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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 6, Issue 07, pp. 1631-1636 July, 2015

RESEARCH ARTICLE

BIOREMEDIATION ORGANOPHOSPHATE PESTICIDES (MALATHION AND PROFENOFOS) BY SELECTED INDIGENOUS BACTERIA IN RAWA PENING LAKE WATERS, DISTRICT SEMARANG, INDONESIA

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ARTICLE INFO

Article History:
Received 16th April, 2015
Received in revised form
25th May, 2015
Accepted 08th June, 2015
Published online 31st July, 2015

Key words:

Organophosphat,
Bioremediation,
Quantitative,
Qualitative,
RPL-5, TRA-5 and RPL-1.

ABSTRACT

Bioremediation of pesticide Organophosphat (Profenofos and Malathion) and utilize Indigenous bacteria from Rawa Pening is an effort to restore the ecological balance of the water due to leaching of Profenofos and lathiMaon. In the process of Bioremediation bacteria required to elect and selected therefore required a screening process involves the isolation, identification and genetic information to get the bacteria have the ability best degradation. Screening is done by qualitative and quantitative experimental method in the laboratory.

- Qualitative Test is done by observing the color change with Bromtymol Blue as an indicator changes color on solid zobell media enriched with Malathion and Profenofos).
- Quantitative tests done by the method of Gas Chromatography and Mass Spektofotmetry Spectrofotometry (GC-MS).
- Based on the selection of bacteria through qualitative and quantitative testing are the three best bacteria with code RPL-5, TRA-5 and RPL-1. Bacteria with the code TRA-5 has the best substrate degradation abilities of Profenofos and bacteria with code RPL-5 has the best degradation of the substrate ability of Malathion

The next best in the bacterial synergistic test to get the best Consortium bacteria to be used in a test of degradation on a scale of application is limited to water and sediment Rawa Pening on maximum concentrations, as follows:

- Bacterial Consortium with the code RPL-5 and RPL-1 has the best on substrate degradation ability test of water from Rawa Pening with the addition of concentrations of Malathion
- Bacterial Consortium with the code RPL-5 and TRA-5 has the best on substrate degradation ability test of water from Rawa Pening with the addition of concentrations of Profenofos

Identification of bacteria selected and most superior in Malathion degrades and Profenofos done in molecular biology shows the following results:

- The maximum identity, similarity levels of bacteria RPL-1 are 87% and based on the analysis of the phylogenetic tree, the bacterial isolate RPL-1 had the closest kinship with *Oceanobacillus iheyenis*
- The maximum identity, similarity levels of bacteria RPL-5 is 99% and based on the analysis of the phylogenetic tree, the bacterial isolate RPL-5 has the closest kinship with the bacteria *Exiquobacterium profundum*
- The maximum identity, similarity levels of TRA-5 are 98% based on the phylogenetic tree analysis of bacterial isolates, TRA-5 has a very close kinship with *Bacillus firmus*

INTRODUCTION

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.

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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 Pra Research On A General Overview Of The Lake Rawa Pening as follows concentrations of Profenofos in Rawa Pening ranging between 0,0260 ppm –0,286 ppm and than concentrations of Malathion ranging between 0,0366 ppm - 0.12 ppm and than concentrations of Profenofos in Rawa Pening ranging between 0,0260 ppm –0,286 ppm (Isworo, *et al*, 2015). It makes the Baseline of the *Organophosphat Pesticides Bioremediation research (Malathion and Profenofos) by Indigenous Bacteria Selected On Lake Rawa Pening Semarang*.

