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ORIGINAL PAPER

Degradation of lindane and endosulfan by fungi, fungal and bacterial laccases

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Abstract The ability of two white-rot fungi (*Trametes versicolor* and *Pleurotus ostreatus*) and one brown-rot fungus (*Gloeophyllum trabeum*) to degrade two organochlorine insecticides, lindane and endosulfan, in liquid cultures was studied and dead fungal biomass was examined for adsorption of both insecticides from liquid medium. Lindane and endosulfan were also treated with fungal laccase and bacterial protein CotA, which has laccase activities. The amount of degraded lindane and endosulfan increased with their exposure period in the liquid cultures of both examined white-rot fungi. Endosulfan was transformed to endosulfan sulphate by *T. versicolor* and *P. ostreatus*. A small amount of endosulfan ether was also detected and its origin was examined. Degradation of lindane and endosulfan by a brown rot *G. trabeum* did not occur. Mycelial biomasses of all examined fungi have been found to adsorb lindane and endosulfan and adsorption onto fungal biomass should therefore be considered as a possible mechanism of pollutant removal when fungal degradation potentials are studied. Bacterial protein CotA performed more efficient degradation of lindane and endosulfan than fungal laccase and has shown potential for bioremediation of organic pollutants.

Keywords Lindane · Endosulfan · Biodegradation · Fungi · Endosulfan sulphate · Endosulfan ether · Laccase

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Introduction

Lindane (gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane) and endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzo(e)dioxathiepin-3-oxide) are broad-spectrum organochlorine insecticides, used mostly on food crops. In the past, both insecticides were also used as wood preservatives. Technical grade endosulfan is a mixture of two stereoisomers, α- and β-endosulfan, in a 2:1–7:3 ratio. Endosulfan is highly toxic to fish and aquatic organisms, as well as to humans and most animal species, provoking acute and chronic symptoms at relatively low exposure levels (Guerin 2001; UNEP 2009). Lindane toxically affects aquatic organisms and human health as well (Zucchini-Pascal et al. 2009). Several countries have banned or restricted the use of both insecticides; nevertheless they are still in use in some parts of the world (Hernández-Rodríguez et al. 2006; Hussain et al. 2007; Rivero et al. 2012). It is recommended that amount of endosulfan and lindane in lakes, rivers and streams should be less than 74 and 0.98 μg/l, respectively (ATSDR 2000; NTP 2011). Despite restrictions, they accumulate in the environment and due to their potential for long-range transport they can be found in remote areas. Due to their high solubility in lipids, lindane and endosulfan can bioaccumulate easily in the food chain (Lal and Saxena 1982; Sutherland et al. 2002; Weber et al. 2010). Lindane was included in the Stockholm Convention on Persistent Organic Pollutants in 2009 (UNEP 2009) and technical endosulfan was listed in 2011 (UNEP 2011).

White-rot fungi are able to degrade numerous environmental organic chemicals. Their ability to form extended mycelial networks and relatively low specificity of their catabolic enzymes are some of the several reasons that account for their suitability for bioremediation processes (Reddy 1995; Harms et al. 2011). White-rot fungi use their

nonspecific oxidative exoenzymes, which are involved in lignin degradation, for co-metabolic mineralization of various organopollutants (Harms et al. 2011). Mineralization of environmental chemicals is less common in brown-rot basidiomycetes. In the past, several studies concerning mycoremediation of lindane and endosulfan have been performed (Bumpus et al. 1985; Kullman and Matsumura 1996; Mougin et al. 1996; Singh and Kuhad 1999; Shetty et al. 2000; Kim et al. 2001; Siddique et al. 2003; Bhalerao and Puranik 2007; Quintero et al. 2008). Few studies regarding fungal degradation of lindane reported formation of metabolites (Mougin et al. 1996; Singh and Kuhad 1999), while several pathways for fungal metabolism of endosulfan were proposed (Kullman and Matsumura 1996; Siddique et al. 2003; Bhalerao and Puranik 2007; Goswami et al. 2009). Fungal oxidative metabolism of endosulfan most often results in formation of endosulfan sulphate, which is as toxic and persistent as the parent compound. Endosulfan diol is the major product of endosulfan hydrolysis and can further be slowly degraded to less toxic endosulfan ether, endosulfan hydroxyether and endosulfan lactone (Kullman and Matsumura 1996; Goswami et al. 2009).

Laccases are one of the enzymes involved in fungal catabolism of organic pollutants and are expressed constitutively in many basidiomyceteous fungi (Scheel et al. 2000; Aro et al. 2005; Harms et al. 2011). Laccases have a wide substrate range and are able to oxidise various aromatic compounds, in particular phenolic substrates (Thurston 1994; Baldrian 2006; Giardina et al. 2010; Majeau et al. 2010). In the presence of a mediator, laccases are able to oxidise nonphenolic substrates as well (Bourbonnais et al. 1997). Compared to fungal peroxidases, laccases do not require hydrogen peroxide and use readily available molecular oxygen as the oxidant. Because of their high nonspecific oxidation capacity and easy production, they can be used in many biotechnological applications, including bioremediation. Laccase activity was also found in bacteria, where laccases are involved in formation of spore pigment (Hullo et al. 2001; Martins et al. 2002).

Another method for pollutant removal, especially from aqueous solutions, is biosorption. Only a few studies concerning mycoremediation of lindane and endosulfan have considered adsorption of the insecticides onto mycelial biomass as a possible mechanism for their removal (Young and Banks 1998; Ghosh et al. 2009).

