The effect of temperature and pollution concentration on Cypermethrin removal in liquid media by using *Thiobacillus* sp. and *Clostridium* sp.

C Grimaldi, B Iswanto and A Rinanti*

Environmental Engineering Department, Faculty of Landscape Architecture and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia

*astririnanti@trisakti.ac.id

**Abstract.** Cypermethrin is known as synthetic pyrethroid (SP) which works to interfere the nervous system with a molecular formula of C₉H₁₁Cl₃NO₃. The use of pyrethroid pesticides tends to be high and continuous without regard to the rules of pest control can have a negative impact, that is decreasing environmental quality and decreasing biodiversity. Pollution degradation in the soil due to pesticide residues can be done biologically by doing remediation technology. One of the microorganisms that can degrade insecticide residues in the soil is bacteria. *Thiobacillus* sp. and *Clostridium* sp. are insensitive to cypermethrin which has been prove by no inhibition zone formed on a media that contained cypermethrin. Therefor this research the aim is to determine the growth response of bacterial culture using *Thiobacillus* sp. and *Clostridium* sp. as a bioremediation to remove the insecticide cypermethrin in liquid media. Optimization of environment conditions use temperature with a variation (°C) of 25-40 and concentration of cypermethrin with a variation (ppm) of 100-250. cypermethrin analysis using the Gas-Chromatography Mass Spectrometer (GC-MS). This research proves that the bacterium *Thiobacillus* sp. and *Clostridium* sp. capable of removing cypermethrin in liquid media reaching 97% occurs at optimum conditions at 30°C and optimum concentration of 100 ppm.

1. **Introduction**

Pesticide is a toxic natural or synthetic compound and is able to remove pests including insects, nematode, and rats. There are several kinds of pesticide which is insecticide, fungicide and herbicide [1]. Pesticide in contact with soil will accumulate and will not degrade on a short time because of its recalcitrant nature that can cause soil pollution. One kind of pesticide which is insecticide used in Indonesian farming activity is a cypermethrin of pyrethroid class pesticide [2].

Researchers [3] explain that uncontrolled pesticide utilization will produce various health problems and environmental pollution. Pesticide utilization influenced by significant toxic power, volume and exposure level will affect health. Besides that, pesticide utilization on plants will leave residue on the plant, the soil, and surrounding environment. If this pesticide residue is accumulated in the soil, it will also affect the life of soil organism and plants.

Cypermethrin insecticide with chemical formulation of C₂₂H₂₀Cl₂NO₃ is well known as synthetic pyrethroid (SP) that works as neural damager [4]. Result of various researches has shown that pyrethroid can produce numerous side effects on soil biology that involve qualitative and quantitative soil micro flora changes, enzyme activity changes, and soil nitrogen balance changes (N₂ binding deceleration and...
microorganism nitrification also ammonification disruption). Direct and indirect effect of pyrethroid on soil’s micro biologic aspect will further affect plant growth and soil fertility [5].

To decrease environmental and public health risks regarding pyrethroid usage, a quick and effective method to eliminate or diminish insecticide concentration in the environment is required. Insecticide residue countermeasure can be applied through physics process such as direct immobilization by using active charcoal produced either from rice husk or coconut shells [6]. It also can be done through chemical process by using urea compost with active charcoal layer (+zeolite), which is combined in FIO (Filter Inlet and Outlet) tool, placed in paddy fields. We can also have done it through biologic process by remediation technology that involves living microorganism to detoxify or lower pollutant level to a less toxic level.

In between the countermeasure methods used to recover a contaminated environment, biological approach with pesticide degrading bacteria catabolic activity base is likely the most promising and effective strategy [7-10]. Bioremediation that involves living microorganism to degrade pollutant is the most promising, environmental positive, efficient, and low cost process [11]. Microorganism is able to degrade pyrethroid by utilizing it directly as carbon source. Biodegradation process rate in liquid media is influenced by various factors such as temperature, pH, nutrition, pyrethroid concentration, inoculum size, and bacteria strain natures.

More evidence has discovered that environmental negative compound can be degraded by various microorganisms from numerous taxonomical groups [12-16]. One of these numerous microorganisms that is able to degrade insecticide residue in the soil is bacteria. *Thiobacillus* sp. and *Clostridium* sp. bacteria are able to degrade 110 ppm of chlorpyrifos as much as 74% in liquid media and soil within 36 hours under 35°C temperature and pH of 7 [17].

