

# Biodegradation of DDT by Co-cultures of *Pleurotus eryngii* and *Pseudomonas aeruginosa*

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## ARTICLE INFO

### Article history:

Received October 7, 2020

Received in revised form June 10, 2021

Accepted June 30, 2021

### KEYWORDS:

Bioremediation,  
Biodegradation,  
*Pleurotus eryngii*,  
*Pseudomonas aeruginosa*,  
DDT

## ABSTRACT

Dichloro-diphenyl-trichloroethane (DDT) is a synthetic insecticide that widely used around the world, which has a negative effect on human health and the environment. The objective of this research was to investigate the ability of bacterium *Pseudomonas aeruginosa* in co-culturing with white-rot fungus *Pleurotus eryngii* to degrade DDT. The various volume of *P. aeruginosa* (1 ml  $\approx$  1.5 x 10<sup>9</sup> CFU) were added into 10 ml of *P. eryngii* culture for a 7-days of incubation. Approximately 82% of degradation of DDT were obtained from co-cultures with the adjunct of 10 ml of *P. aeruginosa* during the 7-day incubation period, which had the best ratio of optimization of 0.57. The confrontational assay showed that *P. aeruginosa* gave no effect on the growth of *P. eryngii* (0.39 cm/day). DDD (1,1-dichloro-2,2-bis(4-chlorophenyl) ethane), DDE (1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene) were detected as metabolic products from the DDT degradation by co-cultures bacterium *P. aeruginosa* and fungus *P. eryngii*. This study indicated that bacterium *P. aeruginosa* can be used to enhance DDT degradation by white-rot fungus *P. eryngii*.

## 1. Introduction

An increasing population has resulted from the industrialization grew rapidly in consort with an accumulation of synthetic chemical. Extensive agriculture has massively used pesticides to solve the attack of pests and to control insects that causing malaria, dengue, and plague (Sudaryanto *et al.* 2007). Organochlorine pesticides (OCPs) are the primary substance in the manufacture of pesticides, which 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) was among them. DDT was one of the persistent organic pollutants (POPs), which was banned since the 1970s. DDT is a dangerous compound, and all of the residues have low levels of decomposition, thus that it becomes one of the serious threats that can endanger the environment and humans. The depositions of DDT and its metabolites absorbed onto soils, sediments and accumulate in animal tissue. Due to this fact, the contamination of DDT has caused significant concern in environmental and food safety.

The most practical, safe, efficient, and economical method that is proven to be the most promising today is biodegradation. This method can degrade a compound to be less or non-toxic. Fungi and bacteria are very often used in the biodegradation process. Many studies show the successful use of various types of fungi and bacteria to degrade DDT or toxic compounds. Both are used separately or in co-cultures. Determination of optimal biodegradation conditions in a laboratory-scale through controlled and manipulation systems has provided essential information to distinguish between biotic and abiotic processes, the length of time of degradation, secondary metabolite products, and different degradation pathways. Recently, wood-rot fungi from group white-rot fungi (WRF) such as *Ganoderma lingzhi* (Boelan and Purnomo 2019), *Xerocomus chrysenteron* (Huang and Wang 2013), *Trametes versicolor* U97 (Sari *et al.* 2013), *Phanerochaete chrysosporium* (Purnomo *et al.* 2008; Zheng *et al.* 2012), *Pleurotus ostreatus* (Purnomo *et al.* 2010a, 2017a), *Pleurotus eryngii* (Purnomo *et al.* 2019a), *Phlebia lindtneri*, and *Phlebia brevispora* (Xiao *et al.* 2011), *Stenotrophomonas* sp. (Gilligan *et al.* 2003) as well as brown-rot fungi (Purnomo *et al.* 2010c,

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2011a, 2011b) could degrade 30-70% of DDT for 7-60 days incubation period, which relatively consumed long incubation times. Therefore, improvement and development of biodegradation methods using fungus need to be prepared.

The use of surfactants to enhance the biodegradation process of organic pollutant compounds has shown significant results. Several types of synthetic surfactants such as Tween 80 (Gonzalez *et al.* 2018), Triton X-100 (Villa *et al.* 2010), Tween and Brij combination (Rios *et al.* 2012), mixed surfactant (SDBS-Tween 80; Wang *et al.* 2018), rhamnolipid, sophorolipid, and trehalose lipid (Teh and Hadibarata 2014) showed the ability to enhance degradation of DDT and pyrene on contaminated soil and water. However, the use of synthetic surfactants has caused another problem, such as the presence of residues, which can lead to an increase in the potential for toxicity, which is harmful to human health and the environment (Purnomo *et al.* 2019c, 2020c). Thus, biosurfactants could be used as an alternative to enhance biodegradation because they were easily degraded, low toxicity (generally non-toxic), and can be produced from substrates of low economic value or waste (Wahyuni *et al.* 2017). A lot of research literature showed that the use of biosurfactants has a sufficient ability to improve the biodegradation process (Nawfa *et al.* 2019).

