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Environmental Bioremediation of DDT-Contaminated Soil by Using the Mushroom Extract of *L. edodes*

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Abstract

Fungal extract of *L. edodes* was evaluated to degrade DDT (dichlorodiphenyltrichloroethane). Two grams of the dry weight of soil was artificially contaminated with 50 µL of 5 mM DDT in Dimethyl formamide (final amount: 0.25 µmol). The DMF evaporate was allowed to room temperature for 3 days and then mixed two grams of dry-weight fungal extract of *L. edodes* was added to the contaminated soil. The samples were tested and analyzed by gas chromatography. Manganese peroxidase activity, lignin peroxidase activity, and laccase activity of fungal extracts were analyzed. The fungal extract significantly degraded DDT in the mixture. It was also observed that the extract has higher ligninolytic enzyme activity which has played a role in DDT degradation.

Keywords: Biodegradation, Mineralization, DDT, *Lentinus edodes*, Contaminated soil.S

Introduction

The most important ecological problem on the planet is that pollution has become one of the natural resources such as air, water, and soil due to heavy metals (1, 2). In the United Nations environment program in 2001, the governing council issued a treaty to eliminate or restrict the production and use of persistent organic pollutants (POPs). The pollution of the environment with synthetic organic compounds has become a major problem worldwide. Nature of these introduced many novel compounds called xenobiotics materials that do not occur naturally in the biosphere and many of which are not easily degraded by the indigenous microflora and fauna (3). The United States Environmental Protection Agency (USEPA) as a priority several classes of chemicals have been targeted by pollutants due to their toxic effects on the environment and human health. The environment with poor management, of stem already the waste and effluents from households, industries, and agricultural fields further deteriorating (4).

These chemicals include polycyclic aromatic hydrocarbons, Penta chlorophenols, polychlorinated biphenyls, 1,1,1- trichloro–2 (4-chlorophenyl) ethane, benzene, toluene, ethylbenzene xylene, and trinitrotoluene. Recalcitrant environmental contaminants are polycyclic aromatic hydrocarbons (PAH) generated from burning fossil fuels, coal mining, oil drilling, and wood burning (5,6). DDT (dichlorodiphenyltrichloroethane) was a wide gain to one of the first synthetic pesticides. Since the 1940s, DDT has been used for pest control in agriculture and forestry worldwide. Dichloro diphenyl trichloroethane (DDT) organochlorine pesticides were one of the most widely used. However, in 1972 DDT was banned in the United States and most of Europe by the late 1970s. Environmental Protection Agency in the US has classified

DDT and its metabolites as a priority pollutant (7). WRF species have different enzymes produced depending on their genetic makeup and growth conditions. Lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), and laccase include key degradation enzymes. The main mechanism of biodegradation by this group of fungi is the lignin degradation system of enzymes employed. The lignin potential of these enzymes is well documented and xenobiotic degradation (8,9). The availability of WRF fungal inocula is a practical consideration and the use of SMW could be advantageous bioremediation for when using due to the low cost and environmentally-friendly treatment. The white-rot fungi are quite different and well-established methods from other bioremediation technology. (For example, bacteria systems).

GC methods are mainly used for environmental science in identifying organic pollutants in recent sediments, following abiotic and biotic transformations of petroleum-type pollutants, distinguishing the oil pollutant from the native organic substance of recent sediments improving our understanding of migration mechanisms of organic pollutants in soil, water, and air environments, pollutant during soil bioremediation experiments. Today it is very hard to imagine an environmental laboratory GC technique is the most widely used and without at least a gas chromatograph (10). In this study, the ability of mushrooms from *L. edodes* to degrade DDT was investigated in the GC method. The biodegradation ability of the *L. edodes* fungal extract before and after mushroom production was compared. The degradation potential in artificially DDT-contaminated soil for application purposes was also investigated.

