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Effects of Mediators for Ligninolytic Enzyme Production and Kinetic Studies on Degradation of Pentachlorobenzene by *Trametes versicolor* U80

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Abstract Pentachlorobenzene is one new persistent organic pollutants (POPs) that has been recently added to the Stockholm Convention on Persistent Organic Pollutants. Based on this reason, one treatment having ability to degrade this compound is needed. The microbiological process by using white-rot fungus was used in this experiment. Free cell of *Trametes versicolor* U80 degraded pentachlorobenzene 43 % in liquid medium at 40 days incubation. The rapid initial uptake of pentachlorobenzene was obtained in the first 20 days. The results based on ionization potential and the partial least square function indicated that both enzymatic systems of lignin peroxidase and P-450 monooxygenase involved in the degradation of pentachlorobenzene. By using addition of Tween 80, $MnSO_4$, and veratryl alcohol, degradation of pentachlorobenzene could be improved. Based on kinetic study, the use of 1 % of Tween 80 showed the highest degradation rate (2.0619/day) and the degradation of pentachlorobenzene by 50 % can be shortened up to 24 days. Application of *T. versicolor* U80 in soil and bioreactor degraded pentachlorobenzene 43 and 50 % at 40 days, respectively. *T. versicolor* U80 shows good capability degrading pentachlorobenzene in soil and bioreactor although it is lower than in liquid due to the difference of pollutant accessibility and transfer oxygen. Finally, strain *T. versicolor* U80 can be proposed as an excellent candidate for remediation application in pentachlorobenzene pollution.

Keywords *Trametes versicolor* · Pentachlorobenzene · Lignin peroxidase · Tween 80 · Kinetic study

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1 Introduction

In addition to the original Stockholm Convention on Persistent Organic Pollutants (POPs), nine new POPs have been recently added, including pentachlorobenzene. This compound has also been proposed for inclusion in the POPs protocol of the Longrange Transport of Atmospheric Pollutants Convention of the UNECE. In the past, pentachlorobenzene was used to reduce the

viscosity of polychlorinated biphenyls (PCBs) products during heat transfer and it was also used in electrical equipment mixed with PCBs (Bailey et al. 2009). This compound can also be produced as a byproduct by industrial processes using chlorine and carbon. Pentachlorobenzene 0.4 ng/L was found in water and sediment in the Yangtse River near Nanjing, China (Jiang et al. 2000). Because of its persistent, long-range transportable nature and toxic biological effects, the presence of pentachlorobenzene in the environment should get attention. Bailey et al. (2009) stated that based on its characteristics, the natural degradation of pentachlorobenzene in the water and soil is estimated to be months to years. Based on this reason, one treatment having ability to degrade this compound is needed.

The microbiological process for degradation of toxic organic pollutant is now considered as a promising method for the problem of environmental pollution. Bacteria has the ability to degrade di- and trichloro benzene even though it has low activity to degrade highly chlorinated benzenes (Takagi et al. 2009). White-rot fungus *Trametes versicolor* has ability to degrade 1,2,3-trichlorobenzene and 1,2,4-trichloro benzene by using P-450 monooxygenase (Marco-Urrea et al. 2009). However, white-rot fungi have been extensively studied for pollutant removal because they mainly produce ligninolytic enzymes, i.e., laccase, lignin peroxidase, and manganese peroxidase. Unfortunately, few researches have studied pentachlorobenzene degradation.

Laccase is *N*-glycosylated extracellular multi copper oxidases that play a key role in the depolymerization of lignin (Wells et al. 2006; Hadibarata and Nor 2014). Manganese peroxidase is a low molecular weight diffusible ligninolytic oxidant, which oxidize Mn^{2+} to Mn^{3+}. It was secreted in carbon and nitrogen limited media and enhanced with supplementation of Mn^{2+} and veratryl alcohol (Asgher et al. 2008). Lignin peroxidase is capable of mineralizing a variety of aromatic compounds (Shrivastava et al. 2005). This enzyme is effective for degradation of pollutant in the presence of hydrogen peroxide and mediators. The role of ligninolytic enzymes in the degradation of pentachlorobenzene mainly depends on the composition of the culture medium and its mediators. The redox mediators have the potential to mediate oxidation reaction between a pollutant and an enzyme and enhance the enzymatic activity (Yamanaka et al. 2008; Jamal et al. 2011).