Biosurfactant-producing bacteria have increased the ability of fungi to degrade DDT in co-cultures. The co-cultures of *P. eryngii* and *Ralstonia pickettii* (Purnomo *et al.* 2019a), *P. ostreatus* and *Pseudomonas aeruginosa*, *P. ostreatus*, and *Bacillus subtilis* (Purnomo *et al.* 2017a), *G. lingzhi* and *B. subtilis* (Boelan and Purnomo 2018), *G. lingzhi* and *P. aeruginosa* (Boelan and Purnomo 2019), *P. brevispora* and *B. subtilis* (Purnomo and Fajriah 2017), *Fomitopsis pinicola* and *B. subtilis* (Sariwati *et al.* 2017), *F. pinicola* and *P. aeruginosa* (Sariwati and Purnomo 2018), *F. pinicola* and *R. pickettii* (Purnomo *et al.* 2020a), *Daedalea dickinsii* and *R. pickettii* (Setyo *et al.* 2018) have been effective in degrading large amounts of DDT about 70-100%. Although some co-cultures of surfactant-producing bacteria and fungi were able to lessen large quantities of DDT, the metabolite compounds produced from the process still have high levels of toxicity such as DDE and DDD. However, no information has been reported about the bioremediation of DDT by co-cultures of *P. eryngii* with *P. aeruginosa*, and a broader assessment of the degradation of DDT by co-cultures

is needed to get the best combination of co-cultures in the DDT biodegradation process.

## 2. Materials and Methods

### 2.1. Material

*P. eryngii* NBRC 32798 and *P. aeruginosa* NBRC 3080 were purchased from NITE Biological Resource Center, NBRC; Chiba, Japan. Pyrene, DDT, DDD (1,1-dichloro-2,2-bis(4-chlorophenyl) ethane), DDE (1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene), and DDMU (1-chloro-2,2-bis(4-chlorophenyl) ethylene) were provided from Tokyo Chemical Industry Co. (Japan). Methanol, sodium sulfate anhydrous, and dimethylsulfoxide (DMSO) were obtained from Merck, Millipore (Germany). *N*-hexane and acetone were purchased from Anhui Fulltime Specialized Solvent and Reagent Co., Ltd. (China).

### 2.2. Cultures Growth Preparation

The stock of *P. eryngii* was retained on potato dextrose agar plates (PDA, Difco, UK) that had been incubated at 30°C. The 1-cm diameter of mycelium was conveyed into 10 ml potato dextrose broth (PDA, Difco, UK) medium in a 100-ml Erlenmeyer flask. *P. eryngii* was furthermore pre-incubated for 7 days at 30°C. One colony of *P. aeruginosa* were inoculated on nutrient agar (NA, Difco, UK) incubated at 37°C for stock culture. One colony of bacteria was inoculated into 100-ml Erlenmeyer flasks containing 50 ml nutrient broth (NB, Difco, UK). *P. aeruginosa* were incubated for 21 h at 37°C with a shaker (11 ×g; Purnomo *et al.* 2013, 2014, 2017b).

### 2.3. Biodegradation Experiment and Metabolite Analysis

After 7 days pre-incubation of the *P. eryngii*, the different concentrations of 1, 3, 5, 7, and 10 ml of *P. aeruginosa* (1 ml ≈ 1.5 × 10<sup>9</sup> CFU) cultures were separately added. Each flask was augmented with 50 µl of DDT 5 mM with a final concentration of 12.5 µM. Besides, the volatilization of the substrate and provide aerobic conditions, oxygen was implemented and protected with a glass stopper. For the control treatment, three cultures were treated using an autoclave (121°C, 20 min) to killed the fungal culture, subsequently the preparation process. Furthermore, all of the procedures were managed in triplicates. The cultures were incubated at 25°C for 7 days incubation.

