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Bioremediation To Reduce Pesticide Pollution On Agricultural Land

Prabang Setyono[1], Hashfi Hawali Abdul Matin[1], Tarzan Sembiring[2], Febriana Kinanthi Putri[1,]*, Ekaputra Agung P.[2]

[1]Sebelas Maret University, Faculty of Mathematics and Natural Sciences, Department of Environmental Science, Jl.Ir.Sutami No 36A Surakarta, 57126, Indonesia

[2]National Research and Innovation Agency, Jl.Sangkuriang Bandung, 40135, Indonesia

Abstract

Environmental pollution until this moment still become crucial environmental problem. Environmental pollution can occur anywhere, one of them is in agriculture sectoral. Environmental pollution in the agriculture area caused by usage of chemical pesticide for managing agriculture. Using chemical pesticide can leave residue that raises pollution. Bioremediation become one of the solutions for the problem. Besides it is environmental friendly, bioremediation is also easy to apply and cheap. This study aim to examine the potency of *Bacillus altitudinis , Bacillus subtilis, Pseudomonas b68, and Pseudomonas b70* as biological agent for bioremediation of pesticide residues in soil Biological agent was incubated in liquid medium polluted pesticide type fungicide for 15 days on some variation concentration pesticides (100, 200, 300) mg/L. Concentration of pesticides and values Optical density (OD) is measured every 3 days with spectrophotometer UV -Vis on long 280 nm and 578 nm waves. The Results showed that the bioagents lower pesticides concentration. and can growing under polluted pesticides conditions The effectiveness and decline in pesticide residue by *Bacillus altitudinis* ranged from 49.91-59.33%; *Bacillus subtilis* (50.06-60.51%); *Pseudomonas b68* (81.32-86.13 %); *Pseudomonas b70* (50.02- 62.1 %). The bioagents produced decreases in the concentration of pesticides, increase in OD value, and decrease in pH indicate. The results affirmed that *Bacillus altitudinis, Bacillus subtilis*, *Pseudomonas b68, and Pseudomonas b70* are effective as bioagents in the remediation pesticides polluted soils

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Corresponding author:

Febriana Kinanthi Putri, Sebelas Maret University, Faculty of Mathematics and Natural Sciences, Department of Environmental Science, Jl.Ir.Sutami No 36A Surakarta, 57126, Indonesia.

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Introduction:

Environmental pollution can occur on agricultural land. One of the causes of environmental pollution on agricultural land is the use of pesticides. Pesticides are all chemical substances and other materials used to control pests [13]. The field of agricultural crops is a field that is exposed to pesticides periodically. The amount and type of pesticides used in the agricultural sector varies. One type of pesticide used in the agricultural sector is a fungicide with the active ingredient difenoconazole. Difenoconazole belongs to the fungicide group to fight and prevent the growth of fungi in plants [9]. Diphenoconazole can be very well absorbed by plant roots and delivered to other parts of the plant via xylem tissue

[15] . This fungicide with the active ingredient difenoconazole can be applied to various types of plants such as vegetables, fruits and other plants. Aside from being a fungicide, difenoconazole can also function as a plant growth regulator. According to [19] diphenoconazole can be used as a growth regulator in rice plants and increases rice yields between 4.42% -15.21% or around 260 kg/ha-1 ton/ha. This makes the use of difenoconazole attractive to farmers for agriculture.

The use of difenoconazole pesticides on plants can leave residues either on fruit, soil or water due to direct contact with pesticides or carried by the wind. According to Shen, et al (2021) excessive use of difenoconazole can cause adverse effects for aquatic organisms in aquatic ecosystems. [29] stated that there were pesticide residues on melons sprayed with difenoconazole. Diphenoconazole residues can also be found in rice plants, paddy soil, and paddy water (Wang et al., 2012). [42] found difenoconazole residues in wheat plants and soil. [27] stated that difenoconazole is persistent in water and soil environments, and is toxic to organisms that live in these environments. Based on research by [32] pesticide residues that contaminate the soil can reduce populations of mycorrhizal fungi and earthworms which play a role in maintaining soil and plant fertility. In addition, accumulated pesticide residues will be dangerous when human contamination occurs [40].

The potentials of pesticide residues to cause environmental pollution has become problem of increasing concerns worldwide. The polluted environment must be repaired so that it is not damaged. The method of recovering a biologically polluted environment can be an alternative choice. This concept was initiated by Gayle's thought in 1952 that when there are harmful organic compounds in the environment, there will be microbes that are able to decompose the pollutant so it does not pollute the environment. The method of biologically restoring the environment is commonly known as bioremediation [11]. Bioremediation is regulated in Minister of Environment Decree Number 128 of 2003. Bioremediation has many advantages. This method is widely accepted and applied by the community because it is considered inexpensive and environmentally friendly. The bioremediation process utilizes biological agents that act as bioremediators. The biological agents in the form of microbes perform a function in decomposing waste substances. Bioremediation is considered a method that is safe, easy, and affordable in helping to restore polluted environmental conditions [21].