Oil palm empty fruit bunches (OPEFB) contains lignin that used for pre-grown source of white-rot fungi during degradation of pollutant (Sari et al. 2014). A study for degradation of pentachlorobenzene in soil is necessary to be conducted because the possibility of this pollutant is settled in soil. However, the extremely low solubility of pentachlorobenzene should get the attention. On the other hand, for technical application, immobilized fungi to degrade organopollutant compounds have been also developed. Immobilized fungi offers advantages such as easy recovery, easy packaging, short retention time, and protection of cells from pollutants (Sari et al. 2015).

Although biodegradation of organochlorine compounds has been widely observed, the effect of mediators on degradation of pentachlorobenzene has not been extensively investigated. Kinetic studies were performed by determination of the residual pentachlorobenzene concentration in samples collected during degradation. This is the first study to evaluate the ability of *Trametes versicolor* to degrade pentachlorobenzene based on its degradation kinetics. In this study, the potential of free cells and immobilized fungus of *T. versicolor* U80 to degrade pentachlorobenzene in liquid medium (batch and bioreactor) and soil was evaluated. This study also investigated the enzymatic activities involving the degradation of pentachlorobenzene after addition of several mediators. In order to understand the fate of pentachlorobenzene, the biodegradation mechanisms for its decomposition were clarified.

2 Materials and Methods

2.1 Chemicals and Preparation of Fungus

Pentachlorobenzene and all solvents were purchased from Wako Pure Chemical Industry, Osaka, Japan. The structure of pentachlorobenzene is shown in Fig. 1. Oil palm empty fruit bunches (OPEFB) was obtained from PTPN V, Sumatera, Indonesia. *T. versicolor* U80 was collected from decaying wood which is native to Matsuyama, Japan and preserved in the Faculty of Agriculture, Ehime University, Japan. This strain cultured on a malt extract agar medium was stored at 25 ° C for several days and kept at low temperature 4 °C.

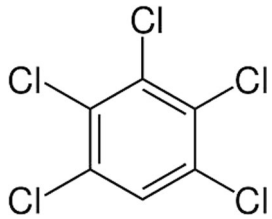

Fig. 1 Structure of pentachlorobenzene

2.2 Degradation Test with Pentachlorobenzene

A malt extract liquid medium containing (in g/L) malt extract (20), glucose (15), and polypeptone (1) was used for degradation experiments (Sari et al. 2015). Three 5-mm plugs were added into the Erlenmeyer flask containing 20 mL of medium. Each inoculated flasks was pre-incubated for 7 days. At the incubation stage, 0.1 mM of pentachlorobenzene diluted in dimethy lformamyde was added to the Erlenmeyer flasks. As the control, fungal culture after pre-incubation was autoclaved and pentachlorobenzene was added. The mediators' ligninolytic activity enhancer was used to know the change of enzyme activity affecting degradation of pentachlorobenzene. Several concentrations of Tween 40 and Tween 80 (0.5, 1, and 1.5 %) were added to the cultures of *T. versicolor* U80 in liquid medium, respectively. Furthermore, 0.1 mM of $CuSO_4$, 0.05 mM $MnSO_4$, or 0.1 mM veratryl alcohol-0.1 mM H_2O_2 were added to the cultures in liquid medium.

Soil from paddy field in the Faculty of Agriculture, Ehime University, was used for this experiment. It was air-dried and sieved through a fraction passing Ø 3-mm mesh and stored at room temperature before use. Thirty grams sterilized soil was mixed with 3 g of *T. versicolor* U80 pre-grown in OPEFB, 10 ppm pentachlorobenzene diluted in DMF, 5 % (*w*/*w*) glucose, and 10 % (*w*/*w*) nutrient source called shiitake no sato. Control treatment was performed with only soil medium and 10 ppm of pentachlorobenzene without inoculated fungus.