A comparison between the amount of DDT degradation by co-cultures and degradation by fungal and bacterial particularly was described by an optimization ratio to illustrate the existence of a synergistic relation between fungi and bacteria. After an additional incubation for 7 days, the culture was substituted with 50  $\mu$ l of pyrene 5 mM to a final concentration of 12.5  $\mu$ M/flask. The cell debris was washed by added 20 ml of methanol and 5 ml of acetone and then homogenized following centrifuge at 3,024  $\times$ g (10 min) to remove the cells. The supernatant was filtered by a glass fiber filter. The organic fractions were collected by extraction with 200 ml *n*-hexane (Purnomo *et al.* 2010b). The organic fractions were dehydrated by using anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), evaporated, and diluted with methanol. The concentration of DDT recovery was estimated by analyzing the collected supernatant using HPLC (Shimadzu, Japan). The supernatant was analyzed using isocratic elution with 82% of methanol in 0.1% trifluoroacetic acid as a mobile phase at a flow rate of 1 ml/min. Retention times of DDMU, DDE, and DDT were 11.3, 12.2, and 14.7 min, respectively. The recovery of DDT and its metabolic products was calculated by compared the peak areas with pyrene. Undetected metabolite products by HPLC were further analyze using GCMS by dissolving the latter sample in *n*-hexane. The column (30-m fused DB-5MS, 0.25 mm diameter) was run with He (helium) at a steady flow rate of 1.0 ml per min. The oven temperature was set at 80°C for 3 min, continually increase to 320°C at 12°C  $\text{min}^{-1}$ . End temperatures were held at 300°C for 5 min (Purnomo 2017; Purnomo *et al.* 2019c).

#### 2.4. Interaction Assay of Fungal-Bacteria

Pre-incubation of *P. aeruginosa* cultures were put (20 ml) into tube, followed by centrifuged (10 min at 3,024  $\times$ g) to separate the dregs. The biomass was washed with 20 ml of sterile demineralized water, homogenized, and centrifuged (10 min at 3,024  $\times$ g) to get the bacterial cells. The fungal disk was placed at the center of the plate that contains PDA, at a distance of 3.5 cm from the fungal disk, the bacterial cells (10  $\mu$ l) were inoculated into the plate. The plate was incubated at 25°C in the dark. After day 4th of incubation, the mycelial growth was started to measure (Purnomo *et al.* 2019a).

#### 2.5. Statistical Analysis

To demonstrate the significant differences between control and treatments, Student's t-test was used at

a significance level of  $p < 0.05$ . Standard errors and mean values were also estimated. The analysis was accomplished using SPSS 22 program for Windows (SPSS Inc., USA; Rizqi and Purnomo 2017).

### 3. Results

#### 3.1. Interaction Assay

The purpose of the confrontation assay experiment was to find out the interactions that occur between fungi and bacteria. As shown in Figure 1, there are diverse interactions between *P. eryngii*-*P. aeruginosa* (co-cultures) after 12 days of incubation. It is apparent from this Figure (2A and 2B) that *P. eryngii* could grow on the media which *P. aeruginosa* used *P. eryngii* mycelium to expand (yellow circle). *P. aeruginosa* did not have a significant effect on the growth of *P. eryngii* either on the line with bacterial confrontation ( $0.39 \pm 0.06$  cm/day) or on the line without bacterial confrontation ( $0.41 \pm 0.02$  cm/day) compare with control ( $0.38 \pm 0.04$  cm/day), there is no enhance or inhibit effects. These results indicate that there is no synergy relationship between *P. eryngii* and *P. aeruginosa*.

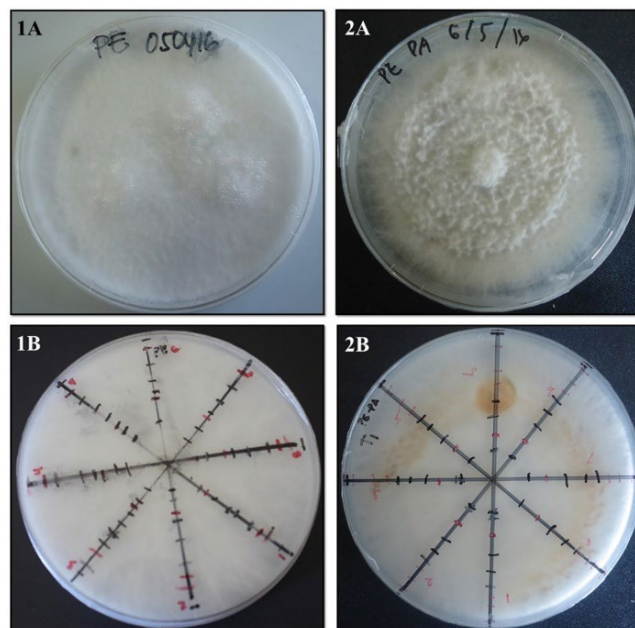


Figure 1. Growth of *P. eryngii* after 12 days incubation. (1A) culture without bacteria (control-front side), (2A) co-cultures of *P. eryngii* and *P. aeruginosa*-front side, (1B) culture without bacteria (control-back side), (2B) co-cultures of *P. eryngii* and *P. aeruginosa*-back side

### 3.2. The Removal of DDT

Biodegradation of DDT by co-cultures was additionally analyzed by co-culturing *P. eryngii* with *P. aeruginosa*. After 7 days of incubation, co-cultures degraded approximately 82% of DDT at concentrations 10 ml (Table 1). These outcomes showed that the expansion of bacteria brought about an expanding DDT degradation rate by *P. eryngii*.