Materials and Methods

Preparation of culture for bioremediation: *L. edodes* fungi were obtained from a mushroom producer's Urban platter (Mumbai, India). The culture medium was made from 15 kg birch sawdust and 1kg rice bran, and the moisture content was adjusted to approximately 60 % using water. The medium was put into 2kg, polyethylene containers and then autoclaved by a high-pressure steam sterilizer at 120˚C for 2hr. Ten grams of fungi mycelium was inoculated into the medium and incubated at 20 ˚C for 28d.

Degradation of DDT by fungal inocula from L. edodes: The fungal substrate from *L. edodes* production was used. Two grams of the dry weight of fungal inocula were put into a 100 mL flask and then 50 µL of 5 mM DDT in DMF (final amount: 0.25 µmol) was added. The cultures were incubated statically at 25˚C for 28 d, 5Mm DDT sample is a control sample.

Degradation of artificial DDT-contaminated soil: Two grams of the dry weight of soil was autoclaved (121 ˚C 10 min), then the sterilized (SL) soil was put into 100 ml flasks and artificially contaminated with 50 µL of 5 mM DDT in DMF (final amount: 0.25 µmol). The DMF evaporate was to allow for 3 d at room temperature. Two grams of the dry-weight fungal substrate from *L. edodes* were added to the contaminated soil and mixed. The mixture (moisture content 66%) was incubated statically for 28 d at 25˚ C. 2g, of un-sterilized (USL) soil was added to 5mM DDT in a control sample.

Analytical method GC-analysis: The samples with 40 mL of methanol and 120 mL of acetone were homogenized. After filtration through a 1.0 mm pore glass fiber filter, the filtrates were mixed and evaporated then extracted with 250 mL n-hexane. Gas chromatography (GC) determined the metabolic products after being extracted with ethyl acetate. The program to increase from 80 to 320 ˚C at 20 min was oven temperature. The following relation based on the total area of each sample per control analyzed using identical conditions of percentage was calculated by the biodegradation (11).

$$\frac{\text{Some of the peak areas of the tested sample}}{\text{Some of the peak areas of a control sample}} \times 100$$

Analysis of enzyme activity: Samples of

mushrooms (10 g wet weight) were mixed with 30 ml distillate water and then blended until homogeneous. After centrifugation, the supernatant was taken (3000 × g, 10 min) (12). Enzyme's activities were determined as described below.

1. Manganese peroxidase activity

2. Lignin peroxidase activity

3. Laccase activity

Manganese peroxidase activity: Manganese peroxidase activity was assayed as described with some changes, using a reaction mixture containing 5mM Guaiacol and 2Mm $MnSO_4$ in 50 mM sodium succinate buffer pH 4.5, 10 mM H_2O_2, and 100 µL enzyme filtrate in a total volume of 1.5 mL. Hydrogen peroxide was added last to initiate the reaction after incubating the mixture for 5 minutes. The monitored increase in oxidation of guaiacol (2-methoxy phenol) absorbance was the substrate at 465nm using a UV-Visible spectrophotometer. One unit of MnP activity was defined as the amount of enzyme that oxidized 1.0µmole of 2-methoxy phenol per minute (13).

Lignin peroxidase activity: Lignin peroxidase activity was assaying mixture of 1.5 mL containing 10 mM 2,2-casino-bis (3-ethyl- benzothiazoline)-6-sulfonate (ABTS) in 20 mM citrate buffer pH 3.5, 10 mM H_2O_2, and 100 µL enzyme filtrate. H_2O_2 (300 µL,50 mM) was added last to initiate the reaction after incubating the substrate and enzyme for 5 minutes. The followed in absorbance was increased spectrophotometrically at 310 nm where the oxidation of veratryl alcohol to veratraldehyde (3,4-dimethoxybenzaldehyde) took place. One unit of LiP was defined as the amount of enzyme that oxidizes1.0µmole of veratryl alcohol per minute at pH 3.5 and 30˚C (14).

