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Enhanced Biodegradation of DDT by White-Rot Fungus *Phlebia brevispora* TMIC34596 in a Nutrient-Rich Medium at Short Incubation Time

Erly Grizca Boelan[1], Adi Setyo Purnomo[2*], Fajriah[2], Ichiro Kamei[3], Maria Aloisia Uron Leba[1], Hildegardis Missa[4], Anselmus Boy Baunsele[1], Aloysius Masan Kopon[1]

[1]*Department of Chemistry Education, Faculty of Teacher Training and Education, Universitas Katolik Widya Mandira, Kupang 85211, Indonesia*
[2]*Department of Chemistry, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember, Kampus ITS Sukolilo, Surabaya 60111, Indonesia*
[3]*Department of Forest and Enviromental Sciences, Faculty of Agriculture, University of Miyazaki 1-1 Gakuen-kibanadai-nishi, Miyazaki, Japan*
[4]*Department of Biology Education, Faculty of Teacher Training and Education, Universitas Katolik Widya Mandira, Kupang 85211, Indonesia*

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ABSTRACT

1,1,1-Trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) is a persistent organic pollutant that remains a global environmental concern due to its high toxicity and recalcitrance. This study investigated the ability of the white-rot fungus *Phlebia brevispora* to degrade DDT in a nutrient-rich Potato Dextrose Broth (PDB) medium under a short incubation time. The fungus degraded 64.25% of DDT within 7 days. Metabolites identified by GC/MS were DDE (1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene), DDD (1,1-dichloro-2,2-bis(4-chlorophenyl) ethane), and DDMU (1-chloro-2,2-bis(4-chlorophenyl) ethylene), indicating both reductive dechlorination and dehydrogenation pathways. The results demonstrate that *P. brevispora* has strong potential for rapid biotransformation of DDT in nutrient-rich environments.

1. Introduction

DDT is the first synthetic insecticide that had an important role in eradicating malaria mosquitoes during World War II. DDT residues are still found in the environment, especially in locations close to human activities such as agricultural areas and waters. Several methods for the DDT remediation process have been applied. In general, chemical and physical processes are faster than biological processes, but they disrupt the condition of the affected soil and require relatively high costs and a lot of energy. Biodegradation is the safest, most efficient, and cheapest method of degrading certain organic pollutants.

White-rot fungi (WRF) are known for their ligninolytic enzyme systems capable of degrading complex organic pollutants, including DDT. The genus *Phlebia* is particularly notable for its degradation potential. Previous studies reported that *P. brevispora* (PB) degraded 30% of DDT after 21 days in low-nitrogen medium (Xiao *et al.* 2011). To enhance efficiency, this study assessed the degradation ability of PB in a nutrient-

*Corresponding Author
E-mail Address: adi_setyo@chem.its.ac.id

rich PDB medium to achieve rapid degradation within only 7 days.

2. Materials and Methods

2.1. Materials

The PB TMIC34596 strain was obtained from the collection of the Microbial Chemistry Laboratory, ITS Surabaya, Indonesia. DDT, pyrene (Tokyo Chemical Industry Co., Japan), potato dextrose agar (PDA), potato dextrose broth (PDB), dimethyl sulfoxide (DMSO), distilled water, sodium sulfate (Na_2SO_4), methanol (Merck, Darmstadt, Germany), acetone, and *n*-hexane (Anhui Fulltime, China) were of analytical grade.

2.2. Biodegradation Experiment

The fungus was pre-incubated for 7 days in PDB medium at 30°C (the wet weight of the fungal biomass was 0.3 g in 10 mL of PDB). Subsequently, 10 mL of fresh PDB and 50 µL of 5 mM DDT (in DMSO) were added to each culture flask. Flasks were flushed with oxygen and sealed to minimize DDT volatilization. Cultures were incubated statically for 7 days at 30°C (Boelan & Purnomo 2018, 2019; Purnomo *et al.* 2019).

