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Biodegradation of Polybrominated Diphenyl Ethers in Liquid Media and Sewage Sludge by *Trametes versicolor*

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ABSTRACT: Polybrominated diphenyl ethers (PBDE) are widely employed as flame retardants and constitute a group of emerging pollutants of high persistence. The degradation of different PBDE commercial mixtures (penta-, octa- and deca-BDE) by the white-rot fungus *Trametes versicolor* under aerobic conditions was studied. This work demonstrates the capacity of *T. versicolor* to degrade three different PBDE commercial mixtures in aqueous phase, obtaining final removal efficiencies of 87±6, 85±13 and 67±7% for deca-, penta- and octa-BDE mixtures (and minimal degradation of 73±5, 38±13, and 28±7%), respectively. The intracellular enzymatic complex cytochrome P-450 is proposed as the enzyme involved in the first step of deca-BDE degradation by *T. versicolor.* In an effort to assay the application of the fungus in real contaminated matrices, the study successfully demonstrated the ability of *T. versicolor* to degrade pre-existent deca-BDE from sewage sludge in a solid-phase treatment, reaching 86% elimination at the end of the process. The findings support the potential use of the ligninolytic fungus in bioremediation of PBDE.

Key words: Polybrominated diphenyl ethers, White-rot fungi, Degradation, Transformation product, Sludge

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are included in the group of commercially produced chemicals referred to as brominated flame retardants, which are used in applications such as electronic manufacturing, textile industry and furniture production, where they constitute between 5 and 30% of the final product (Birnbaum and Staskal, 2004). Among all the existing PBDEs, the three products that have been most frequently used correspond to the commercial mixtures of penta-BDE, octa-BDE and deca-BDE and consequently, the PBDE congeners present in these mixtures are the most frequently detected in environmental samples (Agrell *et al.,* 2004; Eljarrat *et al.,* 2004; Hale *et al.,* 2006; Wang *et al.,* 2009). The penta-BDE and octa-BDE mixtures were banned in the European Union in 2004 and North American manufacturers ceased their production that year (Vonderheide *et al.,* 2008). Since July 2008, in the EU the use of deca-BDE has been banned in electrical and electronic applications. However, the production and usage of deca-BDE mixtures in other parts of the world is on the rise. During long time deca-BDE was considered as a practically non-toxic compound due to its very low solubility, which makes difficult its advancing up in the food chain. But in recent years, several studies have shown photodegradability of deca-BDE, even resulting in the production of lower brominated PBDEs after exposure to UV light (Ahn *et al.,* 2006; Christiansson *et al.,* 2009), which are more bioavailable and therefore more toxic than deca-BDE. The photodegradation products may correspond partially to congeners also present in penta-BDE and octa-BDE mixtures. For this reason, the congeners

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present in the three commercial mixtures studied in this work should be considered as priority pollutants among all the PBDEs. Several studies have demonstrated the ability of different microorganisms to degrade PBDEs under anaerobic and aerobic conditions. Most of them were focused on bacterial processes under anaerobic conditions (Gerecke *et al.,* 2005; He *et al.,* 2006; Lee and He, 2010; Robrock *et al.,* 2008; Shin *et al.,* 2010), which degrade different PBDE congeners by reductive debromination. The anaerobic debromination can cause an increase of the toxicity due to the increase of PBDEs solubility, as the process decreases the number of bromides in the molecule (Kuramochi *et al.,* 2007). Regarding aerobic degradation of PBDEs, few reports have been published and most of them correspond to degradation of the less brominated compounds, such as mono-BDEs or di-BDEs. To date, deca-BDE debromination by aerobic bacteria has only been reported for *Lysinibacillus fusiformis* strain DB-1 (Deng *et al.,* 2011). On the other hand, several strains of *Sphingomonas* sp. have shown the ability to transform different PBDE congeners, from mono-BDEs to tri-BDEs, under aerobic conditions by enzymatic hydroxylation reactions that produce the corresponding hydroxylated PBDEs (OH-PBDEs) (Kim *et al.,* 2007; Schmidt *et al.,* 1992). Similarly, studies by Robrock et al. (2009) with aerobic bacterial strains capable of degrading polychlorinated biphenyls, demonstrated the aerobic degradation of PBDEs with up to six bromines, including the major congeners present in the penta-BDE commercial mixture. Subsequently, the implication of biphenyl and ethylbenzene dioxygenases of *Rhodococcus jostii* RHA1 in PBDEs transformation was described and a dihydroxylated-BDE was detected as the primary metabolite of 4-bromodiphenyl ether (Robrock *et al.,* 2011).

