Chlorpyrifos removal by *Thiobacillus* sp. and *Clostridium* sp. in liquid medium

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Abstract. The bioremediation research of chlorpyrifos has been done to remove the concentration of chlorpyrifos using the natural bacterial *Thiobacillus* sp. and *Clostridium* sp.. The efficiency of chlorpyrifos removal was determined by the temperature and contact time. The research was done by adding 100 ppm of chlorpyrifos into the Stone Mineral Salt solution (SMSs) under controlled condition, then each bacterial are added as much as 10% (v/v) with pH 7. To obtain optimum efficiency, this study was conducted with temperature variation (°C) 25, 30, 35, 40 and contact time (hours) 12, 24, 36, 48. Based on Gas Chromatography Mass Spektrofotometer (GC-MS) analysis, the efficiency of removal at temperature (°C) 25, 30, 35, 40 are 43%, 68%, 71% and 52% respectively, while the removal efficiency at contact time (hours) 12, 24, 36, 48 are 43%, 49%, 74%, and 36%. The result showed that 74% removal efficiency by *Thiobacillus* sp. and *Clostridium* sp. obtained at 35°C in 36 hours of contact time. Thus, the mixed culture of *Thiobacillus* sp. and *Clostridium* sp. are able to synergize for removing the chlorpyrifos at 100 ppm.

1 Introduction

Soil contamination is a condition where the source of artificial pollutants enter into the soil and can change the soil structure so that the soil ecosystem can not function properly. Some waste that can contaminate the soil is leachate waste containing chemicals and insecticides not only contaminate the soil but also the ground water and harm for the aquatic ecosystem. The accumulation of insecticides residue causes the pollution to the soil even the groundwater and surface water [1]

Surface water that contaminate by pesticides has negative effects on aquatic milieu, for example growth of fish [2]. The track of pesticides to entry the aquatic ecosystem begin from precipitation, surface flow, atmospheric deposition to the aquatic ecosystem but also the non target organism that associated with agriculture [3-5]. Pesticide residues are also harmful to human health due to the absorption and accumulation into the food chain [6].

Chlorpyrifos is an effective non-systemic insecticide to kill various insects of plant and fruit pests [7]. Chlorpyrifos attack the insect nervous system by inhibiting the activity of acetylcholinesterase if ingested and inhaled into the respiratory system [8]. Chlorpyrifos is not only use in agriculture, but also to kill insects such as cockroaches and flies.

The hazard of chlorpyrifos residues can be manage through 3 processes, 1) Physical process using direct immobilization with activated charcoal either from rice husk or coconut shell [9], 2) Chemical process using active charcoal urea fertilizer with zeolite combined in

Fio (Inlet and Outlet Filters) placed on paddy fields, 3) Biological processes using bioremediation by microorganisms.

Bioremediation is the process of decomposing organic or inorganic pollutants using microorganisms such as fungi (*Trametes hirsutus*) and some types of bacteria in controlling contamination under controlled conditions into a harmless substance [10-11]. The advantage of bioremediation is to produce harmless products such as carbon dioxide, water and cell biomass also the hazardous compounds may change to harmless compounds [12]. The disadvantage of bioremediation is a requirement of controlled monitoring, and even the remediation process is slower than the chemical remediation.

Bioremediation can be applied by utilizing microorganisms such as fungi and bacteria. Several studies have proven that *Pseudomonas aeruginosa* can degrade residual chlorpyrifos [13]. The success rate of chlorpyrifos biodegradation depends on the concentration of chlorpyrifos in contaminated soil, the physiological microorganisms that will degrade chlorpyrifos, and the environmental conditions.

Based on these descriptions, this laboratory scale research is meant to study investigate potential of *Thiobacillus* sp. and *Clostridium* sp. as remediator of chlorpyrifos contamination in liquid medium [14].

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2 Methods

2.1 Bacterial growth analysis

Cultivation of *Thiobacillus* sp. and *Clostridium* sp. was done in batch culture using Stone Mineral Salt Solution (SMSs) medium, with composition 0.5 g CaCO₃; 2.5 g NH₄NO₃; one g Na₂HPO₄·7H₂O; 0.5 g KH₂PO₄; 0.5 g MgSO₄·7H₂O; and 0.2 g of MnCl₂·7H₂O per liter. The composition contained in the erlenmeyer are 70% (v/v) of the SMSs medium, *Thiobacillus* sp. and *Clostridium* sp. each of them 10% (v/v) with a controlled pH 7, and 10% (v/v) with 10⁻² dilution of molasses. Glucose, glycerol and molasses are added as carbon sources to increase microbial growth in this bioremediation [15].