In the present research, two white-rot fungi and one brown-rot fungus were exposed to lindane and endosulfan and their degradation dynamics in aqueous solution were studied. Additionally, adsorption of insecticides onto fungal biomass was studied in order to ascertain whether the insecticides are removed via fungal metabolism or via uptake by the fungal biomass. Finally, fungal and recombinant bacterial laccase were tested preliminarily for their ability to degrade lindane and endosulfan, in order to study their degradation potentials.

Materials and methods

Chemicals and fungal cultures

Stock solutions of lindane (15 mM) and endosulfan (2.5 mM) were prepared in acetone. Final concentration of lindane and endosulfan in the experiments were 30 and 6 μM, respectively. All chemicals used in the experiment were p.a. grade.

Trametes versicolor, *Pleurotus ostreatus* and *Gloeophyllum trabeum* were obtained from the ZIM collection at the University of Ljubljana, Biotechnical Faculty, Department of Wood Science and Technology and were grown on potato dextrose agar (Difco Laboratories). For the study of lindane and endosulfan bioremediation, liquid culture medium as described by Hadar and Cohen-Arazi (1986) was used: glucose 20 g, L-asparagine 0.65 g, yeast extract 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KH_2PO_4 1.0 g, KCl 0.5 g, distilled water 1 l. The production of ligninolytic enzymes was enhanced with addition of veratryl alcohol, a fungal secondary metabolite, to the liquid fungal cultures (final concentration 2 mM) (Faison and Kirk 1985; Faison et al. 1986; Scheel et al. 2000).

Fungal cultures were grown in Erlenemyer flasks containing 50 ml of sterilised liquid medium and inoculated with three mycelial plugs (9 mm in diameter), which were excised from 7-day-old agar cultures of respective fungi. The liquid fungal cultures were kept agitated (100 rpm) on a shaker at 25 °C in the dark for 21 days. Degradation and adsorption experiments were carried out separately and all experiments were carried out in triplicate. Inoculum for both assays was prepared as described above.

Laccase from *T. versicolor* was purchased from Sigma-Aldrich (cat. no. 51639, specific activity ≥10 U/mg) and recombinant protein CotA, a laccase from *Bacillus subtilis* PS209, was kindly provided by Prof. Dr. Ines Mandič-Mulec from University of Ljubljana, Biotechnical Faculty, Department of Microbiology.

Exposure of liquid fungal cultures to lindane and endosulfan; lindane and endosulfan extraction

Fungal cultures were grown for 21 days and lindane (30 μM) or endosulfan (6 μM) was added to the medium 5 days after inoculation. Uninoculated liquid media with addition of insecticides and liquid fungal cultures without lindane and endosulfan were kept as controls.

Extraction of lindane and endosulfan from the medium was done at 1, 3, 6, 9, 13 and 16 day after the addition into the medium. Liquid fungal culture (50 ml) was removed from the Erlenmayer flask and the flask was washed thoroughly with hexane (50 ml), which was then added to the fungal culture. The obtained mixture was homogenised (T25 Digital Ultra-Turrax, 11,000 rpm, 30 s) and then centrifuged (7,000 rpm, 5 min). The recovery of lindane and endosulfan was 85 and 93 %, respectively. The upper, organic phase was removed and analysed.

Adsorption experiment

After 21 days of growth, fungal cultures were autoclaved (121 °C, 30 min) and afterwards lindane and endosulfan were added. A 1 ml fraction of liquid medium without fungal biomass was taken from each sample after 1, 4 and 24 h and insecticides were extracted with 1 ml of hexane.

Treatment with fungal and bacterial laccases

Lindan and endosulfan degradation was studied using fungal and bacterial laccases. The reaction mixture (5 ml) contained 4 ml McIlvaine buffer (130 mM, pH 3), 1 ml fungal laccase from *T. versicolor* (1 U/ml) and 10 µl of lindane (final concentration 30 µM) or 12 µl of endosulfan (final concentration 6 µM). After 4 days, samples were extracted with 5 ml of hexane. Mixture for degradation with bacterial laccase was prepared from 40 µl of McIlvaine buffer (140 mM, pH 4.5), 10 µl of CotA (0.32 U/ml) and 0.5 µl of lindane (final concentration 0.51 µM) or endosulfan (final concentration 0.08 µM). Extractions were performed with 50 µl of hexane after 4 days. Solutions without laccase were considered as controls.

GC analyses of the extracts

Amount of lindane and endosulfan was quantified using gas chromatography (GC). GC analyses were performed by a Hewlett Packard 6890 Series chromatograph (Hewlett Packard) equipped with ECD detector and HP-1 capillary column (30 m × 0.25 mm × 0.25 µm). The injector and detector temperatures were 150 and 350 °C, respectively. The initial column temperature was 70 °C for 1 min, then increased linearly at 30 °C min^{-1} to 300 °C and held for 5 min. The helium carrier gas flow rate was set to 3.7 ml min^{-1} and 1 µl of solution (lindane extracts diluted in hexane, 1:10) was injected. The retention times of lindane, α-endosulfan and β-endosulfan were 10.9, 8.0 and 8.4 min, respectively.

All experiments were carried out in triplicate.

