（様式５）(Style5)

学位論文全文に代わる要約
Dissertation Abstract

氏名：
Name
Ajeng Arum Sari

学位論文題目：
Title of Dissertation
Biodegradation of Persistent Organic Pollutants (POPs) and Dyes by Fungi Screened from Nature
（天然から選抜した菌による残留性有機汚染物質および染料の生分解）

学位論文要約：
Dissertation Abstract

Environmental pollution is defined as the substances or energy which introduced by man into the environment (water, air, and soil) that cause effects such as hazards to human health, harm to living resources and ecological systems, damage to structures, or interference with legitimate uses of the environment. Most Persistent Organic Pollutants (POPs) are organochlorine compounds used for diverse applications in industrial and agricultural area. Based on Stockholm Convention, 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) has already been banned in most developed countries but some developing countries still use DDT to control agricultural pests and insects that carry diseases such as malaria. Mostly POPs are persistent, strong mutagenicity, and toxicity in environment as well as for human health.. On the other hand, more than 10,000 commercial dyes and about 7 x 10^5 metric tons/year of dyes are used intensively for textile dyeing, paper printing, leather dyeing, and other applications where 10-15% of the used dyes enter the environment through waste water contaminated with dyestuff. This waste water usually contains chemicals including dyes that may be toxic, mutagenic or carcinogenic for microorganisms and aquatic animals and leading to potential hazard to human. POPs, dyes, pesticides, polyaromatic hydrocarbons (PAHs), and other pollutants are becoming issues of public concern where treatments to reduce these toxicity in the environment are necessary to be conducted. Biodegradation is treatment utilizing metabolic potential of organisms, i.e. yeast, fungi or bacteria, to destroy or render harmless

various contaminants.

Recently, the biological methods using microorganisms such as bacteria, fungi, yeast, actinomycetes, and algae are receiving more attention since they are considered as environmental friendly, low cost, reduce the enormous water consumption, and they degrade hazardous contaminant into less toxic compound. White-rot fungi have versatile enzymes which attack lignin and other organic polymers where such multi enzymes can be also used to degrade pollutants. Potential enzyme involving in the degradation of pollutants are P-450 monooxygenase, dioxygenase, and extracellular ligninolytic enzymes such as manganese peroxidase (MnP), laccase, and lignin peroxidase (LiP). Optimization and application of biodegradation to increase the degradation of pollutants can be conducted in many ways such as addition of surfactant (Tween 80), mediators e.g. $MnSO_4$ and hydroxybenzotriazole (HBT), and utilization of lignocellulosic waste.

This study was focused on the biodegradation of organopollutants by fungi screened from nature. In the first chapter, several white-rot fungi from Matsuyama, Japan were isolated to get the potential fungus performing ability to decolorize RBBR and to degrade several organopollutants by using a rapid and easily screening on agar medium. These selected fungi were used to degrade organopollutants in several media which explained in the next chapters. In the second chapter, the degradation of DDT in liquid medium and soil was determined. As addition, the kinetic models of microbial growth and the degradation of DDT by *Trametes versicolor* U97, effect of inducers and inhibitors on degradation, and metabolic products were determined. Utilization of a newly agricultural residue, oil palm empty fruit bunch, as pre-grown source of *T. versicolor* U97 to degrade DDT in batches of liquid media, bioreactor process, and soil was conducted. Next, the decolorization of RBBR by free cell of *T. versicolor* U97 was determined. As addition, effect of inducers and metabolic products were determined.

Thirty strains of 24 edible white-rot basidiomycetes fungi from decaying wood that are native to

Matsuyama, Japan were tested on RBBR agar medium. Only 7 fungi clearly exhibited dye decolorization ability with a wide range of degree, around 0.66-1.1 cm/d. The high decolorization was obtained with U80, U97, M25, and M31 where the decolorization was noticeable on day 2 and achieved a maximum of 100% decolorization on 9 cm diameter of petri dish by 7-8 d. Decolorization of RBBR was occured because of redox reaction between RBBR dye and ligninolytic enzymes of white-rot fungi. RBBR-decolorizing activity is a simple method for a multienzyme system and can be a valuable tool for xenobiotic biodegradation studies as well as an indication of the physiological conditions of white-rot fungi during bioremediation. On agar medium containing DDT, pentachlorobenzene, and reactive green 19, respectively, the selected fungi showed the different abilities of growth. On DDT and reactive green 19 agar medium, the highest growth was obtained by strain U97 while the highest growth on pentachlorobenzene agar medium was obtained by strain U80. After DNA analysis, the sequence of U97 and U80 ribosomal DNA including ITS region showed identity with *Trametes versicolor* in different strain.

The ability of *T. versicolor* U97 to degrade DDT was determined in malt extract liquid medium. About 0.1 mM DDT was degraded by approximately 73% during the 40 d incubation period (Fig. 1).