Laccase activity: Laccase activity was determined by reaction assay mixture1.5 mL contained 0.5 mM of 2,2-casino-bis (3-ethyl-benzothiazoline)-6-sulfonate (ABTS) in 100 mM sodium acetate buffer pH 5.0 and 50 µL of the enzyme filtrate. Oxidation of ABTS was observed by the formation of intense blue-green color which was monitored by measuring the increase in absorbance at 420 nm, using a UV-Visible spectrophotometer (15).

Statistical analyses

All data presented in the study as mean± SD& SE were statistically analyzed by MS Excel for windows version 2019. Mean and standard deviation & standard error were calculated.

Results and Discussion

Degradation of DDT by fungal substrates from L. edodes: Since high levels of residual nutrients and nutrients enzymes, it could be used advantageously as a low-cost bioremediation tool to degrade DDT remains in a fungal substrate. DDT degradation to the ability of various cultivated for their fungi was screened. Fungal substances of *L. edodes* received from an urban platter (Mumbai, India). The results indicated that fungal substrate was significantly better at degrading DDT. The results of the high-resolution gas chromatographic analysis of three samples show different results of DDT degradation. For GC analysis selected samples are DDT+ Control (without fungal substrate), and DDT+Sample (with fungal substrate *L. edodes*) one-week incubation period at 25˚C. DDT+Sample (*L. edodes*) 4-week incubation period at 25˚C. The results revealed that all samples are showing the highest ability to degrade DDT. This suggests that the use of fungal substrate from *L. edodes* might be the most effective for degrading DDT.DDT was degraded by 19% and 29% during the 7d incubation with *L. edodes*. After 28 d during the incubation period, *L. edodes* fungal substrate was degraded by DDT at 43% and 55% respectively. When compared to the control the experimental mushroom shows a degradation range of 19-55%.

Figure 1 (a): The Fungal substrate of DDT degrades 7d incubation (b) The fungal substrate of DDT degrades 28 d incubation.

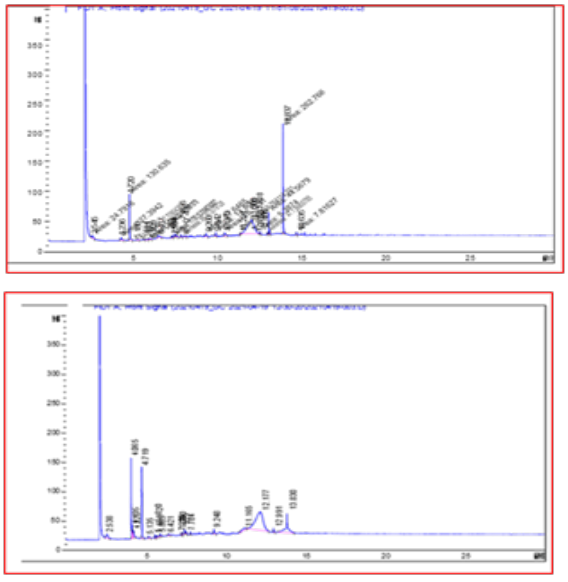
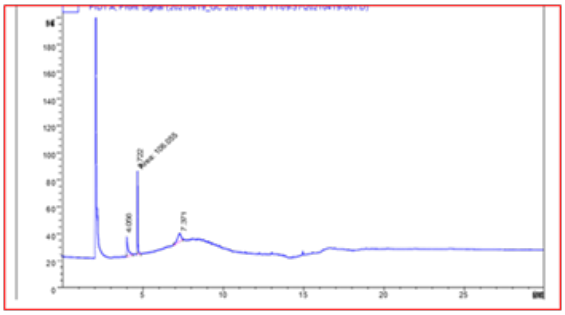


Figure 2 (a): DDT+ Control sample (with fungal substrate) (b): DDT + Sample (*L. edodes*) 7 d incubation period



