Dichloro Diphenyl Trichloroethane (DDT) Insecticide Polluted Soil Remediation by Bacteria Consortium Utilization

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Dichloro Diphenyl Trichloroethane (DDT) Insecticide Polluted Soil Remediation by Bacteria Consortium with Co-Substrate Utilization

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Abstract. Dichloro Diphenyl Trichloroethane (DDT) is an organochlorine insecticide that are largely used on agriculture and health sectors. DDT is an organic pollutant that are difficult to be dissolved and tend to survive in the environment for a long time because it is highly stable and persistent. DDT insecticide residue is still discovered in the soil even though its utilization has been stopped years ago and still negatively impacting human life and the environment through bio accumulation and bio magnification. Bioremediation is a potential method to remove recalcitrance compound such as DDT. The objective of this study is to gather and analyze information on DDT degradation by bacteria consortium with co-substrate addition. *Pseudomonas putida* and *Pseudomonas stutzeri* bacteria consortium were utilized to remediate DDT with glucose, sucrose, and yeast extract as co-substrates. During 72 hours of observation, the three co-substrates namely yeast extract, glucose, and sucrose are able to degrade 75%, 56% and 39.55% of 10 ppm DDT. Yeast is the most ideal co-substrate to assist bacteria consortium growth and to degrade DDT. The implementation of pilot scale land treatment bioremediation is planned to be at $50m^3$, with dimension of 20m length, 5m wide, and 0.5m high. The efficiency of the removal reached 90% with 67 hours of detention time.

1 Introduction

The Increasing population growth demands a rapid and sustainable agricultural sector production. Up until now, insecticides are still widely used by around 95.29% of Indonesian farmers because its high effectiveness, easy application on agricultural crops, and high success rate in avoiding crop failure [1]. However, excessive insecticide utilization will create problems towards the ecosystem and human health [2], because certain type of insecticides will leave residue that could last for decades [3].

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DDT is hazardous for the environment because of its non-polar nature that enables it to not dissolvable in water but dissolvable in fat. The higher the number of trapped insecticides in fat (lipophilic) the higher its non-polar nature. Besides that, DDT is also quite stable and persistent which makes it harder to dissolve and last longer in the environment [4]. DDT has a long half-life, resulting in bioaccumulation and biomagnification in the food chain, which is 7 to 12 years. DDT and its main derivatives 1,1-dichloro-2,2 bis-(4-chlorophenyl) ethane (DDD) and 1,1-dichloro-2,2-bis-(4-chlorophenyl) ethylene (DDE) are found in water, soil, air and river sediments [5].

Considering that insecticides are difficult to degrade in the soil, a technology is needed that can overcome the accumulation of insecticide residues in the soil. Overcoming the accumulation of insecticide residues can be done by remediation. Bioremediation is a method of environmental recovery by utilizing microbes such as fungi, microalgae, and bacteria. The advantages of the bioremediation method are that the costs used are relatively lower than physical-chemical remediation technologies and are safe for the environment. Another advantage of bioremediation is that it can detoxify pollutants into CO_2, and H_2O and biomass, which means that bioremediation can remove pollutants permanently [6], [7].

Although many bacteria have been shown to be capable of degrading DDT, their efficiency is relatively low, especially when DDT is used as the sole carbon source or due to limited substrate availability. Recent studies have shown that the addition of nutrients can stimulate the growth of microorganisms in the ecosystem [8], [9]. Based on that, the objective of this research is to analyze DDT insecticide bioremediation by bacteria consortium with co-substrate utilization as a basic for DDT insecticide bioremediation on pilot scale.

2 Method

This research was implemented in a number of stages. The main reference for secondary data is [9] and a number of supporting journal articles.

2.1 Bacteria consortium cultivation and sensitivity test against ddt

DDT degrading bacteria consortium is isolated from DDT contaminated soil sampled from Menasagere Village, Mandya District in India. 10 ppm of DDT addition is implemented periodically for 24 hours. The cells were examined with a spectrophotometer with density level of O.D(600). This bacteria consortium can be used to degrade DDT.

