Chlorpyrifos Removal Improvement In Liquid Media By Aspergillus Fumigatus

Candra Dwi Anggreini, T. Tazkiaturrizki, Astri Rinanti

Abstract : The purpose of this research was to remove the chlorpyrifos exposed to liquid media by the Aspergillus fumigatus fungi. Chlorpyrifos is a toxic organophosphate insecticide with a molecular formula of $C_9H_{11}Cl_3NO_3PS$, while A. fumigatus was cultivated in potato dextrose agar (PDA) media, incubated at the a temperature of 37^oC, with pH of 7 in 7 days. Furthermore, the research was initiated by variating the concentration of the microorganism at 0.5%, 1.0%, and 1.5%, impacting biodegradation into the Erlenmeyer, containing the media (PDB), pH 7, and 10% chlorpyrifos, to determine the best removal efficiency. In addition, the Erlenmeyer was place at the shaker incubator, and a rotation speed of about 180 rpm, with temperature of 25^oC was maintained. Sampling was conducted every day in 5 days, and also on the 3rd, 5th, 7th, and 9th day, in order to determine the contact timing needed to generate highest efficiency, using Gas-Chromatography Mass Spectrometry (GC-MS) method. Furthermore, optimal removal was up to 99.65%, with the 1.5% A. fumigatus being exposed for 9 days in the liquid media. This research, therefore, shows the microorganism to impact degradation, as well as the potential to protect waters of environmental pollution against the impact of organophosphate pesticide.

Keywords : biodegradation, an organophosphate insecticide, chlorpyrifos, Aspergillus fumigatus, removal efficiency, biodegradator

1 INTRODUCTION

Food necessity always rises in line with the elevation in the world population, which creates the need for an increase in agriculture and farming activities. Moreover, compost and pesticides are of high demand, in an attempt to control growth of plants [1, 2], hence, about 30% of produce molder and loss is incurred due to pest effects. However, an over-use of pesticides causes a significantly destructive effect, being a chemical used to kill, destroy, and control, or repulse the harmful vermin that affect agriculture, wood product, animal, food, and human. Furthermore, they are classified into herbicide, insecticide, fungicide, and rat exterminator, where insecticides, especially of organophosphate base, are used by most developing countries, and approximately, 3 million people are exposed to pesticide, and 0.2 million pass away as a result [3]. Meanwhile, an overuse causes the loss of land fertility, due to the lack of nutrients, toxic substance accumulation, and the low organism activity [4]. Organophosphate insecticide encompasses phosphate acid esters, including the aliphatic derivative, fenil, and heterocyclic, all of which are grossly used worldwide [5]. In addition, most possess the same structure, by containing three forester bonds, commonly known as fosfotriester, further, used to control the masticator and inhalator at several food planting [6, 7]. Furthermore, chlorpyrifos is a type, which is used mainly and continuously by farmers, which is not appropriate with the rule, as it causes the environmental damage, land quality degradation and human health turmoil [8]. According to WHO classification, chlorpyrifos, with the molecule formula $C_9H_{11}C1_3NO_3PS$, is included to second-grade pesticide with medium toxicity, which was implemented on farmlands in 1965, with a half-life of about 10-20 days.

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This is a heavy molecule, weighing about 350.6 g/mol, with a melting point of 43.5-41°C, and the solvent in water was a 1.2 mg/L, at the temperature of 25°C. Moreover, they are easy to dissolve in most organic diluents, as it possesses the high land absorption coefficient during storage, at the normal relative stable condition [9]. Furthermore, chlorpyrifos are degraded naturally by the microorganism in the environment, as they generate the main toxic compound conversion, which develops into the lack of toxic product or not. This biodegradation level, and early toxic early amount, is an important factor that influences the residue concentration in the environment [10, 11]. Biodegradation is a general process to lose organic pollutant because of the low cost and risk to the natural organism, which significantly influence the metabolism of pesticide, as it is also capable of increasing the microorganism production, through genetic engineering. In addition, they are fully able to isolate most of the aliphatic, aromatic, and heterocyclic compounds [12, 13], via the tendency to utilize them in energy conservation and mineralization [14]. Biodegradation depends on several factors, including temperature, solar radiation, land types, pH, oxygen accessibility, or the presence of its receptors, nutrition, chemical structure, and the target compound availability. In addition, the Chlorpyrifos is also an important influencing factor, therefore, environmental engineering takes the essential role to biodegradation them more rapidly [1, 15]. Pseudomonas sp., Paracoccus sp., and Bacillus pumilus possess the ability to degrade the chlorpyrifos, while fungi agents are highly limited, although they are very significant in the biogeochemical cycle, via their role as xenobiotic compound destroyers in the environment [16]. Fungi confer several advantages than bacteria in the process, as they form hypha, and mycelia texture, used to transport water, nutrition, and electron acceptor. Furthermore, they grow through pores containing air, therefore, penetrating land aggregate, and also secrete the modification enzyme of extracellular lignin, capable of diffusing into the contaminant without moving, then subsequently adsorbs into the land particle. Meanwhile, the enzyme is also able to degrade some organic compounds and several chemical substances, thus, enhancing mixing with the land, due to its low specification. These are some bacteria and fungi thas had been ever used in chlorpyrifos biodegradation [17, 18].