Much research on bioremediation has been carried out, such as research by [34] who isolated the microbes *Pseudomonas putida B-2187* and *Rhodococcus erytropolis Ac-859* for bioremediation of soil contaminated with crude oil. [31] isolated rhizosphere microbes for oil-contaminated soil bioremediation. [2] utilized the microbial *Pseudomonas aeruginosa* for bioremediation of oil-contaminated soil. [26] isolated *Bacillus* genus microbes for bioremediation of Pb heavy metal accumulation. Although there have been many studies, most of the application of bioremediation focuses on the restoration of oil-contaminated land in the oil and gas industries and heavy metal. In the future, the application of bioremediation technology has the potential to be carried out in the agricultural sector so that the target pollutants are not only petroleum hydrocarbons but also other compounds such as pesticides.

This study evaluated the bioremediation of pesticide compounds especially for fungicide with the active ingredient difenoconazole by utilizing biological agents in the form of microbes (bacteria). The aim is to evaluate the potential of *Bacillus subtilis, Bacillus subtilis, Pseudomonas b68*, and *Pseudomonas b70* as a biological agent for bioremediation of pesticide compounds

MATERIALS AND METHODS

This study uses an experimental method. The limitation of this study is the ex-situ application of bioremediation of triazole fungicide-type pesticides with difenoconazole active ingredients on a laboratory scale using mineral salt liquid media (MSM). The research design used was a completely randomized design (CRD) of 3 replications with 2 treatment factors consisting of types of microbes (bacteria) as biological agents and different concentrations of Pesticides Score 250 EC. So that the total trials in these 9 studies were 45 trials. The tools used are microbiological tools, Chemical Oxygen Demand (COD) test kits according to SNI 6989.2: 2019, and a pH meter. The materials used include chemicals, pesticides with a trademark score of 250, cultures *Bacillus subtilis, Bacillus subtilis, Pseudomonas b68*, and *Pseudomonas b70.*

How the research works Liquid mineral salt media (MSM) with the following composition (grams/liter): KH2PO4 6.0; (NH4)2SO4 6.0; NaCl 0.5; MgSO4.7H2O 2.4; CaCl2.2H2O 1.6. 0.36 g/L glucose was added. Pour 100 ml of liquid media into a 250 ml Erlenmeyer and add a fungicide-type pesticide with the active ingredient difenoconazole in various concentrations of 100 ppm, 200 ppm and 300 ppm. Then sterilized by autoclaving at 121°C for 15 minutes. The propagated pure microbial culture was added to each aseptically prepared experimental medium. Then incubated at room temperature. The control is a sample without microbes. Pesticide concentration, optical density value at 578nm absorbance, and pH were observed every 3 days until the 15th day. The concentration of pesticides is obtained from the standard curve equation. COD value is calculated at the beginning and end with SNI 6989.2: 2019, pH is calculated using a pH meter.

The data obtained were then analyzed quantitatively with the ANOVA test to determine the difference in variance between the independent variable and the dependent variable. If there is a significant difference where H0 is rejected, then proceed with the Duncan Multiple Range Test (DMRT) at the 5% level to find out the real difference in the effect of microbial species on reducing pesticide contamination.

RESULTS AND DISCUSSION

Environmental pollution can occur on agricultural land. One of the causes of pollution on agricultural land is the use of pesticides. The use of pesticides on agricultural land is usually to eradicate pests and plant-disturbing organisms. Most agricultural activities still use conventional concepts so that the use of pesticides cannot be avoided. One type of pesticide used is difenoconazole pesticide from the triazole group. Triazole pesticides are widely used by farmers to eradicate fungi (Korlina, Latifah and Andri, 2016). This fungicide changes the structure of the fungal cell membrane by inhibiting the synthesis of ergosterol. Triazole pesticides have a complex molecular structure that makes them difficult to degrade in the environment (Tchamitchian, Tavares and Blot, 2022). In this study, microbial-based bioremediation was carried out to reduce pesticide concentrations.

The pollutant in the form of a triazole pesticide with the active ingredient diphenoconazole was made artificially with several concentration ranges (mg/L), namely 0, 100, 200, and 300 which was mixed with liquid media and then inoculated with microbes that act as biological agents in bioremediation. The bioremediation process lasted for 15 days to see the growth of bacteria based on optical density (OD) and pesticide concentration (mg/L). Table 1 shows the effectiveness of reducing pesticide concentrations after 15 days.

Table 1 showed the effectiveness of biological agents to reduce pesticide residues after 15 days of bioremediation treatment. Overall the effectiveness of the bioagents to reduce pesticides compounds exceeds that of the control treatment. The effectiveness of reducing pesticides at a concentration of 0 mg/

Table 1. Effectiveness of Pesticide Reduction After 15 Days of Bioremediation Treatment

Pesticide Concentration (mg/L)		Pesticide Reduction Effectiveness (%)				
		Bacillus height	*Bacillus subtilis*	*Pseudomonas b68*	*Pseudomonas b70*	Control
0		0,00^{a}	0,00^{a}	0,00^{a}	0,00^{a}	0,00^{a}
100		49,91^{b}	56,68^{cde}	85,43^{f}	50,02^{bc}	0,00^{a}
200		56,16^{cde}	50,09^{bc}	81,32^{f}	54,04^{cd}	0,00^{a}
300		59,33^{de}	60,51^{de}	86,13^{f}	62,1^{e}	0,00^{a}