2.2 DDT degradation with co-substrate addition

Erlenmeyer flask filled with DDT, acetone, and bacteria consortium was incubated in a rotary shaker with 180 rpm rotation speed on 26-30°C room temperature for 24, 48, and 72 hours. To study co-metabolic degradation of DDT, 0.5% of glucose, sucrose, and yeast extract as co-substrates were added.

2.3 Pilot scale implementation recommendation

To determine the suitability of bacteria consortium utilization with co-substrate addition to degrade DDT in soil, a pilot scale study was conducted. 10 grams of utilized soil in the research was mixed with liquid media and prepared for the next stage.

3 Result and discussion

3.1 DDT insecticide bioremediation with co-substrate utilization

DDT insecticide degradation occurred on optimum environment which is at 30°C temperature [11] and pH level of 7 [12]. The utilized co-substrates are glucose, sucrose, and yeast extract. Consortium growth graphic that includes all three co-substrates is shown in **Fig. 1**.

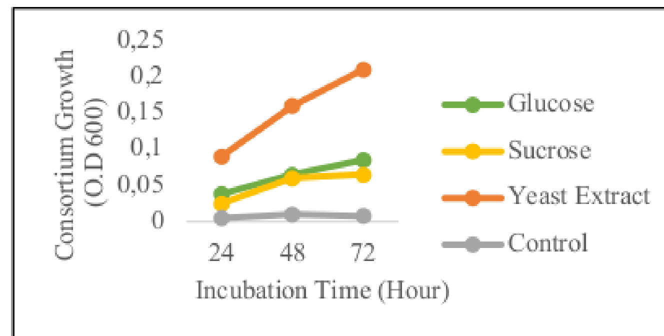

Fig. 1. Consortium growth with three co-substrates added.

During 72 hours of incubation, the bacteria are able to grow with the existence of glucose as co-substrate at 0.085 $O.D_{600}$, sucrose co-substrate at 0,065 $O.D_{600}$, and yeast extract co-substrate at 0,21 $O.D_{600}$ [10]. The existence of co-substrates is proven to be able to improve bacteria activity and enhance DDT degradation process. Glucose co-substrate was able to improve DDT degradation up to 56% for 72 hours of incubation time (**Fig. 2**). Glucose co-substrate was able to degrade DDT up to 39.5% for 72 hours of incubation time (**Fig. 3**). Yeast extract co-substrate was able to degrade up to 78.4% of DDT with the same incubation period (**Fig. 4**) [11].

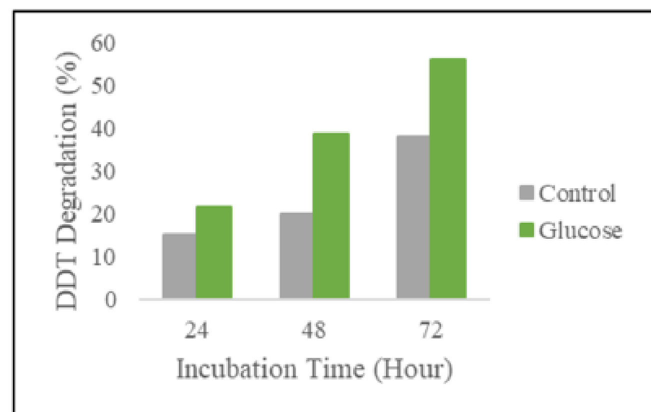

Fig. 2. Degradation of 10 ppm DDT with glucose as co-substrate [9]

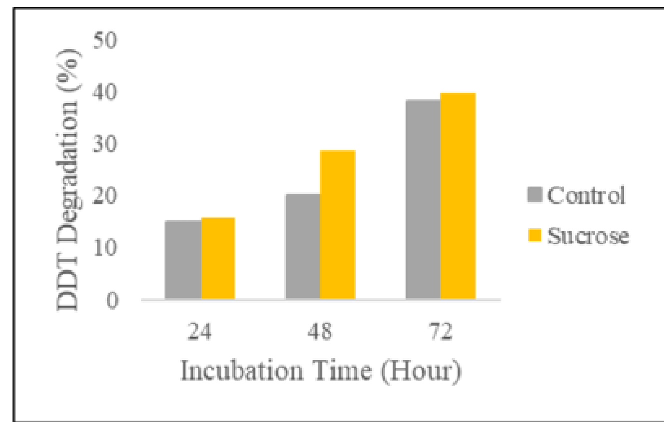

Fig. 3. Degradation of 10 ppm DDT with sucrose as co-substrate [9]

Substances that contain nitrogen such as yeast extract are able to support bacteria consortium growth better than simple sugar. Even though sucrose shows lower performance to degrade DDT than any other co-substrates, the degradation result is better than control (**Fig. 5**).

