The effect of temperature and concentration of *Aspergillus fumigatus* on chlorpyrifos removal

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**Abstract.** Chlorpyrifos is a toxic organophosphate type of insecticide with a molecular formula of C9H11Cl3NO3PS. Bioremediation is an environmental friendly method used to combat this insecticide through microbial enzymatic activity. This study therefore aims to eliminate chlorpyrifos with temperature variations and concentrations of *Aspergillus fumigatus*. *Aspergillus fumigatus* fungi acts as bioremedian to degrade the activity of chlorpyrifos using temperature ranges of 25-35°C at a 0.5-1.5% concentration rate. However, using the Gas-Chromatography Mass Spectrometer (GC-MS) method, optimum chlorpyrifos degradation was achieved at 25°C with a 1.5% concentration for 5 days in potato dextrose broth (PDB) liquid media. This shows that *A. fumigatus* has the ability to remove chlorpyrifos with an efficiency of 95.92% from its initial concentration of 80 ppm.

1. **Introduction**

Organophosphate group of insecticides, comprises of all phosphoric acid esters which include aliphatic, phenyl, and heterocyclic derivatives, are the most widely used types of insecticides all over the worldwide [1]. Majority have the same structure, consisting of three phosphoester bonds often called phosphotriesters and used to control chewing and suction pests on various food crops [2,3]. Chlorpyrifos is one of the organophosphates insecticides widely used by farmers to control various types of plant pests. However, the continuous use of insecticides that are not in accordance with the standard rules resulting in environmental damage, decrease land quality with adverse effects on human health [4].

Chlorpyrifos with a molecular formula of C9H11Cl3NO3PS and 350.59 g/mol molecular weight, is toxic organophosphate type of insecticide. Since the 1960s, it has been extensively used in the agricultural sector to control pests in cotton, cereals, vegetables and fruits [5]. It has a low solubility rate in water, which is approximately 2 mg/L, and easily soluble in most organic solvents, with a high soil absorption coefficient and its storage under normal conditions is relatively stable [6,7].

Bioremediation is a method used to clean chemical pollutants by reducing their concentration and toxicity in order to restore the right ecosystems [8]. Microorganisms use contaminants as carbon sources and terminal electron acceptors, which allows it to utilize these compounds for energy conservation and mineralization [9]. The success of bioremediation is influenced by several factors, namely the concentration of microorganisms, pH, temperature, soil type, pollutant concentration, and nutrition [10].

The main processes in bioremediation include biodegradation, biotransformation, and biocatalyst. The occurrence of bioremediation, which produces enzymes by microorganisms modify toxic pollutants
by changing their chemical structure and reducing activation energy required to start the reaction. In this process biotransformation or bio detoxification occurs in less toxic or non-toxic compounds [11].

Previously, several types of bacteria such as *Pseudomonas* sp., *Paracoccus* sp., and *Bacillus pumilus* have the ability to degrade chlorpyrifos, however, there is limited information on the use of fungi as a degrading agents. This study, therefore aims at analyzing the use of fungi in degrading microbes owing to its importance to the biogeochemical cycle and role as a degradator of xenobiotic compounds in the environment [12].

Fungi has several advantages as bioremedians rather than bacteria in degrading pollutants in the environment, due to its ability to form hyphae, a fungi which forms mycelia tissue and used to transport water, nutrients, and electron acceptors in mycelia. Unlike bacteria, fungi grows through air-filled pores and penetrate soil aggregates. It secretes extracellular lignin modifying enzymes that are able to diffuse into contaminants without displacement which is then adsorbed into soil particles. In addition, because these enzymes have low specificity, they are able to degrade various organic compounds and mix varieties of chemicals in the soil [13,14].

*Aspergillus fumigatus* plays an important role in recycling carbon and nitrogen in nature. Its ability to utilize various sources of carbon and nitrogen to support its growth has made *A. fumigatus* an important part of the nutrient recycling ecosystem. Similarly, its remarkable ability to grow efficiently in a variety of environmental conditions and utilize a variety of substrates to meet its nutritional needs contribute to its role as bioremedian agents [15]. From the above description, the purpose of this study is to determine the potential of *A. fumigatus* fungi in degrading chlorpyrifos, its temperature and concentration as a bioremedian which provides the highest efficient removal of chlorpyrifos.

2. Material and methods
This research was carried out in stages as in figure 1.

![Figure 1. Research flow chart.](image)

### 2.1. Preparation of chlorpyrifos
Chlorpyrifos pollutants are obtained from insecticides containing 200 g/L of active ingredients. To obtain 80 ppm, concentration dilution was carried out by dissolving 0.5 ml of pure chlorpyrifos in 1 L of distilled water.

### 2.2. Cultivation of *A. fumigatus*
*Aspergillus fumigatus* is obtained from the Environmental Biology/Microbiology Laboratory, Trisakti University, Jakarta. *Aspergillus fumigatus* was cultivated in Potato Dextrose Agar (PDA) media, incubated at 37°C with a pH of 7 for 7 days. To obtain concentrations of 0.5%, 1%, and 1.5%, the A.
fumigatus biomass of 0.5, 1 and 1.5 mg was added to each Erlenmeyer containing 100 ml of Potato Dextrose Broth (PDB) media.

2.3. Effect of temperature on chlorpyrifos degradation
The first step to determine the efficiency of chlorpyrifos removal is to carry out temperature variations (Figure 3). As much as 1% of A. fumigatus as a source of enzymes is inserted into Erlenmeyer containing PDB media, pH 7, and 10% chlorpyrifos. Erlenmeyer is placed on the shaker incubator, with a rotating speed of 180 rpm and examined for 5 days at temperatures of (°C) 25, 30, and 35.

