DEGRADATION OF DDT BY *TRAMETES VERSICOLOR* U97 PRE-GROWN IN SEVERAL TYPES OF OIL PALM EMPTY FRUIT BUNCH

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Introduction

In the past time, dichlorodiphenyltrichloroethane (DDT) was heavily used in agriculture and for control the spread of vector-borne human diseases. Considering its health negative effects on wildlife and human health including headache, nausea, vormitting, confusin, and tremors, DDT has already been banned but a limited exemption for controlling mosquitoes which are vectors that carry malaria[1]. Bioremediation is one technology utilizing the metabolic potential of white-rot fungi (WRF) secreting extracellular enzyme to reduce the toxicity of organopollutant compound which has similarity structure with lignin. On the other hand, the utilization of agricultural residue, oil palm empty fruit bunch (EFB), in the environmental technology area is still limited. EFB contains 30% cellulose and 37% lignin which used as substrates for growth of WRF. It is also used to enhance the ligninolytic activity e.g. lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. Therefore, utilization of fungi cultivation in EFB is a challenging opprtunity in environmental pollution treatment. Confirming the optimum treatment of EFB related to growth and enzymatic system of WRF and pollutant degradation is useful for application of bioremediation in upscale. This study aimed to determine the mechanism of EFB as pre-grown source of *Trametes versicolor* U97 to degrade DDT in batch of liquid media. The optimization of EFB for cultivation of fungi to degrade DDT in liquid medium under agitation and non agitation was also conducted. To date, degradation of DDT in bioreactor has been conducted. This study also identified the metabolic products during degradation of DDT.

Materials and methods

Preparation of oil palm empty fruit bunch and culture conditions: Oil palm empty fruit bunch (EFB) was obtained from PTPN VIII palm oil field (Banten, Indonesia) in collaboration with Research Center for Chemistry, Indonesian Institute of Sciences. EFB is provided into three types: powder, 30 mesh, and fiber. Several 5-mm disks of an actively growing fungus on malt extract agar medium were transfered to a 3 h autoclaved EFB combined with 10% (w/w) glucose, 15% (w/w) shiitake no sato, and 50%-60% (v/w) distilled water, and then incubated for approximately one month.

Degradation of DDT in batch: Distilled water and malt extract liquid medium (malt extract 20 g/l, glucose 15 g/l, and polypeptone 1 g/l) were used as media. 3 g of growing fungus in each type of EFB was transfered to 20 ml of sterilized liquid medium. Each inoculated flask was supplemented with 0.1 mM of DDT diluted in 1 ml *N*,*N*-dimethylformamyde (DMF). For optimization, inoculated flasks were conducted under agitation (60 rpm) and non agitation (0 rpm). To know adsorption mechanims of EFB, removal of DDT in erlenmeyer flask containing medium, DDT, and EFB was also measured. The control treatment was performed only containing medium and DDT. The incubation time was 10, 20, and 30 d.

Degradation of DDT in bioreactor: A glass column with a working volume of 45 ml was used as the bioreactor. For the inoculum, 10 g of growing fungus in EFB 30 mesh was put into the column and the bioreactor was filled up with 100 ml distilled water using the same concentration of DDT used in batch. The flow rate used was 1 ml/min (Fig. 1). After the reactor was set up for circulation, samples were also incubated for 10, 20, and 30 d.

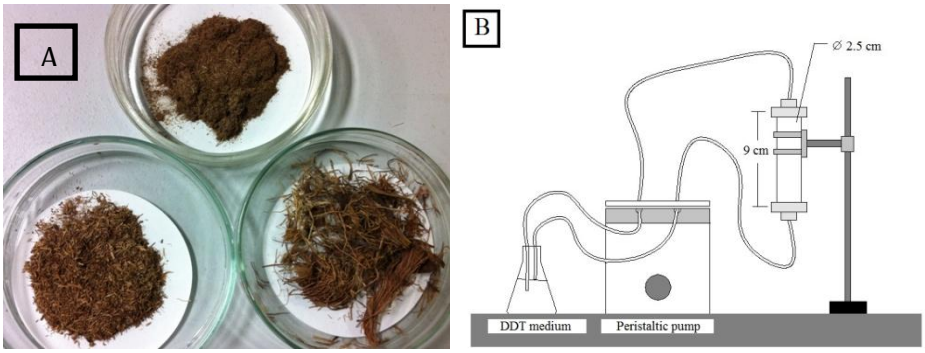

Fig. 1. Appearance of oil palm empty fruit bunch and bioreactor. A: Three types of oil palm empty fruit bunch (upper: fiber, left: 30 mesh, right: fiber); B: Schematic diagram of bioreactor