Results

Mycoremediation of lindane by liquid fungal cultures

The amount of lindane in liquid cultures of *T. versicolor*, *P. ostreatus* and *G. trabeum* was observed for 16 days (Fig. 1). The amount of lindane was the highest in liquid cultures of *G. trabeum* and the lowest in liquid cultures of *T. versicolor*, where less than 10 % of initial amount of

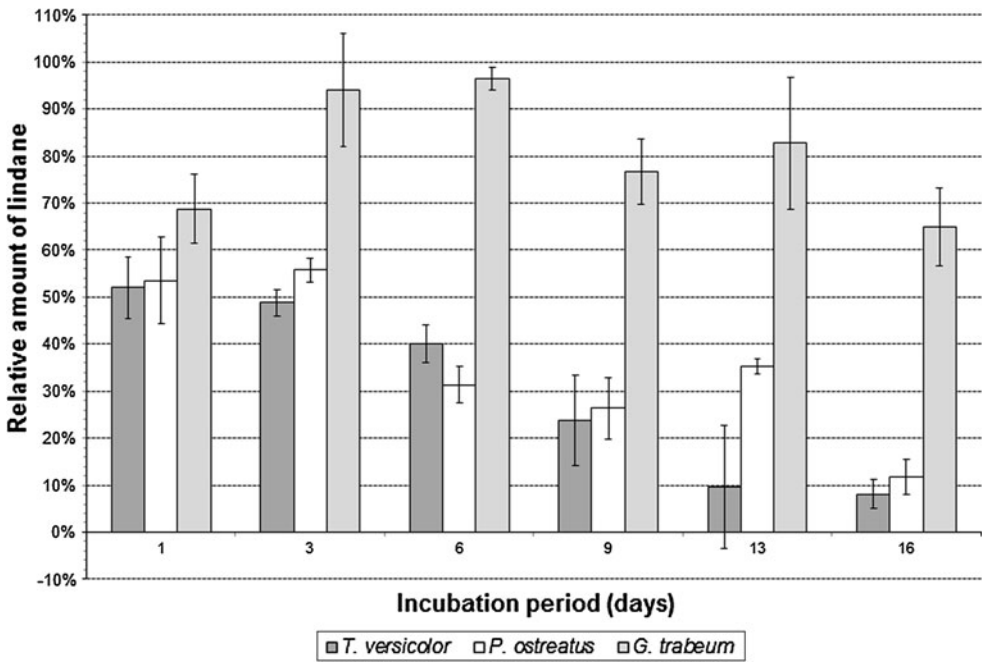

Fig. 1 The relative amount of lindane according to initial amount in liquid fungal cultures. *Error bars* represent standard deviation

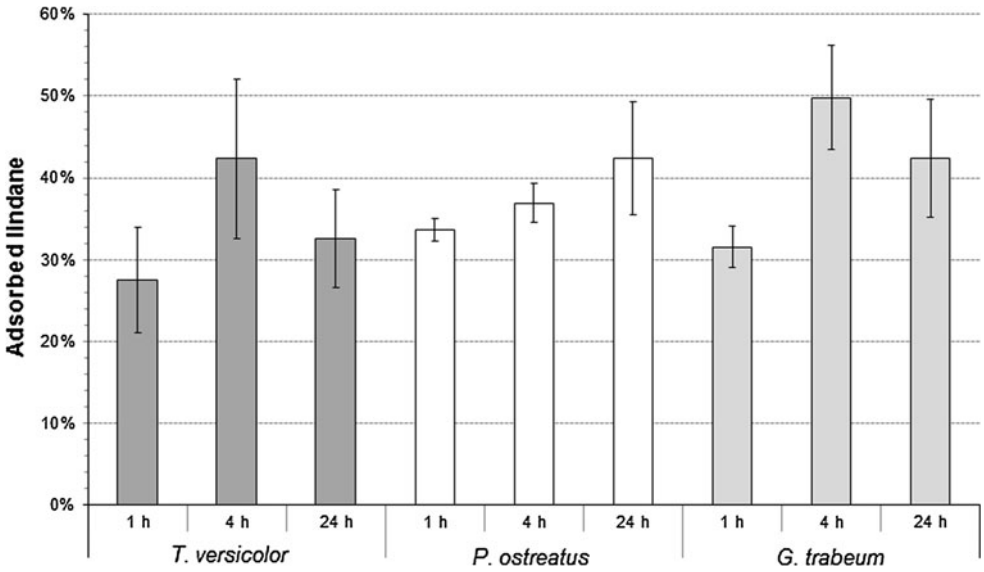

Fig. 2 Adsorption of lindane onto biomass of examined fungi. *Error bars* represent standard deviation

lindane was determined after 16 days. In solutions of both white-rot fungi, *T. versicolor* and *P. ostreatus*, amount of lindane decreased with time. After 1 day, the amount of lindane was already significantly lower, around 50 %, and constantly decreased. In the presence of brown-rot fungus *G. trabeum* the amount of lindane was higher (69 % after 1 day) and further increased in the next days, but remained roughly the same until the end of the experiment.

Adsorption of lindane onto biomass of *P. ostreatus* increased during the experiment, reaching its highest value of 42 % after 24 h (Fig. 2). The same amount of lindane was also adsorbed onto biomass of *T. versicolor* after 4 h. The amount of adsorbed lindane was overall the highest when biomass of *G. trabeum* was used and reached maximum at 4 h (50 %).

Mycoremediation of endosulfan by liquid fungal cultures

Degradation dynamics of endosulfan isomers in the liquid fungal cultures was also observed for 16 days. Concentration of endosulfan isomers decreased with the increase of incubation period in cultures of *T. versicolor* and *P. ostreatus* and gradually additional peaks, corresponding to endosulfan metabolites endosulfan ether and endosulfan sulphate, appeared on the chromatograms (Figs. 3, 4). Extremely low concentrations of both endosulfan isomers were detected in cultures of *T. versicolor* and *P. ostreatus* after 1 day and 3 days of incubation and they were no longer detected after longer incubation periods. Endosulfan sulphate and endosulfan ether were detected already after 1 day of exposure as well as in all other samples with longer exposure periods, and their concentrations increased with time. In liquid cultures of both fungi, amount of these metabolites was the highest after 16 days of exposure.