2.3. Recovery of DDT and Metabolite Identification

The recovery process was carried out to determine the amount of degraded DDT by comparing the peak area of DDT and the internal standard (pyrene). After incubation, 50 µL of 5 mM pyrene (internal standard) and 20 mL of methanol were added. Samples were centrifuged (3,000 rpm, 10 min), filtered, and the filtrate extracted with *n*-hexane. The organic phase was dried using anhydrous Na_2SO_4 and concentrated. DDT concentrations were quantified using HPLC (Shimadzu LC-20AT, Inertsil ODS-3 column, 82% methanol mobile phase). Metabolites were identified using GC/MS (Agilent 7890A/5975C system) in EI mode at 70 eV with a 30-m column and oven program of 100–250°C (Maulianawati *et al.* 2021; Boelan *et al.* 2024).

The percentage recovery of DDT is calculated using the linear regression equation of the standard DDT curve, y = 0.004x, where y is the ratio of the peak areas of DDT and pyrene, and x is the percentage recovery. To calculate the amount of DDT degraded, the following equation is used:

$$\% \text{ DDT Degradation} = \text{Control} - x$$

2.4. Statistical Analysis

All experiments were performed in triplicate. Statistical differences were analyzed using Student's t-test with $p<0.05$ considered significant (Maulianawati *et al.* 2021).

3. Results

In this study, the ability of PB to degrade DDT was evaluated under liquid culture conditions in PDB medium. DDT degradation by PB in PDB medium reached 64.25% within 7 days, while the control recovery was 96.70%. Figure 1 shows the chromatogram of GC-MS analysis results for metabolite products of DDT biodegradation by PB for 7 days of incubation period in PDB medium. It showed the presence of four major peaks corresponding to DDT, DDD, DDE, and DDMU. Metabolite identification was based on retention times and characteristic ion fragments compared to reference spectra (Table 1). The metabolites detected included DDT (14.8 min), DDD (13.4 min), DDE (12.2 min), and DDMU (11.3 min) (Figure 2). The predominant product was DDD, suggesting that reductive dechlorination is the main pathway under these conditions.

4. Discussion

In a previous study, Xiao *et al.* (2011) reported that PB could be degraded by 30% in Kirk's basal medium, low nitrogen medium. In this study, PDB medium was used as a medium for DDT degradation by PB, where this PDB medium contains more complete nutrients compared to low nitrogen medium, so that PB is more suitable for growth and can degrade DDT at a higher rate. These results indicate that the composition of the growth media influences increasing the ability of PB to degrade DDT. Purnomo *et al.* (2008) when screening 12 species of Brown-Rot Fungi (BRF) in degrading DDT, showed that PDB medium can provide good fungal cell growth and high DDT degradation results