DDT analysis: After incubation time, the sample was filtrated and extracted thrice with ethyl acetate. It was purified by column chromatography with C200 silica gel eluted with hexane:dichloromethane (3:1). Gas chromatography/mass spectrometry (GC/MS) analysis was performed on an GC-MS Shimadzu QP-2010, equipped with a TC-1 column (30 m, id: 0.25 mm). The carrier gas was helium delivered at a constant flow rate of 1.5 mL min^{-1} with a column pressure of 100 kPa and interface temperature of 120℃. The temperature program was started at 120℃ for 1 min, raised at 20℃ min^{-1} to 180℃, then 2℃ min^{-1} to 210℃, and 5℃ min^{-1} to 310℃, and maintained at 310℃ for 3 min to allow the eluting peak to exit the column. A recovery of 81%-88% was obtained for this analysis.

Metabolites detection: For the identification of metabolites, the retention time of samples were compared with standard by SIM mode of GC/MS with programme as same as described above. 4-chlorobenzoic acid, dichlorobenzophenone, DDE, DDD, and DDT were detected at 4.06 min, 11.95 min, 16.89 min, 19.08 min, and 21.48 min, respectively.

Enzymatic activity analysis: After each culture period, the sample from the liquid medium was filtrated through a 0.2-µm membrane filter. The supernatants were measured for enzymatic activities by absorption using a Spectrophotometer (Shimadzu UV-1600). Laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP) activity were assayed using the method from a previous report[2].

Results and discussion

Degradation of DDT by Trametes versicolor U97 in batch: White-rot fungi (WRF) degraded cellulose and lignin by a ligninolytic enzymatic system and synthesized relevant hydrolytic system where lignocellulosic substrates are used as energy source for growth[3]. Unusually, in this experiment, a distilled water medium was used to clarify the ability of *Trametes versicolor* U97 to utilize oil palm empty fruit bunch (EFB) without any source from the medium. The results showed that it degraded DDT by 68% and 75% in water and malt extract liquid medium over a 30 d incubation period, respectively (Table 1). The ability of *T. versicolor* U97 to degrade DDT in water showed that this fungus was capable of utilizing lignin, cellulose, and hemicellulose as carbon and energy sources for its growth[4]. However, the presence of glucose caused increasing enzyme activity and degradation. The presence of EFB stimulated secretion of ligninolytic activity where production of ligninolytic systems by *T. versicolor* U97 during degradation DDT were higher than without the presence of DDT, meaning secondary metabolism occured. Levin and Forchiassin[5] reported that lignin peroxidase (LiP) acitivity from *Trametes trogii* can be detected after sawdust as a stimulator of ligninolytic systems was added to a culture containing glucose as a carbon source. The control treatment in liquid medium results in the removal of DDT 18%. This means that only a small percentage of EFB was used in the adsorption mechanism. Wood sawdust can be used to remove DDT from aqueous solutions using an adsorption technique which is affected by the nature of the adsorbent e.g. specific area, porous structure, and surface properties, as well as the nature of the pollutant[6]. As a chlorinated and hydrophobic molecule, DDT can be directly bound adsorbed to the specific surface of the

adsorbent particles. However, the presence of natural organic matter which induces a pore blockage competes with pesticide adsorbed on the adsorbent surface. Therefore, there are three EFB mechanisms during degradation of DDT: adsorption, carbon source utilization, and stimulation ligninolytic systems used as secondary metabolism. The batch experiment could have practical importance in the expolitation of *T. versicolor* U97 pre-grown in EFB for the removal of DDT in bioreactors.

Table 1. Removal of DDT in batch and enzyme activity of *T. versicolor* U97 at 30 days

DDT presence	Treatment	Removal of DDT (%)	Enzyme activities (U/l)		
			Laccase	MnP	LiP
With DDT	U97 pre-grown in EFB in water	68	517	373	131
	U97 pre-grown in EFB in malt extract medium	75	334	285	137
	Empty fruit bunch in batch (adsorption)	18	-	-	-
Without DDT	U97 pre-grown in EFB	-	304	284	89

Optimization of oil palm empty fruit bunch for degradation of DDT by T. versicolor U97: The results in Table 2 and Fig. 2 show that the shape of EFB affected the degradation rate and enzyme activity of fungi. By using EFB 30 mesh, the presence of manganese peroxidase (MnP) and LiP could be improved and then degradation of DDT was higher than other shapes. The higher ligninolytic activities secreted by *T. versicolor* U97 pre-grown in EFB coincided with higher degradation of DDT. Furthermore, the higher density of EFB has an effect of decreasing induction of oxygen used for fungus growth, and then affected to degradation of DDT. On the other hand, the fiber of EFB which has lower density has easier to loss during mycelia growth and degradation of DDT[7].

