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Use of Fungal Humus for 1,1,1-Trichloro-2,2-*bis*(4-chlorophenyl)ethane (DDT) Polluted Soil Treatment

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This paper, describes the use of fungal humus, the residual compost waste generated by mushroom production, for treatment of DDT polluted soils instead of the use of fungi. The capacity of using the fungal humus from culture of Japanese and Vietnamese fungi such as *Pleurotus sajo caju, Pleurotus ostreatus, Hipsizygus marmorenus, Lentinus edodes* to treat DDT polluted soils has been studied. This work focused only in the efficiency of DDT biodegradation by the fungal humus. The obtained data showed that the ratio of the fungal humus and soils mixture being from 1/10 to 1/5 for 30 days, may degrade DDT in the soils with the high efficiency of 50-70 %. The activity of ligninolytic enzymes in the experiment such as lignin peroxidase (LiP) and mangan peroxidase (MnP) has had an influence on the efficiency of DDT degradation in the soil.

Key Words: Biodegradation, DDT, Soils.

INTRODUCTION

Trichloro-2,2-*bis*(4-chloropheny)ethane (DDT) is a persistent environmental organic pollutant[1] known since 1970s. Many methods such as chemical, physical and biological techniques have been used for treatment of the soil polluted with DDT[2-4]. The degradation of this organic compound in soil environment has become a primary interest of many researchers worldwide[5-7]. Many researchers[8-10] have demonstrated that extensive biogradation of DDT and its metabolites occur in some bacteria. They have elucidated the pathway for the DDT biodegradation. The major bacterial pathway appears to involve an initial reductive dechlorination of trichloromethyl group, then undergoes further dechlorination, oxidation, decarboxylation and mineralization to form CO_2, H_2O and others. The biodegradation of DDT in the soil by white rot fungi has shown high efficiency in the degradation process[3]. White rot fungi like *Phanerochaete chrysosporium* may allow for faster degradation of DDT and other recalcitrant organic compounds. The aim of this study focused on the treatment of DDT in the soil, thus we only focus on the biodegradation efficiency of DDT by the fungal humus instead of fungi itself. This paper also focused on role of fungal sorts, enzyme activity and ratio of humus/soil.

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EXPERIMENTAL

In this study, two different types of fungi, one from Japan and another from Vietnam were employed. The former called *Pleurotus sajor caju* (C4), *Hipsizygus marmoreus* (Ht) are supplied by Prof. T. Morinaga, Prefectural University in Hiroshima, Japan. The latter called *Pleurotus ostreatus* (F), *Lentinus edodes* (Lth) are supplied by the Center for Biology Technology, Vietnamese Agriculture Genetic Institute, in Hanoi, Vietnam.

DDT, DDE, DDD samples were purchased from Merck Company. The purity of these chemicals sample and all solvents, such as *n*-hexane, acetone, methanol, ethanol, chloroform used in the analysis was confirmed by with chromatographic analysis.

Polluted soil samples: The total DDT polluted soil samples consist of 36.3 mg/kg in which DDE 1,1-dichloro-2,2-*bis*(4-chlorophenyl)ethene, DDD 1,1- dichloro-2,2-*bis*(4-chlorophenyl)ethane, DDT are 2.5, 16.9 and 16.9 mg/kg, respectively.

Cultivation of fungi: All kinds of fungi in this study were incubated in Center for Biology Technology, Agriculture Genetic Institute, Hanoi, Vietnam. The culture process consisted of the following steps.

First step: The materials used in fungus culture are composed of potato, green peas, maize, rice bran, glucose, agar, water. The mixture is pulverized and sterilized by autoclaving at 0.8 to 1.1 atmospheres for 80 min. The cultivation of *Hipsizygus marmoreus* and *Pleurotus ostreatus, Lentinus edodes* was established in Petri dishes, 10 cm diameter, by inoculating the medium with spores. The incubation of the fungi of *Hipsizygus marmoreus* and *Pleurotus ostreatus, Lentinus edodes* was performed at 24 ± 1 ºC for 16 d and 26 ± 1 ºC for 8 d, respectively.

Second step: Materials used in this case are composed of $CaCO_3$ powder (1 %) and water-soaked rice (99 %). The culture media were sterilized by autoclaviation at 0.8 to 1.1 atmospheres, for 100 min. The cultivation of these fungi was established by inoculating the medium with spores. The incubation of the fungi of *Hipsizygus marmoreus* and *Pleurotus ostreatus, Lentinus edodes* were performed at 24 ± 1 ºC for 35 d and 26 ± 1 ºC for 15 d, respectively.