Apart from bacteria, it is known that white-rot fungi (WRF) are also able to transform PBDEs. Hundt et al. (1999) demonstrated the ability of the WRF *Trametes versicolor* to degrade mono-BDE-4 by an initial hydroxylation reaction and the formation of a hydroxylated mono-BDE as a transformation product. Likewise, Zhou et al. (2007) achieved high removal of deca-BDE by a not specified WRF strain, however they only quantified removal and not degradation was demonstrated in that publication.

In the present study, the degradation of high bromination degree PBDEs (penta-BDE, octa-BDE and deca-BDE commercial mixtures) by *T. versicolor* was assessed in aqueous phase under aerobic conditions. Moreover, the formation of several potential transformation products and the possible role of fungal enzymatic systems in PBDEs degradation were studied. Finally, the degradation of pre-existent deca-BDE by *T. versicolor* was assessed in sterilized sewage sludge by means of a solid-phase treatment.

MATERIALS & METHODS

T. versicolor (ATCC #42530) was maintained by subculturing on 2% malt extract and 1.5% agar slants (pH 4.5) at 25 ºC. Subcultures were started every 30 d. Malt extract was obtained from Scharlau Co. (Barcelona, Spain). *T. versicolor* pellets were produced by inoculating 250 mL of malt extract medium with 1 mL of mycelial suspension, prepared as previously described (Vilaplana *et al.,* 2008). Defined medium contained 15 g/L glucose, 498 mg/L N as ammonium tartrate, 10 and 100 mL/L micro and macronutrient solutions (Kirk et al. 1986), respectively, 1.168 g/L 2,2-dimethylsuccinate buffer, 500 mg/L surfactant Tween 80 (Sigma-Aldrich, Barcelona, Spain) and pH was adjusted to 4.5. The other surfactants used in deca-BDE degradation experiments were Tween 20 (500 mg/L - Sigma-Aldrich), BS-400 (5% v/v - IEPSorbents, Madrid, Spain), and GoldCrew (14% v/v - Environmental Chemical Solutions, Gig Harbor, WA).

Octa-BDE and deca-BDE (98%) technical mixtures were purchased from Sigma-Aldrich (Barcelona, Spain), whereas penta-BDE mixture was purchased from Wellington Laboratories (Ontario, Canada).

PBDE Analytical Standard Solution, deca-BDE-209 and $^{13}C_{12}$-deca-BDE-209 (98%) standard solutions were purchased from Cambridge Isotope Laboratories (Andover, MA). The components of the PBDE Analytical Standard Solution were: three mono-BDEs (BDE-1, -2 and -3), seven di-BDEs (BDE-7, -8, -10, -11, -12, -13 and -15), eight tri-BDEs (BDE-17, -25, -28, -30, -32, -33, -35 and -37), six tetra-BDEs (BDE-47, -49, -66, -71, -75 and -77), seven penta-BDEs (BDE-85, -99, -100, -105, -116, -119 and -126), six hexa-BDEs (BDE-138, -140, -153, -154, -155 and -166) and three hepta-BDEs (BDE-181, -183 and -190). Nine OH-PBDE standards were purchased from Accustandard (New Haven, CT): 3'-OH-tri-BDE-28, 3-OH-tetra-BDE-47, 5-OH-tetra-BDE-47, 5'-OH-penta-BDE-99, 6'-OH-penta-BDE-99, 6-OH-penta-BDE-85, 6-OH-penta-BDE-100, 3-OH-hexa-BDE-154 and 6-OH-hexa-BDE-157. 4'-OH-tri-BDE-17, 6-OH-tetra-BDE-47 and 4-OH-penta-BDE-90 were kindly provided by Dr. Göran Marsh (department of Environmental Chemistry, Stockholm University, Sweden). Standard solutions were prepared and further diluted with isooctane or acetonitrile to obtain mixed fortifying and calibration standard solutions for all compounds. All solvents were of analytical grade. Purified laccase from *T. versicolor* was obtained from Fluka (Madrid, Spain).