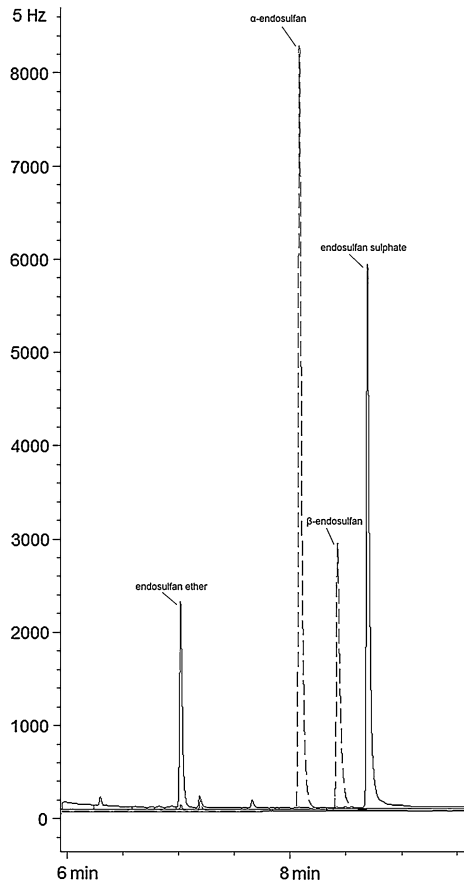

Fig. 3 Chromatogram of endosulfan solution without the presence of fungal culture (*dashed line*) and chromatogram of endosulfan solution exposed to *T. versicolor* for 16 days (*solid line*). Retention times were: for α-endosulfan 8.0 min, β-endosulfan 8.4 min, endosulfan sulphate 8.7 min and endosulfan ether 7.0 min

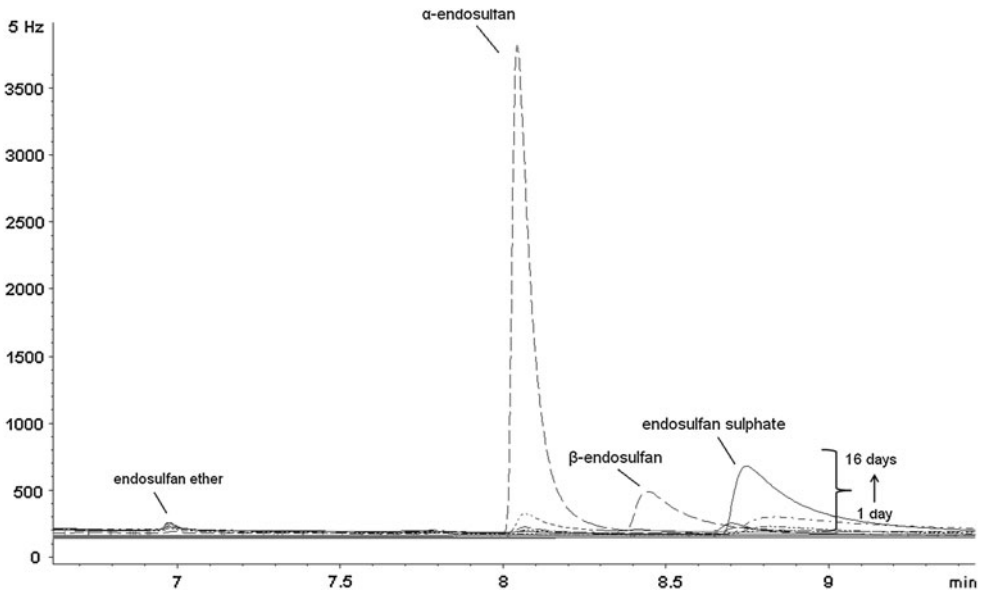

Fig. 4 Chromatogram of endosulfan solution without the presence of fungal culture (*upper dashed line*) and chromatograms of endosulfan solution exposed to *P. ostreatus* for different times