2.4. Effect of concentration of A. fumigatus on chlorpyrifos degradation
After the optimum temperature is reached, which produces the highest chlorpyrifos allowance, the study continues to determine how much A. fumigatus produces the highest chlorpyrifos removal efficiency (Figure 5). Similar to previous stages, 0.5%, 1%, and 1.5% A. fumigatus were inserted into Erlenmeyer containing PDB media, pH value of 7, and 10% chlorpyrifos. It is placed in a shaker incubator with a rotating speed of 180 rpm and observations are carried out for 5 days.

2.5. Efficiency of chlorpyrifos removal and chlorpyrifos removal kinetics
Chlorpyrifos removal efficiency is calculated using the formula as follows [16]:

\[
\text{Removal efficiency (\%)} = \frac{C(a) - C(b)}{C(a)} \times 100\%
\]

\(C(a)\) : initial concentration of chlorpyrifos (ppm)
\(C(b)\) : final concentration of chlorpyrifos (ppm)

In addition, its removal effect was calculated using the Monod equation, which is the first order kinetics model as follows [17]:

\[
C_t = C_0 \times e^{-kt}
\]

\(C_0\) is the initial concentration of chlorpyrifos at \(t_0\) and \(C_t\) is chlorpyrifos concentration at \(t\) (day).

3. Results dan discussion

3.1. Optimization of temperature in removal of chlorpyrifos by A. fumigatus
Temperature optimization is the first step to determine good conditions and suitable for the removal of chlorpyrifos by A. fumigatus. The temperature effects of the study on chlorpyrifos removal are presented in table 1.

| Temperature variations (°C) | Average of chlorpyrifos (ppm) | SD  | Chlorpyrifos removal (%) |
|-----------------------------|--------------------------------|-----|--------------------------|
| 25                          | 5.26                           | 0.79| 93.43                    |
| 30                          | 10.40                          | 1.12| 87.00                    |
| 35                          | 19.81                          | 1.37| 75.24                    |

With the initial concentration of chlorpyrifos at 80 ppm and A. fumigatus at 1%, the chlorpyrifos was removed within 5 days at temperatures of (°C) 25, 30, and 35 which are 93.43%, 87.00%, and 75.24%, respectively (table 1). Figure 2 illustrates the Calculation of chlorpyrifos residues in this study using the GC-MS method [18] and observational data in Table 1 can also be arranged in the form of bar graphs in figure 3.
Figure 2. Chromatogram measurement of chlorpyrifos residue at temperature variations with GC-MS method.

Figure 3. Chlorpyrifos removal at temperature variations.

From the temperature variations conducted, it can be seen that A. fumigatus grows in a temperature range of 25-35°C in PDB media containing 10% chlorpyrifos with pH value of 7. Table 1 shows that the temperature of 25°C is optimum for A. fumigatus to be able to remove chlorpyrifos because it produces the highest allowance efficiency of 93.43%. This result proves that fungi are able to become good bioremedian because it grows in a temperature range of 30-40°C with minimum degrees above the freezing point of pH 4-7 [19,20].

3.2. Optimization of the concentration of A. fumigatus in chlorpyrifos removal

After obtaining the optimum temperature used to extract chlorpyrifos from A. fumigatus, it concentration was optimized. The results can be seen in table 2.

Table 2. Optimization of the concentration of A. fumigatus in chlorpyrifos removal.

| Concentration of A. fumigatus variations (%) | Average of chlorpyrifos (ppm) | SD | Chlorpyrifos removal (%) |
|---------------------------------------------|-------------------------------|----|--------------------------|
| 0.5                                         | 12.64                         | 1.32| 84.20                    |
| 1.0                                         | 5.26                          | 0.79| 93.43                    |
| 1.5                                         | 3.26                          | 0.34| 95.92                    |
From Table 2, the results can be seen when *A. fumigatus* is 0.5%, 1%, and 1.5%, respectively, it is capable of eliminating chlorpyrifos at 84.20%, 93.43%, and 95.92%. These results were obtained from the initial concentration of chlorpyrifos at 80 ppm in PDB media, with a pH value of 7, 25°C temperature for 5 days. Chlorpyrifos residue measurements using the GC-MS method are shown in Figure 4. These results indicate that the higher the concentration of *A. fumigatus*, the higher the amount of chlorpyrifos set aside as shown in figure 5.

**Figure 4.** Chromatogram measurement of chlorpyrifos residue in concentration of *A. fumigatus* variations with GC-MS method.

**Figure 5.** Chlorpyrifos removal in concentration of *A. fumigatus* variations.

The results of this study proves that *A. fumigatus* plays an important role in recycling carbon and nitrogen owing to its growth support [15]. It grows efficiently in PDB contaminated with chlorpyrifos owing to its ability to utilize its pollutants as a carbon source for growth.

By using the Monod equation, with the first kinetics model, the value of the chlorpyrifos removal reaction rate by *A. fumigatus* (k) is 0.64/day from the initial concentration at 80 ppm and the final chlorpyrifos concentration (Ct: 5 days) of 3.26 ppm. This proves the existence of enzymatic activity carried out by removing chlorpyrifos, thereby, making it capable of working in chlorpyrifos contaminated environments.

4. **Conclusion**

About 1.5% of *A. fumigatus* has the ability to remove chlorpyrifos in PDB media up to 95.92% from the initial concentration of chlorpyrifos 80 ppm at a temperature of 25°C for 5 days. Therefore, it can be a good bioremedian alternative to remediate chlorpyrifos contaminated environments.
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