In-vivo degradation experiments were performed in 100 mL amber serum bottles (Wheaton, Millville, NJ) to avoid PBDEs photodegradation during the process. First, each bottle was filled with the appropriate volume of the corresponding PBDE mixture stock solution in dichloromethane to reach a concentration of 10 mg/L in the experimental bottles in the case of deca-BDE mixture and 5 mg/L for penta-BDE and octa-BDE mixtures. The experimental bottles were left in the fume hood until the dichloromethane had completely evaporated. Then, defined medium (10 mL) and the amount of *T. versicolor* pellets equivalent to 3.5 g dry weight (dw)/L were added to each bottle. The bottles were incubated in an orbital shaker (135 rpm, r = 25 mm) at 25ºC. All experiments were carried out in triplicate. For those microcosms that were tested with the cytochrome P-450 inhibitor, 1-aminobenzotriazole (ABT) was added to a final concentration of 5 mM in 10 mL of defined medium with the amount of pellets equivalent to 3.5 g dw/L and deca-BDE mixture at a concentration of 2 mg/L, as described above. The bottles were incubated at 25ºC on an orbital shaker (135 rpm) for 38 h. Experiments included triplicates of uninoculated and heat-killed controls in all cases. Heat-killed controls consisted of autoclaved *T. versicolor* pellets cultures that had been pre-grown for 7 d under identical conditions to those of the experimental cultures. Degradation at a specified time interval was calculated by comparing concentrations in the heat-killed controls with those in the experimental bottles. Removal values at each time interval were calculated comparing concentrations in uninoculated controls with the concentrations in experimental bottles. Triplicate unitary samples were sacrificed at each time point for analysis.

Role of laccase in deca-BDE degradation was investigated using 100 mL amber serum bottles containing 10 mL of a purified laccase solution (final activity of 200 U/L) at pH 4.5. Laccase solution included 500 mg/L surfactant Tween 80. Deca-BDE was added at a final concentration of 2 mg/L. Effect of different mediators on laccase, such as ABTS [2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)], 1-hydroxy-benzotriazole (HOBT), violuric acid (VA), and 3,5-dimethoxy-4-hydroxyacetophenol (DMHAP), was tested at a final concentration of 0.8 mM (except for HOBT, 1 mM) into the reaction mixture. The bottles were incubated on an orbital shaker (135 rpm) at 25ºC for 38 h. Each treatment was carried out in triplicate.

For sample preparation, each experimental bottle (except for the samples corresponding to OH-PBDEs analysis) was subjected to a liquid-liquid extraction with 10 mL of dichloromethane. After sealing each bottle with Teflon-coated grey butyl rubber stoppers (Wheaton, Millville, NJ) and aluminium crimps (Baxter Scientific Products, McGaw Park, IL), the mixture was agitated in an orbital shaker (150 rpm) for 90 min. After that, 1 mL of the organic phase was filtered through a Millex-HV 0.45 µm filter (Millipore, Madrid, Spain) and transferred to a 2 mL amber glass vial (Agilent Technologies, Palo Alto, CA).