Note: Identical letters indicate no significant difference in Duncan's test

L was 0%, which means that there was no decrease because in no pesticide contaminants were added. At a concentration of 100 mg/L, the highest effectiveness against the pesticides was 85.43% for which pesticide residue concentration was 14.57 mg/L by biological agents *Pseudomonas b68*. While the lowest effectiveness was found in the treatment *Bacillus altitudinis* with a decrease in effectiveness of 49.91% or the final concentration 50.09 mg/L. The effectiveness of reducing pesticides concentration of 200 mg/L was obtained for *Pseudomonas b68* which equated to 81.32% while the residue concentration was 37.36 mg/L, while the effectiveness of reducing the lowest pesticide treatment Bacillus subtilis was 50.09% or the final concentration was 99.82 mg/L based on the value of effectiveness of decrease concentration.

For the treatment with concentration of 300 mg/L the lowest reduction effectiveness of pesticides was in the treatment without biological agents of 0.00% for which the final concentration was fixed (or no change from the initial conditions). The highest reduction was in biological agents *Pseudomonas b68* with the final concentration being 41.61 or a decrease of 86.13%. *Pseudomonas b68* showed a higher reduction effectiveness value compared to other microbes (Table 1). A large number of cells will increase microbial biomass. Increasing biomass will increase the ability of microbes to decompose pollutant substances and can increase soil and plant fertility [20]. [14] shows that the greater the number of cells, the more the ability of microbes to decompose pollutants.

The effectiveness of bioagent to reduce pesticide concentrations is presented in Table 1. It is showed that bioremediation can reduce the concentration of pollutants in the form of pesticides. Biological agents (microbes) can degrade pesticides into harmless substances in the environment. In the bioremediation process, microbes will degrade triazole pesticides by using the element carbon (C) as a food source and nitrogen (N) will be bound by microbes to produce carbon dioxide (CO2) and water (H2O) which are harmless in the environment (Wu et al., 2016). The process of degradation by microbes involves hydrolyzation of bond chains in pesticide compounds and use them as the only food source [23]. [10] stated that in static conditions, liquids have surface tension which makes it difficult for two liquids with different densities to blend together. Microbes that are inoculated into liquid media have the potential to produce biosurfactants, namely environmentally friendly surfactants that can reduce surface tension. A decrease in surface tension can increase the solubility of pesticide contaminants so that microbes can attach to the contaminants and carry out the degradation process by using the contaminants as a food source [22]. Genus *Bacillus* and *Pseudomonas* considered to be able to produce biosurfactants so that they can be applied for bioremediation [4, 30]. [15] stated that the degradation process can be through

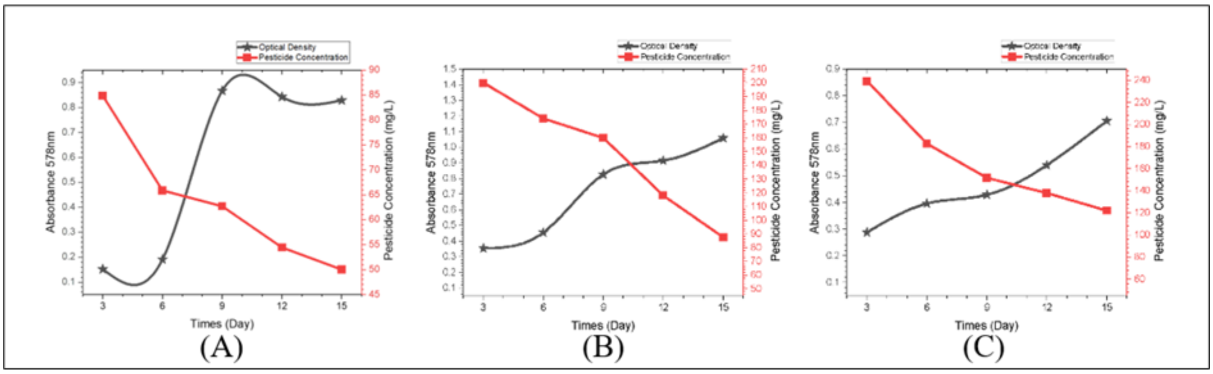

Figure 1. Graph of OD Value and Pesticide Concentration *Bacillus altitudinis*: A) Pesticide Concentration 100mg/L; B) Pesticide Concentration 200mg/L; C) Pesticide Concentration 300mg/L.

enzymatic reactions, namely the entry of compounds into the body of microorganisms in a certain way, which can first be in the form of attachment to the substrate. It follows that through series of physiological and biochemical reactions under the action of various enzymes, pesticides will eventually be degraded into smaller molecular compounds that have low toxicity or are less toxic (Wu et al., 2016). The graphs of OD values and pesticide concentrations are as follows:

Based on Figure 1, the treatment of pesticide concentration of 100 mg/L showed a change in pesticide concentration, namely the pesticide concentration decreased significantly on day 6 to 66.87 mg/L, tended to be stationary on day 9, and continued to decrease until day 15th. The reduction of pesticides at various concentrations of 200 mg/L was not very significant until the 9th day then decreased significantly on the 12th day and continued to decrease until the 15th day. While the rate of change of pesticide concentration in the treatment was 300 mg/L, namely the concentration of pesticides decreased from the 3rd day and but the decreases were not significant on the 9th to the 15th day. Growth phase *Bacillus altitudinis* at various pesticide concentrations based on the OD value measured at a wavelength of 578 nm (Figure 1) it can be observed at a pesticide concentration of 100 mg/L, the adaptation period required by *Bacillus altitudinis* 6 days, experiencing growth (*log phase*) on the 9th day, entered the 2nd day stationary phase and inclined *death phase* 15th day. At a pesticide concentration of 200 mg/L, the adaptation time was 6 days, then the growth phase (*log phase*) started on the 9th day and increased until the 15th day. Meanwhile, at a concentration of 300 mg/L, *Bacillus altitudinis* required adaptation time for 9 days, began to experience growth (*log phase*) on day 12, and continued to increase until day 15.*Bacillus altitudinis* required a longer adaptation period as the pesticide concentration increases. The higher the concentration of contaminants, the heavier the microbes that will be required to in carry out metabolic degradation of the compounds hence the need for time to adjust [28].

Figure 2 shows changes in the rate of reduction of pesticides and OD values for 15 days by biological agents*Bacillus subtilis*. Based on the figure, in the treatment of 100 mg/L pesticide concentration, a decrease in pesticides occurred from the 3rd day and then tended to be stationary on the 9th to 12th day and decreased until the 15th day. In the 200 mg/L treatment, the pesticide concentration decreased significantly until the 9th day, tended to be stationary on the 12th day, and still decreased until the 15th day. While the decrease in pesticides at a concentration variation of 300 mg/L, namely the concentration of pesticides decreased until the 12th day and then tended to be stationary until the 15th day. The OD value measured at a wavelength of 578nm can be observed at a pesticide concentration of 100 mg/L requiring an adaptation period of 6 days, experiencing growth*(log phase)* on the 9th day, and tend to be

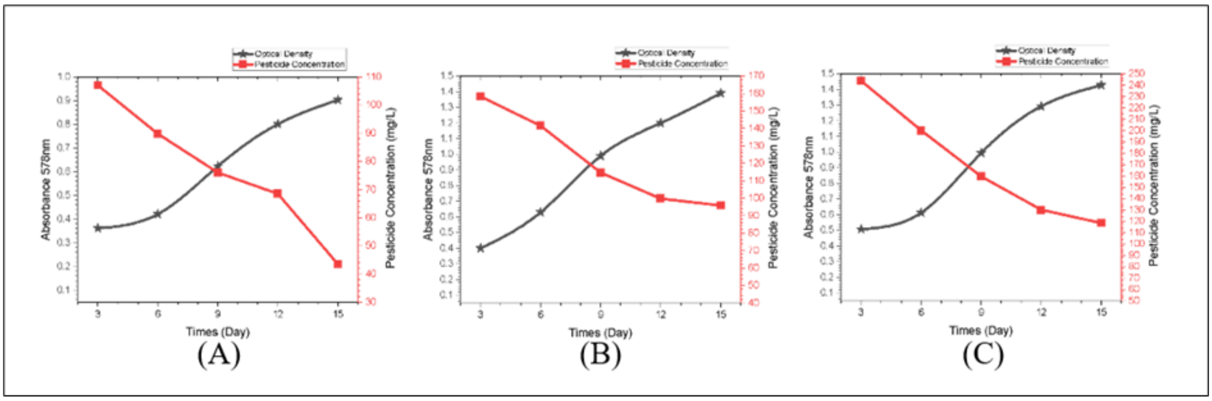

Figure 2. Graph of OD Value and Pesticide Concentration*Bacillus subtilis* Note: A) Pesticide Concentration 100mg/L; B) Pesticide Concentration 200mg/L; C) Pesticide Concentration 300mg/L.

stationary on the 15th day. At a pesticide concentration of 200 mg/L, the 6th day showed an increase *(log phase)* and continued to increase significantly until the 15th day. Meanwhile, at a concentration of 300 mg/L,*Bacillus subtilis* needed time of adaptation for 3 days, began to experience growth*(log phase)* on the 6th day, and continued to increase significantly until the 15th day. Growth *Bacillus subtilis*as the pesticide concentration decreased. This shows that *Bacillus subtilis* can use pesticides as a carbon source to produce ATP. Bakar, et al (2015) stated that *Bacillus subtilis* can use organic compounds for its growth.

Figures 1&2 show that bioremediation with *Bacillus altitudinis* and *Bacillus subtilis* reduced the concentration of pesticides with different abilities. Pesticide concentrations of 100 mg/L and 300 mg/L, using *Bacillus subtilis* reduced the concentration of pesticides more than *Bacillus altitudinis* with final concentrations of 43.32 mg/L and 118.45 mg/L. Whereas *Bacillus altitudinis* can lower pesticides more than *Bacillus subtilis* at a pesticide concentration of 200 mg/L it becomes 87.67 mg/L. *Bacillus subtilis* is the microbe that is most frequently encountered and widely applied because of its easy adaptability. In the process of bioremediation of pesticides, the only food source for microbes is pesticide compounds. Ability *Bacillus subtilis* in adapting makes it possible to survive by using pesticides as a food source. There are several influencing factors *Bacillus subtilis*in degrading pesticides such as including temperature, initial pH value, inoculum ratio, initial pesticide concentration, various inorganic salt species, carbon source, and nitrogen source. [38] stated that *Bacillus subtilis* can degrade glyphosphate pesticides with an initial concentration of 10,000 mg/L with a degradation rate of around 65%, a temperature of 35°C, and a pH of 8.0.

