AEROBIC AND ANAEROBIC DEGRADATION OF POLYBROMINATED DIPHENYL ETHERS IN SEWAGE SLUDGE

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Introduction

Numerous organic compounds have been found in various ecosystems as a result of human activities all over the world. Brominated flame retardants (BFRs) are anthropogenic chemicals that are added to a wide variety of consumer/commercial products in order to improve their fire resistance. They are used as flame retardants in thermal insulation building materials, upholstery textiles and electronics. Based on up-to-date technologies there are about 20-25 classes of BRFs in the current production with at least three major classes: tetrabromobisphenol A (TBBPA) and its derivates, polybrominate diphenyl ethers (PBDE) and hexabromocyclododecanes (HBCDs)[1]. The production and use of BRFs has grown dramatically along with the growth in the use of synthetic polymers and the introduction of more rigorous fire safety requirements. Subsequent studies, primarily in Europe, North America and Japan indicate that BRFs are ubiquitous in sediments and biota and that their levels appear to be increasing rapidly [2,3,4]. Despite their heavy usage, there have been few studies concerning the transformation of these compounds [5,6,7]. Current knowledge suggests that these chemicals will accumulate in the environment and their fate is attributed to the structural stability and subsequently limited degradation[8]. The ability of soil microorganisms to degrade these POPs (persistent organic pollutants) is an important component of determining the fate of PBDEs in the contaminated environment. The purpose of the present study is to extend the knowledge of PBDEs degradation in industrially contaminated sewage sludge samples under aerobic and anaerobic conditions. This will help us to estimate their potential risks for the environment and humans.

Material and methods

Incubation under aerobic conditions

Two sewage sludge samples collected in waste water treatment plant from Hradec Kralove and Brno were chosen for the monitoring of PBDEs degradation. The samples were pooled in jars and stored on ice during the transport and then stored under 4 ^{o}C for no longer than 4 weeks. Slurries were prepared by mixing 15 g of wet sewage sludge and 35 ml of mineral medium[9]. For aerobic degradation three conditions were tested: A) sewage sludge mixed with mineral medium; B) yeast extract was added to the final concentration of 50 μg/ml; C) yeast extract (50 μg/ml) and 4-bromobiphenyl (0.6 μg/ml) for a priming effect. Flasks were incubated at 28 ^{o}C in the incubator (150 RPM) for 3 months in the dark. Control experiments with heat-sterilized sludge (autoclaved at 120 ^{o}C for 60 min, twice 24 h apart) were performed simultaneously. The negative effect of autoclaving on PBDEs composition and content was confirmed. Incubation and sterile control experiments were processed at the same time under identical conditions. Three parallel flasks for each tested condition were always determined for analysis of the PBDEs content.

Incubation under anaerobic conditions

Sewage sludge samples were resuspended in medium of Shelton and Tiedje[10] used for dehalogenators in volume ratio 40:60. 30 ml of suspension was filled up to 50 ml glass serum bottles. The degradation under two conditions was investigated. Subsequent nutrients were added: A) starch (20 mg), yeast extract (50 mg) and B) starch (20 mg), yeast extract (50 mg) and 4-bromobiphenyl. The bottles were tightly capped and incubated in the incubator at 28 ^{o}C (150 RPM) for 6 months in the dark. Control experiments with heat sterilized samples were performed as described for aerobic cultivation.

Analysis
Dry sample (sediment/sewage sludge) was mixed with anhydrous sodium sulphate to form a free flowing powder and transferred into Soxhlet extraction thimbles. Extraction was performed by dichlormethane in Soxhlet apparatus. The crude extract was carefully evaporated and the sample was consequently dissolved in solvent mixture cyclohexane-ethylacetate (1:1, v/v) that was used as a mobile phase in gel permeation chromatography (GPC) employing Bio Beads S-X3 column for separation of interfering co-extracts. Liquid – liquid extraction with an isooctane was used for isolation of BDE 209 from culture medium with sewage sludge samples. The mixture was shaken for 2 h to extract the BDE 209 into isooctane phase. The solvent phase isooctane was properly diluted and transferred to a glass vial and analysed by GC/MS-NCI.