Figure 2 demonstrated the DDT degradation by the single culture of *P. aeruginosa*, and co-cultures *P.*

*eryngii* and *P. aeruginosa*. The addition of *P. aeruginosa* into culture *P. eryngii* had a different effect on the degradation of DDT. The addition of 1 and 3 ml of *P. aeruginosa* decrease the degradation rate of DDT by *P. eryngii*. Additionally, the measure of degradation of DDT by *P. eryngii* was increased when added with *P. aeruginosa* at 5, 7, and 10 ml compared with that without the inclusion of bacterium. The inclusion of 10 ml of *P. aeruginosa* in *P. eryngii* culture brought about the most elevated degradation of DDT, about 82%. On the opposite side, the debasement pace of DDT by co-cultures was lower than *P. aeruginosa* culture only. The ratio of optimization (RO) was used to express the effectivity of the co-cultures on the biodegradation process (Table 1). The co-cultures at 10 ml *P. aeruginosa* (RO ≈ 0.57) was selected for a further experiment on the variation of time and identification of metabolic products.

Figure 3 provides the experimental data of the difference of time adjunct of *P. aeruginosa* at 0, 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup> days incubation. The variation of time addition bacteria into the fungal culture shows a fluctuating result, in which DDT degradation was approximately 71, 46 82, and 65% at 0, 1<sup>st</sup>, 3<sup>rd</sup>, and

Table 1. Biodegradation of DDT in PDB medium during a 7-days incubation period

Volume of bacteria	DDT degradation (%)		Ratio of Optimization
	<i>P. aeruginosa</i> *	Co-culture	Co-culture
( <i>P. eryngii</i> only)*	43.12±1.18		
1 ml	75.17±2.06 <sup>b</sup>	33.11±1.98 <sup>a</sup>	0.26 <sup>a</sup>
3 ml	82.13±2.34 <sup>c</sup>	42.10±0.98 <sup>b</sup>	0.34 <sup>b</sup>
5 ml	86.15±0.51 <sup>c</sup>	72.22±1.45 <sup>c</sup>	0.56 <sup>a</sup>
7 ml	90.20±0.16 <sup>d</sup>	71.34±3.09 <sup>c</sup>	0.52 <sup>c</sup>
10 ml	65.98±0.87 <sup>a</sup>	82.37±3.48 <sup>d</sup>	0.57 <sup>c</sup>

HPLC was used to evaluate the results. The data is presented as mean±S.D. (n = 3). No substantially different data on each column followed by the same lowercase (p<0.05); 1 ml of *P. aeruginosa* ≈ 1.5 x 10<sup>9</sup> CFU