A 45-mL glass column was used for the bioreactor. Growing fungus in malt extract medium was homogenized 10,000 rpm for 10 min and then used for the inoculum (Sari et al. 2015). 1.5 % sodium alginate was mixed with crude fungal and then droply added into 0.1 M $CaCl_2$ diluted in water. The beads were added into the column, and the bioreactor was filled up with 100 mL pentachlorobenzene solution (final concentration 0.1 mM). The flowrate used was 1 mL min^{-1}. All the samples were incubated in the dark place at 25 °C for 10–40 days.

2.3 Pentachlorobenzene Degradation Analysis

After harvesting, the culture from liquid medium and bioreactor was extracted using ethyl acetate three times. On the other hand, the sample in soil was extracted by using soxhlet apparatus with dichloromethane for 16 h. All samples were purified by column chromatography with C200 silica gel (hexane/dichloromethane, 9:1). GC-MS Shimadzu QP-2010 was dissolved used to analyze samples. It was equipped with a TC-1 column (30 m, id 0.25 mm), helium as a carrier gas, a flow rate 1.5 mL min^{-1} with column pressure 100 kPa, and interface temperature at 120 °C. The temperature program was started at 120 °C hold for 2 min, raised to 180 °C with a rate 20 °C min^{-1}, then 2 °C min^{-1} to 210 °C, then 5 °C min^{-1} to 310 °C, and finally maintained for 3 min to allow eluting peak to exit the column. Aliquot of 1 μL of sample was injected into the chromatographic system.

2.4 Enzyme Assays

Lignin peroxide (LiP), manganese peroxide (MnP), and laccase were assayed by determination of absorbance of sample using a Shimadzu UV/Vis-1600 spectrophotometer. In the harvest time, the culture from liquid medium was filtered on a filter paper. On the other hand, 5 g of the culture from soil was diluted with 30 ml distilled water, and then homogenized at 10,000 rpm for 10 min. After mixing, the mixture was filtrated (Sari et al. 2013). MnP activity due to oxidation of 2,6-dimethoxyphenol malonate buffer in $MnSO_4$ solution was assayed at 470 nm (Takano et al. 2004). LiP activity was determined by monitoring the formation of 2 mM H_2O_2 and LiP buffer at 310 nm (Collins et al. 1997). Laccase activity was determined by syringaldazine oxidation at 525 nm (Zavarzina and Zavarzin 2006). The enzyme activities were expressed in unit/l.

2.5 Statistical Analyses

All data were expressed as mean ± SD (standard deviation) from triplicate experiments. Partial least square and linear regression to obtain kinetic study were calculated by using MINITAB 17.

3 Results and Discussion

3.1 Degradation of Pentachlorobenzene in Liquid Medium and its Mechanisms

In control experiment, pentachlorobenzene was degraded only 5 %, so that the adsorption mechanism by using the dead fungal was not occurred. The result explains that biosorption was not playing a role in the bioremoval mechanism. Figure 2 shows that pentachlorobenzene was degraded by approximately 43 % during the 40-day incubation period. The rapid degradation of pentachlorobenzene was obtained in the first 20 days, and then followed by a slower rate up to 40 days. The slower degradation rate was caused by the decreasing of nutrient source. A few informations providing degradation of pentachlorobenze by white-rot fungi were obtained. Nineteen strains of basidiomycetes were found to be capable of growing in soil contaminated with hexachlorobenzene (HCB) with concentration range of 5000–50,000 mg of HCB kg^{-1} soil. *Psilocybe castanella* CCB444 and *Lentinus zeyheri* CCB274 were the most capable of degrading HCB in soil during a 65-day study period (Matheus et al. 2000). *T. versicolor* (ATCC42530) has able to degrade 1,2,3- and 1,2,4-trichlorobenzene in initial concentration 6 mg L^{-1}. After 7 days of incubation, the percent degradation of 1,2,3-and 1,2,4-trichloro benzene were 91.1 and 79.6 %, respectively (Marco-Urrea et al. 2009).

