Summary

The Problems of pesticide pollution on the environment, especially in the waters of Lake Rawa Pening into a top priority and need to get the attention that carefully, especially by Stakeholders or interested parties, given concerns over the nature of the persistent pesticides and can cause the onset of bio-accumulation due to exposure to pesticides occurs in long periods on living beings and bio-magnification in the food chain in an ecological system, so the need to do a research on content of organophosphate pesticides residues on the waters of Lake Rawa Pening and how to overcome it. Bioremediation technology with the indigenous bacteria is also taking advantage of the most efficient, inexpensive and effective in addressing the problems of pollution pesticide *Malathio*n and *Profenofos* on Lake Rawa Pening.

Legal basis the activities of Bioremediation in Indonesia is a decision of the Minister of the environment of the Republic of Indonesia No. 128 of 2003, concerning the procedures and technical requirements And is the biological waste management (Bioremediation) is done with the use of Indigenous Microorganisms. Hence the need for screening of bacteria that have the most good degradation capability of the pesticide Malathion and Profenofos as bioremediation technology development steps

Based on the results of research regarding the pre pesticide residues contained in the water and sediments of Lake Rawa Pening, then the results obtained the highest pesticide residues are a residue of the pesticide Malathion

and Profenofos group Organophosphat. Analysis of pesticide residues using the Gas chromatography method by using test samples taken from the three-point sampling is representative. The presence of pesticide residues in Lake Rawa Pening is made possible due to uncontrolled pesticide use that lasts a long time. A survey of the workplace directly to farmers around the Lake Rawa Pening about the dominant pesticides used by farmers around the Lake Rawa Pening obtained results are pesticides of *Profenofos* with trademark Curacron It makes the Baseline of the ***Organophosphat Pesticides Bioremediation research (Malathion and Profenofos) by Indigenous Bacteria Selected On Lake Rawa Pening Semarang***.

The research is divided into 2 phases i.e. phase pra research and research.

1. Pre-research.

   The purpose of the pre Study intended to find an overview of the initial conditions of Lake Rawa Pening, and became a major research base line. Pre research starts with a simple survey conducted with a simple interview methods directly to farmers around the Lake Rawa Pening followed by sampling the water and sediments of Lake Rawa Pening at random and analyzed in the laboratory of Integrated Environmental Farm Research Center Pati, Central Java with Gas Chromatography method.

2. The Research Phase,

   a. Research covers the stages of exploration against bacteria the most superior in degrade pesticides (Malathion and Profenofos) and test the ability of the qualitative and quantitative degradation

b. Selected Bacteria then identified in morphology, biochemistry and molecular biology. Identification is done in the laboratory of the Integrated Dipenogoro University, Semarang and Bio Science Laboratories, Jakarta

Research Results

A. Pre Research As A Baseline Study Of Bioremediation

1. A Simple Survey Results Against Farmers Around Lake Rawa Pening

   The survey results show that the type of pesticide Organophosphat-Profenofos with trade mark Curacron most widely utilized by farmers around Rawa pening (50%), then the Carbamate (16%), Deltamethrin (8%), Lamdacloprid (6%), Fentoat (5%), Karbosulfan(5%), Carbofuran (5%) and Lamda Sihalotrin (4%).

2. The results of the Analysis Of pesticide residues In water and Sediment of Lake Rawa Pening

   Based on the results of the analysis of water and sediment of Lake Rawa Pening as follows:

   a. Concentrations of Profenofos in water ranging between 0,0260 ppm Rawa Pening (point sampling Galeh River) – 0.08 ppm (the confluence of the Tuntang and the river Tambak Rejo)
   b. Concentration of Profenofos on a sediment range between 0.12 ppm (the sampling point is the confluence of the Tuntang and the river Tambakrejo) –0,286 ppm (the sampling Point on the river. Galeh)
   c. Concentrations of Malathion in water ranging between 0,0366 ppm (the sampling point of Confluence. Long and a showdown) - 0.0521 ppm (the

sampling point is the confluence of the Tungtang and the river Tambakrejo)

d. Sediment concentrations of Malathion in the ranges 0,0567 ppm (the sampling point of confluence. a showdown, the river Panjang and the river Renggas) – 0.12 ppm. (a sampling point River Galeh)