Cultivation of fungi: The fungal humus amounts of 0.8-1 kg/bag, consisted of 0.2 kg of rice bran flour and 90 mL of saturated water of lime were incubated at 55- 65 ºC for 4-5 d in polyethylene bags and then were sterilized heating at 100 ºC for 12 h, followed by 125 ºC for 2.5 h. The cultivation of fungi was established by inoculating the medium with 8 g of fungal mycelium collected from the second step. The cultures were incubated at (24 ± 1) ºC for 60-65 d for *Hipsizygus marmoreus* at (26 ± 1) ºC for 30-35 d for *Pleurotus ostreatus*, *Lentinus edodes*.

The experiments of DDT degradation by fungal humus. After a different cultured time intervals (corresponding to a defenite value of ligninolytic enzyme activity), an amount of pulverized fungal humus was collected and mixed uniformly with the DDT-contaminated soil in the ratio of from 1/10 to 1/1 by weight and then placed

under an atmosphere of air with relative humidity 80 %, 30 ºC to allow degradation to procced. The efficiency of DDT-degradation was determined during a 30 day experiment.

Analytical procedure

DDT analysis: DDT and its products of degradation such as DDE, DDD have been analyzed by gas-liquid chromatography (model 370; Varian, Sunnyval, California). The instrument equipped with an electron capture detector and a digital integrator (model 3390; Hewlett-Packard Co.). The soil samples after 30 days treatment with fungal humus (15 g) were collected and pulverized to analyze the total DDT remained. The degradation efficiency of DDT (E) by fungal humus is calculated as follows:

$$E = \frac{[DDT]_i - [DDT]_f}{[DDT]_i} (\%)$$

where the initial and final concentration denoted $[DDT]_i$, $[DDT]_f$, respectively.

Analysis of enzyme activity: Lignin peroxidase (LiP) activity was measured using the usual method as described[11]. In this method, the increase of absorbance at 310 nm, due to the oxidation of the veratryl alcohol to veratryl aldehyde is measured. The reaction mixture contained: 2.2 mL of sodium tartrate buffer (50 mM, pH 4.5 at 25 ºC, 40 µL of veratryl alcohol (2 mm) and 240 µL of culture supernatant. The reaction was initiated by the addition of 20 µL of H_2O_2 (0.2 mM). One enzyme activity was defined as quantity of enzyme that produced 1 µmol of oxidized product.

Manganese peroxidase (MnP) activity was measured[11] by method based on the oxidation of Mn(II) to Mn(III) and used as substrate 2.5 mL of phenol red (0.01 %) and $MnSO_4$ (0.1 mM) in sodium succinate buffer (0.1 M). The reaction mixture contained: 2.5 mL of substrate and 200 µL culture supernatant. The reaction was initiated by the addition of H_2O_2 (0.1 mM). After an incubation of 2 min at 30 ºC, the reaction was completed by the addition of NaOH (5 M). The absorbance was measured at 610 nm. One enzyme activity was defined as quantity of enzyme that produced 1 mol of oxidized product.

RESULTS AND DISCUSSION

The variation of DDT amounts and its products in degradation process of soils for 30 days are presented in Table-1.

The obtained results show a different decrease of DDE, DDD and DDT amounts in polluted soils treated by fungal humus. The biodegradation efficiency of these chemicals depends on the ratio of humus/soil and the sorts of fungi. The ratio of fungal humus/soil has an influence on the degradation efficiency of the total DDT. The ratio of 1/10 of the fungal humus to soil caused a decrease of DDT of 45.4-55.4 %, while the ratio of 1/5 caused this decrease of DDT of 58.5-70.5 % after 30 days. The DDD degradation is more difficult than DDE and DDT. This is reasonable concordance between the theory and practice. The DDD may be the first result of DDT biodegradation process.

TABLE-1
DEGRADATION EFFICIENCY OF DDT IN SOIL BY FUNGAL HUMUS CULTURES OVER FOR 30 DAYS

Samples	Humus/soil	DDE		DDD		DDT		DDT total	
		A	B	A	B	A	B	A	B
Mo	Without hums	2.5	0	16.9	0	16.9	0	36.3	0
M1/C4	1/10	0.7	72	9.6	43.2	6.0	64.5	16.2	55.4
M2/F	1/10	0.7	72	12.0	29.0	5.2	69.2	17.8	50.1
M3/Lth	1/10	0.8	68	11.2	33.7	6.0	64.5	18.5	49.0
M4/Ht	1/10	0.9	64	12.5	26.0	6.4	62.1	19.8	45.4
M5/C4	1/5	0.4	84	9.6	43.2	0.7	95.8	10.7	70.5
M6/F	1/5	0.5	80	9.8	42.0	1.5	91.1	11.8	67.5
M7/Lth	1/5	0.9	64	9.9	41.4	4.3	74.5	15.1	58.4
M8/Ht	1/5	0.7	72	13.1	22.5	3.1	81.7	16.9	53.4

A = After treatment (ppm); B = Efficiency (%).

