Bull. Environ. Contam. Toxicol. (2006) 77:882–887

DOI: 10.1007/s00128-006-1226-1

Bulletin of Environmental Contamination and Toxicology

Dehalogenation of Lindane by *Penicillium camemberti*

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Received: 19 July 2006/Accepted: 16 November 2006

Lindane is an organochlorine insecticide that has been used on a variety of soil-dwelling and plant-eating insects. Its common usage includes crops, warehouses and public health to control insect-borne diseases. It is also used in lotions, creams, and shampoos for the control of lice and mites in humans at present.

Kidd and James (1991) were reported that exposure to lindane damages the kidneys and liver and it is a probable carcinogen and a suspected endocrine disruptor, interfering with the natural function of estrogen, androgen and thyroid hormones. It was also stated that lindane is extremely persistent in most soils, with a half-life of approximately 15 months and is very stable in both fresh and salt water environments and is resistant to photodegradation.

The first published report on the degradation of chemicals by white rot fungi showed that these fungi degrade DDT, PCB, lindane, dioxin, and benzo[a]-pyrene (Bumpus and August 1987). Kumar and Sharma (1992) were reported that fungi, *F. oxysporium* and *P. chyrysosoporium*, as well as bacteria, *P. cepacia* and *P. putida*, are used to degrade environmental pollutants like pentachlorophenol (PCP), lindane, DDT, and 2,4-dichlorophenol.

An earlier study of Taşeli et al. (2004) showed that *P. camemberti* effectively treats pine-based (softwood) pulp and paper plant bleachery effluents. It was also reported by Taşeli and Gökçay (2005a) that chlorinated organic compounds like PCP, 2-chlorophenol and trichloroacetic acid those are known to be rich in pulp and paper plant bleachery effluents were effectively degraded by the fungus. The aim of this study is to examine the ability of *P. camemberti* to degrade lindane ($C_6H_6Cl_6$) that are known to be exist in the pulp and paper plant effluents.

MATERIALS AND METHODS

The *P. camemberti* isolate used in this study was isolated from chlorination-stage acidic effluents of SEKA Pulp and Paper Plant in Kastamonu in Turkey. The isolated fungus was identified through elaborate biochemical tests (Pitt 1993).

For determination of biodegradation, the basal medium (2 g/l of acetate, 2 g/l KH_2PO_4, 0.5 g/l $MgSO_4$, 0.1 g/l $CaCl_2$, 0.12 g/l NH_4Cl and 0.001g/l thiamine) was

supplemented with lindane (Sigma Chemical Co., 98% of purity) that was dissolved in ethanol before it was added to the batch system. Some physical and chemical properties of lindane are given in Table 1 (Hadzi-Pazov et al. 2004). The pH was adjusted to 5 and temperature to 25±2°C since in an Author's earlier study, the optimum temperature and pH was found as 25±2 °C and 5, respectively (Gökçay and Taşeli 1997). Batch culturing was carried out in 500 ml conical flasks that were inoculated with 10 ml of a spore suspension (optical density of 0.5 at 650 nm) and shaking flasks were incubated on a rotary shaker at 80 rpm.

Table 1. Physical and chemical characteristics of lindane.

Chemical formula	$C_6H_6Cl_6$
Appearance	White powder
Smell	Unpleasant, like naphthalene
Particle size	40-80 microns
Molecular weight	290,9
Thickness	D=1.95
Melting point	183 °C
Solubility	Insoluble in water at 20°C (10 mg-l), moderately soluble in ethanol, ether, benzene acetone, slightly soluble in mineral oils.
EPA toxicity classification	Class II

Adsorbable organic halogens (AOX) analyses were carried out according to German DIN 38409 Norm. The soluble organics were first adsorbed onto pure activated carbon particles and then filtered on polycarbonate filters, washed with a nitrate solution and combusted in the AOX analyzer's furnace. The chloride release was detected and recorded as AOX.