The condition has been demonstrating the process of degradation of *Malathion* and *Profenofos* which lasts long enough and demonstrating that the conditions of the water and sediment Rawa Pening already polluted the pesticide Organophophat (*Malathion and Profenofos*)

B. Primary Research On Bioremediation Of Pesticide

The main research aims to get the best indigenous bacteria and analysis of the ability of indigenous bacteria best degrade pesticides (*Malathion and Profenofos*) qualitative and quantitative

1. Qualitative test of Indigenous Bacteria Selected as Malathion and Profenofos degrading

At the initial stage of qualitative test obtained 117 bacteria that can grow on media Zobell which was later reselected by growing the bacteria isolated on media Zobell has been modified by eliminating the yeast extract and glucose as an energy source and bacteria with *Malathion* and *Profenofos* as a replacement source of energy to determine the occurrence of degradation added bromtymol blue as an indicator

The results of isolation followed by growing the bacteria on solid media Zobell. Bacteria that have indicated the existence of degradation ability of

yellow color on solid Zobell's media. The color yellow appears after incubation for 7 to 14 days.

Test the degradation continued in liquid media with zobell incubation period 3 days. The results of the Screening of bacteria that have the capability of Malathion And degradation of the Profenofos Of the liquid Media Zobell minimum With Bromo Tymolblue with a concentration of 25 ppm, 50 ppm, 75 ppm and 100 ppm.

Based on observations of color change from blue to yellow (bromtymol blue indicator) where only up to 75 ppm, at concentrations of 100 ppm, the color change is not visible.

Selection result retrieved 8 bacteria on degradation of Malathion has the ability of bacterial code TR-A-7, TR-A-5, TR-A-6, RP-L-1, RPL-2, RP-L-3, RP--L-5 and RP-L-6, While the bacteria that have the ability of degradation of Profenofos(> 75 ppm) is a bacterium with TBR-A-6, GL-L-1, TR-A-5, TR-A-6, RP-L-1, RP-L-3, RP-L-5 and RP-A-1

2. **A quantitative test of Indigenous Bacteria Selected have the ability to degrade the Malathion and Profenofos.**

The best bacteria test results, qualitative test in subsequent quantitatively by comparing the ability of bacterial degradation of selected bacteria using the spectrophotometry method based on the absorbance of bacteria test by specific wave length. Results of Malathion are scanning ƛ = 400 nm and Profenofos ƛ = 730 nm. According to Venugopal et al (2011) that the maximum wavelength by using a spectrophotometer to Malathion is 760 nm while according to

Ramika et al (2012) stated that the wavelengths are used to test compounds the degradation of profenofos (Curacron 500 EC) is 365 nm.

Quantitative test begins with the creation of a standard curve serves to know the conversion of absorbance to the concentration of a solution of Malathion and Profenofos.

The equation is to be used in the calculation for a qualitative test

a. Measuring results of Absorbance *Profenofos* (ƛ = 400 nm) and Bacterial Degradation Test Results.

   The initial concentration of the degradation of Profenofos is 74.75 ppm based on a standard curve equation of Profenofos formula y = 0.004 x + 0.021. Based on the ability of bacterial degradation of *Profenofos* substrate obtained on 3 bacteria best with the code TRA-5 = 58.13%, RPL-1 = 43% and RPL-5 = 38.44% ‘

b. Measuring results Absorbance *Malathion* (ƛ = 730 nm) and Bacterial Degradation Test Results

   The initial concentrations of Malathion were 76.5 ppm (linear equation y = 0.008 x + 0.002) and based on, a percentage of Malathion degradation, then selected the best bacteria with code RPL-5 = 40.99% degradation, TRA-5 = 40,37% degradation and RPL-1 = 20.50% degradation capability

3. A quantitative test of the Indigenous Bacterial Consortium is selected that has the ability to degrade Malathion and Profenofos