T. versicolor U80 secreted all enzymes during the absence and presence of pentachlorobenzene on days 10 and 20. The enzyme activity increased 5- to 10-fold in the presence of pentachlorobenzene at 20 days, and this means that catalysis of the decomposition of the recalcitrant aromatic compounds by *T. versicolor* U80 is affected by ligninolytic enzymes. This result was also in line with the rapid degradation at those days.

The pollutant oxidation by white-rot fungi is not rapid but efficient, but they are very nonspesific (Hammel 1994). Organopollutants are usually chemically resistant because of delocalization of their energy and, moreover, the dense clouds of Π-electrons on both sides of the ring structures make them highly resistant to nucleophilic attack (Cajthaml and Svobodová 2012). Therefore, ligninolytic enzymes are needed to breakdown this compound through reaction with electron in the ring structure of aromatic compounds. However, these enzymes require the presence of H_2O_2 as the electron acceptor to oxidize organopollutants (Ruiz-Dueñas and Martínez 2009). *Phanerochaete chrysosporium* degraded several polycyclic aromatic hydrocarbons (PAHs) by using LiP and MnP (Hammel 1994). LiP oxidizes certain PAHs directly based on their ionization potential, whereas MnP co-oxidizes them indirectly during enzyme-mediated lipid peroxidation. The mechanism involves applying the required energy to remove one-electron, so-called ionization potential and transforms substrates into radical, which used to degrade pollutant compounds. Pentachlorobenzene has

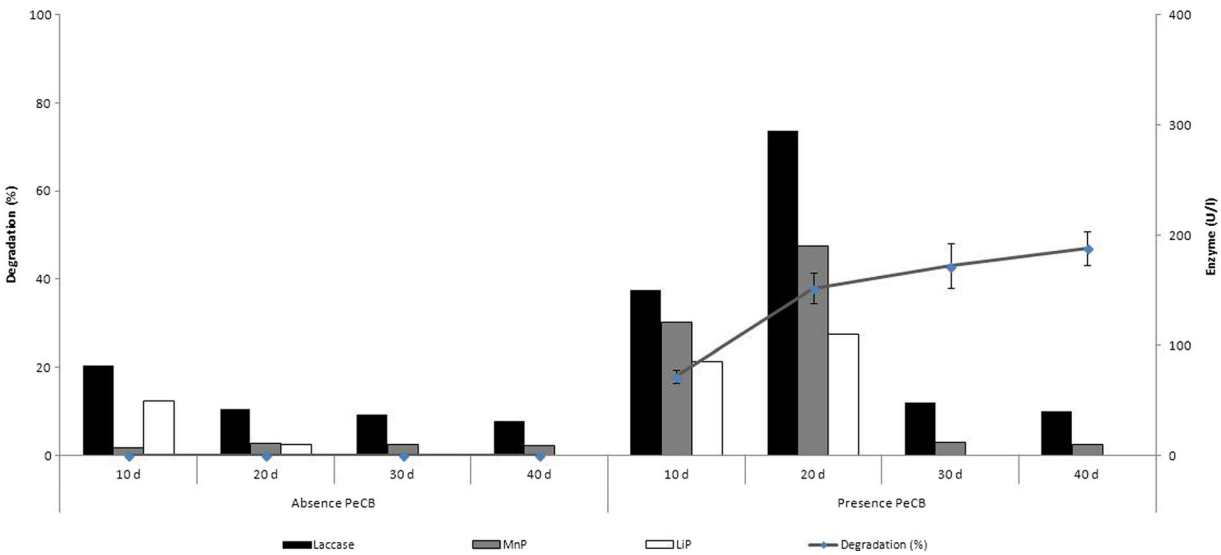

Fig. 2 Degradation of pentachlorobenzene by *T. versicolor* U80 and its enzyme activity

Table 1 The effect of different parameters on pentachlorobenzene degradation by *T. versicolor* U80