Bacterial growth was calculated using the Total Plate Count method observed in Petri dishes that contained Nutrient Agar (NA) and calculated by Colony Counter by the formula:

$$\text{Numbers of Colonies /ml or /gram} = \text{Numbers of Colonies per petri dishes} \times \frac{1}{\text{dillution factor}} \quad (1)$$

2.2 Chlorpyrifos removal test in liquid medium

Chlorpyrifos removal test in liquid medium was done in two stages, as in the research design in Fig 1. The test was done by adding 10% (v/v) chlorpyrifos in the erlenmeyer containing the bacteria *Thiobacillus* sp. and *Clostridium* sp. The environmental conditions are controlled by pH 7, 100 ppm of chlorpyrifos. Temperature and contact time were varied with the aim of finding the optimum conditions for *Thiobacillus* sp. and *Clostridium* sp. to remove the highest concentration of Chlorpyrifos. In the first stage of chlorpyrifos removal was tested with 4 variation of temperature i.e. 25, 30, 35 and 40 (°C) with pH 7. Chlorpyrifos concentration was 100 ppm and contact time was 48 hours. The results of the first stage to be the basic of the second stage with contact time variation 12, 24, 36, and 48 by pH 7. Chlorpyrifos concentration was 100 ppm and the temperature used was the optimum temperature from the first stage.

Fig. 1. Research Design.

2.3 Analysis of Chlorpyrifos Removal in Liquid Medium

Chlorpyrifos removal in liquid medium was analyzed using Gas Chromatography - Mass Spectrometry (GC - MS) method [16]. Chlorpyrifos concentrations can be calculated by the formula:

$$R = \frac{\frac{Au}{Ab} \times cb \frac{Vb}{Vu} \times Ve}{Wu} \quad (2)$$

Information:

R: Concentration of residual pasticide (ppm)

Au: Sample chromatogram area

Ab: The standard chromatogram area

Cb: The standard concentration (ng / µl)

Vb: Standard volume injected (µl)

Vu: The volume of the injected sample (µl)

Ve: The final volume of sample extract (µl)

Wu: Weight of sample (g)

3 Results and discussion

3.1 The ability test of *Thiobacillus* sp. and *Clostridium* sp. in Chlorpyrifos

The diameter zone of inhibition aims to determine the sensitivity of a bacteria to an antibiotic compound or pathogen compound [17]. The ability of bacteria *Thiobacillus* sp. and *Clostridium* sp. to survive in chlorpyrifos has been tested by exposing *Thiobacillus* sp. and *Clostridium* sp. into a Petri dish that contained Nutrient Agar (NA) and chlorpyrifos on a paper disc as in Fig 2.

Fig. 2. The ability of *Thiobacillus* sp. and *Clostridium* sp. In Chlorpyrifos

Based on Figure 2, there is no inhibition zone that is formed until the observation of the fifth day. This proved that *Thiobacillus* sp. and *Clostridium* sp. are able to live and grow in an environment that contained chlorpyrifos.

3.2 Chlorpyrifos removal in stage I

In stage I, the sample was analyzed by tri replicated with the results as shown in Fig. 3.

Fig. 3. % Removal at temperature optimization.

Fig 3 shows that the highest chlorpyrifos removal occurred at 35 °C is 71% and the lower removal occurred at 25°C is 43%. The removal of chlorpyrifos continues to increase along with the addition of temperature from 25 °C to 35 °C. But at 40°C the chlorpyrifos removal decreased to 52%. The result of the temperature optimization from 25, 30, 35, 40 (°C) occurred at 35°C. This happened because that temperature is suitable for the highest growth of *Thiobacillus* sp. and *Clostridium* sp. to remove the highest chlorpyrifos. This proved that *Thiobacillus* sp. and *Clostridium* sp. were able to remove chlorpyrifos at the optimum temperature, which is 35°C.

Research [14] shows that chlorpyrifos with a concentration of 1000 mg l⁻¹ can be degraded up to 89% with an optimum temperature of 35 °C by *Bacillus pumilus* C2A1. The highest percentage of chlorpyrifos removal by *Thiobacillus* sp. and *Clostridium* sp. occurred at 35 °C reaching 71%. This removal is 70% higher than the removal at 40 °C which reached 52%.

3.3 Chlorpyrifos removal in stage II

In stage II, the sample was analyzed by tri replicated with the results as shown in **Fig. 4**.

Fig. 4. % Removal at Contact Time Optimization.

Fig 4 shows that the highest chlorpyrifos removal occurred at 36 hours is 74% and the lower removal occurred at 48 hours is 36%. The removal of chlorpyrifos continues to increase along with the addition of contact time from 12 hours 36 hours. But at 48 hours the chlorpyrifos removal decreased to 36%. The result of the contact time (hours) from 12, 24, 36, 48 occurred at 36

hours. This happened because the optimum growth of *Thiobacillus* sp. and *Clostridium* sp. occurred at 36 hours. Whereas, if the contact time is extend to 48 hours, the growth of *Thiobacillus* sp. and *Clostridium* sp. is decreasing along with the chlorpyrifos removal. This proves that *Thiobacillus* sp. and *Clostridium* sp. were able to remove chlorpyrifos at the optimum contact time, which is 36 hours. The previous study [18] showed that the removal of 150 mg l⁻¹ of chlorpyrifos by *Bacillus cereus* was 80% occurring at contact time for 5 days.

4 Conclusions

The results of this study, show that *Thiobacillus* sp. and *Clostridium* sp. were able to grow well in liquid medium that contained 100 ppm of chlorpyrifos. In addition, *Thiobacillus* sp. and *Clostridium* sp. were able to remove chlorpyrifos up to 71% at 35°C. Furthermore, the removal may reach 74% if contact time is shortened from 48 hours to 36 hours and set on pH 7.

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