Removal of deca-BDE at environmental pre-existent concentrations was evaluated in sewage sludge collected from the WWTP of El Prat de Llobregat, in Barcelona, Spain. The plant has a total treatment capacity of two million equivalent inhabitants. It is a typical biological activated sludge plant with sludge anaerobic digestion and thermal drying. Dry sludge obtained from the final stage of processing, i.e., after thermal drying (~10% water content) was employed in sterile solid-phase systems inoculated with *T. versicolor* previously grown on wheat straw pellets (Rodríguez-Rodríguez *et al.*, 2011). Biopiles were incubated at 25 °C and continuously moisturized and homogenized for 42 d. To act as a control, raw sludge (i.e., fungus free) received the same processing as the treated sludge. One gram dw of sewage sludge was spiked with internal standards (BDE-77, BDE-181 and ^{13}C-BDE-209). A pressurized liquid extraction (PLE) was carried out using a fully automated ASE 200 system (Dionex, Sunnyvale, CA, USA). The temperature of cell extraction was heated to 100ºC. The solvent extraction was hexane: dichloromethane (1:1) mixture and the pressure reached 1500 psi. Then, extracts were treated with concentrated sulphuric acid (3x3 mL) in centrifuge tubes and subsequently purified with solid-phase extraction (SPE) alumina cartridges. Samples were finally concentrated to full dryness and re-dissolved in toluene prior instrumental analysis (Eljarrat *et al.*, 2008). For the analysis of PBDE congeners a previously described method by gas chromatography coupled to a mass spectrometer using negative ion chemical ionization (GC-NICI-MS) was applied (Eljarrat *et al.*, 2004). Furthermore, analyses by GC-EI-MS were carried out to obtain more structural information about other possible brominated products.

GC-combustion-isotope ratio mass spectrometry system (GC-CIRMS) analysis was performed to determine the mineralization of $^{13}C_{12}$-deca-BDE by the fungus, as previously described (Marco-Urrea *et al.*, 2008). The GC-CIRMS consisted of a GC (Agilent 6890N) coupled to a Thermo-Finnigan (Bremen, Germany) Delta Plus mass spectrometer through a FinniganMat GC combustion-III interface. $^{13}C/^{12}C$ ratios of headspace CO_2 were expressed as relative deviations δ‰ (delta per mille). The $\delta^{13}C$ value is defined as $\delta^{13}C=(R_s/R_r-1) \times 1000$, where R_s and R_r are the $^{13}C/^{12}C$ ratios in the sample and the international standard Vienna Peedee Belemnite, respectively.

Hydroxylated metabolites (OH-PBDEs) were searched by liquid chromatography coupled to a linear ion trap tandem mass spectrometrer (LC QqLIT-MS-MS). The chromatographic separation was done with a LC system Symbiosis Pico (Spark Holand, Emmen, Netherlands) using a C18 BetaBasic column (100mm x 2.1mm, 3um particle size) from Thermo Scientific, preceded by a C18 guard column (2.1 mm x 10 mm) from Waters. Water was used as solvent A and acetonitrile as solvent B at a 200 µL/min flow rate. Mass spectrometric analyses were performed with a hybrid triple quadrupole/linear ion trap Applied Biosystem MSD Sciex 4000 QTRAP (Applied Biosystems, Foster City, CA) working in atmospheric pressure chemical ionization (APCI) mode. Data acquisition was performed in selected reaction monitoring (SRM) in order to increase sensitivity. Two SRM transitions were monitored for each OH-PBDE degree of bromination, the most intense for quantification purposes and the second one for confirmation purposes. Transitions were 421>79 and 421>81 for OH-tri-BDEs; 501>79 and 501>81 for OH-tetra-BDEs; 579>79 and 579>81 for OH-penta-BDEs and 654>79 and 654>81 for OH-hexa-BDEs.

RESULTS & DISCUSSION

The capacity of the fungus *T. versicolor* in the form of pellets to degrade penta-BDE, octa-BDE and deca-BDE commercial mixtures in aqueous phase was evaluated. Table 1 shows the degradation and removal of deca-BDE mixture after 7 d and octa-BDE and penta-BDE commercial mixtures after 14.5 d. In the case of deca-BDE, degradation and removal values of 73 ± 5% and 87 ± 6% were achieved, respectively. The difference between those values indicates that a significant mass of pollutant (about 14%) was adsorbed on the fungus. It is remarkable that such efficiency was obtained in the presence of Tween 80, employed as a surfactant to increase the bioavailability of such hydrophobic compounds.