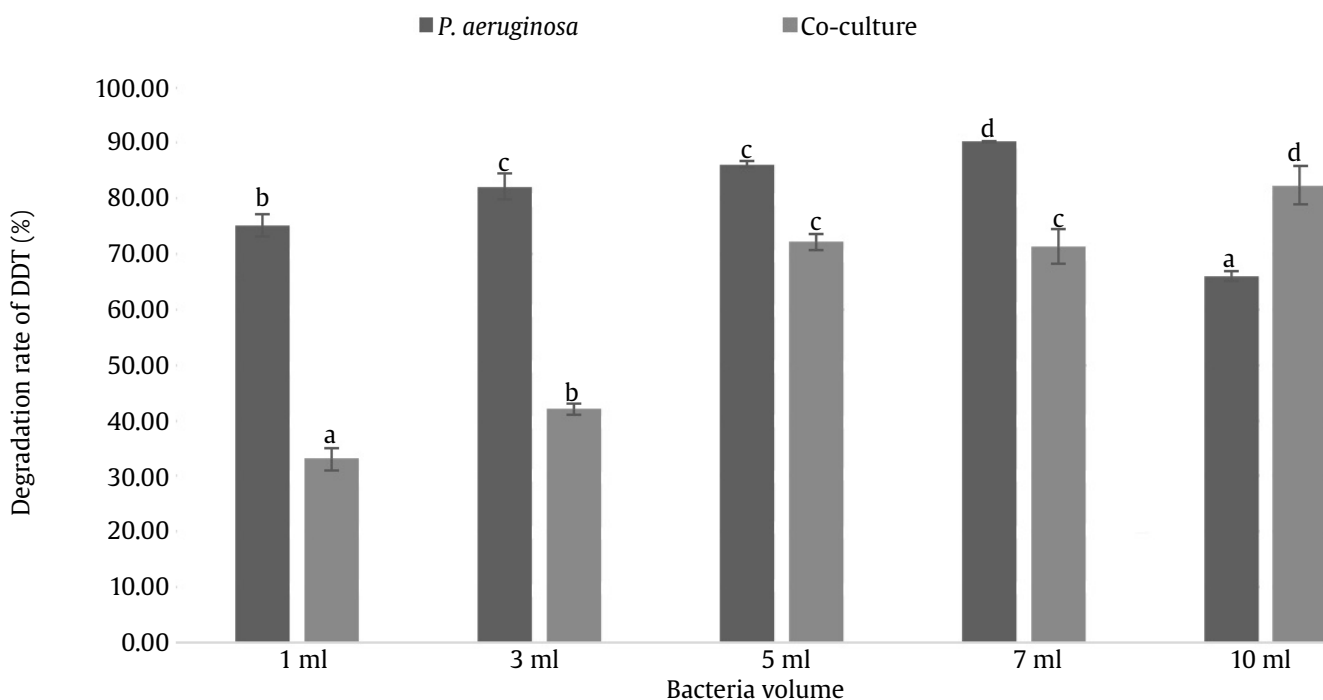


Figure 2. Effect of the addition of *P. aeruginosa* into *P. eryngii* culture in DDT degradation during 7 days of incubation. The data were determined by HPLC. Data are presented as mean and standard deviations (n = 3). The same lowercase letter on each bar indicates no significant differences (p<0.05)

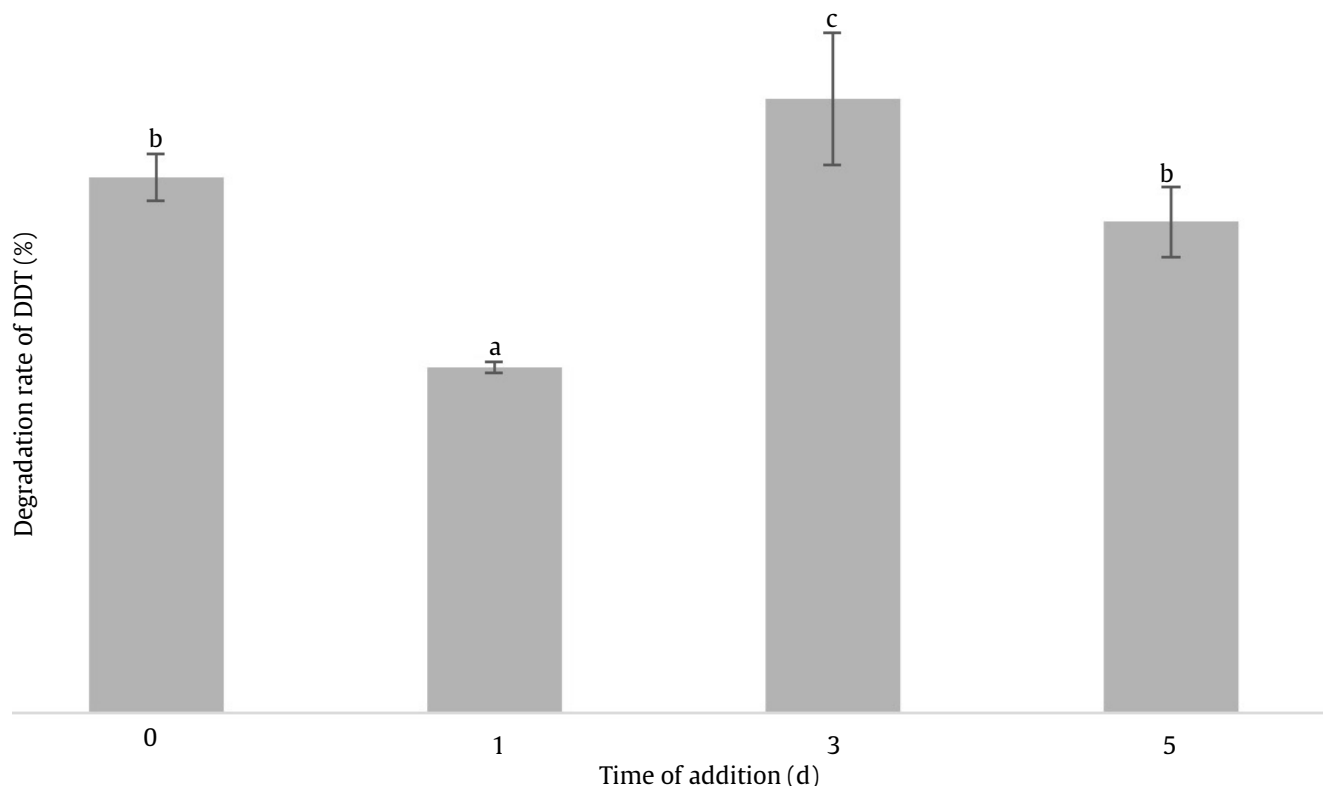


Figure 3. The effect of different time addition of *P. aeruginosa* on DDT degradation by co-cultures. HPLC was used to evaluate the results. The data was presented as mean  $\pm$  standard deviation ( $n = 3$ ). No substantially different data on each bar followed by the same lowercase letter ( $p < 0.05$ )

5<sup>th</sup> days incubation, respectively. The lowest DDT degradation value was about 46% in co-cultures with the adjunct of bacteria on the first day (46%), while the best adjunct time was on the fifth day (82%).