Gas chromatography analyses were carried out by Perkin Elmer Autosystem 1020 Plus Gas Chromatograph. Firstly, gas chromatographer was calibrated with standard mix solution including target compounds. The calibration procedure was repeated prior to samples every 5 samples. Secondly, effluent samples were first pre-conditioned with methanol and then were passed through C18 solid phase extraction columns. Organics retained on the C18 column were eluted with freshly distilled chloroform. The collected chloroform phase was dried by passing through anhydrous Na_2SO_4 and further concentrated down to 0.1 ml in a micro Kuderna Danish concentrator. The concentrated samples were then injected to and analyzed using the gas chromatographer with electron capture detector and CP Sil-5 capillary column. (30m, 0.25mmID, 0.25 micron film thickness).

RESULTS AND DISCUSSION

The capability of *P. camemberti* to degrade lindane was examined both in shaking and non-shaking batch cultures by using acetate as primary carbon source. Acetate was chosen as primary carbon source for the fungus since, in an earlier study the highest biomass (dry weight/l) concentration was achieved by acetate. The other carbon sources tested were peptone, maltose, acetate, methanol, glucose and phenol (Gökçay and Taşeli 1997).

The rationale behind using shaking and non-shaking batch cultures was to examine the appropriateness of the fungal system to the suspended growth treatment process (conventional systems) or attached growth treatment process (packed bed column reactor).

Firstly, four 500 ml conical flasks containing 0.001 M lindane was supplemented with basal medium (2/l of acetate, 2 g/l KH_2PO_4, 0.5 g/l $MgSO_4$, 0.1 g/l $CaCl_2$, 0.12 g/l NH_4Cl and 0.001g/l thiamine). pH was adjusted to 5 and 2 of them were inoculated with 10 ml of a spore suspension of fungus (optical density of 0.5 at 650 nm) and other 2 of them was inoculated with *P. camemberti* cultures that had been boiled for 10 min. (control experiments) which was reported to be acceptable practice for sterilization of fungi (Kuritz and Wolk 1995).

Non-shaking flasks (one for control experiment and the other for lindane degradation experiment) were incubated at 25±2°C. However, shaking flasks were supplemented with 0.05% Tween 80 which was reported to be somehow enhancing the enzyme activity and acts as both a surfactant and cooxidant for the enzyme system (Hofrichter et al. 2001) and were incubated on a rotary shaker at 80 rpm at 25±2°C.

Degradation of lindane by fungus was examined by both AOX and gas chromatography analysis. The AOX contents of the flasks were measured on first day and 10 day of incubation. 10 day of incubation was chosen since it was proved by the earlier studies that 10 day was the optima for the fungus (Taşeli et al 2004; Taşeli and Gökçay 2005a).

The experiments with lindane serving as co-substrate to the fungus revealed the results tabulated in Table 2. AOX removals of 58% and 53% were achieved in shaking and non-shaking flasks containing lindane in 10 days, respectively.

Table 2. Results of first set of experiments conducted with lindane.

Condition	Shaking(*)	Non-shaking(**)
Lindane (AOX) removal in 10 days	58 %	53 %

(*) 0.001 M lindane + *P. camemberti* + mineral salts + 2 g/l acetate + 0.05 % Tween 80, pH 5, 25±2°C, shaking (80 rpm)

(**) 0.001 M lindane + *P. camemberti* + mineral salts + 2 g/l acetate, pH 5, 25±2°C, non-shaking

The gas chromatography analysis performed in shaking and non-shaking flasks supports AOX analysis since Figure 1 revealed that non-shaking (Figure 1-B) suggests the presence of more breakdown products than shaking (Figure 1-C). In addition, lindane degradation is better under shaking conditions since there is a significant reduction in the total peak area.

In order to examine the effect of acetate on the removal of lindane and also to confirm the first set of experiments, a second set of experiments was conducted under shaking conditions with different acetate concentrations. Lindane (measured as AOX) removals of 57%, 62% and 70% were achieved with 0.5 g/l,