TABLE 1
MICROBIAL DEGRADATION OF CHLORPYRIFOS

Microbes	*Species*
Bacteria	*Eterobacter sp.* *Flavobacterium sp.* *Pseudomanas diminuta* *Micrococus sp.*
Fungi	*Phanerochaete chrysosporium* *Aspergillus sp.* *Trickoderma harzianum* *Penicilum brevicompactum*

Abiotic chlorpyrifos degradation, such as chemical handling, photodecomposition, volatilization, and combustion, is always inefficient, expensive, and dangerous for the environment, so it is biodegradable by enzymes, microorganisms, and which can be used for chlorpyrifos, easy to use and equipped. The process of chlorpyrifos biodegradation is shown in Figure 1 [17].

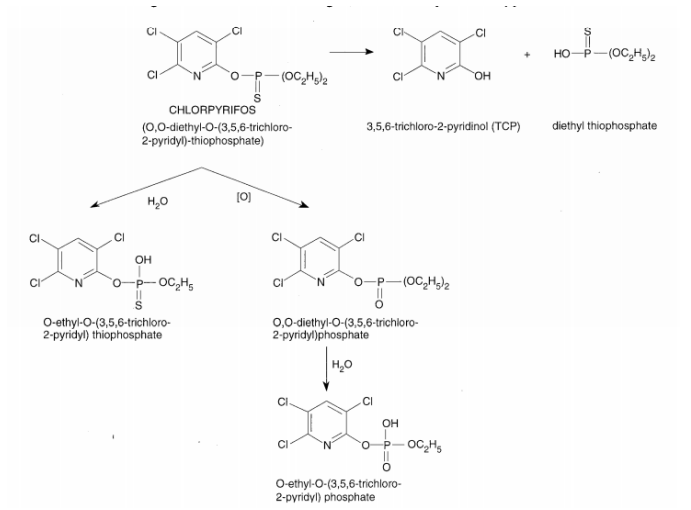

Fig. 1. *Biodegradation Pathways of Chlorpyrifos*

A. fumigatus is fungi with an important role in carbon and nitrogen recycling in nature, due to their ability to utilize various sources to support personal growth. Furthermore, they efficiently grow within various environmental conditions and also to utilize various substrates in the process of fulfilling the nutrition necessity [19]. Based on the explanation above, the purpose of this research is to evaluate A. fumigatus and also the contact time needed for it to give the highest chlorpyrifos removal efficiency.

2 RESEARCH METDHOLOGY

2.1 Chlorpyrifos Preparation

Chlorpyrifos pollutant was obtained from an insecticide that contains it as an active, at the concentration of 200 g/l. This was subjected to dilution with 0.5 ml of pure sample into 1 L aquades, in other to achieve 50 ppm.

2.2 Aspergillus fumigatus Cultivation

A. fumigatus was obtained from the collection of Biological Laboratory/Environmental Microbiology, Universitas Trisakti, Jakarta, cultivated in potato dextrose agar (PDA) media, incubated at the temperature 37°C with pH 7 for 7 days. In addition, to obtain 0.5%, 1.0%, and 1.5%, respectively, 0.5, 1.0 and 1.5 mg of the fungi biomass was added to each Erlenmeyer, containing 100 ml PDB.

2.3 The influence of A. fumigatus Concentration as the Chlorpyrifos Biodegradator

The first step involves conducting the concentration variation of A. fumigatus, in order to determine its ability to produce the highest chlorpyrifos removal efficiency, using the serial dilutions as the enzyme source, to which 10% chlorpyrifos was added and maintained at pH 7. Furthermore, Erlenmeyer was placed in the shaker incubator with a rotation speed of 180 rpm, and 25°C, therefore, sampling continually occurred every day for 5 days.

2.4Contact Time Influence at the Chlorpyrifos Degradation

The optimum concentration of A. fumigatus obtained was used to produces the highest removal efficiency, and subsequently the contact time investigation was conducted in order to generate the most significant chlorpyrifos removal efficiency. The preparation technique was similar to the previous step, but Erlenmeyer was put at the shaker incubator at a rotation speed of 180, and temperature of 25°C, therefore, sampling was conducted on the 3rd, 5th, 7th, and 9th day.

