STUDYING THE USE POSSIBILITY OF CONSUMABLE MUSHROOM CULTIVATING HUMUS TO TREAT 2,4-D, 2,4,5-T AND 2,3,7,8-TCDD

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Introduction

The increasing use of herbicides, particularly compounds containing chlorine leads to pollution of many world water and soil ecological systems. Among biotechnological solutions used for decomposition of pollutants containing chlorine in soil, micro organism solution was likely widely studied.[1-3] Versus, the use of mushroom in biological treatment was less concerned though many practical studies clearly indicated that mushroom, especially white rotten mushroom may decompose very much pollutants, of which, pesticides, herbicides and dioxin compounds.[4-10] Whiterot fungus seem to be more successful as they may adapt to and eliminate toxins of some herbicides and chemical compounds containing chlorine and phenol usually toxic for other organisms.[6] Decomposability of artificial organic compounds, in which 2,4-dichlorophenoxyaxetic acid (2,4-D), 2,4,5-trichlorophenoxyaxetic acid (2,4,5-T) and polichlorodibenzo-p-dioxins (PCDDs) in soil by whiterot fungus - *Phanerochaete Chrysosporium* - was suggested in some published studies.[4-10] However, study results on use of humus in rotten mushrooms for treatment of soil contaminated with above compounds were few published.

In this paper, we introduce the study results on the possibility of use of humus for cultivating some consumable mushrooms such as oyster-mushroom - *Pleurotus Sajo Caru*, pearl-mushroom - *Hipsizygus Marmoreus* of Japan origin, white oyster - mushroom - *Pleurotus Ostreatus*, thin top mushroom - *Litoned Edodes* of Vietnam origin for treatment of simultaneously contaminated soil with 2,4-D, 2,4,5-T and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

Materials and methods

Mushroom species

Japan-originated mushrooms: *Pleurotus Sajo caru* (C4) and *Hipsizygus Marmoreus* (Ht) supplied by Prof. T. Morinaga of Hiroshima Prefectural University, Japan.
Oyster mushrooms - *Pleurotus Ostreatus* (F), thin-top mushrooms (Lth) supplied by Phytobiological Technological Center - Vietnam Agricultural Genetic Institute.

Contaminated soil

Contaminated soil (DN3) directly taken from one among Vietnam hotspots with primarily and respectively concentration 18,7 ppm (2,4-D), 46,3 ppm (2,4,5-T) and 18,0 ppb (TCDD).

Chemicals

Standards 2,4-D, 2,4,5-T with 98-99% purity supplied by Merck firm.
Solvents for extraction and chromatographic analysis 2,4-D, 2,4,5-T such as n-hexane, acetone, methanol, ethanol, chloroform with grade for chromatography.

Humus preparation for mushroom cultivation

Mushroom-seeds cultivated in Phytobiological Technological Center - Vietnam Agricultural Genetic Institute - Hanoi, Vietnam. Procedures include following main processes.

- Seed multiplication class 1:

Material preparation for creating environment for seed multiplication class 1 (potato, bean sprouts, fresh mushrooms, maize flour, rice bran, glucose, water, agar). Sterilising environment by autoclave at 0.8 - 1.1 at in 80 minutes; Seedling at $24^{o}\pm1^{o}C$ in 16 days (for pearl mushroom), $26^{o}\pm1^{o}C$ in 8 days (for oyster and thin-top mushroom).

- Seed multiplication class 2:
Material preparation for environment creating for seed multiplication class 2 (water soaked paddy, $CaCO_3$ powder). Sterilising environment by autoclave at 0.8-1.1 at in 100 minutes; Seedling at $24^oC \pm 1^oC$ in 35 days (for pearl mushroom), at $26^oC \pm 1^oC$ in 15 days (for oyster mushroom and thin-top mushroom).

- Mushroom cultivating:
Preparation of material for cultivating (pulverised corncob, limewater, maize flour, $CaCO_3$); incubating at $55^oC \pm 65^oC$ in 4-5 days. Packing in 0.8-1 kg/bags; sterilising at 100^oC in 12 hrs and 125^oC in 2.5 hrs; Seedling with class 2 seeds, 8 g of seed/bag, to be spread evenly on surface; cultivating fiber sapling at $24^oC \pm 1^oC$ in 60-65 days (for pearl mushroom), at $26^oC \pm 1^oC$ in 30-35 days (for oyster and thin-top mushroom); seedling fruit-shape at $13^oC \pm 18^oC$ in 10-15 days.

Treatment method
Taking a quantity of cultivating mushroom humus with different cultivating periods (i.e different lignin decomposing enzyme action) then grinding and blending with contaminated soil at 1/10 to 1/1 ratios subject to weight.