(c): DDT + Sample (*L. edodes*) 28d incubation period

Analysis of enzyme activity: Bioremediation activity is dependent on the ability of the fungal species colonized in the substrate, to produce oxidative ligninolytic enzymes such as laccase, MnP, and LiP. These enzymes of lignin are responsible for the degradation as well as pollutants (16). Therefore, the ligninolytic enzyme activities of 3 fungal substrates *A. bisporus, L. edodes,* and *T. heimii* were determined. Among the showed high activities for both the fungal substrate and the MnP activity ligninolytic enzymes. The degradation of some pollutants by white-rot fungi has been correlated with the ligninolytic activity of the fungi and lignin peroxidase (LiP) activity is particularly important (17). *L. edodes* fungal substrate showing DDT degradation in MnP, LiP, and Laccase activity is higher than in other fungal substrates. MnP activity of *L. edodes* was 47.7- 80.5%, *A. bisporus* was 38.4.-67.5% and *T. heimii* was 35.8-65.4%. LiP enzyme activity of *L. edodes* was 43.5-75.19%, *A. bisporus*40.7-67.3%, &*T. heimii* was 32.7-61.10%. Ligninolytic enzymes laccase was showing degradation activity of *L.edodes* 37.58-70.3%, *A. bisporus* 28.7-57.5%, &*T. heimii* 25.9-49.5%. However, since the ligninolytic enzyme activities were significant in the fungal substrate. The results indicate that the higher mineralization of DDT by *L. edodes* in comparison to other fungal substrates is caused by the higher ligninolytic enzyme activities. However, the ability of ligninolytic enzymes *in vitro* needs DDT degradation further investigation.

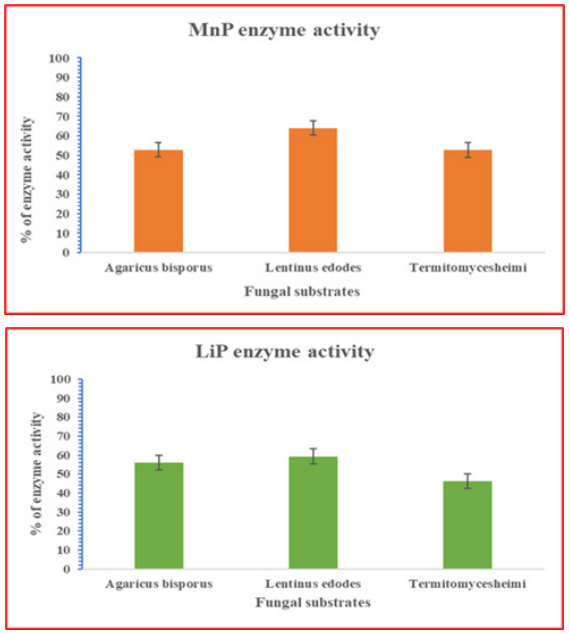
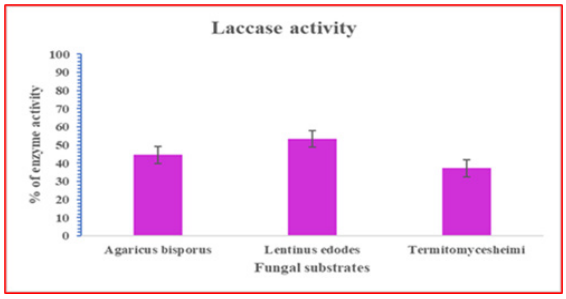


Figure 3 (a): MnP enzyme activity of three fungal substrates (b): LiP enzyme activity of three fungal substrates. The error bars indicate the standard deviation of three measurements. Mean values of triplicate independent experiments SD±SE.



(c): Laccase enzyme activity of three fungal substrates The error bars indicate the standard deviation of three measurements. Mean values of triplicate independent experiments SD±SE.

Conclusion

This study investigated the degradation potential of the fungal substrates and are degrading ability of DDT chemicals. According to the GC results. *L. edodes* fungal substrate degrades DDT by 19-29% in 7 d. Results of GC analysis showed that fungal substrate degrades DDT by 43-55% in 28 d. The fungi producing ligninolytic enzymes MnP, LiP, Laccase, and target enzyme synthesis may play an important role in degrading the DDT chemical of the major role in bioremediation development. In the present study, the results of allowing us to conclude that *L. edodes* is good for the degradation of DDT.

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