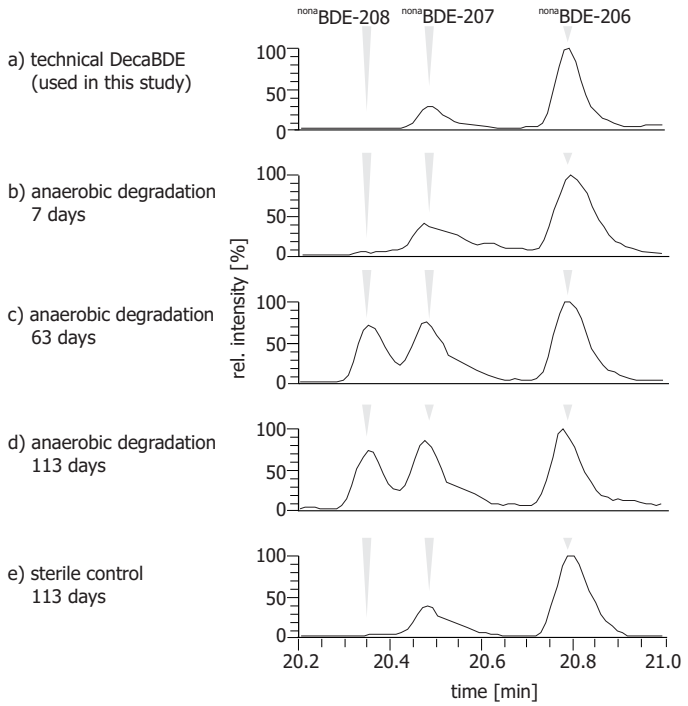

Figure 2 GC/HRMS mass traces of nonabrominated diphenyl ether congeners (m/z 719.425 + 721.423). Retention times of ^{nona}BDE208, -207 and -206 were 20.35, 20.48 and 20.78 minutes, respectively.

Conclusions

^{deca}BDE-209 undergoes debromination to nona- and octabromodiphenyl ethers under anaerobic (i.e. methanogenic) conditions in sewage sludge. However, the degradation rate ($<3 \times 10^{-3}$ d^{-1}) was more than a factor of hundred smaller as the one observed for α-HCH (4×10^{-1} d^{-1}). After 114 days, greater than 75% of ^{deca}BDE-209 remained.

As degradation rates are expected to vary greatly between individual anaerobic systems (sludge, sediment, soils), a rate constant that would be valid for other systems cannot be deduced. However, our results provide clear evidence that ^{deca}BDE-209 is not completely persistent in the anaerobic environment and that formation of lower brominated congeners has to be considered in future risk assessments.

References and Acknowledgment

1. Bromine Science and Environmental Forum, "*Major Brominated Flame Retardants Volume Estimates Total Market Demand by Region in 2001*", http://www.bsef-site.com/docs/BFR_vols_2001.doc.
2. Buser, H. R.; Müller, M. D. *Environ. Sci. Technol.* 1995, *29*, 664-672.
3. Bedard, D. L.; Van Dort, H. M. *Appl. Environ. Microbiol.* 1998, *64*, 940-947.
4. "EUR 20402 EN - European Union Risk Assessment Report bis(pentabromophenyl) ether, Volume 17", European Commission, 2002.
5. Wu, Q.; Bedard, D. L. et al. *Environ. Sci. Technol.* 1999, *33*, 1148-1148.
6. Deweerd, K. A.; Bedard, D. L. *Environ. Sci. Technol.* 1999, *33*, 2057-2063.

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