Pesticide concentration with treatment *Pseudomonas b68* showed a decrease until the 15th day. Growth phase *Pseudomonas b68* at various concentrations of pesticides based on OD values measured at a wavelength of 578nm can be seen in Figure 3. At a pesticide concentration of 0 mg/L, the OD graph shows an increase but not too significant. On the 9th day it has shown decreased growth *(death phase)*. In the decreased growth phase, it means that the microbe enters a stationary phase close to death (Olawale et al., 2020). The adaptation period needed at a pesticide concentration of 100 mg/L is 6 days, experiencing growth *(log phase)* on the 9th day, and tends to rise until the 15th day although not too significant. At a pesticide concentration of 200 mg/L, the 6th day showed an increase *(log phase)* and continued to increase significantly until the 15th day. Meanwhile, at a concentration of 300 mg/L, *Pseudomonas b68* takes 6 days of adaptation time, begins to experience growth*(log phase)* on the 9th day, and continued to increase significantly until the 15th day. The adaptation period needed by microbes at each

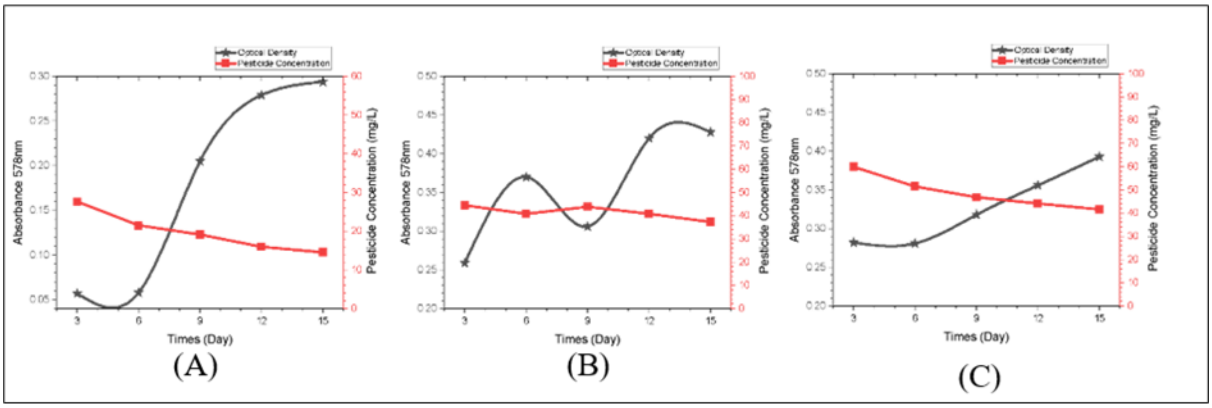

Figure 3. Graph of OD Value and Pesticide Concentration*Pseudomonas b68*: A) Pesticide Concentration 100mg/L; B) Pesticide Concentration 200mg/L; C) Pesticide Concentration 300mg/L.

concentration is different. The length of time the microbes are in the growth phase is also different. The growth phase indicates that the microbes have been able to use pesticides as a food source. In this case the availability of food sources still exists so that growth occurs [9].

The concentration of pesticides with *Pseudomonas b70* treatment showed a decrease until the 15th day. Overall pseudomonas b70 showed growth and decreased concentrations of pesticides until the 15th day (figure 4). At a concentration of 200 mg/L, there was a decrease in growth on the 12th day. The decrease on the 12th day could mean that the microbes were in a resting phase or an adaptation process to use pesticides as food or a carbon source. Oktavia, et al (2018) stated that during the adaptation phase, microbes usually prepare themselves to digest new substrates as a food source so they can grow . The increase in the concentration of pesticides can be caused because the glucose that was added at the beginning as a food source has been completely digested by the microbes, then the microbes have to adapt to using pesticides as a food source. The adaptation process results in a decrease in the ability of microbes to degrade pesticides resulting in an increase in pesticide concentrations. This is also supported by the opinion of [35] which states that microbes need adaptation time when they are in an environment that is not suitable or in an environment that is different from the environment they lived in before. At the end of the process or the 15th day at a pesticide concentration of 200 mg/L, *Pseudomonas b68* showed a better reduction than *Pseudomonas b70* with a final concentration of 37.36 mg/L.