Agilent 6890 (Agilent, USA) gas chromatograph equipped with a single quadrupole mass analyser Agilent 5975 XL operated in negative chemical ionization mode (GC/MS-NCI) and DB-XLB capillary was employed for routine analysis of PBDEs and HBCD in purified extracts. The GC conditions (PBDEs and HBCD) were as follows: capillary column DB-XLB column (30 m x 0.25 mm i.d. x 0.1 μm film thickness, J & W Scientific, Folsom, USA), column temperature program: from 105°C (hold 2 min) to 300°C at 20°C/min (hold 5 min); carrier gas: helium (Linde, Prague, Czech Republic) with constant flow 1.5 ml/min; injection temperature: 275°C; injection volume: 1 μl using pulsed splitless injection mode (splitless time: 2 min). Mass selective detector with quadrupole analyzer was operated in a selective ion-monitoring mode (SIM) in a negative chemical ionization (NCI). Monitored ions (m/z) were 79, 81, 159 and 161 (PBDEs); 79, 81, 158 and 160 (HBCD). Ion m/z 79 was used for quantification of all target analytes. Methane which was used as a reagent gas (purity 99.995%, Linde, Prague, Czech Republic) was set at a pressure 2 x 10.4 mbar. Ion source temperature was 150°C and quadrupole temperature 105°C. The presence of BDE 209 was monitored using the same GC/MS-NCI employing a shorter capillary column (BD-XLB (15 m x 0.25 mm i.d. x 0.1 μm film thickness J & W Scientific, USA). The temperature program was as follows: from 80°C (hold 2 min) to 280°C at 20°C/min and to 320°C at 5°C/min (hold 5 min); carrier gas: helium with constant flow 3 ml/min; injection temperature: 285°C; injection volume: 1 μl using pulsed splitless injection mode (splitless time: 2 min). Monitored ions (m/z) were 485 and 487; ion at m/z 487 was used for quantification.

Results and discussion:

Experiment set up
To simulate the real environmental conditions the degradation was studied in the industrially contaminated sewage sludge samples and degradation was achieved by indigenous microflora present in contaminated samples. The sewage sludge samples (Figure 1) from two locations (Hradec Kralove and Brno) were chosen to monitor the degradation of PBDEs under aerobic and anaerobic conditions. These two industrially contaminated samples were different in the content and composition of PBDEs. The total concentration of ten monitored congeners (BDE 28, 47, 49, 66, 85, 99, 100, 153, 154, 183) were 920.9 and 220.4 ng/g dry weight in Hradec Kralove and Brno, respectively. The major contaminating congener BDE 209 achieved the levels 685.3 ng/g dry weight in Hradec Kralove and 1402.6 ng/g dry weight in Brno.

Degradation of PBDEs under aerobic conditions
Degradation of PBDEs by indigenous microflora was performed under aerobic conditions. The effect of additional nutrients and priming effect of 4-bromobiphenyl to the degradation had no significant effect as shown in Figure 1. The removal of 10 congeners and BDE 209 corresponded to 30% and 20% decrease, respectively. These results describe the first aerobic degradation of decabrominated congener BDE 209 by microorganisms.

There are only two studies describing the aerobic degradation of PBDEs. Previous researches have shown that mono- and di-BDEs are subjected to the aerobic attack of dioxygenase by *Sphingomonas* sp. when bromocatechol and bromophenol are formed[11]. In the second study penta-BDEs mixture (DE-71) was biodegraded by consortium of soil microorganisms[7]. Pentabrominated congener BDE 99 was debrominated to tetrabrominated BDE 47 congener but formation of other by-products was not detected. Due to the mixture of PBDEs congeners in sewage sludge the identification of product was very difficult. Never less we detected two new formed unknown products during the degradation which identification is in a progress.

Degradation of PBDEs under anaerobic conditions

Shortly after incubation, gas production indicating methanogenic conditions started in all samples excluding autoclaved controls. Under both investigated conditions decrease in total concentration of 10 congeners was detected but the degradation was faster when the priming effect of 4-bromodiphenyl ether was exploited (Figure 2). In sewage sludge sample from Hradec Kralove the removal was higher (up to 50%) than in Brno (up to 30%) probably due to the higher initial concentration of 10 congeners and their availability for degradation. It is known that PBDEs are due to hydrophobic character strongly bound to solid particles such as soil, sediment, and sewage sludge. The removal of PBDEs under anaerobic conditions is dependent on the degree of bromination. Hence, lower brominated congeners (octa- and penta-) were debrominated, the significant removal of BDE 209 was detected only in sewage sludge sampled from Brno. The amount of BDE 209 in sewage sludge from Hradec Kralove remained constant even after six months of cultivation under both tested conditions (data not shown).