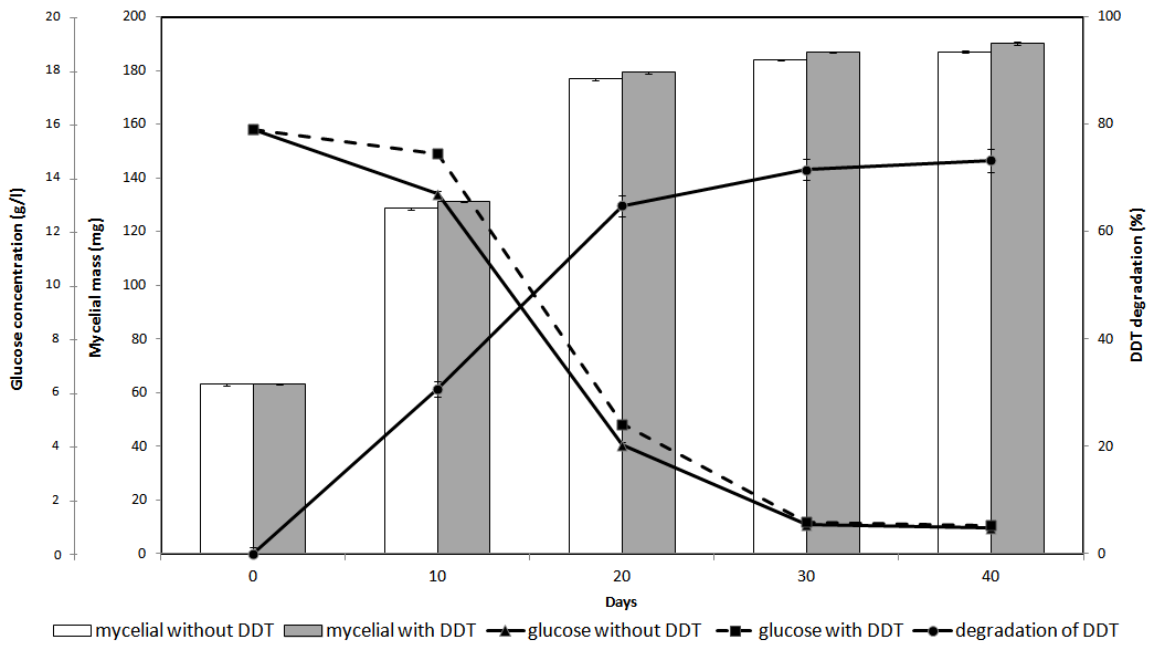

Figure 1. Growth curve of *T. versicolor* U97 without and with DDT

Result of glucose consumption and mycelial dry weight with and without DDT showed no significant difference meaning that DDT was not the carbon source for growth of *T. versicolor* U97. DDT was degraded by secreted enzyme of *T. versicolor* U97 triggered by a limitation of glucose, called secondary metabolism. The fungus may not use the pollutant as an energy source but may transform DDT. DDT is like a lignin compound, rich in carbon, but it is not a growth substrate. DDT was possibly degraded by secreted enzyme *T. versicolor* U97, called secondary metabolism, triggered by a limitation of glucose. *Trametes versicolor* U97 secreted all enzymes with the absence and presence of DDT in cultures on days 15 and 30. However, only 1,2-dioxygenase (115.7 U/L) and LiP (98.7 U/L) were present in high amounts in the presence of DDT. The enhanced LiP and 1,2-dioxygenase activity during cultivation could thus be the result of enzyme induction caused by the presence of small amounts of DDT. 1,2-dioxygenase and LiP levels on addition of DDT were higher, indicating that these enzymes may play important roles in DDT degradation.

Several inducers were used to enhance the ligninolytic activity. The addition of 0.1 mM $CuSO_4$ to the reaction mixtures, which increased laccase activity 300 fold, had no effect on DDT degradation. No improvement