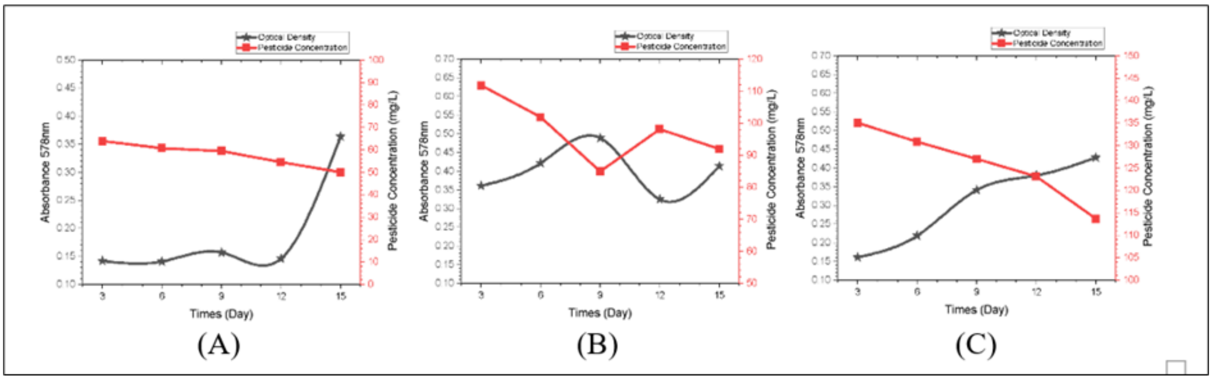

Figure 4. Graph of OD Value and Pesticide Concentration*Pseudomonas b70*: A) Pesticide Concentration 100mg/L; B) Pesticide Concentration 200mg/L; C) Pesticide Concentration 300mg/L.

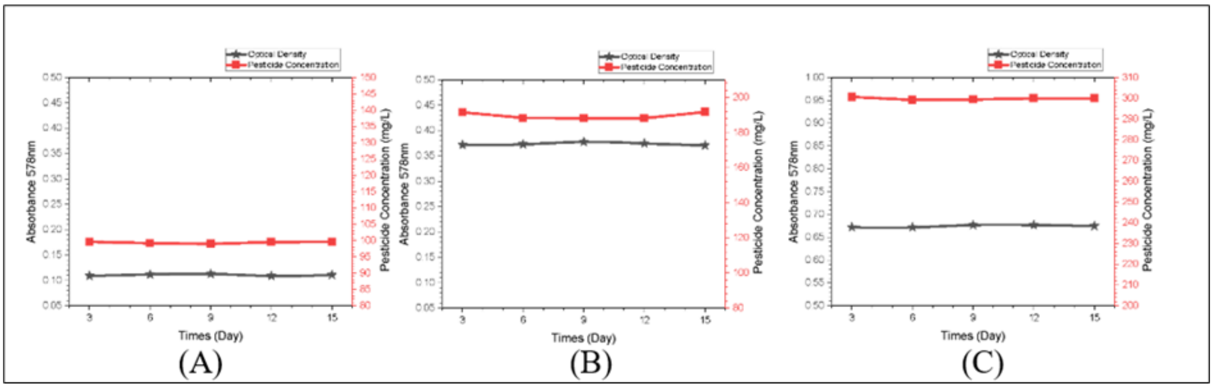

Figure 5. Graph of OD Values and Control Pesticide Concentrations: A) Pesticide Concentration 100mg/L; B) Pesticide Concentration 200mg/L; C) Pesticide Concentration 300mg/L

Meanwhile, the rate of change of pesticide concentration in the treatment of 300 mg/L showed a decrease until the 15th day. *Pseudomonas b68* showed a greater reduction with a final concentration of 41.62 mg/L compared to *Pseudomonas b70* with a final concentration of 113.69 mg/L. Microbes of the genus *Pseudomonas* degrade pesticides through enzymatic reactions. *Pseudomonas* can produce 3 enzymes that play a role in the degradation process, namely AtzA, AtzB, and AtzC. These three enzymes have their respective functions where AtzA plays a role in catalyzing hydrolysis reactions, AtzB acts as a catalyst in dehydrochlorination reactions, and AtzC plays a role in catalyzing acidic compounds. Of the three enzymes, AtzA is the most important enzyme in the biological degradation of pesticides because it turns pesticides into non-toxic compounds in the environment [12].

Treatment without the use of biological agents for 15 days as a whole did not show changes in pesticide concentrations and OD values (figure 5). Based on the experimental results, treatment with biological agents can reduce pesticides. Bioremediation with potential microbes of more than 1 species simultaneously is more efficient for degrading xenobiotic compounds than single microbes [3] Góngora-Echeverría et al., 2020). The growth of various biological agents is inversely proportional to the decrease in pesticide concentrations. When the growth of biological agents increases, the levels of pesticides will decrease and vice versa. The biological agents differed in their ability to reduce pesticides and multiply [39] opined that the ability of biological agents to reduce pesticides and multiply is influenced by microbial species, pH, temperature, and metabolic capabilities. The value of COD (Chemical Oxygen Demand) is one of the parameters that was also measured at the beginning and end of this study. The following table shows the effectiveness of reducing COD from the research conducted:

Table 2 showed the reduction effectiveness of *Chemical Oxygen Demand* (COD) after 15 days of bioremediation treatment. The effectiveness of reducing COD of each type of biological agent at various concentrations was different (table 2). The effectiveness of reducing COD at the highest pesticide concentration of 0 mg/L was 85.1% by Pseudomonas b68 44 with the final COD value being 32.89 mg/L. At a pesticide concentration of 100 mg/L, the highest effectiveness in reducing COD was 97.52% or the final COD value was 107.33 mg/L by the biological agent Pseudomonas b68 followed by Bacillus subtilis, Pseudomonas b70, Bacillus altitudinis. The effectiveness of reducing COD at the highest concentration of 200 mg/L pesticides was the biological agent Pseudomonas b68 of 98.53% or the final concentration was 138.44 mg/L. Then in the treatment of pesticide concentrations of 300 mg/L the highest effectiveness in reducing COD was in the biological agent Pseudomonas b68 with the final concentration being 160.67 or a decrease of 98.6%. This is in line with the results of research conducted

Table 2. Effectiveness of COD Reduction After 15 Days of Bioremediation Treatment

Pesticide Concentration (mg/L)		COD Reduction Effectiveness (%)				
		Bacillus height	*Bacillus subtilis*	*Pseudomonas b68*	*Pseudomonas b70*	Control
0		13,1[a]	3,77[a]	85,1[c]	62,43[b]	0,00[a]
100		93,80[c]	94,65[c]	97,52[c]	94,3[c]	0,00[a]
200		97,46[c]	96,46[c]	98,53[c]	96,53[c]	0,00[a]
300		96,65[c]	96,82[c]	98,60[c]	96,88[c]	0,00[a]

Note: Identical letters indicate no significant difference in Duncan's test

by [36] that there was a decrease in the COD value of up to 38% due to the bioremediation process with indigenous microbes for 7 days. Research conducted by [24] showed that microbes can reduce COD levels with an effectiveness of 30.09% for 6 days. Factors that can cause a decrease in COD include increasing microbial biomass in an object [36]. The results of changes in the pH of the liquid media during the 15 days of the experiment are shown in Figure 6.

The results showed the pH value in Figure 6, the final pH of *Bacillus subtilis* is 5.3 (A), which means it is higher than *Bacillus altitudinis*. This can affect the degradation ability and growth of *Bacillus subtilis* better than *Bacillus altitudinis*. Likewise, the final pH value of *Pseudomonas b68* was 5.5 which was higher than the final pH of *Pseudomonas b70* so that the ability to degrade and grow *Pseudomonas b68* was better than *Pseudomonas b70*. Overall the final pH value of treatment with biological agents

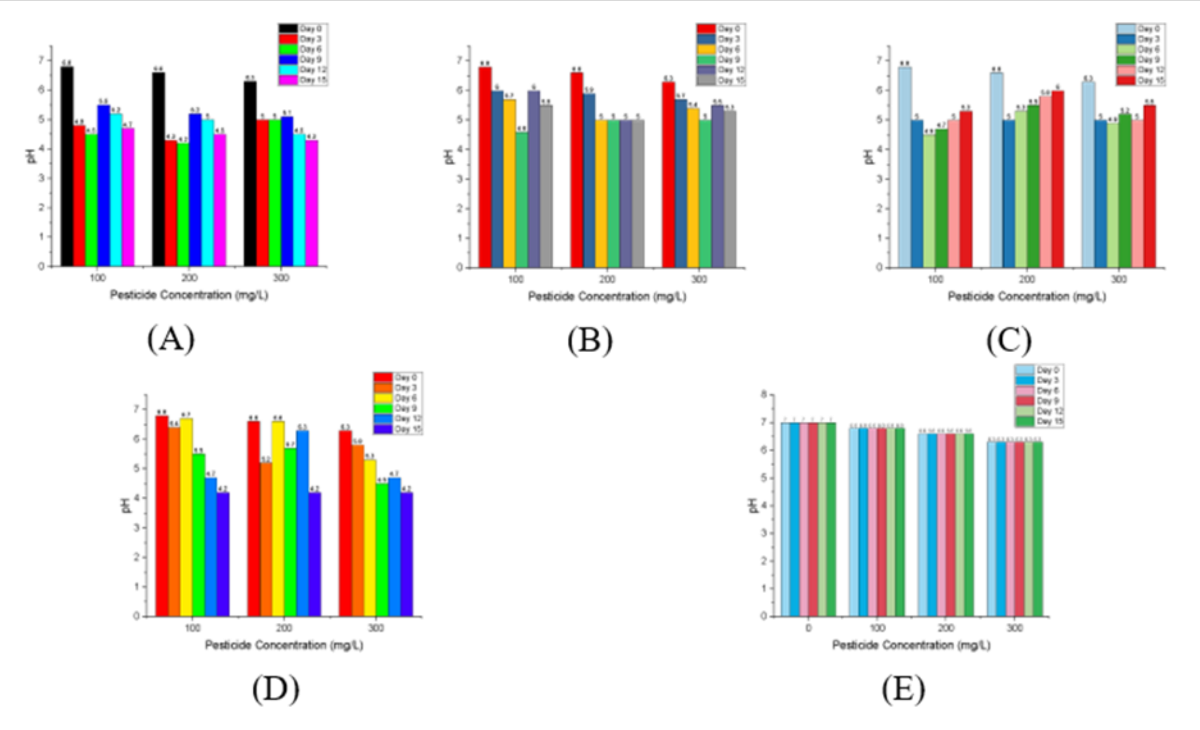

Figure 6. pH measurement: A) *Bacillus altitudinis*; B) *Bacillus subtilis* C) *Pseudomonas b68*; D) *Pseudomonas b70*; E) Control

