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DEGRADATION OF PCBs BY WHITE ROT FUNGI, METHYLOTROPHIC AND HYDROCARBON UTILIZING YEASTS AND BACTERIA

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SUMMARY

Of the white rot fungi tested, two strains alone and other three in combination with specific yeasts or bacteria degraded PCBs in polluted soil within interval 20-30%. When all organisms were applied together · PCB degradation achieved was about 50%.

INTRODUCTION

Degradation of xenobiotics contaminating the environment has been studied intensively mostly employing bacteria (see e.g. Chaudhry and Chapalamadugu, 1991). Since 1985, when Bumpus *et al.* and Eaton demonstrated that the white rot fungus *Phanerochaete chrysosporium* has the ability to degrade, besides the lignin molecules, also several chloroaromatics, this organism became a model of concentrated research (Bumpus and Aust, 1987; Sanglard *et al.*, 1986; Mileski *et al.*, 1988; Bumpus, 1989; Haemmerli *et al.*, 1986; Hammel *et al.*, 1986). Besides *Ph. chrysosporium*, few other white rot fungi have been examined for their chloroaromatics degrading ability. White rot fungi growing on straw were successfully used for clean-up of the soils

contaminated by different aromatic and chloroaromatic pollutants (Hüttermann *et al.*, 1988, 1989a,b; Loske *et al.*, 1990).

In practice, pollution is often caused by more than one type of chemical compounds, for instance combination of chloroaromatics and crude oil products. In such cases, use of a mixture of different microorganisms may be more effective resulting into more complete degradation of diverse recalcitrant compounds polluting the environment (Volfová *et al.*, 1991). Upto now, however, when a mixture of several degraders was employed, they were usually of the same ecological nature (see e.g. Chapatwala *et al.*, 1992). In this paper the effectiveness of the selected white rot fungi individually and in combination with other microorganisms to degrade PCBs in soil is evaluated.

MATERIALS AND METHODS

Microorganisms and inoculum development

White rot basidiomycetes

Hirneola nigricans (Sw.:Fr.) Donk [syn. *Auricularia polytricha* (Mont.) Sacc.], a strain of cultivated mushroom native of Vietnam; *Lentinus edodes* (Berk.) Sing., strain 4, a culture of cultivated mushroom native of Japan; *Pleurotus ostreatus* (Jacq.:Fr.) Kumm., strain 3004; *Phanerochaete chrysosporium* Burds., strain ME-446; Px-strain, a culture originated of Vietnam; artificially grown-up fruit bodies resemble that of the basidiomycete *Lentinus tigrinus* (Bull.:Fr.) Fr.

Cut wheat straw was moistened with distilled water to 65% humidity, taken in polypropylene bags and sterilized. After inoculation with a grain spawn the cultures were incubated at 24°C (*Pl. ostreatus*, *L. edodes*, and Px strain), 32°C (*H. nigricans*) and 37°C (*Ph. chrysosporium*). Such 3-4 week old cultures were used in the experiments.

Methylotrophic and hydrocarbon utilizing yeasts and bacteria

Candida lipolytica (Munk *et al.*, 1969); *Candida boidinii* (Volfová *et al.*, 1992); a non-pathogenic *Pseudomonas* sp., strain B2, isolated from soil.

Individual strains were grown separately as shake flask cultures at 30°C in a medium containing 3.5 g NH_4Cl; 1 g KH_2PO_4; 0.25 g $MgSO_4.7H_2O$; 0.1 g NaCl; 10 mg $ZnSO_4.7H_2O$; 100 mg yeast extract and tap water to 1 l. The medium for *C. boidinii* was supplemented with 10 µg/l biotin and 10 g/l methanol. Carbon source for *C. lipolytica* and *Pseudomonas* sp. was 10 g/l of n-alkane mixture (C_{16}-C_{18}). pH of all media at the beginning of cultivation was set to 5.0. 48 hours old culture was used as inoculum.

Preparation of soil samples and their microbial treatment

Soil contaminated with PCBs (Delor 106 - a commercial technical mixture, its degree of chlorination corresponds to Arochlor 1260) originated from a field in south Moravia, (Czech Republic), where the pollution was caused by a dye-making factory. After estimating the water content and adjusting original alkaline reaction to pH 6.7, the soil was dried at room temperature, crushed, mixed thoroughly and sifted through 2.5 mm sieve.

100 g soil samples were taken, and moistened, in 250 ml Erlenmeyer flasks, plugged with cotton-wool stoppers and pasteurized in a thermostat (3 times, 6 hours at 80°C with one day interval); original water content was then readjusted with sterile distilled water. 10 g of fungal and/or 10 ml of yeast/bacterial inoculum was added to these flasks and incubated in dark, 80% humidity at 24°C.

For the biotreatment of soil in bulk, 750 g combined fungal infested straw comprising of 150 g of each strain, and 500 ml mixture of yeast and bacterial inocula in equal proportions was added to 5 kg of non-pasteurized soil in a glass container. The incubation took place in a greenhouse without temperature regulation, only regular watering of the soil surface was performed.