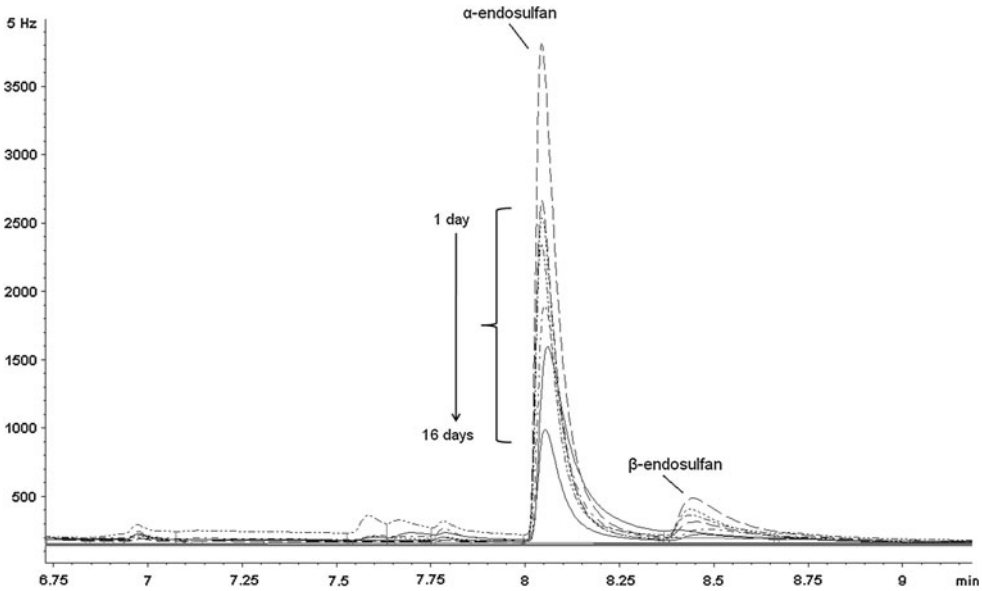

Fig. 5 Chromatogram of endosulfan solution without the presence of fungal culture (*upper dashed line*) and chromatograms of endosulfan solution exposed to *G. trabeum* for different times (the peaks are descending with increase of exposure time)

Removal of endosulfan in the presence of *G. trabeum* was different (Fig. 5). Chromatographic peaks for both endosulfan isomers decreased with time, but no additional peaks appeared.

A significant amount of both endosulfan isomers was adsorbed onto biomass of *G. trabeum*, around 70 % after 24 h (Fig. 6). Differences in individual isomer adsorption were observed at exposure to dead biomass of *P. ostreatus*, as 41 % of α-endosulfan and 57 % β-endosulfan was adsorbed after 1 h and 77 % of α-endosulfan and 74 % of β-endosulfan was adsorbed after 24 h. The greatest variations in amount of adsorbed endosulfan were observed in the presence of *T. versicolor*. After 1 h minimal adsorption occurred, but after 4 h 50 % of α-endosulfan was adsorbed, while adsorption of β-endosulfan remained minimal. Similar situation occurred after 24 h.

Laccase treatment

Degradations of lindane and endosulfan by fungal and bacterial laccases are shown in Fig. 7. Amount of lindane was lower in solutions treated with bacterial laccase (around 50 %) compared to fungal treatment (over 70 %). Similar results were obtained for endosulfan, where both isomers of endosulfan substantially decreased when bacterial laccase was added, whereas no degradation was observed when fungal laccase was used. The amount of endosulfan present in the solutions was even higher than in

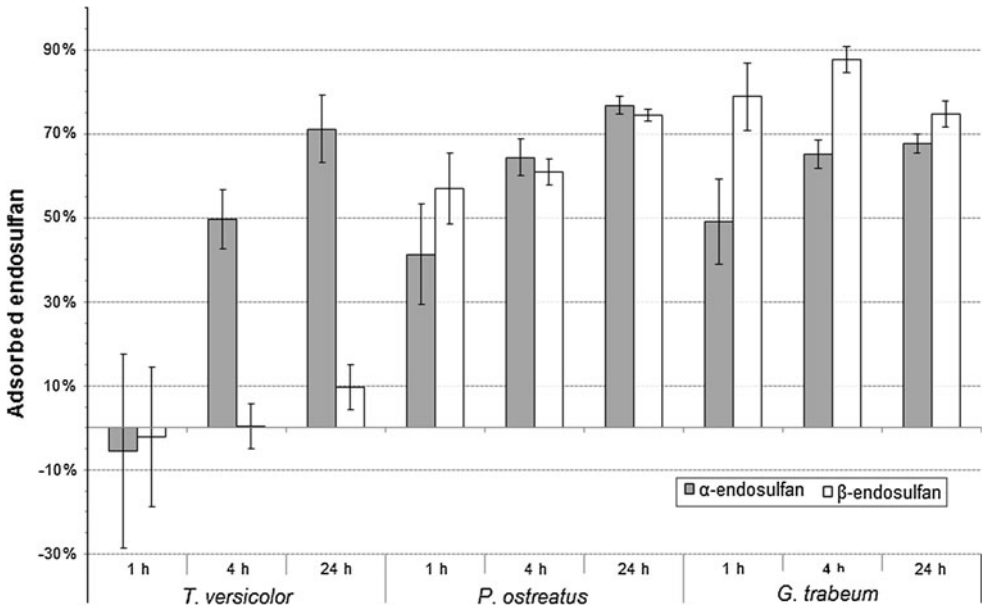

Fig. 6 Adsorption of endosulfan isomers onto biomass of examined fungi. *Error bars* represent standard deviation

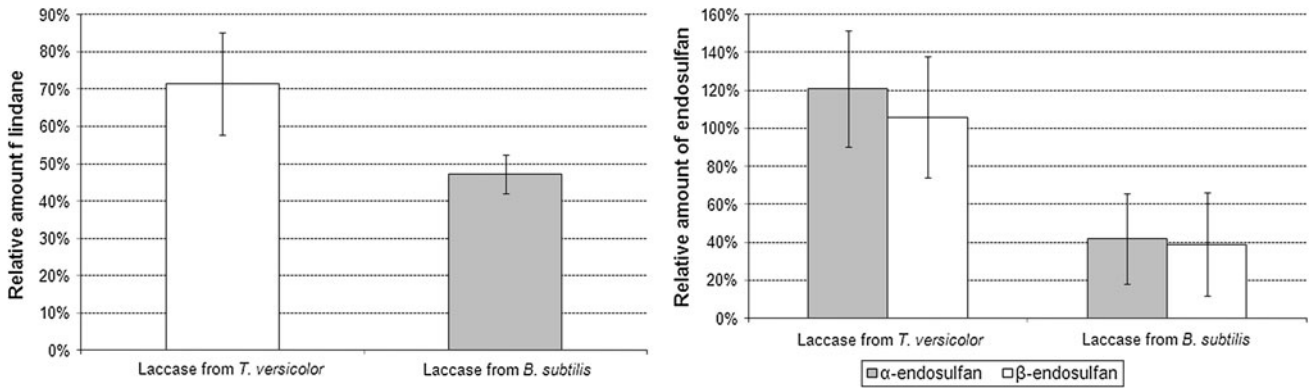

Fig. 7 The relative amount of lindane (*left*) and endosulfan (*right*) according to initial amount after treatment with fungal and bacterial laccase. *Error bars* represent standard deviation

the control solutions, indicating difficulties with extraction method and subsequently poor repeatability of the results.