In the case of the other brominated BDE mixtures, GC-NICI-MS analysis permitted to detect a hepta (BDE-183) and a hexa (BDE-153) congener in the octa-BDE mixture; similarly, other components including one hepta (BDE-183), two hexa (BDE-154 and -153), two penta (BDE-100 and -99) and one tetra congener (BDE-47) were determined for the penta-BDE commercial mixture. Table 1 shows the fungal removal of both mixtures. Remarkably, all the detected congeners were eliminated at high extents; their adsorption on the fungus played an important role in most of the cases, resulting again in marked differences between minimal degradation and removal values. Comparing with adsorption results from deca-BDE, it is evident that the less brominated compounds are more easily adsorbed, which could be ascribed to their greater solubility and bioavailability to interact with biomass in aqueous phase.

Table 1. Degradation and removal of deca-BDE and the detected components of the octa-BDE and penta-BDE commercial mixtures by *T. versicolor*

	Degradation (%)	Removal (%)
Deca-BDE	73 ± 5	87 ± 6
Octa-BDE mixture components		
BDE-183	31 ± 7	62 ± 8
BDE-153	5 ± 12	98 ± 22
Total octa-BDE mixture congeners	28± 7	67 ± 7
Penta-BDE mixture components		
BDE-183	46 ± 35	48 ± 22
BDE-154	49 ± 9	99 ± 9
BDE-153	62 ± 18	93 ± 18
BDE-100	33 ± 11	90 ± 11
BDE-99	38 ± 17	79 ± 17
BDE-47	33 ± 10	90 ± 10
Total penta-BDE mixture congeners	38 ± 13	85 ± 13

Despite deca-BDE was considered for a long time as a practically non-toxic compound due to its low availability, the significant proportion of mass pollutant adsorbed on the fungus clearly demonstrates that it is bioavailable in the aqueous phase in the presence of surfactants. Considering that wastewater discharged into the environment often contains different types of surfactants, such as linear or composite polyethoxylated alkylbenzenes sulfonates (Cantero *et al.,* 2005; Terzic *et al.,* 2005), deca-BDE present in these conditions may also represent a hazard for living organisms.

The high deca-BDE removal by *T. versicolor* obtained in the presence of Tween 80 remarks the importance of surfactants to treat low solubility pollutants. Therefore, three other surfactants (Goldcrew, BS-400 and Tween 20) were used in deca-BDE degradation experiments for comparison purposes. Deca-BDE degradation in the presence of Tween 20 was 70 ± 12%, which is very similar to that obtained with Tween 80. It is reported that Tween 80, but not Tween 20, can become a substrate for the production of perlipidic radicals in the presence of laccase (Camarero *et al.,* 2008), thus improving its

oxidative capacity. In this case, the potential of Tween 80 to produce oxidative radicals had no evident effect on deca-BDE degradation by purified laccase, as described in section 3.2. Regarding the other two surfactants, which are used at in-situ bioremediation processes, deca-BDE degradations of 11 ± 11% and 52 ± 13% were obtained in the presence of BS-400 and Goldcrew, respectively. On one hand, it is quite evident that BS-400 is not appropriate to treat deca-BDE in aqueous phase under the experimental conditions tested. On the other hand, the degradation achieved in the presence of Goldcrew was significant and thus, this product is an interesting candidate for further study focused on in-situ bioremediation processes.