Estimation of PCBs

Air dried soil samples (5 g), were extracted for 16 hours in a Soxhlet apparatus with n-hexane:acetone (3:1). After the removal of organic contaminants with H_2SO_4 and drying with anhydrous Na_2SO_4, the analysis of PCBs was performed by GC (chromatograph Varian model 3700 with ECD, capillary column BP-5, 30m x 0.25mm I.D., df 0.25 µm, J+W). The quantification of PCBs was performed by standard procedure, i.e. calculation from the sum of characteristic peaks selected from chromatograms of samples and of the standard Delor 106.

Experiments were performed in duplicates as well as the PCB analyses. In the control sample the measurement was performed 5 times. Soil contained 2.61 ± 0.20 mg PCBs ($\bar{x}$ ± SEM) in 1 kg of dry matter.If indiviual experimental data differed from control in 25% or more, the extraction and PCB estimation was repeated.

PCB estimation in soil samples of the experiment with non-pasteurized soil was performed in 3 different laboratories to compare our data with reference laboratories.

RESULTS AND DISCUSSION

From the data summarized in Fig. 1 it can be concluded that <i>due to high variability in PCB estimation in individual pasteurized soil samples, only the data over 18% of control can be considered as significant; <ii>white rot fungi *H. nigricans* and Px strain degraded PCBs when introduced into soil samples as the only microorganisms; <iii>methylotrophic and hydrocarbon utilizing yeasts and bacteria alone did not degrade PCBs significantly; <iv>higher degradation was performed by combinations: *Pl. ostreatus* + *Pseudomonas B2*, *H. nigricans* + *C. boidinii* and *L. edodes* + *C. lipolytica*, respectively.

PCB degradation in non-pasteurized soil into which all white rot fungi, yeasts and bacteria were incorporated simultaneously is seen in Table 1. After 11 weeks the mean content of PCBs was about 50% of the original one. It also indicates a synergistic effect of the microorganisms involved and give a chance for practical application in a large scale soil clean-up.

The physico-chemical properties of the target soil constitute important abiotic parameters to be taken into consideration for the successful bioremediation program (Lamar *et al.*, 1990). Based on the results presented here, the authors suggest that combination of cultures forms an equally important criterion for the efficient *in situ* degradation of pollutants in the soil. Perhaps, with increasing heterogeneity, interspecies interactions become more diverse with the net outcome of higher and more

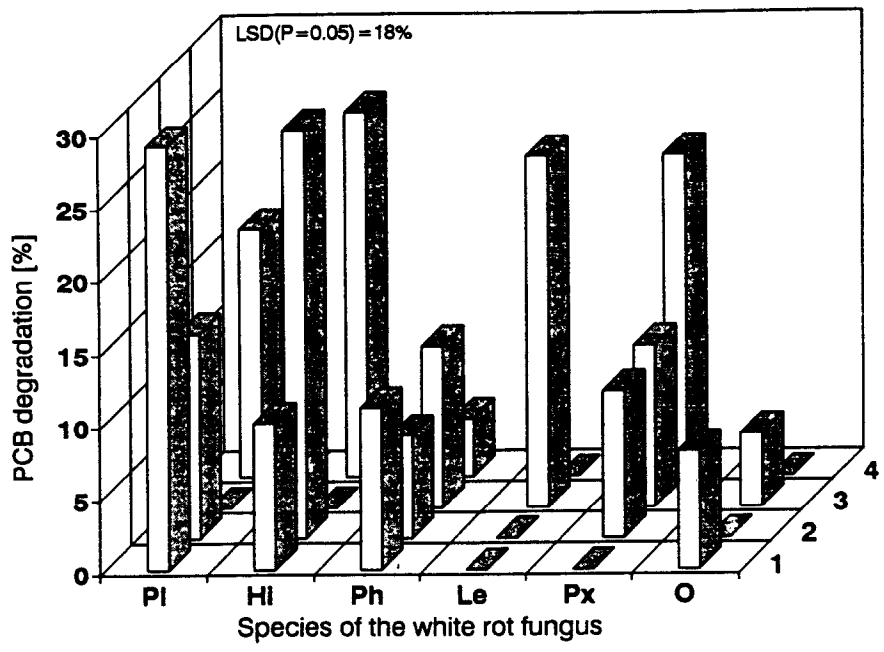

Fig. 1. Degradation of PCBs in pasteurized soil by pure and mixed cultures of selected white rot fungi, yeasts and bacteria after 6 week incubation

1 - *Pseudomonas* B2
2 - *C. boidinii*
3 - *C. lipolytica*
4 - no yeast/bacteria

Le - *Lentinus edodes*
Pl - *Pleurotus ostreatus*
Ph - *Phanerochaete chrysosporium*
Px - strain Px
Hi - *Hirneola nigricans*

Table 1. Degradation of PCBs in non-pasteurized soil by the mixture of white rot fungi, yeasts and bacteria

Incubation Time (week)	PCB degradation (%) estimated in different laboratories		
	1	2	3*
0	0	0	0
3	48	n	16
7	51	n	0
11	60	48	35

n - not estimated * - data of our measurement

complete degradation of the target pollutants.

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