Parameters	Pentachlorobenzene degradation (%)			
	Day 10	Day 20	Day 30	Day 40
Without addition mediators	18.2 ± 1.5	38.5 ± 3.5	43.3 ± 5.1	47.4 ± 3.8
Effect of Tween 40				
0.5 %	15.9 ± 1.4	36 ± 2.9	41.9 ± 3.5	43.9 ± 2.5
1 %	13. ± 1.1	35.4 ± 3.7	40.7 ± 2.1	42.7 ± 1.5
1.5 %	11.3 ± 2.9	31 ± 1.9	35.5 ± 3.8	37.5 ± 3.7
Effect of Tween 80				
0.5 %	25.7 ± 4.7	43.3 ± 4.5	58.0 ± 1.5	61.3 ± 2.9
1 %	28.9 ± 3.9	58.7 ± 1.5	65 ± 3.7	68.9 ± 1.2
1.5 %	22 ± 2.5	38.4 ± 2.8	44.2 ± 2.8	46.5 ± 2.6
0.1 mM veratryl alcohol −0.1 mMH_2O_2	20.3 ± 3.9	50.1 ± 3.5	63.3 ± 2.7	67.2 ± 1.9
0.1 mM $CuSO_4$	15.2 ± 2.9	31.9 ± 2.7	37.6 ± 2.5	40.2 ± 3.5
0.05 mM $CuSO_4$	27.8 ± 2.9	45.3 ± 2.6	55.5 ± 2.9	58 ± 2.2

ionization potential 8.8–9.21 eV. LiP-H_2O_2 catalyzes the oxidation of pentachlorobenzene based on the ionization potential of the pollutant. Since ionization potential LiP 7.35 eV, it seems that pentachlorobenzene was difficult to be degraded by LiP itself. It indicates that degradation of pentachlorobenze is not only affected by LiP. The other possibility is role of P450-monooxy genase. Ligninolytic fungi were able to decompose organopollutants using ligninolytic enzymes and cytochrome P-450 monooxygenase (Cajthaml 2015). To confirm this hypothesis, partial least square analysis was used later.

3.2 Effects of Mediators on Degradation in Liquid Medium

In order to demonstrate enhancement of the ligninolytic activity of *T. versicolor* U80, some mediators were used in this study. The surfactans as mediators, Tween 40 and Tween 80 in several concentrations, were employed on

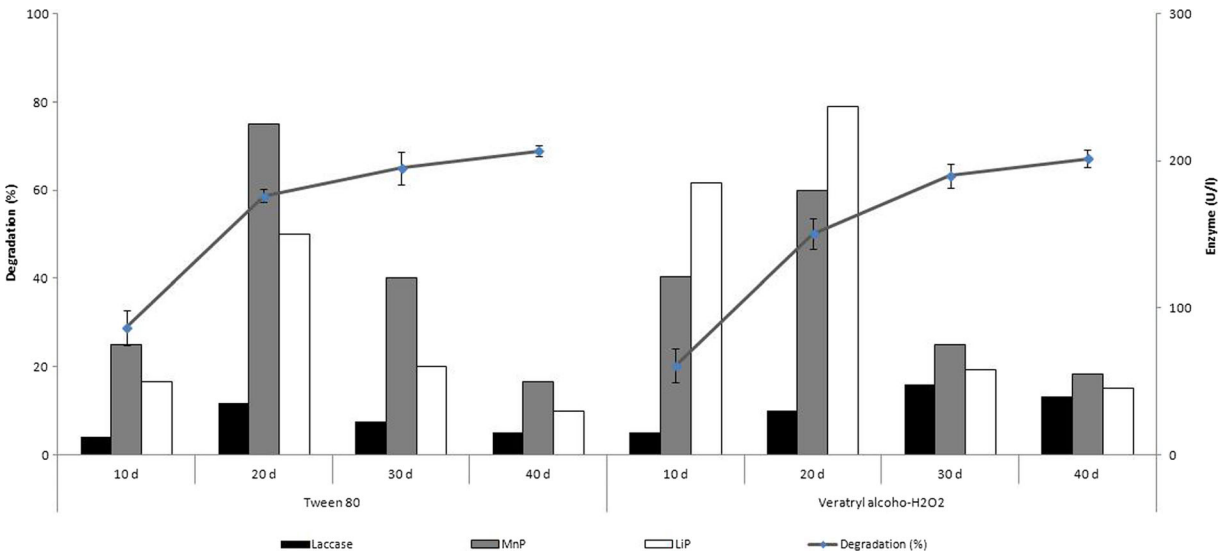

Fig. 3 The effect of optimum parameters on degradation of pentachlorobenzene by *T. versicolor* U80 and its enzyme activity