The role of the extracellular enzyme laccase and the intracellular enzyme cytochrome P-450 in deca-BDE degradation by *T. versicolor* was studied. Laccase is commonly limited to the oxidation of phenolic substrates, but in the presence of appropriate low molecular weight mediators, it is able to oxidize non-phenolic structures (Han *et al.*, 2004; Knutson and Ragauskas, 2004). An enzymatic degradation assay showed that deca-BDE is not influenced by the presence of laccase nor any of the laccase-mediator systems tested (data not shown), which means that this enzyme is not involved in the first step of deca-BDE degradation by the fungus. However, assays with and without ABT, a known cytochrome P-450 inhibitor, showed significant deca-BDE degradation only in inhibitor free-cultures (Table 2), while the amount of pollutant present in cultures containing the inhibitor was similar to that in heat-killed controls. These results suggest that cytochrome P-450 is involved in the first step of deca-BDE degradation by *T. versicolor*. These findings are consistent with the degradation pathway described for mono-BDE-4, also by *T. versicolor* (Hundt *et al.*, 1999). This coincidence could indicate that the cytochrome P-450 may be responsible for the generic degradation of compounds of the family of PBDEs.

Table 2. Effect of ABT, a cytochrome P-450 inhibitor, on deca-BDE degradation at a time of 38 h

	Deca-BDE mass in serum bottles (μg)
Heat-killed controls	12.6 ± 0.3
ABT-free controls	6.7 ± 0.3
Cultures containing 5mM ABT	14.4 ± 0.1

Mineralization of pollutants such as trichloroethylene or tetrachloroethylene has been demonstrated via cytochrome P-450 by *T. versicolor* (Marco-Urrea *et al.*, 2006; Marco-Urrea *et al.*, 2008). For this reason, a $^{13}C_{12}$-deca-BDE degradation experiment was performed using a concentration of labelled deca-BDE of 3 mg/L. However no evident deca-BDE mineralization was achieved under the experimental conditions (data not shown), thus suggesting the accumulation of organic transformation products. Nevertheless, the total absence of deca-BDE mineralization under the degradation conditions tested cannot be assured, as low pollutant mineralization could occur below the detection limit of $^{13}CO_2$ by GC-CIRMS analysis.

As no significant deca-BDE mineralization was obtained during degradation assays, different analyses were performed to detect the formation of metabolites from the transformation of the different PBDE mixtures. First, the formation of less brominated compounds was studied through GC-mass spectrometry analysis of samples at different treatment times. This was the same method used to quantify PBDE substrates. In any case, the formation of less brominated compounds was not detected during the process. Sequential debromination has been regarded as a transformation pathway of deca-BDE by bacterial communities in soil/water systems (Chou *et al.*, 2013) and penta- and octa-BDE technical mixtures by enrichment cultures containing strains of *Acetobacterium* sp and *Dehalococcoides* sp. (Ding and Chow, 2013) but mainly in anaerobic conditions. On the other hand, according to the role of cytochrome P-450 determined above for deca-BDE and previously reported for BDE-150, BDE-151 and deca-BDE in mammals (Badia-Fabregat *et al.*, 2012; Gago-Ferrero *et al.*, 2012; Morck *et al.*, 2003; North, 2004), it was expected that fungal degradation of PBDEs mixtures would yield very similar intermediate metabolites, i.e. OH-PBDEs and methoxy-hydroxilated-PBDEs. Therefore, samples corresponding to 12, 24, 72 and 169 h of treatment were analyzed as described before for OH-PBDEs. Despite the fact that some unknown peaks were observed, they did not match the criteria to be considered as OH-PBDEs. Likewise, the production of methoxy-hidroxylated-PBDEs from the fungal degradation of the PBDEs mixtures could not be demonstrated, as such metabolites were not detected in any sample.

As no extracellular transformation product from deca-BDE was detected, authors searched in the intracellular compartment. However, neither less brominated, hydroxilated nor methoxy hydroxylated congeners were detected. Moreover, it had been described that in these working conditions the addition of a pentose or hexose via glycosidic bond to an hydroxylated group might be a possibility (Badia-Fabregat *et al.*, 2012; Gago-Ferrero *et al.*, 2012). Thus, this option was also explored, searching for conjugated metabolites of OH-PBDEs with a pentose or hexose.

Unfortunately, these products were not found in any sample.