To investigate the metabolites of DDT, co-cultures were incubated with 5 mM of DDT for 7 d. Figure 4 and 5 showed the products obtained from co-cultures which identified by GC-MS. The co-cultures resulted in DDE as a dominant metabolite and trace amount of DDD. These sorts of metabolites were not quite the same as the metabolites delivered by a single culture of *P. eryngii* and *P. aeruginosa* (data not shown). The transformation of DDT into DDE and DDMU was

carried out by *P. eryngii*, while *P. aeruginosa*, forming DDT to DDD and DDMU, and co-cultures of *P. eryngii* and forming DDT to DDE.

To find out the DDT degradation pathway in co-cultures, DDD and DDE were used as substrates (Table 2). However, the amount of recovery of each substrate did not match with the metabolites detected in HPLC. It is suspected, HPLC cannot detect some metabolites or the amount of metabolites was limited. DDD and DDE could be degraded approximately 8 and 53% by co-cultures, respectively. This condition showed that the structure of DDD is quite difficult to be degraded by co-cultures.

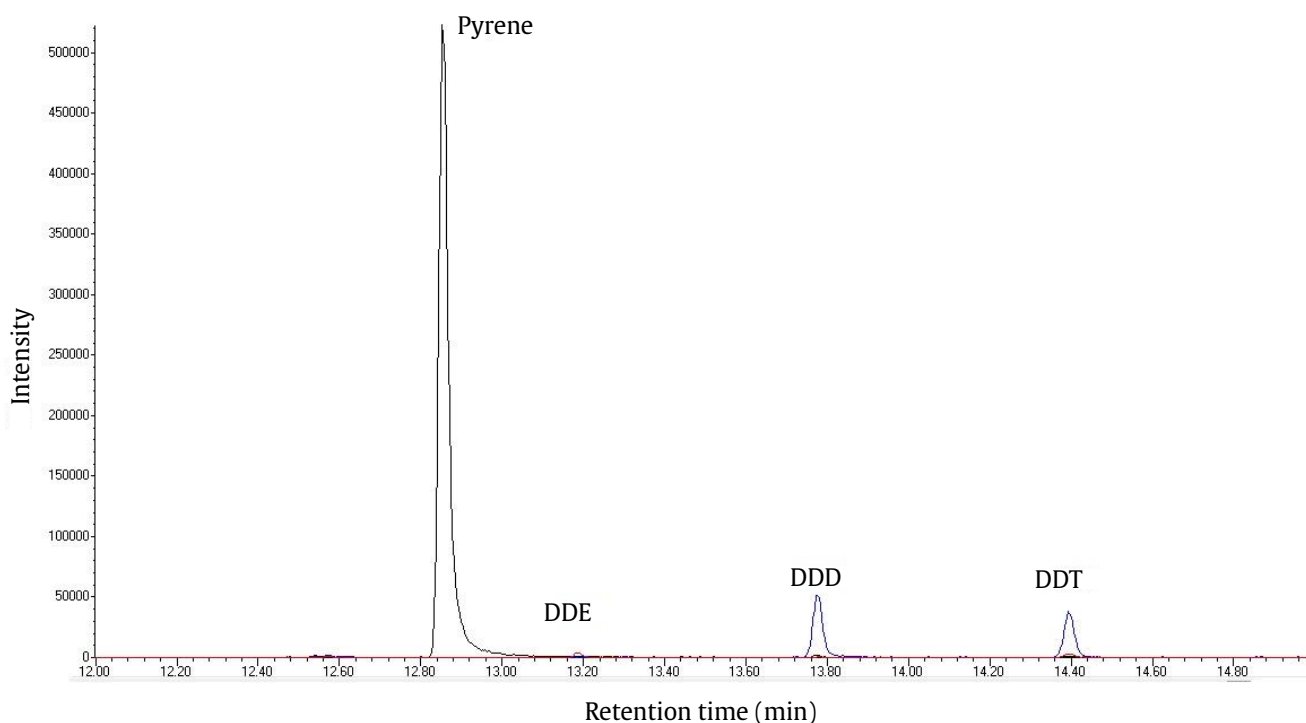


Figure 4. GC/MS chromatogram of DDT degradation by co-cultures of *P. eryngii* and *P. aeruginosa*