MATERIALS AND METHODS

- Screening process begins with the qualitative test by growing bacteria from the waters of the Rawapening on the media the zobell enriched with pesticides (Malathion and profenofos). Bacteria that can grow up to show that the bacteria can do against degradation of pesticides (Malathion and Profenofos)
- Test of degradation against indigenous bacteria selected by observing the ability organophosphat (profenofos and malathion) and the method of spectrophotometer (UV), to get the best from these selected bacteria. Spectrophotometer (UV). Spectrophotometer (UV) is used in the research was spectrophotometer (UV) SP 3000 Plus was quantified
- Quantitative test begins with the creation of a standard curve serves to figure out conversion absorbent against concentration solution of Malathion and Profenofos.
- Test ability of bioremediation bacteria best done in experimental laboratory with limited application to test the ability of degrading bacteria in samples of water and sediment Rawa pening different methods with GC MS (Gas Chromatography Mass Spectrophotometry). GC - MS is used in the research was Shimadzu GC-2010 Plus type with the Auto Injector type Shimadzu AOC 20i, Rtx-1 column OP Pesticides and Shimadzu GC MS TQ-8030

Gas chromatography system condition in this study as follows;

a) Injector Temperature: 250°C;
b) The carrier Gas/Helium Flow Rate 1ml/min. Constant Rate,
c) Oven Temperatur 80°C,
d) Temperature of the column (280°C),
e) Gas flow: N2/nitrogen (25 ml/min)
f) Detector Temperature (250°C.),
g) Sample 1 ƛl splitless US EPA Method 8141A Standard Mix 1 ƛl 100 ppm.

- The isolation and identification of bacteria conducted in molecular biology to identify genetic information in phylogenetic. Microbial Identification based on 16S rRNA Gene Analysis bacterial species through an agarose gel electrophoresis method. The process of isolation of genome characterized the formation of one tape for each bacterial genome test after observing using UV Transluminator with 16S-rRNA Gene Encoder Ribbon 1.5 kb and compared with Marker (1 KB DNA ladder). DNA Amplification results 16- rRNA sequencing to get on the order of the nucleotide similarity and analyzed using a gene bank with N-BLAST (Basic Local Alignment Search Toll- Free Nucleotode) so that it can be known homology and species of bacteria test.

RESULTS

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

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) were only up to 75 ppm, at concentrations of 100 ppm, the color change is not visible.

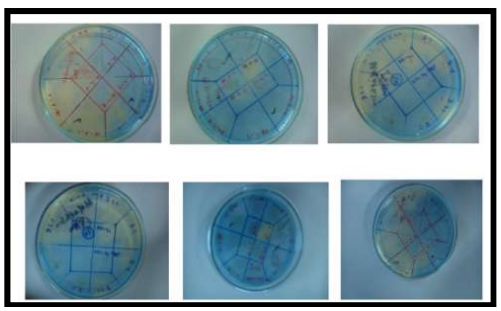

Figure 1. Bacterial degradation of indigenous test on solid media (zobell) so with the indicator bromtymol blue with the addition of Malathion/Profenofoss with a concentration of 10 ppm for 8-14 days

Selection result retrieved 8 bacteria on degradation of Malathion has the ability of bacterial code TRA-7, TRA-5, TRA-6, RPL-1, RPL-2, RPL-3, RPL-5 and RPL-6, While the

bacteria that have the ability of degradation of Profenofos (> 75 ppm) is a bacterium with TBR-A-6, GLL-1, TRA-5, TRA-6, RPL-1, RPL-3, RPL-5 and RPA-1. Figure 1 shows that the change in the color blue to yellow indicates that bacteria was able to do with the degradation of pesticides (Malathion and Profenofos)

A quantitative test of indigenous bacteria selected have the ability to degrade the Malathion and Profenofos

The good 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% ‘

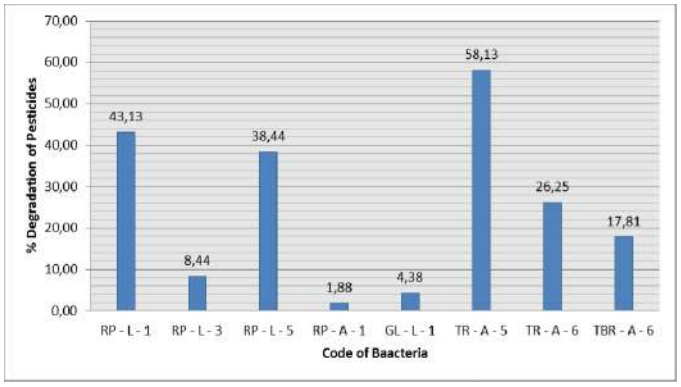

Figure 2. The graph of the percentage of the results of the degradation test is selected bacteria in the substrate Profenofos