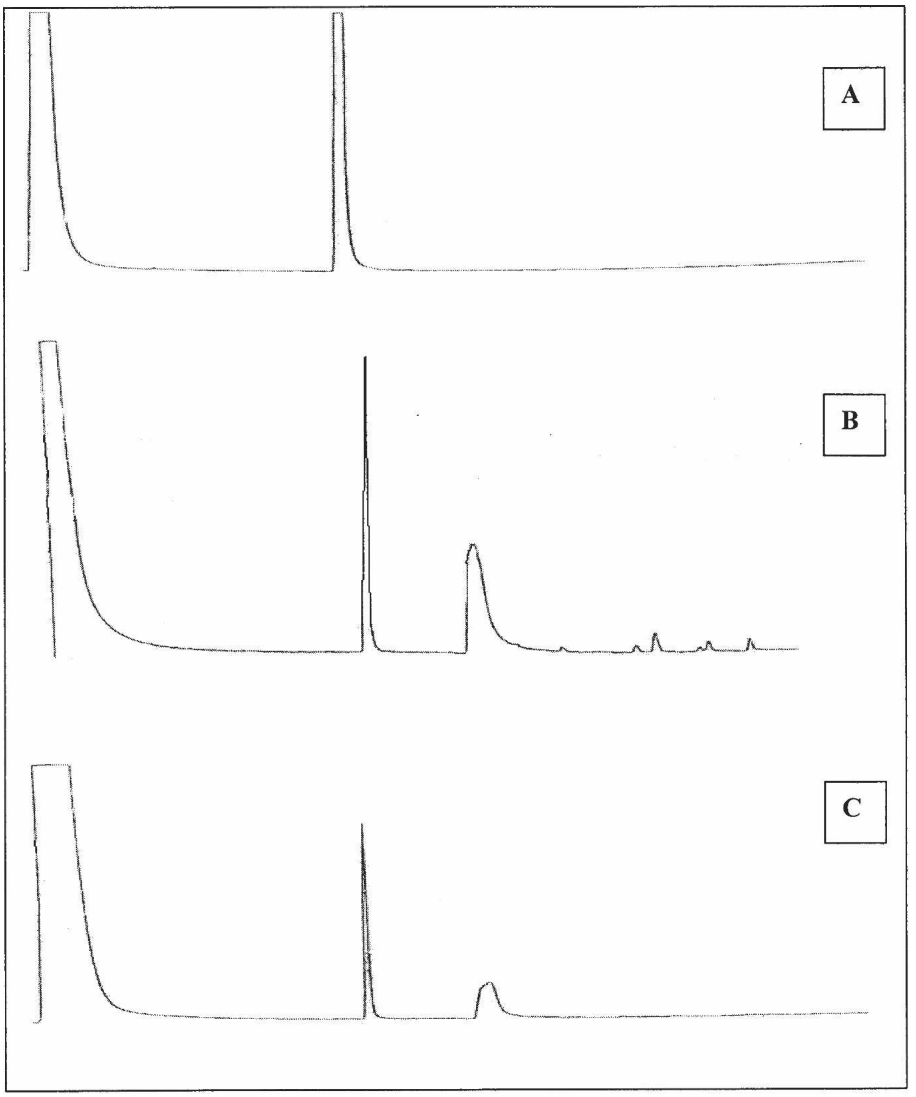

Figure 1. Gas chromatography analysis of lindane before fungal treatment (A) and after fungal treatment (under non-shaking conditions (B) and shaking conditions (C)).

0.2 g/l of acetate and without acetate, respectively (Table 3). The effect of acetate concentration on the removal lindane can also be clearly seen when Figure 2A is compared with Figures 2B, 2C and 2D. The best AOX removal efficiency of 70% was achieved in shaking flasks without acetate but with Tween 80. This result was also in accord with gas chromatogram given in Figure 2D, in which it revealed that considerable reduction in the total peak area is possible following the fungal treatment without acetate.