Sample analysis
The concentration of 2,4-D, 2,4,5-T are determined by HPLC in accordance with US.EPA8321A method; TCCD are determined by GC/MS in accordance with US.EPA8280A method.

Enzyme action determining method
Enzyme lignin peroxidase (LiP) and manganese peroxidase (MnP) are determined in Bio-Chemistry Labs of Food Biology and Technology Institute - Hanoi University of Technology - following the procedures provided in references 11, 12.

Results and discussion
Table 1 shown the 2,4-D, 2,4,5-T residual concentration in contaminated soil samples after 39 days of blending with different humus for mushroom cultivating. From this, the followings may be learned: A quantity of humus for mushroom cultivation added to contaminated soil significantly decreases 2,4-D and 2,4,5-T content contaminated in the soil. Humus mixed with soil at 20% ratio after 39 days may reduce up to 90% of 2,4-D and primary 2,4,5-T. In all cases, an increase of humus content in soil leads to treatment efficiency increase which differs with different pollutants. Vietnam oyster mushroom, and thin-top mushroom also may decompose 2,4-D and 2,4,5-T equivalently to Japanese oyster or pearl mushrooms if treated at high action times of lignin decomposing enzymes (LiP and MnP).

Influences of LiP and MnP on 2,4-D and 2,45-T decomposing efficiency by mushroom-cultivating humus are shown in Table 2, where humus samples M1, M5, M7 are taken at the end of fruit state tapling or fruit state tapling formation. Other samplings were taken at other periods. From table 2, if enzyme actions in humus decrease, 2,4-D and 2,4,5-T treatment efficiency also decreases. Just for this, selecting appropriate for humus sampling for treatment has an extremely important signification for technological solution. Experiment shows that enzyme actions vary with mushroom development cycle. Generally, LiP, MnP actions are often higher for humus when mushroom fibers grow crowdly over all the support (at fiber germinating or fruit germinating period end). In humus samplings with on-going fruit formation enzyme high actions may be disclosed. However, in unfavourable environment humidity for fiber development (mostly 10-15 days after fruit-crop, enzyme actions often strongly reduce. So taking humus at this time for treatment shall lower efficiency.

Influence of subbase (support) for mushroom cultivation on humus treatment efficacy is also studied. 2 main subbases were studied: corncob and ground straw. LiP, MnP enzymes analysis results show that enzyme action in humus samples using these 2 subbases has minor difference and ground raw may also give 2,4-D and 2,4,5-T treatment efficiency equivalent to that with corncob use. With a 1-5 humus/ soil blending ratio, after 10 days, 70% of 2,4-D and 2,4,5-T contaminated in earth may be eliminated. This has a practical meaning as humus for

mushroom cultivation is based on raw - a more decomposable material compared with corncob and often used as fertilizer in agriculture, and this subbase shall bitterly improve soil quality.

Besides 2,4-D and 2,4,5-T, the possibility of use of humus for cultivating some mushrooms is studied for decomposing TCDD often prevent in polluted soil samples taken in hotspots. Treatment result on 2,4-D and 2,4,5-T and TCCD is shown in Table 3. This shows that a mix ratio 1/2 to 1/1 of humus and soil may obtain decomposition efficiency up to 90% (for 2,4-D and 2,4,5-T) or 50%-60% (for TCCD) after 39 days of-treatment.

Conclusion

Humus in some consumable mushrooms, such as oyster mushroom *Pleurotus ostreatus*, *Pleurotus Sajo caru*, Pearl-mushroom *Hipsizygus Marmoreus* and thin-top mushroom *Litoned Edodes*, may rapidly decompose artificial, toxic and organic compounds such as 2,4-D and 2,4,5-T and TCDD contaminated in the soil. Decomposition of pollutants efficiency is impacted by lignin degradation enzymes (LiP, MnP) available in humus for mushroom cultivation. Therefore, in order to obtain high decomposition efficiency, appropriate time for taking humus samples with high LiP and MnP actions should be selected. Subbases as cheap and popular agricultural wastes such as corncob, dry raw may be used to create humus for mushroom cultivation with rapid decomposition capacity and at high rate of pollutants such as 2,4-D and 2,4,5-T and TCDD.