Origin of endosulfan ether

In Fig. 8, chromatograms of endosulfan sulphate standard solution at two different injector temperatures (150 and 250 °C) are shown. Retention time of endosulfan sulphate was 8.7 min and at 250 °C injector temperature additional peak at 7.0 min appeared, corresponding to endosulfan ether.

Discussion

In the present study, two white-rot fungi and one brown-rot fungus were tested for their capability to degrade lindane and endosulfan. Lindane was most efficiently removed in liquid cultures of *T. versicolor*, as only 8 % of initial amount of lindane was determined after 16 days of incubation. Similar results were obtained with *P. ostreatus*, where 12 % of initial amount of lindane was determined in the liquid cultures after 16 days of exposure. Amount of lindane decreased with time in solutions of both white-rot fungi and was already quite low after only 1 day of exposure (52 % for *T. versicolor* and 54 % for *P. ostreatus*). Removal of lindane in liquid cultures of *G. trabeum* occurred to a lesser extent and there were greater deviations in amount of lindane at different incubation periods. Taking standard deviations into account, the amount of removed lindane ranged between 10 and 30 %, regardless of incubation period. Amounts of residual lindane after 1 and 3 days in liquid cultures of *T. versicolor* and *P. ostreatus* were similar to those determined in adsorption tests for both fungi. After longer incubation periods, concentration of lindane in liquid cultures of these two fungi

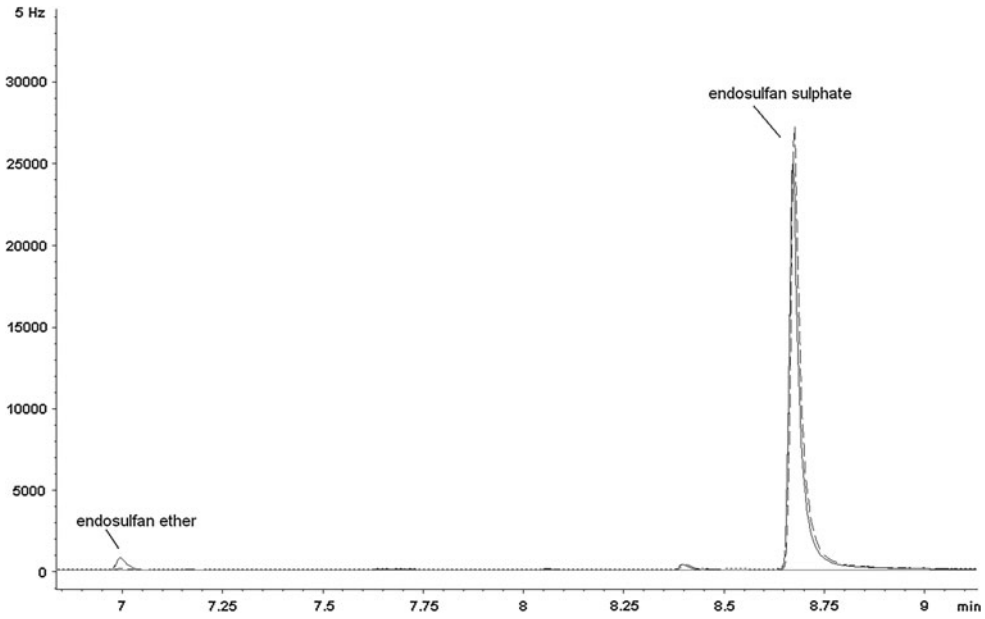

Fig. 8 Chromatograms of endosulfan sulphate standard solution at different injector temperatures (*dashed line*—150 °C, *solid line*—250 °C)

decreased, indicating that removal was due to degradation and not only adsorption. On the contrary, amount of residual lindane in cultures of *G. trabeum* did not steadily decrease with incubation period and concentration of lindane was roughly the same in both degradation and adsorption experiments. This corresponds to our expectations, since *G. trabeum* is a brown-rot fungus and therefore lacks the ligninolytic enzymes, which degrade lignin and most likely organopollutants as well. Removal of lindane was therefore a result of adsorption onto fungal mycelium.

In contrast to the results obtained with lindane, additional peaks appeared on chromatograms when solutions with endosulfan and *T. versicolor* or *P. ostreatus* were analysed. Clearly seen from Figs. 3 and 4, amount of endosulfan decreased considerably compared to control solutions, and two additional peaks, corresponding to endosulfan ether and endosulfan sulphate, appeared. Many mycoremediation studies identified endosulfan sulphate as one of the major endosulfan metabolites (Kullman and Matsumura 1996; Kim et al. 2001; Bhalerao and Puranik 2007; Hernández-Rodríguez et al. 2006; Goswami et al. 2009). Endosulfan sulphate is formed via oxidation of endosulfan, which indicates oxidative pathways were used by *T. versicolor* and *P. ostreatus* to degrade endosulfan.