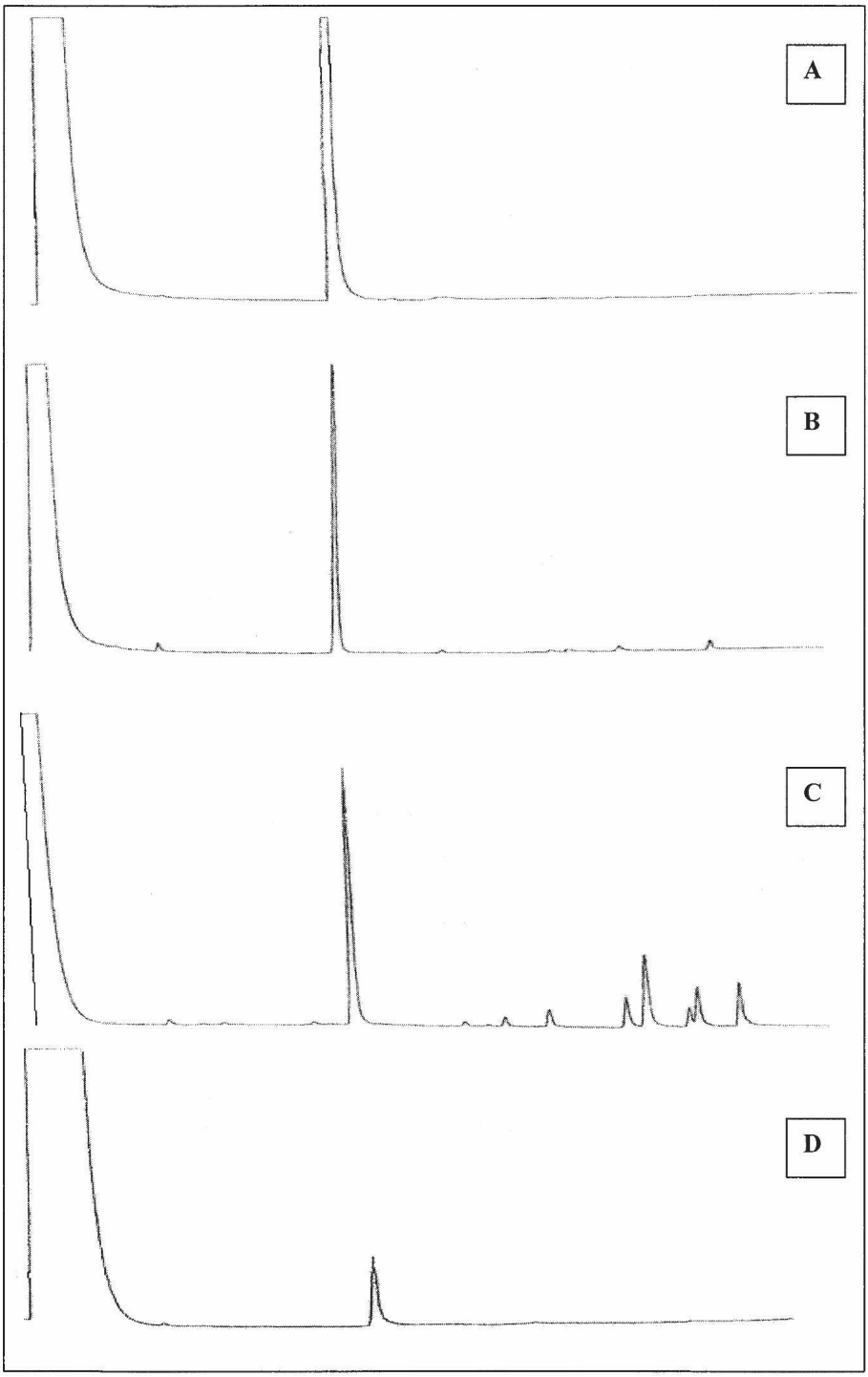

Figure 2. Gas chromatography analysis of lindane under shaking conditions before fungal treatment (A) and after fungal treatment (with 0.5 g/l acetate (B), with 0.2 g/l acetate (C) and without acetate (D)).

Table 3. Effect of acetate concentration on lindane removal under shaking conditions.

Acetate concentration	0.5 g/l acetate	0.2 g/l acetate	No acetate
Lindane (AOX) removal in 10 days	57 %	62 %	70 %

Condition: 0.001 M lindane + *P. camemberti* + mineral salts + 0.05 % Tween 80, pH 5, 25±2°C, shaking (80 rpm)

It should be noted that acetate reduces lindane removal presumably by shifting fungal metabolism to aerobic respiration. In an author's earlier study, it was noticed that at high acetate concentrations, AOX removal was retarded and the metabolism shifted towards aerobic respiration. Moreover, contrary to this, aerobic respiration was suppressed at low acetate concentrations and AOX removal was enhanced. Inorganic chloride removal was also detected using a chloride electrode, and verified findings too (Taşeli and Gökçay 2005b).

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