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. The graph below shows the ability of bacterial degradation test, as follows:

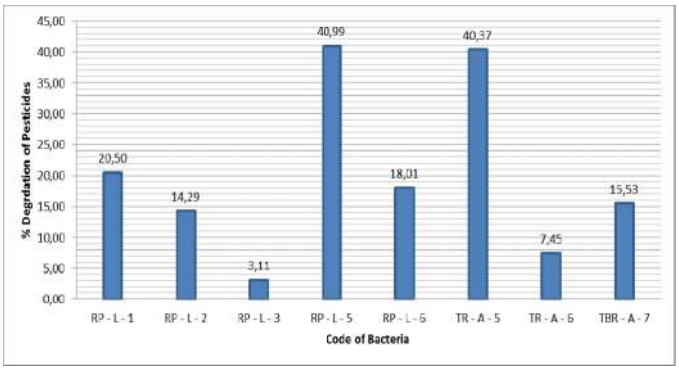

Figure 3. The graph of the percentage of the results of the degradation test is selected bacteria in the substrate Malathion

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:

- Bacteria RPL-1 and RPL-5 no drag zone
- Bacterial RPL-5 and TRA-5 no drag zone
- Bacteria RPL-1 and TRA-5 no drag zone
- 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.

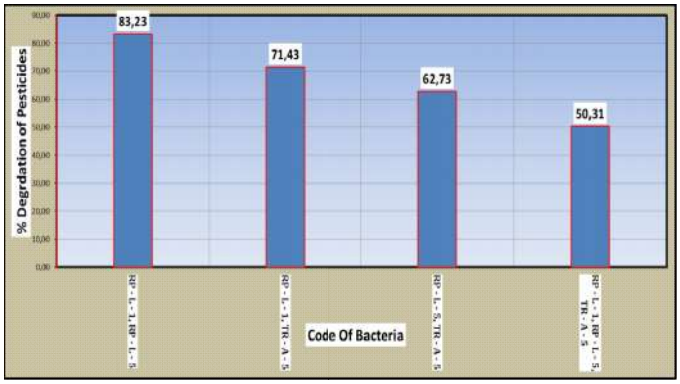

Figure 4. The graph of the percentage of the results of the degradation test is concortium bacteria in the substrate Malathion

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). The graph below shows the ability of bacterial degradation test, as follows:

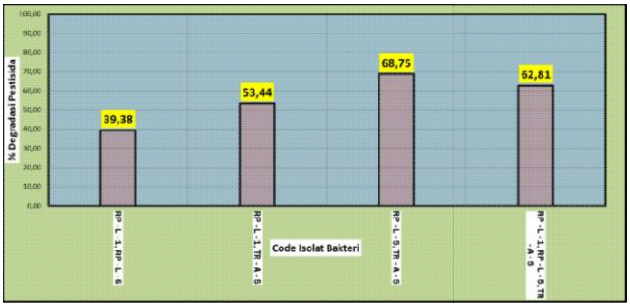

Figure 5. The graph of the percentage of the results of the degradation test is concortium bacteria in the substrate Profenofos

Identification of Bacteria Selected

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.