degradation of pentachlorobenzene in liquid medium (Table 1). The surfactant is easily catabolized without any effect on the production ligninolytic enzymes for degradation of pollutant compounds (Leonardi et al. 2007). The result showed that 1 % Tween 80 improved the degradation 1.5-fold. However, addition of Tween 40 did not improve degradation of pentachlorobenzene.

The bioavailability of a pollutant is one of the factors which determines the success of bioremediation approaches (Leonardi et al. 2007). The objective in using mediators to enhance bioremediation processes through improvement solubilization of pollutant in the liquid phase has been reported (Fava et al. 2004). The structure of a Tween consists of a nonionic polyoxythylene-sorbitan head group and a hydrophobic linear hydrocarbon chain where the difference between Tween 40 (monopalmitate) and Tween 80 (monooleate) is in their hydrophobic. The growth of *T. versicolor* U80 was not affected by the presence of Tween 80. By contrast, Tween 40 suppressed the growth of this strain. Degradation of phenanthrene by *Pleurotus ostreatus* did not improve with addition of Tween 40 at 14 days was also obtained by Marquez-Rocha et al. (2000).

Presence of Tween 80 caused the bioavaibility of pentachlorobenzene to increase more, which has high stability in the media. Tween 80 as surfactants could reduce surface and interfacial tensions and also increase the solubility and mobility of hydrophobic organic compounds (Singh et al. 2007). Addition of 1 % Tween 80 affected on the highest degradation of pentachlorobenzene. After addition of this surfactant, enzyme activity of *T. versicolor* U80 was increased (Fig. 3). The presence of a monounsaturated acryl chain in Tween 80 also could be the possible occurrence of peroxidation reactions in the process (Zheng and Obbard 2001). Venkatadri and Irvine (1990) stated the possible effects of Tween 80 are protecting ligninase from being mechanically inactivated, increasing ligninase activity by hydrolysis mechanism, and increasing an extracellular energy source in its hydrolysis products during secondary metabolism.

Addition of other mediators to enhance the ligninolytic enzymes resulted in the change of degradation of pentachlorobenzene (Table 1). These mediators act as natural diffusible redox mediators of ligninolytic enzymes. The addition of 0.1 mM $CuSO_4$ to the reaction mixtures had no effect on improvement of pentachlorobenzene degradation; even the growth of fungus was not suppressed. It indicated that laccase has no role in this degradation. Laccase is more difficult to degrade in some xenobiotic compounds than LiP because laccase ionization potential is lower than that of Fe^{3+} from LiP (Li et al. 1999). Addition of $MnSO_4$ slightly improved the degradation of pentachlorobenzene. Addition of $MnSO_4$ to induce MnP improved degradation of the pollutant (Zhao et al. 2010; Marco-Urrea and Reddy 2012). MnP acts by oxidizing Mn^{2+} to Mn^{3+} which then diffuses into the pollutant. Reaction between veratryl alcohol and hydrogen peroxide enhanced LiP production in pentachlorobenzene degradation. This result affected in the increasing degradation of pentachlorobenzene (Fig. 3). Veratryl alcohol is naturally present in white-rot fungi as a secondary metabolite. It is useful as a mediator for the degradation of lignin. Furthermore,

Table 2 Degradation kinetic of pentachlorobenzene in liquid medium with *T. versicolor* U80