of DDT degradation in the presence of $CuSO_4$ indicated that laccase has no role in this degradation. Addition of $MnSO_4$ did not also improve the degradation of DDT either. On the other hand, by using veratryl alcohol, the presence of LiP could be improved 2-fold, and then degradation of DDT was improved 82%. The effects of several inhibitors on the degradation of DDT in fungal cultures of *T. versicolor* U97 were investigated. The results in Fig. 3.4 show that after adding inhibitors at optimum concentrations, the degradation rate changed. With 0.1 mM EDTA to inhibit LiP, the presence of DDT was reduced by 70% on day 30. *Trametes versicolor* U97 could still produce 1,2-dioxygenase used for degradation of DDT. NaN_3 was effective in inhibiting LiP even though it could induce 1,2-dioxygenase in the presence of 1 mM NaN_3, DDT was degraded 23%. Using 1 mM $AgNO_3$ which inhibits almost all enzymes, DDT was degraded 8.5%. With the addition of 1 mM piperonyl butoxide, commonly used to inhibit P-450 monooxygenase, DDT was degraded 24%. Furthermore, modeling using partial least squares regression showed the most important enzyme for the degradation of DDT by *T. versicolor* U97 to be LiP. However, there was possiblity another enzyme, P-450 monoxygenase, takes part in the degradation of DDT. During degradation, the culture of *T. versicolor* U97 resulted in DDE as a major metabolite and trace amounts of DDD, 1-chloro-2,2-bis(4-chlorophenyl) ethylene (DDMU), 2,2-bis(4-chlorophenyl) ethanol (DDOH), bis(4-chlorophenyl) ketone (dichlorobenzophenone), and 4-chlorobenzoic acid. DDT to DDE is formed through dehydrogehalogenation later became DDD via hydrogenation. DDT can be directly dechlorinated became DDD. DDD undergoes further dechlorination reaction to yield DDMU, which was then hydroxylation reaction to DDOH. This compound might have been oxidized to dichlorobenzophenone, and then became single-ring aromatic compound, 4-chlorobenzoic acid, via subsequent *meta*-ring cleavage.

Next, oil palm empty fruit bunch was utilized for pre-grown source of white-rot fungi to degrade DDT in soil. Degradation of DDT by *T. versicolor* U97 pre-grown in wood meal and oil palm empty fruit bunch was

compared. Fig. 2 shows that cultivation with oil palm empty fruit bunch resulted in more LiP and higher degradation than with wood meal. For that reason, oil palm empty fruit bunch can be used to replace wood meal as a pre-grown source for white-rot fungi. However, comparing with our previous report, this degradation was still lower than in liquid medium . This is related to sorption phenomena where mass transfer effects limit the accessibility of DDT in soil compared to a liquid medium.

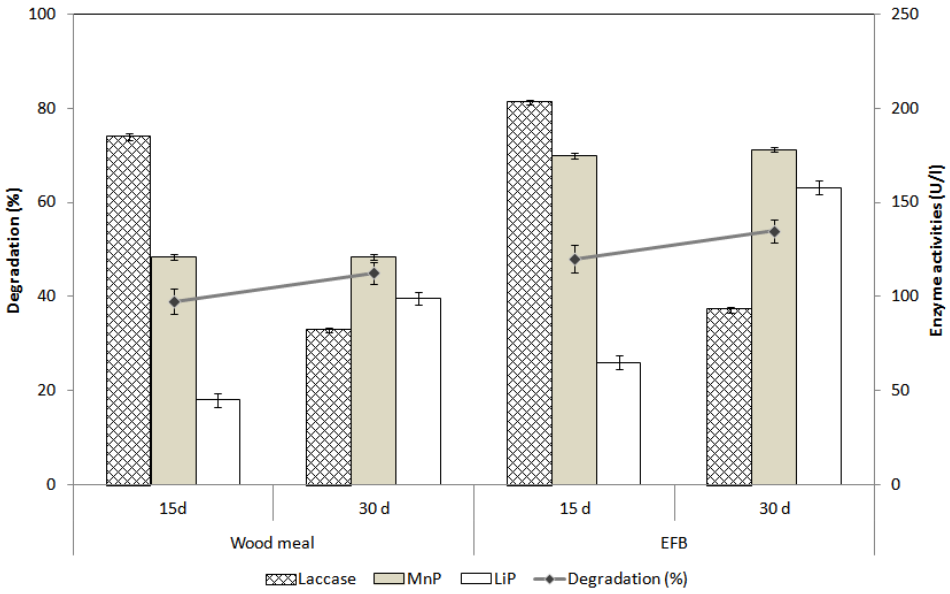

Figure 2. Degradation of DDT in soil and enzyme activities of *T. versicolor* U97 pre-grown in wood meal and oil palm empty fruit bunch

The additives in the form of metal ions e.g. copper and manganese are used to stimulate ligninolytic activity of fungi. Improving the ligninolytic system was attempted by addition of several additives in fungal cultures of *T. versicolor* U97 during DDT degradation. After adding additives, the degradation and enzyme activity produced by the fungus were changed (Fig. 3).