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References:

1. Haugland.R.A. Schlemm R.P, Lyons III, Sferra P.R, and A.M Chakrabarty. *App. Environ. Microbiol* 1990 56:1357-1362
2. Oh, K.H, and O.H. Tuovinen. *J.Ind. Microbiol* 1990 .6:275-278
3. Rosenberg A. and M. Alexander. *J Agric. Food Chem*. 1980, 28: 297-302
4. Ryan T.P. and Bumpus J.A. *Phanerochaete chrysosporium*. *App. Microbiol.biotechnol*, 1989. 31: 302 - 307.
5. Yadav, J.S, Reddy C.A. *App. Environ. Microbiol*. 1993, 59, N-9: 2904-2908.
6. Christopher Bucke. *J. Chem. Techol Biotechnol*, 1998, 4: 356 – 367.
7. Joshi D.K, Gold M.H. *Phanerochaete Chrysosporium. Biochemistry* 1994 33: 10 969 - 10 976
8. Takada S, Nakamura M, Matsueda T, Kondo R, Sakai K. *Phanerochaete sordida*. YK – 624. *App.Environ. Microbiol*. 1996 62: 4323 – 4328.
9. Valli K., Wariishi, H., and Gold, M.H. *J.Bacteria* 1992 174(7): 2131 – 2137.
10. Bumpus J.A., Tien M., Wright D. and Aust S.D. *Science.* 1985 228: 1434 – 1436
11. Paszczynski.A., R.L. Grawford and V.B Huynh. *Enzymol.* 1988 161: 238 - 249
12. Tien, M and T.K. Kirk. *Enzymol.* 1988. 161: 238 – 249.

Table 1: 2,4-D, 2,4,5-T decomposition in DN3 soil sample of humus for mushroom cultivation using cobcorn as subbase

Sample	Fungi	Humus/ contaminated soil	Concentration of remain polutants, ppm		Degradation efficiency, %	
			2,4 - D	2,4,5 – T	2,4 - D	2,4,5 – T
N1	*Pleurotus SajoCaru (C4)*	1/10	5,2	13,8	72,3	70,5
N 2	*C4*	1/5	2,7	4,9	85,6	89,5
N 3	*C4*	1/2	1,8	3,6	90,4	92,3
N 4	*C4*	1/1	1,3	3,2	93,0	93,1
N 5	*C4*	2/1	0,7	1,7	96,2	96,3
N 6	*Hipsizygus Marmoreus (Ht)*	1/5	1,9	4,2	90,0	91,0
N 7	*Ht*	1/2	1,2	2,7	93,6	94,2
N 8	*Pleurotus Ostreatus* (F)	1/5	1,7	3,6	91,0	92,3
N 9	F	1/2	1,2	2,7	93,6	94,2
N 10	*Litoned Edodes* (Lth)	1/5	1,5	2,9	92,0	93,4
N 11	Lth	1/2	1,2	2,1	93,6	95,5
DN3	-		18,7	46,7		

Note: Humus samples are taken at fruit germinating period.

Table 2: Impact of actions of LiP and MnP lignin decomposition enzyme actions on 2,4-D and 2,4,5-T decomposition by mushroom cultivating humus

Samples	Fungi	Concentration of remain polutants, ppm		Degradable effiency, %		Enzyme activityU/g	
		2,4 - D	2,4,5 – T	2,4 - D	2,4,5 – T	LiP	MnPx10^{-3}
M1	C4	1,1	2,4	94,1	94,8	4,5	185
M2	C4	5,9	13,5	68,5	71,1	2,0	31,8
M3	F	2,5	5,5	86,6	88,2	4,0	31,0
M4	F	5,5	11,3	70,6	75,8	3,0	15,0
M5	Ht	1,2	2,0	93,6	95,7	5,0	192,6
M6	Ht	6,1	14,0	67,4	70,0	2,5	45,5
M7	Lth	0,9	1,8	95,2	96,1	5,0	210
M8	Lth	2,5	4,9	86,6	89,5	2,0	45
DN3		18,72	46,3				

Note: Treatment period: 39 days, Mushroom subbase: Ground corncob

Table 3: Treatment result of 2,4-D and 2,4,5-T and TCDD simultaneously contaminated soil

Sample	Fungi	Humus/contaminated soil	Concentration of pollutants			Treatment efficiency TCDD, %
			2,4 - D ppm	2,4,5 – T ppm	TCDD, ppb	
DN3			18,7	46,7	18,0	
S1	C4	1/10	7,0	15,5	15,3	15,1
S2	F	1/10	6,1	14, 8	15,2	15,6
S3	Ht	1/10	4,1	10,8	15,6	13,4
S4	C4+F	1/2	1,6	3,7	11,0	38,7
S5	F	1/2	1,2	2,7	8,1	55,3
S6	C4	1/1	1,3	3,2	7,0	60,7

Note: Treatment period: 39 days, Mushroom subbase: Ground corncob