Sample group	Parameters		
	k (day^{-1})	R^2	$t_{1/2}$ (day)
Without addition mediators	1.3495	0.9487	37.05
Effect of Tween 40			
0.5 %	1.3031	0.9608	38.37
1 %	1.2764	0.9715	39.17
1.5 %	1.0960	0.9627	45.62
Effect of Tween 80			
0.5 %	1.7701	0.9648	28.25
1 %	2.0619	0.9545	24.25
1.5 %	1.3885	0.9508	36.01
0.1 mM veratryl alcohol −0.1 mMH_2O_2	1.9018	0.9797	26.29
0.1 mM $CuSO_2$	1.1937	0.9709	41.89
0.05 mM $CuSO_2$	1.7266	0.9612	28.96

Table 3 Degradation of pentachlorobenzene in soil by *T. versicolor* U80

Days	Degradation (%)	Enzyme activities (U/l)		
		Laccase	MnP	LiP
10	16.3 ± 1.5	315	210	115
20	34.9 ± 2.8	280	40	95
30	40.1 ± 2.9	15	27	29
40	43.4 ± 1.8	12	15	18

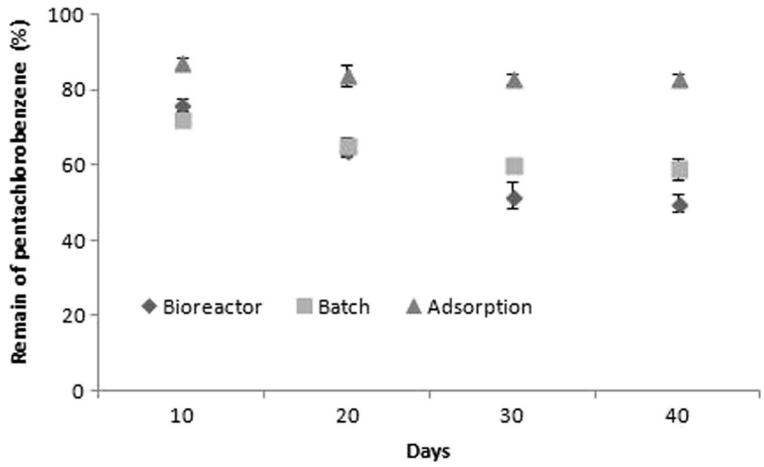

Fig. 4 Degradation of pentachlorobenzene by immobilized of *T. versicolor* U80

it protects LiP against inactivation (Christian et al. 2005). However, due to the short life span of veratryl alcohol cation radical, the addition of veratryl alcohol is needed to improve degradation of pollutants. The formation of a veratryl alcohol-LiP complex and the production of veratryl alcohol radicals may play a role in the enhanced activity of LiP (Vazquez-Duhalt et al. 1994). Furthermore, partial least squares regression showed the most important enzyme for the degradation of pentachlorobenzene by *T. versicolor* U80 was LiP. The regression equation is given below.

$$\text{Degradation}(\%) = 29.4541 - 0.0118\text{Laccase} + 0.0138\text{MnP} + 0.1517\text{LiP} \quad (1)$$

LiP catalyzes the oxidation of non-phenolic aromatic compounds ring opening and side chain cleavage reactions, C-C cleavage in side chain of lignin, cleavage of aromatic ring, oxidation of benzyl alcohols to aldehydes, oxidative dechlorination reactions, and methoxylations. The constant 29.45 is a value that cannot be represented by the ligninolytic enzymes. The study assumed that this enzyme was P-450 monoxy genase. Recent studies shows ligninolytic fungi have P-450 type genes that are useful to attack many pollutant compounds in the early step of degradation (Cajthaml 2015). It catalyzes hydroxylation and reduction. The efficient functioning of an intracellular catabolic system was related with the adsorbed ability of the pollutant compound. The proteins associated with fungal membranes probably play a major role in the steps of degradation (Shary et al. 2008). *Phlebia brevispora* degraded polychlorinated biphenyls (PCBs) by using P-450 monooxygenase and formed the methoxy metabolites *via* hydroxylated PCBs (Kamei et al. 2006). The role of P-450 monooxygenase in degrading pollutant, chlorobenzoates, was also reported by Stella et al. (2013). Purfied P-450 monooxygenase was able to degrade chlorobenzoates upon 1 h incubation through hydroxylated derivatives.