2.5 Chlorpyrifos Removal Efficiency and Removal Kinetic

The analysis of chlorpyrifos residue was performed through GC-MS method, hence, the removal efficiency was calculated, using the adapted formula from Sawyer [20] as follow:

$$\text{Removal Efficiency (\%)} = \frac{C_{(a)} - C_{(b)}}{C_{(a)}} \times 100\% \quad (1)$$

Where:
$C_{(a)}$: chlorpyrifos initial concentration (ppm)
$C_{(b)}$: chlorpyrifos final concentration (ppm)

Meanwhile, the effect of removal was analyzed with the Monod equation, applying the first kinetical model adapted from [21] as seen in the formula:

$$C_t = C_0 \times e^{-kt} \quad (2)$$

C_0 and C_t were the chlorpyrifos concentration at t_0 and t (days), respectively.

3 RESULT AND DISCUSSION

3.1 Optimum Concentration of A. fumigatus through The Chlorpyrifos Degradation

This was the first step to determine the total A. fumigatus required to achieve the most significant chlorpyrifos removal efficiency. Therefore, using the initial chemical concentration of about 50 ppm, in 0.5%, 1.0%, and 1.5% (w/v) concentration of the microorganism, clearance by up to 79.36%, 84.36%, and 90.29%, respectively were observed within 5 days of treatment, and at the temperature 25°C (Table 2 and Figure 2).

TABLE 2
THE TOTAL OF A. FUMIGATUS AT THE CHLORPYRIFOS DEGRADATION PROCESS

A. fumigatus (w/v (%))	*ChlorpyrifosConc. (ppm)*	*SD*	*Chlorpyrifos Removal Efficiency (%)*

0.5	*6.94*	*0.92*	*79.36*
1.0	*5.26*	*0.79*	*84.36*
1.5	*3.26*	*0.34*	*90.29*

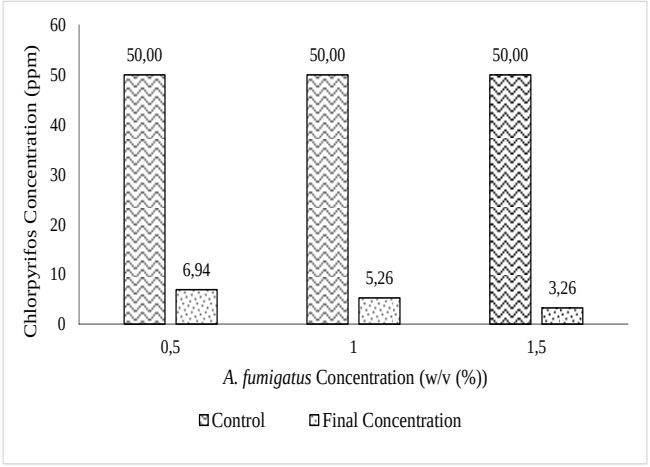

Fig. 2. *Chlorpyrifos Removal at the A. fumigatus Various Concentration.*

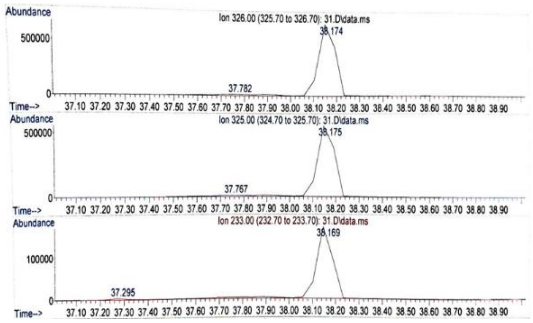

Fig. 3. *Chlorpyrifos Residual Measurement Using GC-MS Method by 1,5% A. fumigatus.*

observed to produce utmost chlorpyrifos removal efficiency of about 90.29%, as seen in Figure 3. Moreover, the results of this investigation is compatible with the theory expressed by [19] stating that A. fumigatus is a fungus that confers an important role in the recycle of carbon and nitrogen in nature, due to its ability to utilize the several sources for supporting growth. In addition, they possess an advantage in the process of biodegradation of pollutant in the environment, when compared with bacteria. Differentially, fungus grows through pores containing air, and subsequently penetrates land aggregate, forming hypha, therefore, materializing the mycelia network. This is used to absorb water, and nutrient in the form of carbon and nitrogen element, forming the chlorpyrifos, as well as the electron acceptor in the mycelia [17, 18].

3.2 Contact Time Optimization at the Chlorpyrifos Degradation

After obtaining the optimum concentration of A. fumigatus, optimization of contact time was assessed, and the results are shown in Table 3.