On the other hand the efficiency of DDT degradation thus depended in humus of the sorts of fungi. As shown in Table-1, the humus collected from the fungi *Pleurotus sajor cajur* or *Pleurotus ostreatus* showed the ability to degrade DDT in the soil with the high efficiency of 50-70 % after 30 days. The humus from two other fungi *Hipsizygus marmoreus* and *Letines edodes* showed a lower degradation efficiency (45-58 %). For the ratio of 1/5, the three fungi *Lentinus edodes*, *Pleurotus sajor caju* and *Pleurotus ostreatus* degraded DDT in the soil by 95.8 %, but for DDD only to 43.2 %. For the *Hipsizygus marmoreus* the fungal humus degraded DDD with the lowest efficiency of 22.5 %. These data show again that DDD is a more recalcitrant of fungal decomposition than DDE and DDT.

The reasons for the different efficiencies of DDT degradation in the soil by the fungal humus fractions were examined. The data showed that the efficiency of DDT degradation little depends on the fungus sorts, while it significantly depends on the activity of ligninolytic enzymes like LiP and MnP in the humus (Table-2).

TABLE-2
ACTIVITY OF LIGNINOLYTIC ENZYME (LiP) MnP/DRY WEIGHT OF HUMUS

Name of samples	Fungi	Activity of enzyme (U/g)	
		LiP	MnP × 10^{-3}
D1	C4	3.6	198.0
D2	F	3.0	161.5
D3	Lth	2.7	135.3
D4	Ht	2.5	110.5

The data in Table-2 show that the activities of enzymes LiP and MnP decreased in the range C4 > F > Lith > Ht and corresponded to the decrease of degradation efficiency of DDT by the humus. Thus the humus with the higher enzyme activity causes higher degradation efficiency. This evidence indicates a important role of ligninolytic enzymes in the degradation of DDT.

By practice showed that the activity of enzymes varies in the culture process of fungi. Therefore, the selection of the suitable time to collect fungal humus is very important problem for this treatment technology. Figs. 1 and 2 show that the activity of ligninolytic enzymes like LiP and MnP is elevated by the time of the fungal growth within the culture bags. For C4 fungus the suitable time to collect the humus from the fungal culture is between 45 to 75 days at which time the activity of ligninolytic enzymes has reached the highest value.

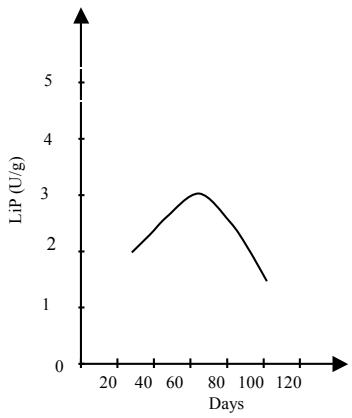

Fig. 1. Variation of LP enzyme activity *vs.* time from culture of *Pleurotus sajo caju* in corncob

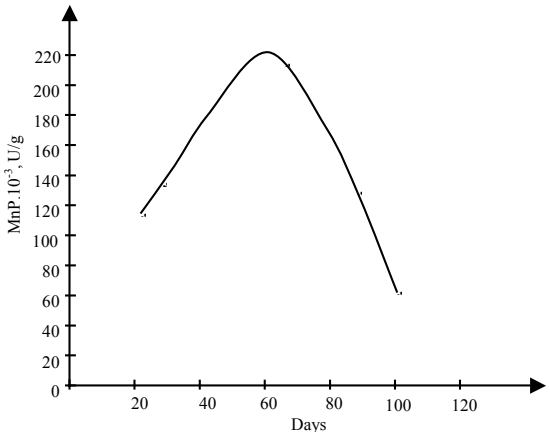

Fig. 2. Variation of MnP enzyme activity *vs.* time from culture of *Pleurotus sajo caju* in corncob

Conclusion

The fungal humus from culture of fungi such as *Pleurotus ostreatus*, *Letinus edodes* (Vietnam), *Pleurotus sajor caju*, *Hypsizigus marmoreus* (Japan) were able to support degradation of DDT in soil.

The activities of the ligninolytic enzymes like LiP and MnP in the fungal humus were positively correlated with the efficiency of DDT degradation in the soil. The selection of the time to collect the fungal humus in the fungus culture process is an important factor in success of this treatment technology.

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