By seeing the ability of *Thiobacillus* sp. and *Clostridium* sp. bacteria to degrade pesticide in the previous researches, this research is aimed to determine *Thiobacillus* sp. and *Clostridium* sp. mixed culture bacteria in degrading cypermethrin insecticide residue in liquid media.

2. Methods

2.1. Stone Mineral Salt solution (SMSs) media preparation

*Thiobacillus* sp. and *Clostridium* sp. bacteria mixed culture growth was conducted by using SMSs media. A liter of aquades to produce SMSs media contains 0.5 grams of CaCO₃, 2.5 grams of NH₄NO₃, 1 gram of Na₂HPO₄·7H₂O, 0.5 grams of KH₂PO₄, 0.5 grams of MgSO₄·7H₂O, and 0.2 grams of MnCl₂·7H₂O.

2.2. Cypermethrin preparation as pollutant source

Cypermethrin insecticide used as pollutant source is a cydamethrin 50 EC that contains 50 g/l of active compound. Pesticide liquidation was conducted to obtain 100 ppm concentration by adding 1 ml of pure cypermethrin into 500 ml of aquades.

2.3. *Thiobacillus* sp. and *Clostridium* sp. bacteria sensitivity test against pollutant

*Thiobacillus* sp. and *Clostridium* sp. was grown in SMSs media with pH of 7 obtained from Trisakti University Environment Microbiology Laboratory collection in Jakarta. *Thiobacillus* sp. and *Clostridium* sp. bacteria was grown by using Gel Nutrient (GN), which later was put on a disc paper that contains 100 ppm of cypermethrin.

2.4. Temperature variation in cypermethrin removal

*Thiobacillus* sp. and *Clostridium* sp. bacteria was involved on a contact with 100 ppm of cypermethrin in an Erlenmeyer flask that contains SMSs and molasses for seven days with temperature variations of 25°C, 30°C, 35°C and 40°C. Erlenmeyer was placed inside an incubator shaker with rotation speed of 180 rpm in predetermined temperature variations.
2.5. **Pollutant concentration variation in cypermethrin removal**

Insecticide variation used to involve *Thiobacillus* sp. and *Clostridium* sp. bacteria on a contact inside Erlenmeyer flask with SMSs and molasses media variations of 100 ppm, 150 ppm, 200 ppm and 250 ppm, for seven days. Erlenmeyer flask was inserted into an incubator shaker with rotation speed of 180 rpm in an optimum temperature (in point 2.4).

2.6. **Cypermethrin removal efficiency**

Cypermethrin removal efficiency measurement was conducted by using the following formulation [18]:

\[
\text{Removal Efficiency (\%)} = \frac{C_o - C_a}{C_o} \times 100\%
\]

\(C_o\) : Original cypermethrin concentration (ppm)
\(C_a\) : Final cypermethrin concentration (ppm)

3. **Results and discussion**

3.1. **Thiobacillus sp. and Clostridium sp. bacteria sensitivity test against cypermethrin**

*Thiobacillus* sp. and *Clostridium* sp. bacteria can grow in a GN media that contains cypermethrin with 100 ppm concentration. *Thiobacillus* sp. and *Clostridium* sp. bacteria ability to survive in cypermethrin has been tested by exposing *Thiobacillus* sp. and Clostridium sp. bacteria in Gel Nutrient (NA) media and cypermethrin in disc paper as seen in Figure 1.

![Figure 1. Bacteria growth test in GN media that contains 100 ppm of Cypermethrin for 48 hours.](image)

This experiment detected no obstruction zone in media that contains cypermethrin so we can say that bacteria are not sensitive against cypermethrin. If the disc paper contains obstruction zone that shows no bacteria growth, it means that the tested compound contains anti microbe compound. On the contrary, if the disc paper does not contain obstruction zone which is free of bacteria growth, it means that the tested compound does not possesses anti microbe power. Because of that, bacteria can grow in the environment that contains cypermethrin, which in this research; it can be utilized to remove cypermethrin.