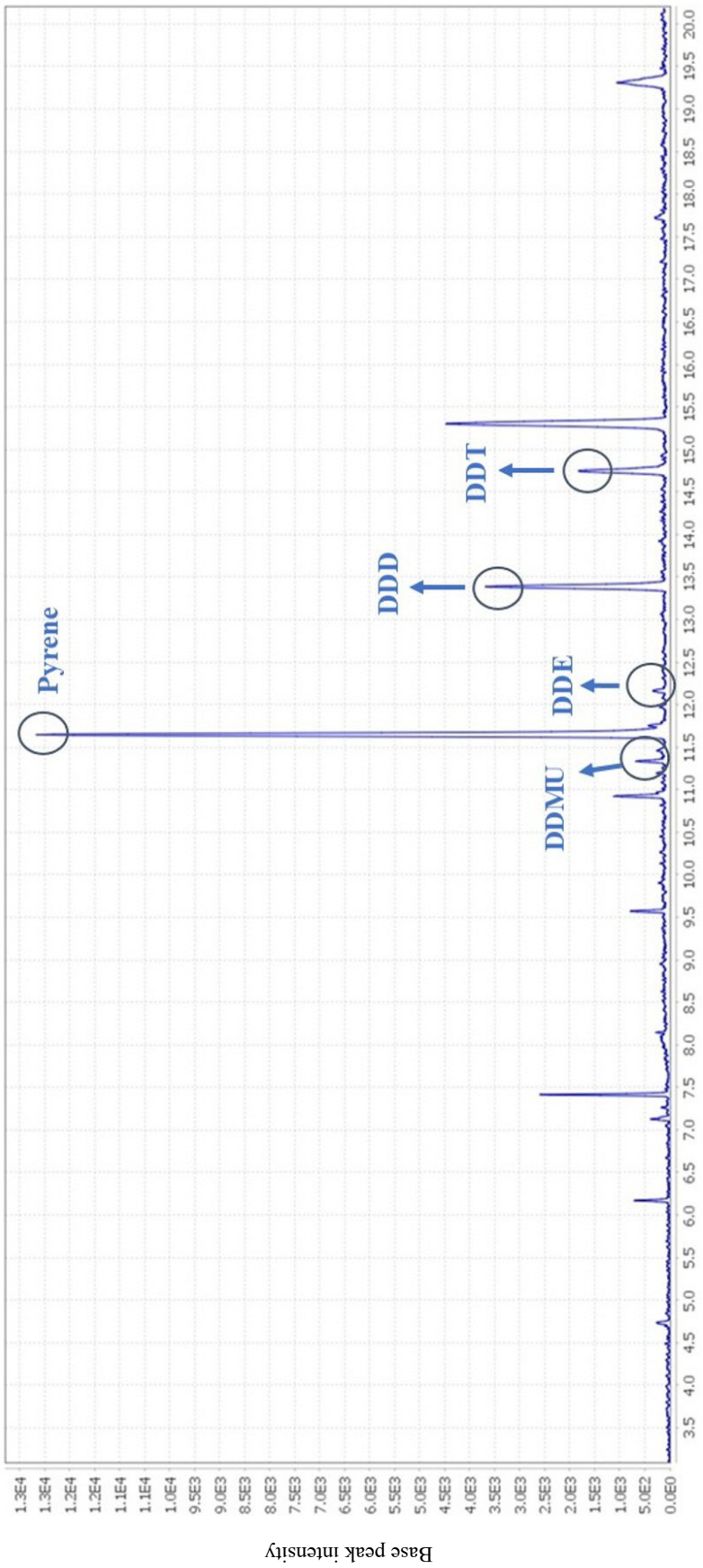
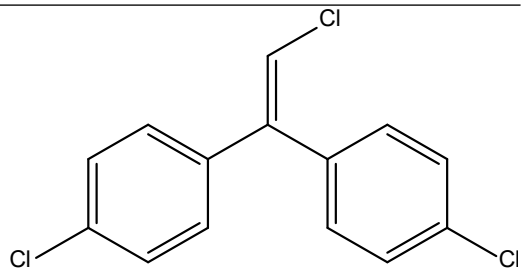
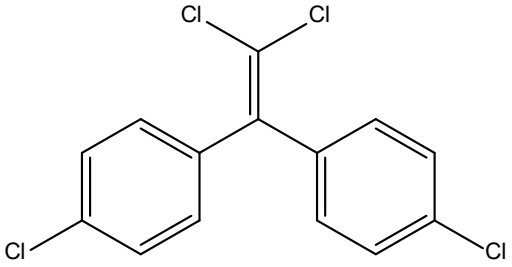
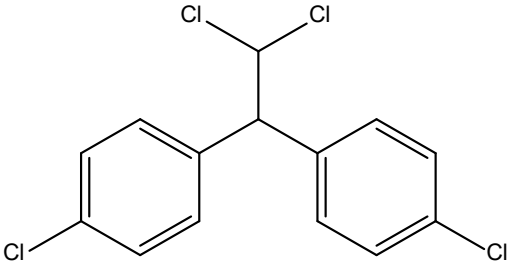
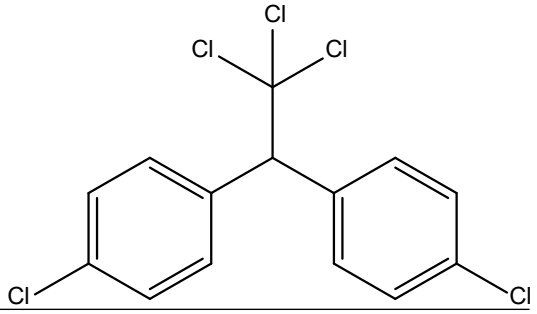


