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学 位 論 文 要 旨
Dissertation Abstract

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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

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Stockholm Convention aims to protect human health and environment by banning the production and use of Persistent Organic Pollutants (POPs) to humankind. These POPs are sometimes used in our daily life such as for pesticides, production of solvents, and pharmaceuticals. The majority of POPs are environmental hormones and now POPs, dyes, pesticides, polyaromatic hydrocarbons (PAHs), and other pollutants are becoming issues of public concern where treatments to reduce toxicity of these pollutants 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. In the preliminary research, several white-rot fungi from Ehime, 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. Furthermore, *Trametes versicolor* U97 was selected for the degradation of DDT, RBBR, and reactive green 19 while *Trametes versicolor* U80 was selected for degradation of pentachlorobenzene. Based on these backgrounds, the objectives of the study are: 1) to characterize the degradation of DDT by *T. versicolor* U97; 2) to investigate the degradation of pentachlorobenzene by *T. versicolor* U80; and 3) to investigate the decolorization of RBBR and reactive green 19 by *T. versicolor* U97.

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. 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. Addition of several additives ($CuSO_4$, $MnSO_4$, veratryl alcohol, EDTA, NaN_3, $AgNO_3$, and piperonyl butoxide) showed variation on degradation and enzyme activities results where the maximum degradation 80% was obtained at 30 d after addition of veratryl alcohol. Modeling of several inhibitors using the partial least squares function in Minitab

15, revealed lignin peroxidase (LiP: 98.7 U/L) that plays a role in the degradation of DDT compare to other enzymes tested. During degradation, *T. versicolor* U97 produced six metabolic products. Oil palm empty fruit bunch (EFB) can be used as an alternative pre-grown source for white-rot fungus to degrade organopollutants. *Trametes versicolor* U97 pre-grown in EFB degraded 58% and 61% of DDT (0.1 mM) in batches and bioreactor of liquid media for 15 d, respectively. During degradation, three mechanisms of EFB were adsorption, carbon source utilization, and stimulation ligninolytic systems used as secondary metabolism. This culture also has ability to degrade DDT in bioreactor even it could not be maintained for 30 d possibly due to continued mycelial growth which reduced the volume and hydraulic retention time. For 30 d incubation, degradation of DDT (10 ppm) in soil by *T. versicolor* U97 pre-grown in EFB was 54%, however, the degradation could be improved to 63% after addition of veratryl alcohol.

Free cell and immobilized fungi of *Trametes versicolor* U80 degraded 43% and 54% of pentachlorobenzene (0.1 mM) in batch and bioreactor of liquid media for 40 d incubation where the relative degradation per-unit biomass was similar during the time of degradation indicating the pollutant was not toxic for the growth of fungal strain. Application of *T. versicolor* U80 in soil could be degraded 43% of pentachlorobenzene (10 ppm) for 40 d. Several metabolic products were produced during degradation. Addition of EDTA and piperonyl butoxide revealed that LiP and P-450 monooxygenase was responsible for degradation in different time. Moreover, the degradation can be improved by addition of Tween 80, $MnSO_4$, and veratryl alcohol. The maximum degradation of 57% and 65% were obtained after addition of veratryl alcohol in batch of liquid medium and soil, respectively.

In liquid medium, *T. versicolor* U97 decolorized 85% of RBBR (100 ppm) for 6 h. HPLC analysis revealed that two peaks were identified as metabolic products. Using mixed mediators consist of Tween 80, hydroxybenzotriazole (HBT), and $MnSO_4$-H_2O_2 87% of decolorization of RBBR (100 ppm) was obtained by immobilized fungi of *T. versicolor* U97 in bioreactor process for 72 h. On the other hand, by using agitation 60 rpm and mixed mediators, immobilized enzymes of *T. versicolor* U97 decolorized 90% of RBBR for 72 h. Immobilized fungi and immobilized enzymes of *T. versicolor* U97 were able to decolorize Reactive green 19 (100 ppm) for 72 h approximately 42% and 21%, respectively. By using mixed mediators, 88% and 82% of the maximum decolorization of Reactive green 19 could be obtained by immobilized fungi and immobilized enzyme of *T. versicolor* U97 in bioreactor, respectively.

In conclusion, *T. versicolor* U97 degraded 73%, 61%, and 54% of DDT in liquid medium, bioreactor, and soil, respectively, by co-metabolism. Free cell and immobilized fungi of *T. versicolor* U80 degraded 43%, 54%, and 43% of pentachlorobenzene in batch and bioreactor of liquid media, and soil for 40 d, respectively. The mixed mediators (Tween 80, HBT, MnSO4, H_2O_2) improved decolorization of RBBR and Reactive green 19 up to 2-4 fold by *T. versicolor* U97. It was considered that *T. versicolor* U80 and *T. versicolor* U97 can be used as alternative fungi to degrade some organopollutants.