3.2. **Thiobacillus sp. and Clostridium sp. bacteria sensitivity test against cypermethrin**

The first step conducted is by determining optimum temperature in predetermined temperature variations, so that in the future, we can produce a suitable temperature condition to remove cypermethrin. Based on that, the temperature variations can be seen in Figure 2.
Cypermethrin can be removed in the temperature of 25°C, 30°C, 35°C and 40 °C with each removal of 89%, 97%, 95% and 74%. This result was obtained in SMSs media with original cypermethrin concentration of 100 ppm with pH value of 7 within 7 days. The highest removal was obtained on 97% level on a 30°C temperature. The obtained optimum temperature is the best condition to conduct cypermethrin removal by utilizing *Thiobacillus* sp. and *Clostridium* sp. bacteria. This result shows that mixed culture *Thiobacillus* sp. and *Clostridium* sp. bacteria can be utilized as a good bioremediation agent because *Thiobacillus* sp. bacteria is a mesophyll microorganism that grow in around 20-40°C temperature [19].

Based on the result of the previous researches, Bacillus amyloliquefaciens AP01 bacteria with optimum result of 30°C and pH of 7 is able to degrade 50 ppm of cypermethrin concentration as much as 45% in 5 days [20]. This result shows that *Thiobacillus* sp. dan *Clostridium* sp. bacteria mix has a better ability to conduct cypermethrin pesticide removal than bacillus amyloliquefaciens AP01 bacteria.

### 3.3. Cypermethrin pesticide pollutant concentration optimization by *Thiobacillus* sp. and *Clostridium sp. bacteria*

After obtaining optimum temperature of 30°C, this temperature was utilized to remove cypermethrin with pollutant concentration level as shown in Figure 3.

From Figure 2, we can see that Thiobacillus sp. and Clostridium sp. bacteria mix can be utilized to remove cypermethrin as much as 63.7%, 59.8%, 57.1% and 46.2% with pollutant concentration variations of 100 ppm, 150 ppm, 200 ppm and 250 ppm in seven days. The largest removal was produced...
in 100 ppm concentration that removes as much as 63.7%. This finding shows that the existence of bacterial enzyme activity can be used in cypermethrin removal.

Cypermethrin concentration addition did not produce a higher removal percentage, but the removal constantly decreases along with the additional cypermethrin concentration. This situation happened because *Thiobacillus* sp. and *Clostridium* sp. bacteria response on concentration above 100 ppm is decreased and unable to conduct optimum growth activity. Biodegradation speed by microbe was getting slower and it needs a preliminary acclimatization period to accelerate degradation process on higher cypermethrin concentration.

*B. subtilis* strain BSF01 bacteria is able to remove 85% concentration of 100 ppm cypermethrin in 30°C temperature and pH of 7 in 7 days [21]. This result proves that *Thiobacillus* sp. and *Clostridium* sp. bacteria possess better abilities from *B. subtilis* strain BSF01 bacteria in 100 ppm concentration cypermethrin removal. Pesticide concentration removal can be conducted by utilizing *Chlorella sorokiniana* micro algae, with highest chlorpyrifos removal efficiency of 99.18% in liquid media that contains 100 ppm on a temperature of 27°C and pH of 7 This result shows that micro algae utilization to remove pesticide is better than bacteria utilization [22,23].

4. Conclusion
*Thiobacillus* sp. and *Clostridium* sp. bacteria are able to eliminate cypermethrin in liquid SMSs media until 97% of original cypermethrin concentration of 100 ppm in 30°C temperature in 7 days. This result shows that *Thiobacillus* sp. and *Clostridium* sp. bacteria mixed culture can act as a decent bioremediation agent to recover a cypermethrin polluted environment.