Test of Molecular Biology *Profenofos*

- The maximum Level similarity, identity test bacteria with RPL-1 code is 87% based on the phylogenetic tree analysis is *Oceanobacillus iheyenis*
- The maximum Level similarity, identity test bacteria with RPL-5 code is 99%, based on the phylogenetic tree analysis is *Exiquobacterium profundum*
- 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, LC 019790, RPL-5, LC 019791 and TRA-5, LC 019792

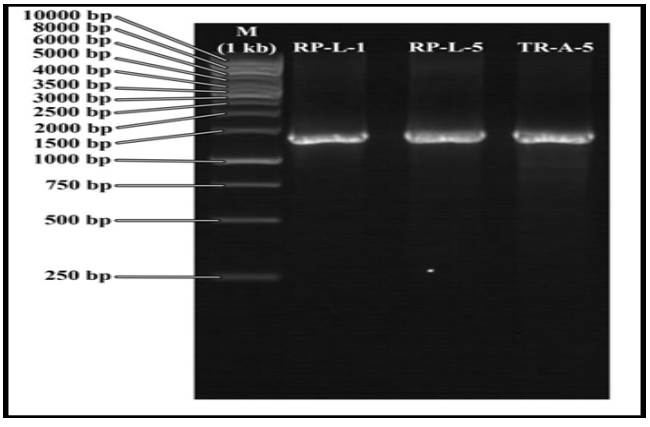

Figure 6. the results of gel electrophoresis the amplification of 16s rRNA-Gene. (M) Marker; (1) RPL-1; (2) RPL-5; and (3) TRA-5

Table 1. The results of sequencing using the primary forward and reverse

No	Kode Isolat	Panjang Basa Nukleotida (bp)	Nama Bakteri	Homologi (%)	Accession Number
1	RP-L-1	1071	*Oceanobacillus heyensis*	87	LC019790
2	RP-L-5	1238	*Exiquobacterium profundum*	99	LC019791
3	TR-A-5	1326	*Bacillus firmus*	89	LC019792

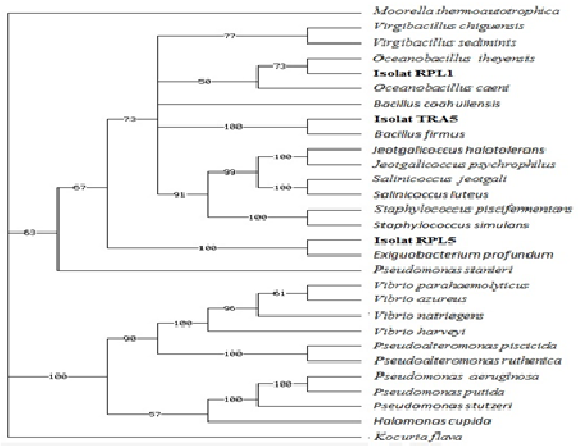

Figure 7. The Phylogenetic Tree construction

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 formis* 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)

Application Phase Limited in the Laboratory

The application stage is carried out in laboratories in non sterile, so expect close to the 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 with spectrophotometry and GC - MS method.

Table 2. Measuring results based on the percentage of the area of Malathion and Profenofos (GC-MS method)

Sample	Consortium Bacteria	t	Malathion Area %	Profenofos Area %
Water Rawa Pening	Code of Concortium RPL-5 dan RPL-1	1	7,37	6,91
		4	2,25	2,21
		8	not detected	not detected
Water Rawa Pening	Code of Concortium RPL-5/ dan TRA-5	1	6,1	4,95
		4	1,19	1,63
		8	not detected	not detected
Sedimentary Rawa Pening	Code of Concortium RPL-5 dan RPL-1	1	10,95	7,24
		4	not detected	6,75
		8	not detected	not detected
Sedimentary Rawa Pening	Code of Concortium RPL-5 dan TRA-5	1	12,92	7,24
		4	0,67	not detected
		8	not detected	not detected

