Fipronil removal at various temperature and pollutant concentration by using *Pseudomonas aeruginosa* and *Brevibacterium* sp. in liquid media

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**Abstract.** Fipronil is one of organochlorine pesticide with persistent nature in the environment. Fipronil can be biologically removed by utilizing bioremediation that utilizes microorganism activity. This research was conducted to remove fipronil in Stone Mineral Salt Solution (SMSs) liquid media by using *Pseudomonas aeruginosa* and *Brevibacterium* sp. with temperature and fipronil concentration as independent variable. Fipronil removal was conducted with temperature variations of 25, 30 and 35°C and fipronil concentration of 30, 40 and 50 mg/L. Based on the conducted Gas Chromatography-Mass Spectrometry (GC-MS) test, an optimal fipronil removal was achieved on 30°C temperature for three days with fipronil concentration of 40 mg/L. This finding shows that *Pseudomonas aeruginosa* and *Brevibacterium* sp. are able to remove fipronil with 65% efficiency, so that this research can be carried further to acknowledge physical parameter that influence fipronil removal in liquid media.

1. **Introduction**
Soil pollution can be defined as a contaminant polluted soil with an over limit concentration according to the regulation [1]. This situation can happen due to industrial or commercial waste leaks, pesticide usage, and the entrance of polluted water on the surface of the soil, waste or chemical transportation accident, or waste water from nearby landfills and direct industrial waste dumping into the soil [2]. Pesticide utilization in farming sector has a potential to pollute soil [3]. Pesticide that enters the soil can cause saturation on the soil. The nature of pesticide that is persistent in the environment for a long time will cause pesticide to settle and create soil degradation [4]. The existence of pesticide in the environment can be seen in Figure 1.

Organochlorine pesticide is a contact insecticide that has a lipophilic nature and possesses low vapour pressure and slow environmental degradation. This nature makes it penetrable, durable and is able to act as a highly effective pesticide [5]. Fipronil with a chemical formula of C$_{12}$H$_{11}$Cl$_{2}$F$_{6}$N$_{4}$O$_{5}$ is a wide-spectrum insecticide included in *phenyl pyrazole* class [6,7]. Fipronil is effective as a control agent for insects that are immunity against organophosphate, carbamate, and pyrethroid insecticides.
Organochlorine pesticide in the environment can be removed by utilizing physics and biological methods. In physics method, active charcoal created from rice husks and coconut shells utilization can lower lindane, eldrin, dieldrine, DDT, endosuphane, and hexaplore concentrations as much as 70 to 90% in the soil [9]. There is also another research which mentions that microbe-enriched urea layered active carbon can also lower eldrin concentration as much as 33.6% in rice fields [10]. Meanwhile in biological method, removal can be conducted by utilizing bioremediation.

Bioresidiation is a process that highly dependent on microorganism to decrease, lower, detoxify, or alter pollutant concentration into a less dangerous concentration [11]. If we look at the process, bioremediation can be divided into three, which is: 1) by natural weakening; 2) by bio stimulation; and 3) by bio augmentation process [12]. Bioremediation has its own advantage which is to be able to turn dangerous compound into a less dangerous compound such as carbon dioxide, water, and biomasses [13], meanwhile its weakness is that it needs a controlled monitoring with a slower process [14].

Biodegradation is a natural way to recycle waste or to break organic matter into an available nutrition for the other organisms. This waste is a hydrocarbon compound that comes from oil, petrochemical, and farming activity and/or industry [15]. In atrazine degradation by *Pseudomonas* sp. bacteria with ADP strain, there are three enzymes involved in several degradation phases. The first enzyme is AtzA that catalyse dechlorination hydrolysis of atrazine into a non-toxic atrazine hydroxyl and act as a main enzyme of atrazine biodegradation. The second enzyme is AtzB that catalyse dehydrochlorination of atrazine hydroxyl to produce N-isopropyl cyanuric amide. The third enzyme is AtzC that catalyse cyanuric acid and isopropylamine so that atrazine can be degraded into CO$_2$ and NH$_3$ [16].

Bacteria as a common microbe used to degrade pesticide in *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria mixed culture can remove endosulphane pesticide as much as 90% [17]. The single culture of *Pseudomonas aeruginosa* can remove chlorpyrifos pesticide for 1, 5, and 7 days respectively in 10, 25, and 50 mg/L [18]. *Brevibacterium frigoritolerants*, *Bacillus aerophilus*, and

![Figure 1. Fate of pesticide in the environment [8].](image)
Pseudomonas fulva consortium can degrade phorate as much as 97.65 – 98.31% [19], meanwhile Bacillus sp., Brevibacterium sp., Pseudomonas putida, Bacillus subtilis, and Rhizobium sp. are able to lower imidacloprid concentration as much as 25.36 – 45.48% from its original 25 mg/L concentration in 25 days [20]. Because of that, this research is aimed to analyze the ability of Pseudomonas aeruginosa and Brevibacterium sp. to remove fipronil residue in liquid media.