Previous results by Hundt et al. (1999) demonstrated the cleavage of monohalogenated diphenyl ethers by *T. versicolor*, resulting in the production of 4-bromophenol. Based on this result and by molecular structure analogy, we evaluated the formation of pentabromophenol as a potential transformation product from the cleavage of deca-BDE. Nonetheless this molecule was not detected in the assays with sampling points from 4 h to 5 d. As it is commercially available, degradation experiments of pentabromophenol with *T. versicolor* (analogous to those using deca-BDE) showed a complete removal in 24 h (Fig. 1). This finding suggests that under our experimental conditions, the degradation rate of pentabromophenol would be higher than its production rate, thus making its potential detection a difficult task. A similar situation could explain the incapacity to detect other metabolites here analyzed.

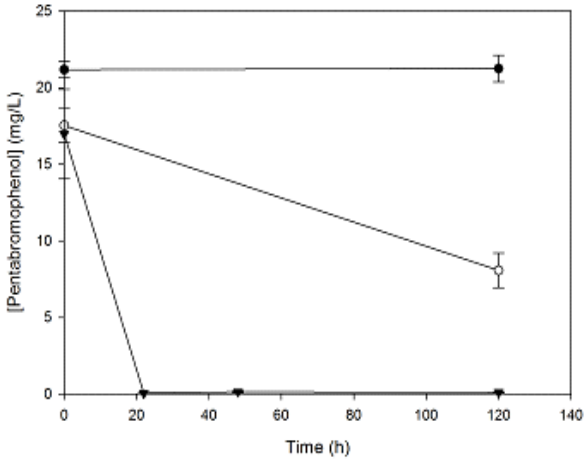

Fig. 1. Time course of pentabromophenol concentration during degradation by *T. versicolor*. Symbols: uninoculated controls (●), heat-killed controls (❍) and degradation (▲). Data plotted correspond to mean values ± standard deviation of triplicates

Sewage sludge was recently reported as one of the major sources of PBDE pollution (North, 2004). Therefore, the potential application of *T. versicolor* for the removal of deca-BDE, the brominated flame retardant found at higher concentrations, was assayed at pre-existent concentrations in sludge. Solid-phase "biopile" systems containing sterilized sludge amended with a fungal inoculum growth in a lignocellulosic substrate were employed (Rodríguez-Rodríguez *et al.*, 2011). Characterization of the raw sludge showed an initial deca-BDE concentration of 285 ng/g dw (± 5%), similar to reports from WWTPs in the Netherlands (mean 350 ng/g dw) (de Boer et al. 2003), but lower than findings in the USA (1183 ng/g dw) (North 2004). After the fungal treatment the remaining concentration was 25.4 ng/g dw (± 14%), equivalent to a reduction of 86%. The scarce reports of analogous treatments with solid wastes refer to anaerobic digestion. Shin et al. (2010) found a deca-BDE removal of 39.8% at bench-scale and around 45% in pilot-scale anaerobic digesters; similarly Gerecke et al. (2005) achieved a 30% decrease in deca-BDE concentration from sewage under bench-scale anaerobic conditions. On the other hand when non sterile sewage sludge was inoculated with *T. versicolor*, high removal values of several brominated flame retardants were obtained; however the synergetic effect due to the presence of other microorganisms did not allow to distinguish the contribution solely of *T. versicolor* in the removal (Rodríguez-Rodríguez *et al.*, 2014) contrary to this work.

CONCLUSION

Although transformation products were not detected, degradation of penta-, octa- and deca-BDE technical mixtures was demonstrated by *T. versicolor* in aqueous systems, in a process mediated by cytochrome P-450. The different analyses performed to detect the formation of products from deca-BDE degradation by *T. versicolor* together with the results obtained from pentabromophenol degradation experiments by the fungus, lead to a main hypothesis on deca-BDE degradation pathway: breakage of ether bond present in deca-BDE molecule obtaining two molecules of pentabromophenol, which are completely degraded by the fungus in a short period of time. Removal of deca-BDE by *T. versicolor* at pre-existent concentrations from sewage sludge supports the potential use of WRF in the elimination of brominated flame retardants from complex matrices.

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