The appearance of endosulfan ether, although in small quantities, was observed in our study. We observed formation of endosulfan ether from endosulfan sulphate at high injector temperature 250 °C. Decreasing the injector temperature to 150 °C decreased the peak area of endosulfan ether, with only slight change in the peak area of endosulfan sulphate. Therefore it can be concluded that endosulfan ether is produced from endosulfan sulphate in injector at higher temperatures. According to this result, we have shown that endosulfan ether could be formed from endosulfan sulphate under high injector temperatures during GC analysis and is not necessarily an endosulfan metabolite produced by fungi. As endosulfan ether was detected in some previous studies where injector temperatures were 220 °C (Kullman and Matsumura 1996; Kim et al. 2001) or 250 °C (Goswami et al. 2009), reconsideration about its origin is required.

Amount of endosulfan also decreased in solutions of *G. trabeum*, but no additional peaks appeared, indicating decrease resulted from adsorption onto mycelial biomass and not from fungal degradation.

The course of lindane removal from the medium via adsorption onto dead biomass of all three fungi was similar. Around 30 % of lindane was removed from the medium of all three species after 1 h and amount of adsorbed lindane differed for approximately 10 % after 4 and 24 h of incubation. This correlates with results by Ghosh et al. (2009), who reported that adsorption process onto *Rhizophus oryzae* biomass was very rapid and reached equilibrium after 1 h. Results of endosulfan adsorption, on the other hand, were less concise. Concerning dead biomass of *G. trabeum*, approximately the same amount of endosulfan was detected in all samples, confirming the fast adsorption process. Amount of removed endosulfan increased with time in both white-rot fungi, with greater differences in samples from *T. versicolor*. Our results indicate that adsorption of lindane and endosulfan onto mycelial surface should be considered when studying fungal degradation of both insecticides and appropriate extraction methods should be used.

We encountered difficulties with endosulfan analyses, especially regarding poor repeatability considering extraction procedures. As reported by Rivero et al. (2012),

interactions between the growing mycelia, fungal enzymes, growth medium, pollutant and its putative metabolites contribute to a complex matrix which makes extraction difficult, therefore leading to great aberrations. In our study, rigorous homogenisation was used for endosulfan and lindane extraction, but comparison of all degradation and adsorption results, especially in solutions of *G. trabeum* and after laccase treatment of endosulfan, raises doubts about the sufficiency of this procedure for the complete extraction of endosulfan. Homogeneity of fungal liquid cultures, as well as of all other biological systems, is hard to achieve, which together with difficult endosulfan analytical procedures adds to poor recoveries and possible inaccurate evaluation of degradation.

Treatments of lindane and endosulfan with laccase from *T. versicolor* were less efficient in reducing the concentration of insecticides compared to bacterial CotA. Less than 30 % of lindane and no endosulfan removal occurred when fungal laccase was applied. Addition of ABTS, a laccase mediator (Bourbonnais et al. 1997), did not improve degradation rates (data not shown). As the activity of bacterial laccase and fungal laccase in the experiments was not the same, different concentrations of lindane and endosulfan were used. Laccase from *B. subtilis* PS209 gives promising results, as both lindane and endosulfan were removed significantly after 4 days. Degradation of lindane was nevertheless more efficient in cultures of *T. versicolor* and *P. ostreatus,* although no endosulfan sulphate was detected when laccases were used. Only a small amount of bacterial laccase was available for this study, but our preliminary results indicate that bacterial laccase CotA from *B. subtilis* PS209 has a good potential to degrade lindane and endosulfan and possibly other organic pollutants as well.

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References

Agency for Toxic Substances and Disease Registry (ATSDR) (2000) Public Health Statement Endosulfan. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA

Aro N, Pakula T, Penttilä M (2005) Transcriptional regulation of plant cell wall degradation by filamentous fungi. FEMS Microbiol Rev 29:719–739

Baldrian P (2006) Fungal laccases—occurrence and properties. FEMS Microbiol Rev 30:215–242

Bhalerao TS, Puranik PR (2007) Biodegradation of organochlorine pesticide, endosulfan, by a fungal soil isolate, *Aspergillus niger*. Int Biodeterior Biodegradation 59:315–321

Bourbonnais R, Paice MG, Freiermuth B, Bodie E, Borneman S (1997) Reactivities of various mediators and laccases with kraft pulp and lignin model compounds. Appl Environ Microbiol 63: 4627–4632

Bumpus JA, Tien M, Wright D, Aust SD (1985) Oxidation of persistent environmental pollutants by a white rot fungus. Science (Wash) 228:1434–1436

Faison BD, Kirk TK (1985) Factors involved in the regulation of a ligninase activity in *Phanerochaete chrysosporium*. Appl Environ Microbiol 49:299–304

Faison BD, Kirk TK, Farrel RA (1986) Role of veratryl alcohol in regulating ligninase activity in *Phanerochaete chrysosporium*. Appl Environ Microbiol 52:251–254

Ghosh S, Das SK, Guha AK, Sanyal AK (2009) Adsorption behavior of lindane on *Rhizopus oryzae* biomass: physico-chemical studies. J Hazard Mater 172:485–490

Giardina P, Faraco V, Pezzella C, Piscitelli A, Vanhulle S, Sannia G (2010) Laccases: a never-ending story. Cell Mol Life Sci 67: 369–385