Acknowledgements

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References:

1. Boer J. *Environ. Chem* 2004; 1:81.
2. Covaci A., A.C. Gerecke, R.J. Law, S. Voorspoels, M. Kohler, N.V. Heeb, H. Leslie, C.R. Allchin and J. Boer. *Environ Sci Technol* 2006; 40: 3679.
3. Hale R.C., M.J. La Guardia, E.P. Harvey, N.O. Gaylor, T.M. Mainor and W.H. Duff. *Nature* 2001; 412:140.
4. Hyötyläinen T. and K. Hartonen. *Trends Anal Chem* 2002; 21: 13.
5. Gerecke A.C., P.C. Hartmann, N.V. Heeb, H.P.E. Kohler, W. Giger, P. Schmid, M. Zennegg and M. Kohler. *Environ Sci Technol* 2005; 39: 1078.
6. Gerecke A.C., W. Giger, P.C. Hartmann, N.V. Heeb, H.P.E. Kohler, P. Schmid, M. Zennegg and M. Kohler. *Chemosphere* 2006; 64: 311.
7. Vonderheide A.P., S. R. Muller-Spitz, J. Meija, G.L. Welsh, K.E. Mueller, B.K. Kinke, J.R. Shann and J.A. Caruso *J Anal At Spectrom* 2006: 21: 1232.
8. Palm A., I.T. Cousins, D. Mackay, M. Tysklid, C. Metcalfe and M. Alaee. *Environ. Pollut* 2002; 117: 195.
9. Zlamalikova J. (2006) Diploma theses, Institute of Chemichal Technology, Prague
10. Shelton D.R and J.M. Tiedje. *Appl Environ Microbiol* 1984; 56: 850.
11. Schmidt S., P. Fortnagel and R. M. Wittich. *Appl Environ Microbiol* 1993, Vol. 59: 3931.

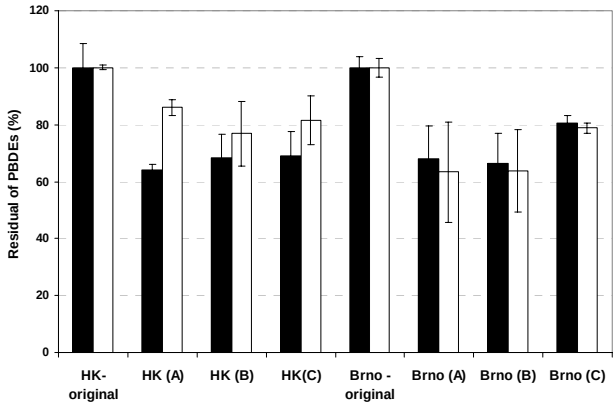

Figure 1: Percentage of residuals of total concentration of 10 congeners
■ (28, 47, 49, 66, 85, 99, 100, 153, 154, 183) and □ BDE 209 in sewage sludge from Hradec Kralove (HK) and Brno (B) after 3 months of cultivation under three conditions: A) sewage sludge; B) sewage sludge and yeast extract; C) sewage sludge, yeast extract and 4-bromobiphenyl.

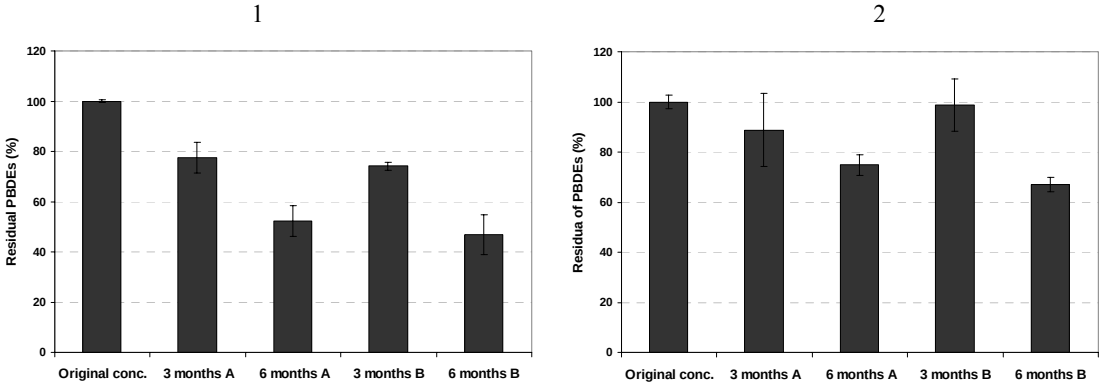

Figure 2.: Percentage of residuals of total concentrations of 10 congeners (28, 47, 49, 66, 85, 99, 100, 153, 154, 183) after 3 and 6 months of degradation under anaerobic conditions in sewage sludge samples
1) Hradec Kralove; 2) Brno; A) starch and yeast extract; B) starch, yeast extract and 4-bromobiphenyl.