Figure 1. GC-MS chromatogram of biodegradation of DDT by PB

Table 1. Metabolite products of DDT biodegradation by PB

Metabolite	GC retention time (min)	Characteristic fragment ions (m/z)	Molecule structure
DDMU	11.3	281, 247, 212, 176	
DDE	12.2	316, 281, 246, 176	
DDD	13.4	318, 235, 199, 165	
DDT	14.8	352, 317, 282, 235, 165	

compared to low-nitrogen and high-nitrogen medium. In another study, Taştan *et al.* (2016) tried to examine the differences in triclosan degradation results by *Penicillium* sp. with various media, including T6 nutrimedia, minimal salt medium with yeast (MSMY), ammonium mineral salts (AMS), and wastewater (WW), where *Penicillium* sp. grown in MSMY media gave the highest results for triclosan degradation. *Phlebia lindtneri* has the ability to degrade DDT by 70% for 21 days of incubation in Kirk's basal medium low nitrogen (Xiao *et al.* 2011).

Xiao *et al.* (2011) had reported that PB can degrade DDT to DDD, and then convert DDD to DDA, followed by DBP during the incubation period of 21 days. In this study, DDD was also detected as the major metabolite, besides DDE and DDMU. The biodegradation pathway of DDT by PB was proposed based on the metabolite profile shown in Figure 3. There are two pathways of DDT degradation, namely, DDT is reductively dechlorinated to DDD and dehydrogenated to DDE. While DDMU can be produced from reductive