Table 2. Degradation of DDT at 30 d by each type of oil palm empty fruit bunch as source for *T. versicolor* U97

Type of EFB	Treatment	Removal of DDT (%)
Powder	Non-agitation	55
	Agitation	63
30 mesh	Non-agitation	75
	Agitation	80
Fiber	Non-agitation	68
	Agitation	73

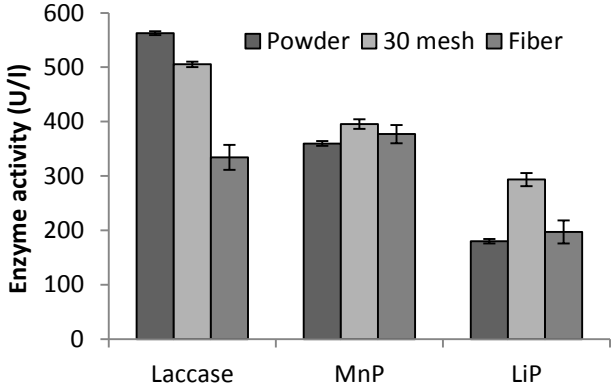

Fig. 2. The maximum enzyme activities of *T. versicolor* U97 pre-grown in each type of oil palm empty fruit bunch during degradation of DDT at 30 d

Degradation of DDT by T. versicolor U97 in a bioreactor: Since *T. versicolor* U97 pre-grown in EFB 30 mesh shows the highest ability to degrade DDT in batch of liquid medium, it was used in bioreactor process. *T. versicolor* U97 pre-grown in EFB 30 mesh degraded DDT 80% over a 30 d incubation period. During incubation, the fungus grew in the reactor in line with the secretion of ligninolytic enzymes (data was not shown).

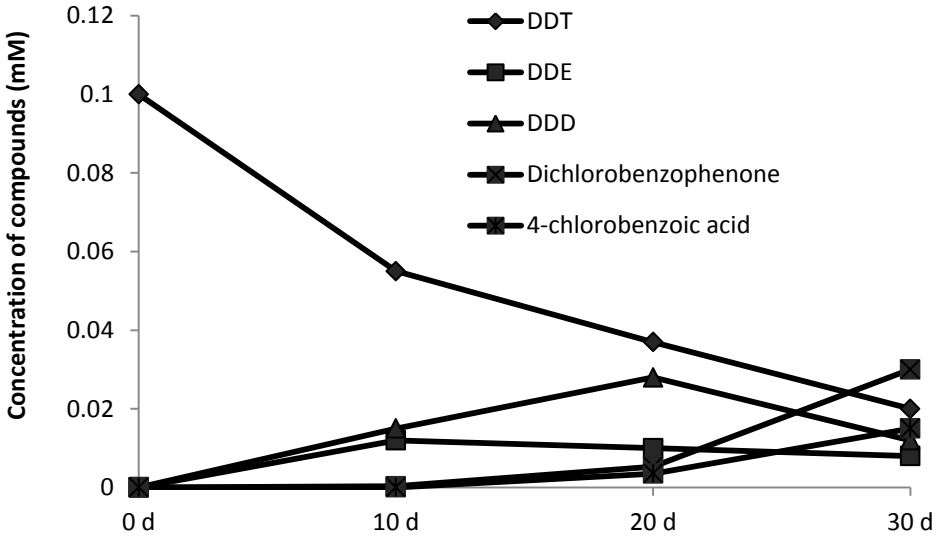

Fig. 3. Degradation of DDT and its metabolic products in bioreactor by *T. versicolor* U97

This figure also shows the products obtained from the degradation of DDT after extraction and analysis by GC-MS in comparison with standards. The culture of *T. versicolor* U97 resulted in DDE, DDD, DBP, and 4-chlorobenzoic acid. DDT to DDE is formed through dehydrohalogenation later became DDD via hydrogenation or DDT directly became DDD by dechlorination. DDD later became DBP through several reactions e.g. dechlorination, hydroxylation, and oxidation, and finally produced single-ring aromatic compound, 4-chlorobenzoic acid, via subsequent meta-ring cleavage[2]. In conclusion, this report shows that *T. versicolor* U97 pre-grown in EFB can successfully degrade DDT in liquid media where 3 mechanisms were occured: adsorption, carbon source utilization, and stimulation ligninolytic systems used as secondary metabolism. Furthermore, optimization of EFB as source for *T. versicolor* U97 to degrade DDT resulted the highest degradation was obtained in EFB 30 mesh and under agitation 60 rpm. The ability of *T. versicolor* U97 degraded DDT in bioreactor shows that EFB can be used as an alternative pre-grown source for WRF to degrade organopollutants.

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