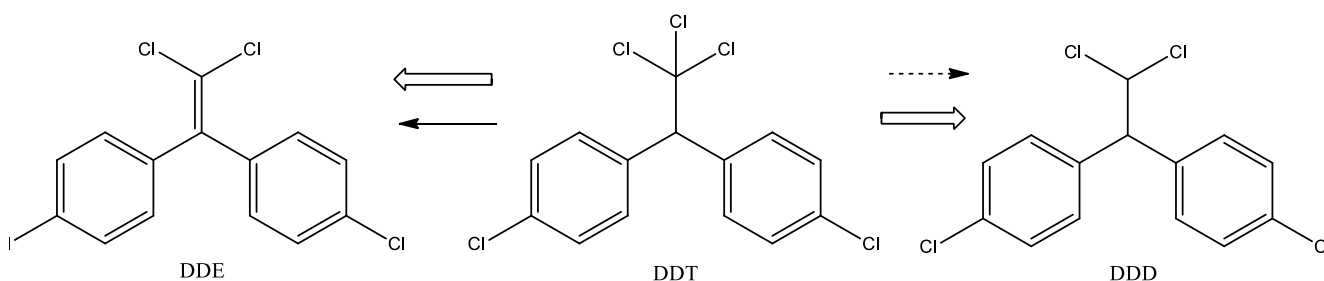


Figure 5. Proposed pathway for DDT degradation by *P. eryngii*, *P. aeruginosa*, and co-cultures. *P. eryngii* single culture (thin black arrows), *P. aeruginosa* single culture (dotted arrows), co-cultures (open arrows)

Table 2. Recovery of DDT and metabolic products by co-cultures of *P. eryngii* and *P. aeruginosa*

Substrate	Degradation (%)	Metabolic product recovery (%)		Total
		DDD	DDE	
DDT	82.37±3.48	14.33	1.48	98.18
DDD	8.95±0.70	87.75	-	96.70
DDE	53.66±10.49	-	43.04	96.70

HPLC was used to evaluate the results. The data is presented as mean ± standard deviation (n = 3).

#### 4. Discussion

Bacteria and fungi have various interactions such as antagonistic, cooperative, synergistic, commensal, and symbiotic were often occur between the microorganisms (Frey-Klett *et al.* 2011).

In this current research, the confrontational assay of *P. eryngii* co-culturing with *P. aeruginosa* had not shown synergistic interaction. As shown in Figure 1, *P. aeruginosa* used the *P. eryngii* mycelium to grow (yellowish color and circle form); still, the growth of *P. eryngii* was average. In this case, *P. eryngii* gave positive interaction for *P. aeruginosa* because the colony of *P. aeruginosa* was spread without scratched the medium with bacteria. Bacteria are microorganisms which in the process of formation of colonies, are influenced by several factors are known as substrate texture, microstructure, and carbon content.

Bacteria are very dependent on the presence of water and usually adhere to the surface of microparticles or solid particles. In the soil

environment, fungi and bacteria occupy the same space to grow. As a result, fungi and bacteria compete with each other to obtain sources of nutrients for cell growth and cause interactions between the two (Effmert *et al.* 2012). Based on this, a possible explanation for this current result might be that *P. aeruginosa* utilizes the surface of mycelium of *P. eryngii* to grow and obtain nutrients in the culture medium. *P. aeruginosa* also used *P. eryngii* as a vector for translocation (Kohlmeier *et al.* 2005).

Prior studies have noted the utilization of *P. aeruginosa* to produce biosurfactant (Kim *et al.* 2010; Nie *et al.* 2010; Zhang *et al.* 2012; Abbasi *et al.* 2012; Al-Wahaibi *et al.* 2014) and capable of degrading DDT (Bidlan and Manonmani 2002). In our previous study, *P. aeruginosa* has shown the potency to degrade DDT (Sariwati and Purnomo 2018), biodecolorization of methyl orange (Purnomo and Mawaddah 2020; Purnomo *et al.* 2020b), in a single culture or co-culturing with fungal. In the current research, co-culturing *P. eryngii* with *P. aeruginosa* significantly increase the biodegradation of DDT in 7 days incubation. Notably that *P. eryngii* culture only, degrade 48% of DDT in 7 days incubation. In general, therefore, it seems that biosurfactants produced by bacteria can reduce surface tension and increase the solubility and bioavailability of DDT in water (Wang *et al.* 2018).