1. The results of the degradation of Malathion-Profenofos by bacterial *Consortium* ***Oceanobacillus iheyenis (RPL-1)*** **and** ***Exiquobacterium profundus (RPL-5)*** on water Rawa Pening) as follows.
   a) The initial concentrations of Malathion 78.33 ppm (area % = 7.37) on the T-1 (0 hours) and T-4 (96 hours) concentration 23.91 ppm (area % = 2.25).
   b) The initial concentration of Profenofos 84.89 ppm (area % = 6.91) on the T-1 and T-4 with concentration 27,15 ppm (area % = 2.21)
   c) That concentration of Malathion and Profenofos in water Rawa Pening was not detected at t = 8, 192 hours.
2. The results of the degradation of Malathion-Profenofos by bacterial Consortium ***Exiquobacterium profundus (RPL-5)*** **and** ***Bacillus formis (TRA-5)*** on water Rawa Pening) as follows
   a) The initial concentrations of Malathion 76.11 ppm (6.10% area) on the T-1 (0 hours) and T-4 (96 hours) at the concentration of 14.85 ppm (% area = 1.19)
   b) The initial concentration of Profenofos 84.44 ppm (% area = 4.95) on the T-1 and T-4 (0 hours and 96 hours) at the concentration of 27.81 ppm (% area = 1.63)
   c) That concentration of Malathion and Profenofos in water Rawa Pening was not detected at t = 8, 192 hours.
3. The results of the degradation of Malathion-Profenofos by bacterial *Consortium* ***Oceanobacillus iheyenis (RPL-1)*** **and** ***Exiquobacterium profundus (RPL-5)*** on sediment Rawa Pening) as follows
   a) The initial concentrations of Malathion 77.67 ppm (% area = 10.92) on the T-1 and T-4 (0 hous amd 96 hours) is not detected concentrations of 0 ppm
   b) The initial concentration of Profenofos 84.78 ppm (% area = 6.75) on the T-1 and T-4 (0 hours and 96 hours) concentrations were 13.82 ppm (% area = 1.1)
   c) That concentration of Malathion and Profenofos in sediment Rawa Pening was not detected at t = 8, 192 hours.
4. The results of the degradation of Malathion-Profenofos by bacterial Consortium ***Bacillus firmus (TRA-5)*** ***Exiquobacterium profundus (RPL-5)*** **on sedimen**t Rawa Pening as follows
   a) The initial concentrations of Malathion 76.33 ppm (% area = 12.92) on the T-1 and T 4 concentration on 3.96 ppm (% area = 0.67)
   b) The initial concentration of Profenofos 83.56 ppm (7.24% area) on the T-1 and T-4 concentrations was not detected (0 ppm)
   c) Capability degradation by bacterial Consortium ***Exiquobacterium profundus*** and ***Bacillus formis*** shown that concentrations of Malathion in the sediments of Rawa Pening have not been detected in T-8 (192 hours) while for Profenofos undetectable at T-4 (96 hours)

Based on the results of the analysis of the obtained results are as follows: Bacterial Consortium ***Exiquobacterium profundus (RPL-5)*** **and** ***Bacillus formis (TRA-5)*** has the best substrate degradation abilities of Profenofos whereas bacterial Consortium ***Oceanobacillus iheyenis (RPL-1)*** **and** ***Exiquobacterium profundus (RPL-5)*** has the best substrate degradation abilities of Malathion

CONCLUSION

1. ***Exiquobacterium profundum*** strains of bacteria have the ability the best substrate degradation of Malathion whereas strains of bacteria ***Bacillus firmus*** has the ability the best substrate degradation of Profenofos
2. The consortium selected bacteria have the ability to better degradation compared to a single bacterial isolates selected.
3. Bacterial Consortium ***Exiquobacterium profundum (RPL-5)*** and ***Oceanobacillus iheyenis (RPL-1)*** has the ability and the degradation of Malathion than the best substrate, substrate Profenofos, while *Consortium* ***Exiquobacterium profundum (RPL-5)*** **and** ***Bacillus firmus (TRA-5)*** has the ability the best substrate degradation of Profenofos than Malathion at both the scale and the scale of laboratory applications.
4. Organic compounds Compounds with a phosphate group became a source of pollutants on Raw Pening organophosphate pesticide compounds not only will but fertilizers with cluster phosphate also allow degraded with good GC MS analysis of detecting Results of compounds 1-2 *Cinnoline and Benzothiophene-3carboxylic Dicarboxylic Acid -Tetrahydro-2-Amino-6-Ethercompound Etyl* results Urea degradation

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