Bacillus altitudinis, Bacillus subtilis, Pseudomonas b68, and Pseudomonas b70 was lower than the control treatment (without biological agents) wherein the control treatment did not occur degradation process so that the final pH value was the same as the initial pH value. *Pseudomonas b68* has the highest final pH among other treatments so that the ability to degrade pesticides is higher. [33] stated that the microbes of the *Pseudomonas* genus can live optimally at pH 5-8. A decrease in pH does indicate the presence of pollutant metabolism carried out by microbes, but a pH that is too acidic is also not good for the environment, especially when applied to the soil can affect soil fertility. Therefore, it is necessary to add a buffer solution if the pH is too low or add organic fertilizer so that the pH value can be stable [39].

The changes in pH (Figure 6) showed decrease in acidic conditions. The decrease in pH can be caused by the metabolic activity of microbes that produce organic acids so that H^+ ions increase [5]. This is in line with research conducted by [22] who carried out bioremediation of endosulfan using fungi and bacteria where the pH value of the media showed a change every time of observation, which showed a decrease, in other words, the pH became acidic. Utari (2018) also conducted bioremediation research with chromium metal contaminants in the soil where the results of soil pH measurements after the bioremediation process also decreased to acid.

Bioremediation of contaminants can provide environmental value added. Environmental Value Added (EVA) is an added environmental value due to a process that occurs where there is an increase or can be said to be better when compared to before the process occurred. In this study, bioremediation of pesticide contaminants can not only be a solution for agricultural land pollution, but also has added value to the environment. EVA is presented in Table 3. EVA or environmental added value from pesticide pollutant bioremediation shows a significant positive effect on reducing pesticide concentrations and COD values (table 3). The decrease in pesticide contaminants with treatment showed a higher percentage than the control at all types of concentrations. The difference in the percentage reduction in pesticide pollution between the control without treatment and the treatment showed a big difference. This shows that bioremediation with the microbes used can reduce pesticide contaminants with their respective percentage values at various pesticide concentrations. Reducing the concentration of pesticides can increase the carrying capacity of the environment. In addition, reducing the concentration of pesticides can reduce pesticide residues both in the environment and in agricultural products. Reducing pesticide residues will improve environmental quality and create healthier food ingredients due to reduced pesticide residues [8].

Bioremediation apart from reducing pesticide contaminants can actually reduce the value of Chemical Oxygen Demand (COD). The difference in COD values between the controls and the presence of treatment shows a very large difference where with the bioremediation treatment the percentage of reduction in COD values is higher. The existence of added environmental value from bioremediation shows that bioremediation can be a solution to the problem of environmental pollution. Bioremediation can reduce pollution thereby supporting environmental sustainability [7].

Bioremediation technology has several environmental prerequisites that must be met when directly applied. There are prerequisites or environmental factors because microbes are very susceptible microorganisms that require optimal conditions to support growth. Technically according to the work method that has been carried out in this study, the prerequisites that must be met are the Initial pH value in the neutral range (pH$\pm$7) and the room temperature is neither too cold nor too hot.

According to Vyatrawan (2016) the prerequisites that affect the effectiveness of bioremediation are physical, chemical and environmental factors. Physical factors include water content and suitability of

Table 3. Environmental Value Added

Variable	Pesticide Concentration (mg/L)	Control (%)	Treatment (%)	Delta (%)
Pesticide Concentration Reduction	100	0,00	49,91-85,43	49,91-85,43
	200	0,00	50,09-81,32	50,09-81,32
	300	0,00	59,33-86,13	59,33-86,13
Decrease in COD Value	100	0,00	93,80-97,52	93,80-97,52
	200	0,00	96,46-98,53	96,46-98,53
	300	0,00	96,65-98,60	96,65-98,60

the number of microbes with contaminants. The chemical factor is the chemical structure of the pollutant compound. While environmental factors such as pH, temperature, nutrition, humidity, and oxygen availability.

Factors that influence microbes to work optimally during bioremediation such as pH, temperature, moisture content (moisture), oxygen, and nutrition. Optimal pH supports growth *Bacillus* ie 6-8, while for *Pseudomonas* namely 5-9 (Leonita et al., 2015). Optimum pH for bioremediation of pesticides is at neutral pH or in the range of 6.9-7.12 [25]. The optimum temperature to support microbial activity is in the range of 10-40°C (Vyatrawan, 2016). The optimal water or humidity content for bioremediation of hydrocarbon-contaminated soil is around 60% [1]. Oxygen supply during the bioremediation process on a field scale can be done by aeration (Thapa et al., 2012).

CONCLUSION

The bioagents (microbes: applied concentrations of 100-300 mg/L) evaluated in this study were able to reduce the concentration of pesticide contaminants in the soil . *Pseudomonas b68* showed a high reduction effectiveness compared other biological agents. However, the potentials of other microbes need to be evaluated considering the number of microbial species.

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