A significant increment of DDT removal rate by *P. eryngii* was obtained after the addition of biosurfactant producing bacteria. The maximal DDT removal rate (82.37%) was achieved in the co-cultures at a concentration of 10 ml (Figure 1). The ability of particular bacterium or fungus to degrade DDT was not equivalent to its co-cultures. *P. aeruginosa* at 7 ml addition could degrade approximately 90% DDT. These results were higher than co-cultures (73%). Co-cultures at 1 and 3 ml, showed that the concentration of *P. aeruginosa* was limited to be able to enhance the DDT removal processed by *P. eryngii*. Another possibility was that *P. aeruginosa* and *P. eryngii* produce some metabolite that inhibits each other to degrade DDT (Wahyuni *et al.* 2016; Ramadhania *et al.* 2018; Sariwati *et al.* 2019). Generally, the addition of *P. aeruginosa* enhanced the degradation rate of DDT by *P. eryngii*, but there is no synergistic relationship between *P. aeruginosa* and *P. eryngii*.

The ratio of optimization (RO) is a well-established approach to ensure the synergistic interaction of fungal and bacteria. All of co-culture showed RO <1 (Table 1), which indicated that *P. aeruginosa* were not

affected in increments of biodegradation of DDT in co-cultures, because the levels of DDT degradation in single culture was higher than co-cultures. This finding is contrary to previous studies, which have suggested that *P. eryngii* and *R. pickettii* have a synergistic interaction with RO >1 (Purnomo *et al.* 2019a).

The variation of time addition of bacteria in *P. eryngii* culture resulted in different degradation rates of DDT. The proliferation of *P. aeruginosa* bacterium at other incubation times influenced increasing the biodegradation ability of DDT by *P. eryngii*. The *P. eryngii* single culture was only able to degrade DDT by 48% during 7 days of incubation. Still, by the addition of *P. aeruginosa* at the beginning of incubation, the degradation rate was 70%. The degradation increased in third-day addition bacteria, with a degradation rate of approximately 80%, but a decrease in the first day and fifth day of addition bacteria. Mentioned the variable of a medium that affected the rate of biodegradation as an example of the obtainability of a xenobiotic compound to the microorganisms, physiological mechanisms of the microorganisms the ability to survive and produce cells by utilizing xenobiotic compounds, and sustainable population of the microorganisms (Purnomo *et al.* 2019b; Khumaidah *et al.* 2019). Based on this, at the beginning of the incubation, the rate of biodegradation is strongly influenced by the presence of bacteria. *P. aeruginosa* bacteria were able to utilize the availability of DDT in the media as a source of nutrients to increase the biodegradation of DDT by *P. eryngii*. The decrease of DDT degradation on the first day of incubation suggested caused by *P. aeruginosa* was unable to regenerate cells or unable to survive due to the presence of *P. eryngii*. According to Li *et al.* (2014), *P. eryngii*, in its growth phase, produced sulfated polysaccharides that could damage the cell walls and bacterial cytoplasmic membrane (Putri *et al.* 2018; Ramadhania *et al.* 2019; Auwalayah *et al.* 2019). The increasing of DDT degradation on day 3 of incubation, DDT was partially degraded by *P. eryngii*, after the addition of *P. aeruginosa*, DDT was available to lessen by bacteria.

#### 4.1. Metabolite Analysis and Degradation Pathway

Our previous study showed DDE and DDMU were metabolite products of DDT degradation by *P. eryngii* (Purnomo *et al.* 2019a). At the same time, DDD and

DDMU were products of DDT biodegradation by *P. aeruginosa* (Boelan and Purnomo 2019). In this current research, after 7 d of incubation, metabolite analysis by GCMS and HPLC showed the addition of *P. aeruginosa* (10 ml) could transform DDT into some metabolites such as DDE and DDD (Figure 4). This finding is contrary to previous co-cultures studies, which suggested that co-culturing of *P. eryngii* and *R. pickettii* could transform DDT into DDE, DDD, and DDMU (Purnomo *et al.* 2019a). This inconsistency may be due to the different interactions that occur between bacteria and fungi. Although *P. aeruginosa* used *P. eryngii* as a vector to mobilize, *P. eryngii* hyphal growth was unstimulated by bacterial metabolites. Several relationships between bacteria and fungi must be considered from various perspectives. Co-cultures of *Penicillium* sp. and three strains of bacterial (*Burkholderia cepacea*, *R. pickettii*, and *P. aeruginosa*) showed a synergistic effect on the elimination of phenanthrene, approximately 68-73% in 18 days (Chávez-Gómez *et al.* 2003). In comparison, a co-culture of biofilm of *Penicillium frequentans* and *Bacillus mycoides* confirmed an ability to degrade polyethylene (Gou *et al.* 2009). Bacteria and fungal had various mechanism of DDT transformation into others metabolite. Degradation mechanism of DDT by *P. aeruginosa* through reductive dichlorination of DDT to DDD, and meta ring cleavage mechanism. White-rot fungal species transform DDT through reductive chlorination, transformation, and mineralization into some metabolite product (Foght *et al.* 2001). The proposed pathway was shown in Figure 5. Co-cultures transformed DDT to DDE through the elimination of chloride ion and transformed DDT to DDD to through reductive dechlorination.

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