References
[1] Ahmad M and Ahmad I 2014 Bioremediation of Pesticides Research Gate 11(5) 125-165
[2] Mantzos N, Karakitsou A, Hela D and Konstantinou I 2015 Science of Total Environment Environmental Fate of the Insecticide Cypermethrin Applied as Microgranular and Emulsi Fi Able Concentrate Formulations in Sun Fl Ower Cultivated Fi Eld Plots Science of the Total Environment
[3] Yuantari M G C 2009 Environmental Economic Study of Pesticide Utilization and Its Effect on Farmers’ Health in Horticulture Area of Sumber Rejo Village Ngablak Sub District Magelang Regency Middle Java Province, Thesis (Semarang: Universitas Diponegoro)
[4] Kementerian Kesehatan RI 2012 Insecticide Utilization Guidance (Pesticide)
[5] Das R, Das S J and Das A C 2016 Effect of synthetic pyrethroid insecticides on N2-fixation and its mineralization in tea soil Eur. J. Soil Biol. 74 9–15
[6] Nugraha A 2008 Active Charcoal Technology to Control Pesticide Residue in Farm Environment (Balai Penelitian Lingkungan Pertanian)
[7] Rinanti A, Nainggolan I J 2018 Petroleum residues degradation in laboratory-scale by rhizosphere bacteria isolated from the mangrove ecosystem IOP Conference Series: Earth and Environmental Science 106(1) 012100
[8] Zhao H, Geng Y, Chen L, Tao K and Hou T 2013 Biodegradation of cypermethrin by a novel Catellibacterium sp. strain CC-5 isolated from contaminated soil Can. J. Microbiol. 59 311–317
[9] Cycon M, Mijowska A, Wójcik M and Piotrowska-Scget Z 2013 Biodegradation and bioremediation potential of diazinon-degrading Serratia marcescens to remove other organophosphorus pesticides from soils J. Environ. Manage. 117 7–16
[10] Akbar S, Sultan S and Kertesz M 2015 Determination of cypermethrin degradation potential of soil bacteria along with plant growth-promoting characteristics Curr. Microbiol. 70 75–84
[11] Singh B K 2009 Organophosphorus-degrading bacteria: Ecology and industrial applications Nature Rev Microbial. 7 156–163
[12] Cycon M, Wojciech M and Piotrowska-Scget Z 2009 Biodegradation of the organophosphorus insecticide diazinon by Serratia sp. and Pseudomonas sp. and their use in bioremediation of
contaminated soil *Chemosphere* 76 494–501

[13] Yang L, Chen S H, Hu M Y, Hao W N and Geng P 2011 Biodegradation of carbofuran by Pichia anomala strain HQ-C-01 and its application for bio-remediation of contaminated soils *Biol Fert Soils* 47 917–923

[14] Lin Q S, Chen S H, Hu M Y, Rizwan-ul-Haq M and Yang L 2011 Biodegradation of cypermethrin by a newly isolated actinomycetes HU-S-01 from wastewater sludge *Int J Environ Sci Tech* 8 45–56

[15] Chen S H, Geng P, Xiao Y and Hu M Y 2012 Bioremediation of b-cypermethrin and 3-phenoxybenzaldehyde contaminated soils using Streptomyces aureus HP-S-01 *Appl Microbiol Biotechnol* 94 505–515

[16] Chen S H, Luo J J, Hu M Y, Geng P and Zhang Y B 2012 Microbial detoxification of bifenthrin by a novel yeast and its potential for contaminated soils treatment *PLoS ONE* 7 e30862

[17] Sunaryo T, Widyatmoko H and Rinanti A 2018 Chlorpyrifos removal by Thiobacillus sp. and Clostridium sp. in liquid medium *MATEC Web of Conferences* 197 13012

[18] Sawyer C N 1994 *Chemistry for Environmental Engineering. Ed ke-4* (Singapore: McGraw-Hill)

[19] Holt J G 1994 *Bergey’s Manual of Determinative Bacteriology. Ninth Ed* (Philadelphia: A Wolters Kluwer Company) pp 562-570

[20] Lee Y S, Lee J H, Hwang E J, Lee H J, Kim J H, and Heo J B 2016 Characterization of biological degradation cypermethrin by Bacillus amyloliquefaciens AP01 *J. Appl. Biol. Chem.* 59 9–12

[21] Xiao Y, Chen S, Gao Y, Hu W, Hu M and Zhong G 2015 Isolation of a novel beta-cypermethrin degrading strain Bacillus subtilis BSF01 and its biodegradation pathway *Appl. Microbiol. Biotechnol.* 99 2849–2859

[22] Habibah R, Iswanto B and Rinanti A 2020 The significance of tropical microalgae chlorella sorokiniana as a remediate of polluted water caused by chlorpyrifos *International Journal of Scientific and Technology Research* 9(1) 4460-4463

[23] Permatasari R, Rinanti A and Ratnaningsih R 2018 Treating domestic effluent wastewater treatment by aerobic biofilter with bioballs medium *IOP Conference Series: Earth and Environmental Science* 106(1) 012048