Quantitative test of bacterial Consortium is selected for the synergism test needs to be done between the selected bacteria. Test result

synergism/antagonisms between the inhibitory zones are properly observed bacteria test obtained the following results:

a. Bacteria RPL-1 and RPL-5 no drag zone
b. Bacterial RPL-5 and TRA-5 no drag zone
c. Bacteria RPL-1 and TRA-5 no drag zone
d. Bacteria RPL-1, RPL-5 and TRA-5 no drug zone

The next test of bacteria selected synergism to get the best consortium bacteria. Test results of three bacterial isolate selected, namely RPL-5, TRA-5 and the RPL-1 is not visible drag zone, therefore bacteria test can be done Consortium with the optimum concentration of inoculum bacteria in degradation process early starter pesticides (*Malathion and Profenofos*) largely determine the optimum bacterial biodegradation test therefore needed testing how much the optimum concentration as a starter culture that will be inoculated at the beginning of the process of bioremediation. Testing to get the best initial starter done on test bacteria concentrations of 1%, 2%,3%, 4% and 5%. Test results showed that the initial starter the most optimum is 3%.

A quantitative comparison of the test results the ability of bacterial degradation of single isolates and bacterial Consortium showed that the Bacterial Consortium (RPL-1 and RPL-5) has the ability best degrdasi (83,23%) in comparison to other bacteria Malathion substrate, whereas Bacterial Consortium (RPL-5 and TR-5) has the best effectiveness with the ability of degradation of 68.75% (Profenofos substrate).

C. Application Phase limited in the laboratory

The a pplication stage is carried out in laboratories in non sterile so expect close to Lake Rawa Pening's condition. Research done by the method of Gas Chromatography and Mass Spectrofotometry, this is to the validity of comparisons and the ability of bacteria best in degradated *Malathion* and *Profenofos.* The research showed that the bacterial Consortium RPL-1 and- RPL-5 has the capability of degradation in *Malathion* and the bacterial Consortium RPL-5 and TR-5 has the capability best degradation on *Profenofos* with spectrophotometry and GC - MS method

D. Identification Of Bacteria Selected

1. Test Morphology and biochemistry

Bacterial identification test done on the morphology and biochemistry to figure out early screening and morphological and biochemical properties of bacteria test. The picture below shows the morphological and biochemical tests

2. Test of molecular biology

a. The maximum Level similarity, identity test bacteria with RPL-1 code is 87% based on the phylogenetic tree analysis is *Oceanobacillus iheyenis*

b. The maximum Level similarity, identity test bacteria with RPL-5 code is 99%, based on the phylogenetic tree analysis is *Exiquobacterium profundum*

c. The maximum Level similarity, identity test bacteria with TRA-5 is 98% based on the phylogenetic tree analysis is *Bacillus firmus*

The bacteria have already registered on: DNA Bank of Japan Data. DDBJ Center. National Institute of Genetics. Research Organization of Information and Systems, Mishima, Shizuoka 411-8540, Japan with accession number: RPL-1 = LC019790, RP-L-5 = LC019791and TR-A-5 = LC019792

Based on the results of research and new strains of bacteria have been found which proved to have the best Of Malathion degradation ability and Profenofos. These bacteria are found in Lake Rawa Pening, so it is a wealth of biodiversity in Indonesia. The success of the bacteria *Ocenobacillus iheyensis, Exiquobacterium profundum* and *Bacillus form is* in bioremediation process is expected to be applied on the bioremediation of pesticides (*Malathion and Profenofos*) in other cases and may help resolve the problem of pollution due to pesticides *(Malathion and Profenofos*)

Suggestion For Further Research

Research on bioremediation of pesticide *Organophosphat* was this *Malathion* and *Prefenofos* by indigenous bacteria of aquatic Rawa Pening lake needed cooperation in a comprehensive manner involving stakeholders related The Enviromental Agency of The Province of Central java, State Minister for Enviromental of Indonesia (Kementerian Negara Lingkungan Hidup), The Local Government of Salatiga, The Local Goverment of Semarang and Universitas Diponegoro in the application test strains od bacteria *Oceanobacillus iheyenis, Exiquobacterium profundus* and *bacillus formis* as agents of bioremediation in Rawa Pening.