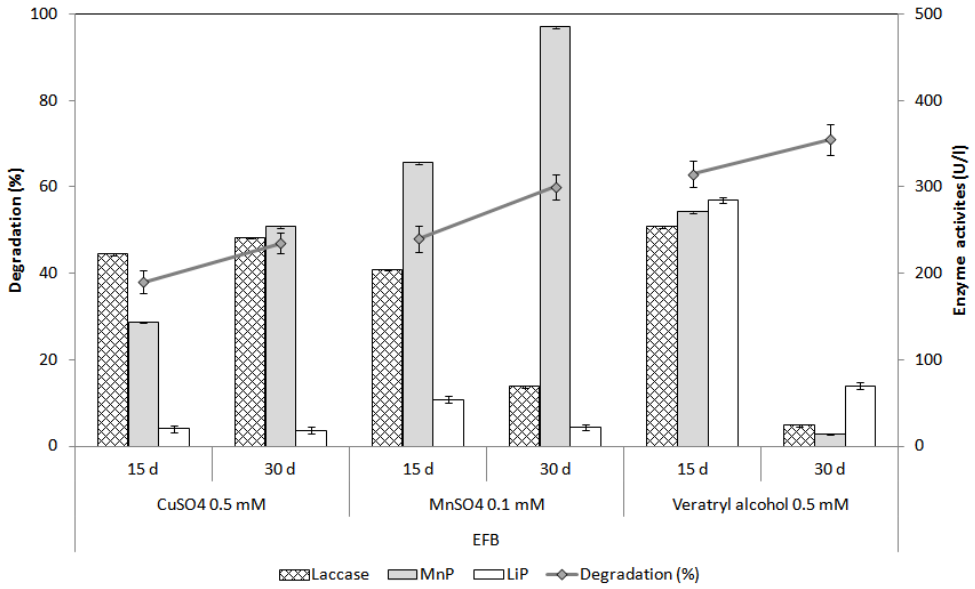

Figure 3. Effects of additives on degradation of DDT in soil and enzyme activities of *T. versicolor* U97 pre-grown in oil palm empty fruit bunch

Addition of a little concentration of manganese has increased the enzyme activity of *T. versicolor* U97 during degradation of DDT. At 30 d, two fold laccase production following addition of $CuSO_4$ did not improve degradation of DDT. Furthermore, addition of veratryl alcohol-H_2O_2 enhanced LiP and MnP production, and then improved DDT degradation by as much as 73%. The catalytic cycle of LiP involves the oxidation of ferric peroxidase (Fe^{3+}) by two electrons from hydrogen peroxide.

Table 1 shows the products obtained from the degradation of DDT by *T. versicolor* U97 at 30 d after extraction and analysis by GC-MS in comparison with standards. The culture of *T. versicolor* U97 resulted in DDE, DDD, dichlorobenzophenone (DBP), and 4-chlorobenzoic acid (4-CBA).

Table 1. Mass balance of DDT and its metabolite products during DDT degradation in soil by *T. versicolor* U97

Treatment	Degradation rate	Concentration of metabolite products (ppm)				
	(%)	DDE	DDD	DBP	4-CBA	Total
-	74	3.5 ± 0.1	1 ± 0.2	0.7 ± 0.3	0.3 ± 0.1	5.5
EDTA	32	2.5 ± 0.2	0.2 ± 0.1	-	-	2.7

Lack of mass balance may indicates that DDT was also metabolized to other products undetectable with GC-MS without (trimethyl silyl) TMS derivatization. The effect of EDTA on the degradation of DDT was investigated. After addition of 0.1 mM EDTA, degradation of DDT was only 32%, coinciding with decreasing LiP and MnP. During addition of EDTA, only DDE and DDD were produced. It confirms that peroxidase enzymes played a role in the degradation of DDT.

RBBR was decolorized above 50% in 3 h after the dye addition. At the end of the 6-h incubation period, *Trametes versicolor* U97 had decolorized 85% of 100 ppm RBBR. All ligninolytic enzymes were produced during RBBR decolorization and the highest enzyme activity of *T. versicolor* U97 was laccase (80 U/L). Furthermore, several inducers were used in fungal cultures of *T. versicolor* U97 to during RBBR decolorization. In addition of $CuSO_4$, decolorization was only 77%, meaning laccase did not play a role in RBBR decolorization. In the presence of $MnSO_4$, which induces MnP, RBBR decolorization was also reduced to 75% in 6 h. Furthermore, addition of veratryl alcohol improved RBBR decolorization became 93%. For detection of metabolic products, after 6 h, one major peak at retention time 2.8 min was occured. After 24 h, another major peak was detected at retention time 18.3 min.

In conclusion, *T. versicolor* U97 degraded 73% and 54% of DDT in liquid medium and soil, respectively, by co-metabolism. *T. versicolor* U97 decolorized 85% of RBBR in 6 h. Among inducers for ligninolytic enzymes, only veratryl alcohol improved RBBR decolorization. It was considered that *T. versicolor* U80 and *T. versicolor* U97 can be used as alternative fungi to degrade some organopollutants.

(注) 要約の文量は，学位論文の文量の約１０分の１として下さい。図表や写真を含めても構いません。
(Note) The Abstract should be about 10% of the entire dissertation and may include illustrations