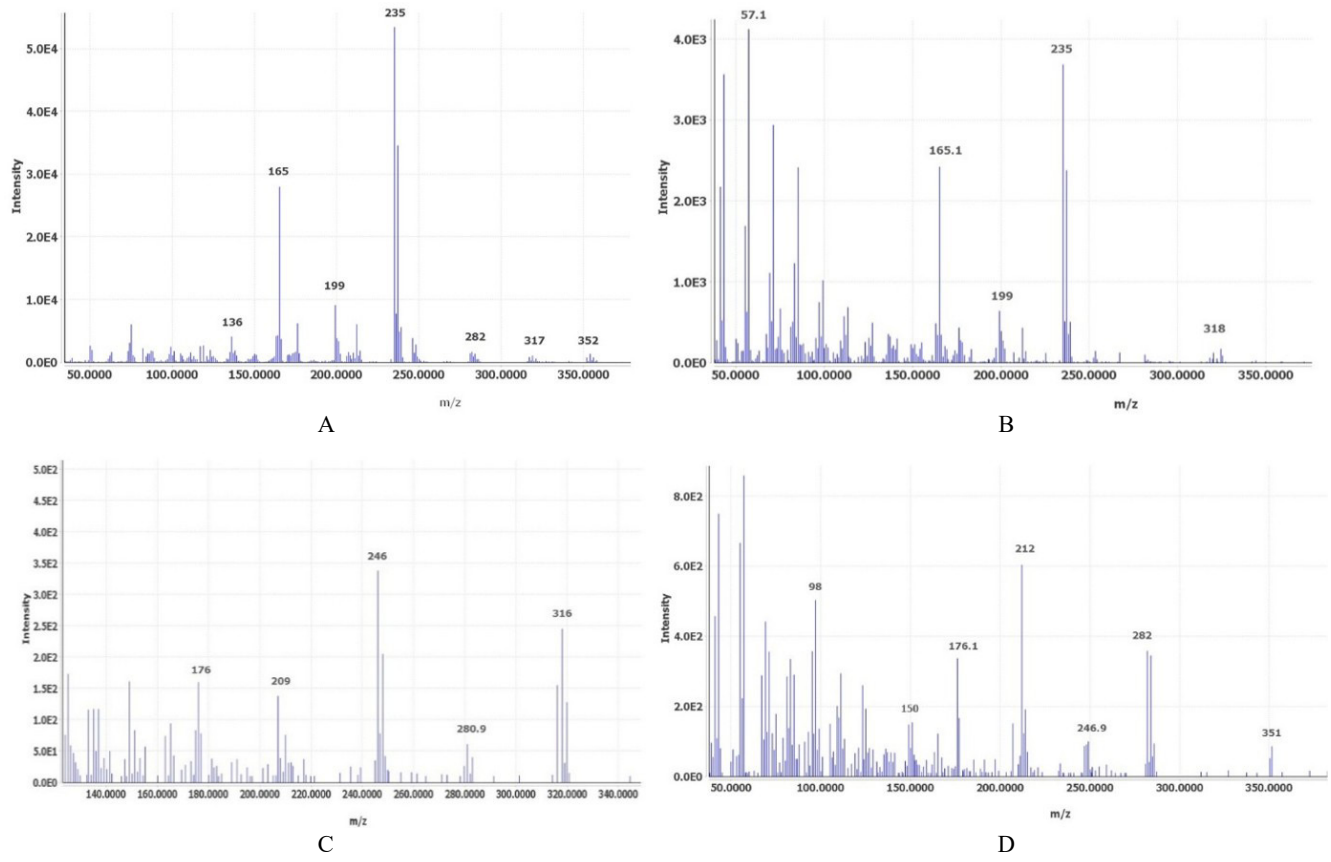


Figure 2. Mass Spectra of: (A) DDT, (B) DDD, (C) DDE, (D) DDMU

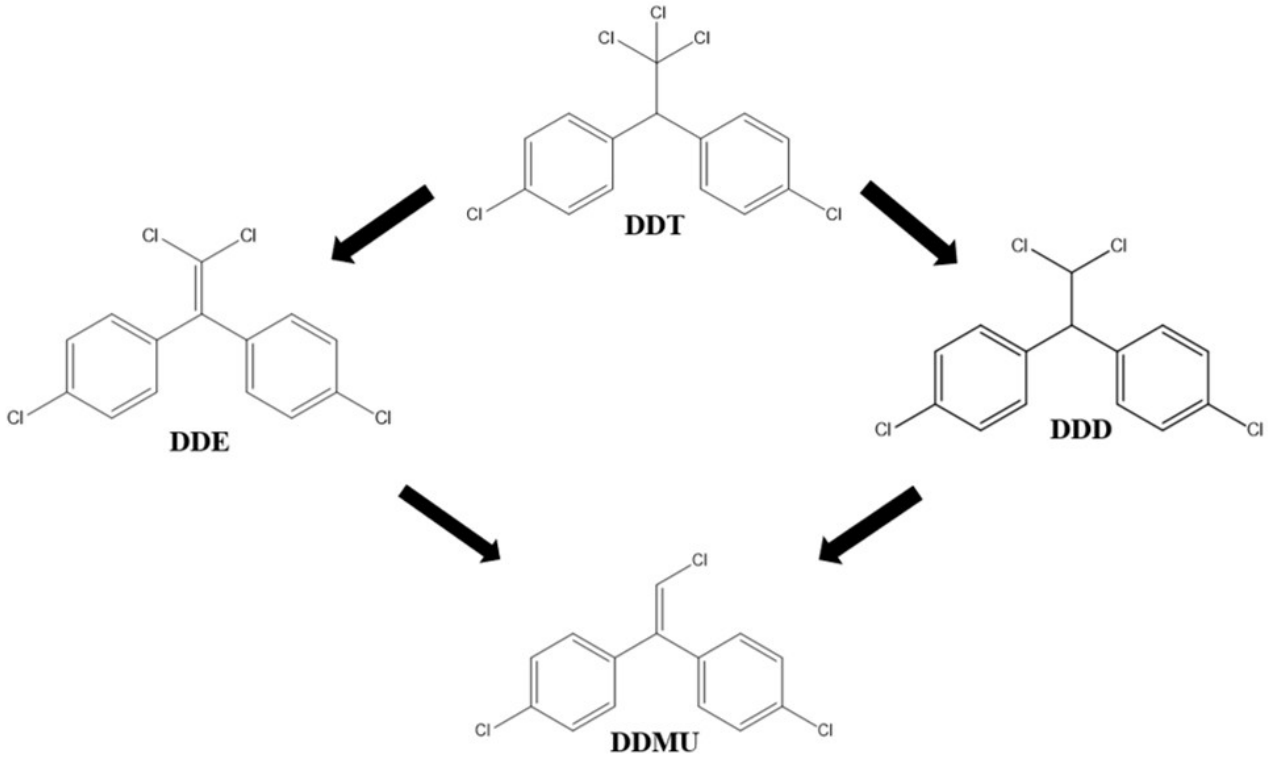


Figure 3. Proposed DDT biodegradation pathways by PB

dechlorination of DDD or dehydrochlorination of DDE.

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