TABLE 3

CONTACT TIME OPTIMIZATION AT THE CHLORPYRIFOS DEGRADATION

Contact Time (days)	*ChlorpyrifosConc. (ppm)*	*SD*	*Chlorpyrifos Removal Efficiency (%)*
3	*12.64*	*1.32*	*62.37*
5	*3.26*	*0.34*	*90.29*
7	*0.37*	*0.01*	*98.91*
9	*0.12*	*0.01*	*99.65*

From Table 2, it can be also presentated in graphic that shown in Figure 4.

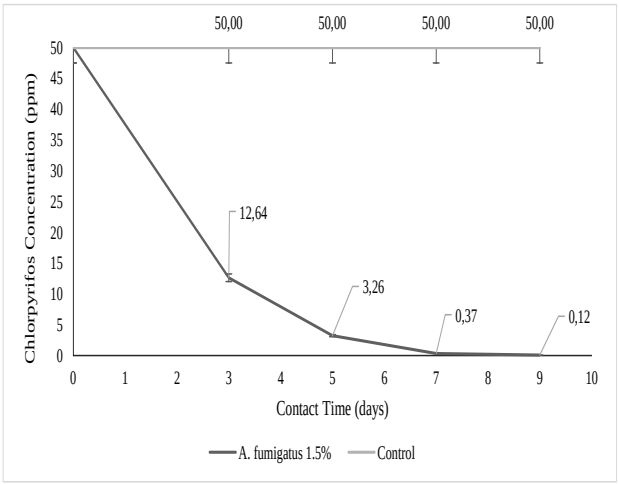

Fig. 4. Chlorpyrifos Removal Efficiency Chlorpyrifos at the Contact Time Variation

From Table 2, it is understood that 1.5% (w/v) of A. fumigatus, which was exposed in 3, 5, 7, and 9 days, was able to eliminate each chlorpyrifos by about 62.37%, 90.29%, 98.91%, and 99.65%. This result is obtained from the initial chemical concentration of about 50 ppm, in PDB media, pH 7, and temperature 25°C. Furthermore, the highest residue removal efficiency was measured on the 9th day, using the GC-MS method, as seen in Figure 5.

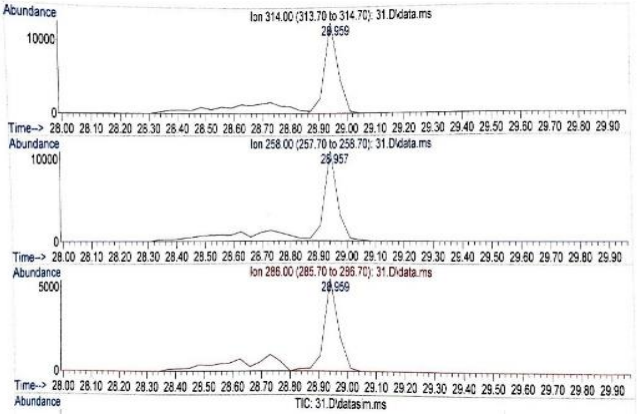

Fig. 5*. Chlorpyrifos Residual Measurement by GC-MS Method Chlorpyrifos on The 9th Day*

Figure 4 shows the highest chlorpyrifos residual removal

efficiency of about 99.65% occurred on the 9th day, due to the high enzymatic activity taking place at the exponential phase of A. fumigatus. However, it was observed to be in the adaptation phase on the 3rd day, hence the enzymatic activity was only capable of removing 62.37% of the chemical. In addition, at the biodegradation process, the produced enzymes modified the toxic pollutant by changing its chemical structure and they hasten the process by reducing the activation energy needed to initiate the reaction. Meanwhile, compound biotransformation or biodetoxification occurs, hence, the biological process, through the use of microorganism is able to effectively reduce harmful chemical substances within the environment [14, 22-23]. Conversely, utilizing the Monod equation with the first kinetical model, the rate constant value of chlorpyrifos removal reaction (k) was estimated to be about 0.43/day, from an initial concentration (C_0) of 50 ppm at t_0, to the final (C_t: 9 days) of about 0.12 ppm. This proves that the enzymatic activity conducted was capable of eradicating the chlorpyrifos, hence, enabling the A. fumigatus to function properly in polluted environment.

4 CONCLUSION

Elevating the concentration of A. fumigatus in a liquid media, promotes the removal efficiency of chlorpyrifos, which is also in line with the increased contact time. Moreover, at a specific temperature and pH, 1.5% (w/v), the microorganism was able to induce the most significant removal efficiency by about 99.65% in 9 days, in comparison with that of the 0.5% (w/v), exposed for 5 days. Furthermore, the k (chlorpyrifos removal rapid constant) was evaluated to be 0.43/day, using the Monod equation. This therefore uncovers the possibility of applying A. fumigatus as a good biodegradation alternative in the rehabilitation of chlorpyrifos polluted environment.

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