2. Research methodology

2.1. Stone Mineral Salt solution (SMSs) media preparation
SMSs media is a liquid phased growth media utilized in this research. One liter of 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. Fipronil preparation as pollutant source
Fipronil that is used as pollutant source comes from Regent 50 SC pesticide that contains 50 gram/L of active compound.

2.3. Pseudomonas aeruginosa and Brevibacterium sp. bacterium cultivation
Pseudomonas aeruginosa and Brevibacterium sp. bacterium was obtained from Trisakti University’s Environmental Microbiology Laboratory collection in Jakarta. Pseudomonas aeruginosa Brevibacterium sp. was growth in SMSs media with pH of 7. In the environment, Pseudomonas aeruginosa can be found in water and soil [18], while Brevibacterium sp. can be found in highly salinated water [21].

2.4. Temperature variation in fipronil removal
Pseudomonas aeruginosa and Brevibacterium sp. bacterium was inserted into an Erlenmeyer flask that contains SMSs media and 40 mg/L of fipronil. Observation was conducted for three days with temperature variations of 25, 30, and 35°C.

2.5. Concentration variation of fipronil removal
Pseudomonas aeruginosa and Brevibacterium sp. bacterium was inserted into an Erlenmeyer flask that contains SMSs media with optimum temperature from previous research (point 2.4). Observation was conducted for three days with pollutant concentration variations of 30, 40, and 50 mg/L.

2.6. Fipronil removal efficiency
Fipronil removal efficiency measurement can use the following formulation [22]:

\[
\text{Elimination efficiency (\%) } = \frac{C_0 - C_a}{C_0} \times 100\%
\]

Co : initial fipronil concentration (ppm)
Ca : final fipronil concentration (ppm)

3. Results and discussion

3.1. Temperature variation in fipronil removal
Temperature determination is the first step to determine optimum condition in fipronil removal by using Pseudomonas aeruginosa and Brevibacterium sp. bacterium. Fipronil removal efficiency in several temperature variations can be seen in Figure 2.
Figure 2. Fipronil removal efficiency in various temperature.

Figure 2 shows that *Pseudomonas aeruginosa* and *Brevibacterium* sp. can survive in 25 – 35°C temperature intervals in SMSs media that contains 40 mg/L of fipronil with pH of 7. Based on figure 2, fipronil removal efficiency tends to increase in 25 – 30°C and decrease on 35°C. The highest fipronil efficiency of 61% occurred on 30°C, which indicates that this level of temperature is the optimum temperature to remove fipronil in liquid media. The result of this research is similar as the results from previous researches [23,24], which is *Pseudomonas aeruginosa* and *Brevibacterium* sp. bacterium can optimally grow in 30°C temperature. This bacterium is a common microbe used to degrade pesticide in the environment. The other researchers are also reported that in 30°C temperature and pH of 7, *Pseudomonas aeruginosa* and *Staphylococcus aureus* mixed culture can remove 40 mg/L of endosulphane pesticide with removal efficiency of 96% in seven days’ incubation time [17]. Therefore, at temperature variation fipronil is used with concentration of 40 mg/L as a fixed variable.

3.2. Fipronil removal concentration variation

After determining optimum temperature, a test to acknowledge fipronil removal in various concentration variations was conducted. *Pseudomonas aeruginosa* and *Brevibacterium* sp. bacterium are able to remove fipronil in concentration variation of 30, 40, and 50 mg/L. Removal efficiency in every concentration variation can be seen in Figure 3.

Figure 3. Fipronil removal efficiency in various fipronil concentration.
Figure 3 shows that in fipronil concentrations of 30, 40, and 50 mg/L, *Pseudomonas aeruginosa* and *Brevibacterium* sp. are able to grow decently in SMSs media with 30°C temperature and a pH of 7. The highest fipronil removal of 65% occurred in 40 mg/L concentration and the lowest fipronil removal of 45% occurred in 30 mg/L concentration. The highest fipronil indicated that 40 mg/L is the optimum concentration to remove fipronil in liquid media.

4. Conclusion

*Pseudomonas aeruginosa* and *Brevibacterium* sp. can remove fipronil in 30 to 50 mg/L concentration in 30°C temperature in three days inside a SMSs media. A further research on the other physical factors such as pH and contact time is required to increase fipronil removal efficiency by using *Pseudomonas aeruginosa* and *Brevibacterium* sp.

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