3.3 Degradation Kinetics of Pentachlorobenzene

Application of kinetic study is conducted to estimate kinetic parameters for growth of fungi on a pollutant and subtrates (i.e., mediators) (Helbling 2015). Degradation rate constants and half live periods of pentachlorobenzene were calculated from the data in Table 1, using first-order reaction model (Zhang et al. 2016). Table 2 shows the calculated results of degradation kinetics. The degradation of pentachloro benzen in liquid medium during 40 days incubation fitted well to the first-order reaction model. It was reflected by the regression coefficient R^2 (0.9487–0.9797). The use of 1 % of Tween 80 showed the highest degradation rate (2.0619/day). On the other hand, the use of 1.5 % of Tween 40 showed the lowest degradation rate (1.0960/day). Our result showed that the different mediators led to the different degradation kinetics. Furthermore, the time needed to degrade pentachlorobenzene by 50 % was 37 days, while after addition of 1 % Tween 80, degradation of pentachlorobenzene can be shortened up to 24 days. These results were consistent with the previous result in section 3.2. Tween 80 enhanced an

extracellular energy to break down pentachlorobenzene during secondary metabolism.

3.4 Degradation of Pentachlorobenzene in Soil

In 30 days, *T. versicolor* U80 pre-grown in oil palm empty fruit bunches (OPEFB) had the ability to degrade pentachlorobenzene in soil by 40 % (Table 3). Previously, OPEFB was used as pre-grown source for *T. versicolor* U97 to degrade DDT (Sari et al. 2013). It was concluded that OPEFB mechanisms during the degradation of DDT were adsorption, carbon source utilization, and stimulation of ligninolytic systems used for secondary metabolism (Sari et al. 2014). Degradation in soil was still lower than in liquid medium, even the concentration of pentachlorobenzene in liquid medium was higher than in soil. The high electrophonic chlorine atoms on the benzene ring make aerobic oxidative degradation of pentachlorobenzene became difficult (Kengara et al. 2013). The low accessibility of pentachlorobenzene in soil because of limitation of mass transfer and low diffusion into organic matter that affected pentachlorobenzene is retained in the soil pores caused desorption hysteresis (Mougin et al. 1997; Fujian et al. 2001; Gao and Jiang 2010). Pollutants may have different concentration-dependent relationships with soil conditions, which influence the fate of organic chemicals in soil (Zhang et al. 2006).

Furthermore, the same result with the enzyme activity in liquid medium *T. versicolor* U80 in soil secreted the high enzyme activities at 10 and 20 days. However, the degradation was not only affected by the enzyme activity but also depended on the medium and accessibility attack enzyme to pollutant.

3.5 Degradation of Pentachlorobenzene in Bioreactor

Continuous flow column system was designed to determine the ability of immobilized microbial strain at the water interface to degrade pentachlorobenzene. Figure 4 shows that degradation by immobilized fungi in the bioreactor was higher than in the batch condition after 10 days, meaning that transfer oxygen in the bioreactor process was higher than in the batch condition, and it will affect on the improvement of pentachlorobenzene degradation. The immobilization system affected the fungal biomass surface charge, thus influencing the adsorption of pentachlorobenzene to it.

4 Conclusion

Free cell of *T. versicolor* U80 degraded pentachlorobenzene by 43 % in liquid medium during 40 days incubation. Based on the ionization potential and the partial least square function, the results indicated that both lignin peroxidase and P-450 monooxygenase enzymatic systems are involved in the degradation of pentachlorobenzene. By using the addition of Tween 80, $MnSO_4$, and veratryl alcohol, the degradation of pentachlorobenzene could be improved. Based on kinetic study, the use of 1 % of Tween 80 showed the highest degradation rate (2.0619/day) and the degradation of pentachlorobenzene by 50 % can be shortened up to 24 days. Application of *T. versicolor* U80 in soil and bioreactor degraded pentachlorobenzene 43 and 50 % at 40 days, respectively. Furthermore, the metabolite products of pentachlorobenzene after the degradation still need further investigation.

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