Goswami S, Vig K, Singh DK (2009) Biodegradation of α and β endosulfan by *Aspergillus sydoni*. Chemosphere 75:883–888

Guerin TF (2001) Abiological loss of endosulfan and related chlorinated organic compounds from aqueous systems in the presence and absence of oxygen. Environ Pollut 115:219–230

Hadar Y, Cohen-Arazi E (1986) Chemical composition of the edible mushroom *Pleurotus ostreatus* produced by fermentation. Appl Environ Microbiol 51:1352–1354

Harms H, Schlosser D, Wick LY (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat Rev Microbiol 9:177–192

Hernández-Rodríguez D, Sánchez JE, Nieto MG, Márquez-Rocha FJ (2006) Degradation of endosulfan during substrate preparation and cultivation of *Pleurotus pulmonarius*. World J Microbiol Biotechnol 22:753–760

Hullo MF, Moszer I, Danchin A, Martin-Verstraete I (2001) CotA of *Bacillus subtilis* is a copper-dependent laccase. J Bacteriol 183: 5426–5430

Hussain S, Arshad M, Saleem M, Zahir ZA (2007) Screening of soil fungi for in vitro degradation of endosulfan. World J Microbiol Biotechnol 23:939–945

Kim YK, Kim SH, Choi SC (2001) Kinetics of endosulfan degradation by *Phanerochaete chrysosporium*. Biotechnol Lett 23: 163–166

Kullman SW, Matsumura F (1996) Metabolic pathways utilized by *Phanerochaete chrysosporium* for degradation of the cyclodiene pesticide endosulfan. Appl Environ Microbiol 62:593–600

Lal L, Saxena DM (1982) Accumulation, metabolism, and effects of organochlorine insecticides on microorganisms. Microbiol Rev 46:95–127

Majeau JA, Brar SK, Tyagi RD (2010) Laccases for removal of recalcitrant and emerging pollutants. Bioresource Technol 101: 2331–2350

Martins LO, Soares CM, Pereira MM, Teixeira M, Costa T, Jones GH, Henriques AO (2002) Molecular and biochemical characterization of a highly stable bacterial laccase that occurs as a structural component of the *Bacillus subtilis* endospore coat. J Biol Chem 277:18849–18859

Mougin C, Pericaud C, Malosse C, Laugero C, Asther M (1996) Biotransformation of the insecticide lindane by the white rot basidiomycete *Phanerochaete chrysosporium*. Pestic Sci 47: 51–59

NTP (2011) Report on Carcinogens, Twelfth edn. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC

Quintero JC, Moreira MT, Feijoo G, Lema JM (2008) Screening of white rot fungal species for their capacity to degrade lindane and other isomers of hexachlorocyclohexane (HCH). Cienc Investig Agrar 35:123–132

Reddy CA (1995) The potential for white-rot fungi in the treatment of pollutants. Curr Opin Biotechnol 6:320–328

Rivero A, Niell S, Cesio V, Cerdeiras MP, Heinzen H (2012) Analytical methodology for the study of endosulfan bioremediation under controlled conditions with white rot fungi. J Chromatogr B 907:168–172

Scheel T, Höfer M, Ludwig S, Hölker U (2000) Differential expression of manganese peroxidase and laccase in white-rot fungi in the presence of manganese or aromatic compounds. Appl Microbiol Biotechnol 54:686–691

Shetty PK, Mitra J, Murthy NBK, Namitha KK, Savitha KN, Raghu K (2000) Biodegradation of cyclodiene insecticide endosulfan by *Mucor thermohyalospora* MTCC 1384. Curr Sci 79:1381–1383

Siddique T, Okeke BC, Arshad M, Frankenberger WT (2003) Biodegradation kinetics of endosulfan by *Fusarium ventricosum* and a *Pandoraea* species. J Agric Food Chem 51:8015–8019

Singh BK, Kuhad RC (1999) Biodegradation of lindane (γ-hexachlorocyclohexane) by the white-rot fungus *Trametes hirsutus*. Lett Appl Microbiol 28:238–241

Sutherland TD, Weir KM, Lacey MJ, Horne I, Russel RJ, Oakeshott JG (2002) Enrichment of a microbial culture capable of degrading endosulphate, the toxic metabolite of endosulfan. J Appl Microbiol 92:541–548

Thurston CF (1994) The structure and function of fungal laccases. Microbiology 140:19–26

UNEP (2009) Report of the conference of the parties of the Stockholm convention on persistent organic pollutants on the work of its fourth meeting. UNEP/POPS/COP.4/38, vol. 8, May 2009

UNEP (2011) Endosulfan included under the Convention. Fifth meeting of the conference of the parties to the Stockholm Convention on Persistent Organic Pollutants. Available at http://chm.pops.int/Convention/COP/Meetings/COP5/tabid/1267/mctl/ViewDetails/EventModID/870/EventID/109/xmid/4351/Default.aspx. Accessed 15 October 2012

Weber J, Halsall CJ, Muir D, Teixeira T, Small J, Solomon K, Hermanson M, Hung H, Bidleman T (2010) Endosulfan, a global pesticide: a review of its fate in the environment and occurrence in the arctic. Sci Total Environ 408:2966–2984

Young E, Banks CJ (1998) The removal of lindane from aqueous solution using a fungal biosorbent: the influence of pH, temperature, biomass concentration and culture age. Environ Technol 19:619–625

Zucchini-Pascal N, de Sousa G, Rahmani R (2009) Lindane and cell death: at the crossroads between apoptosis, necrosis and autophagy. Toxicology 256:32–41