Out of the three types co-substrates, the best 10 ppm DDT degradation occurred when yeast extract was utilized as carbon source. Based on observation, 75% of 10 ppm DDT were degraded after 1% of yeast extract co-substrate was added without additional degradation level on more than 1% utilization (**Fig. 6**). The low value of yeast extract will slow bacteria growth and main enzyme synthesize, and high co-substrate concentration level will lower degradation efficiency because of competitive inhibition [13].

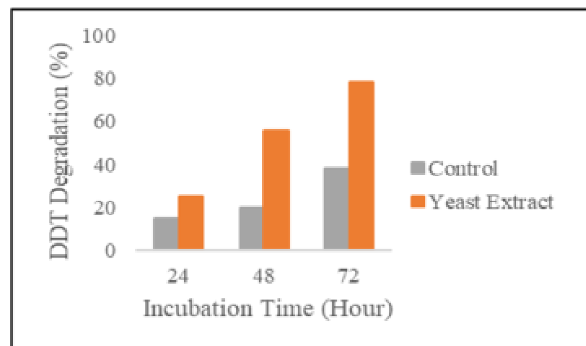

Fig. 4. Degradation of 10 ppm DDT with yest extract as co-substrate [9]

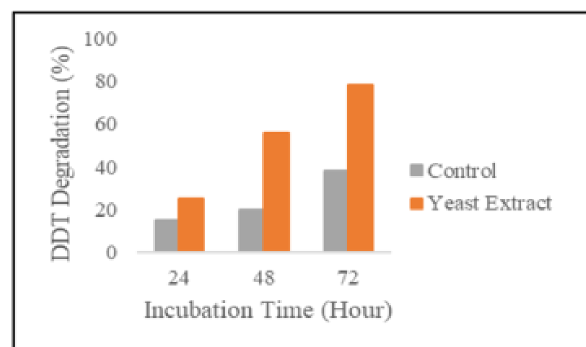

Fig. 5. Degradation of 10 ppm DDT with all three co-substrates

Aerobic DDT degradation by bacteria was started by oxygenation process to form 2.3-*dihydrodiol* DDT, which would lead to structure breaks that cause complete degradation and the formation of 4-*chlorobenzoic acid* (4-CBA) [14].

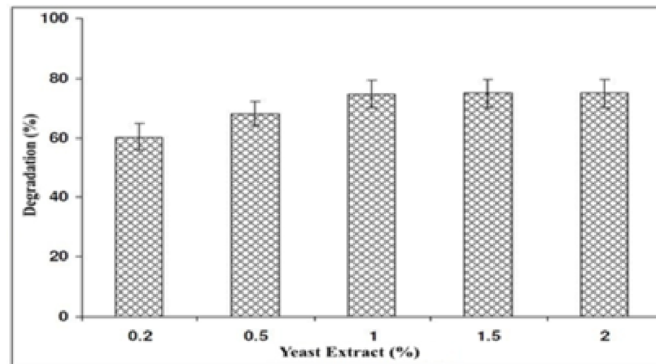

Fig. 6. The influence of yeast extract level difference on DDT degradation [9]

3.2 Bacteria consortium growth rate

Because the data only consist of 3 incubation periods which are 24, 36 and 72 hours, 2 additional incubation periods were added which at 54 and 96 hours to complete the data [9]. **Fig. 7** shows that DDT utilization rate as substrate (q) has value range of 0.067-1.430/hour. *Pseudomonas putida* and *Pseudomonas stutzeri* specific mix culture bacteria growth (μ) is between a range of 0.013-0.0261/hour. The relationships between q and μ values produced total growth (Y_T) level against DDT at 0.016 hour^{-1}, a value of $1/Y_T$ was obtained from slope value of 60.83, and the obtained bacteria death constant (Kd) was at 0.0029 hour^{-1}. The relationships between x_{max} and substrates (S) are presented in a graphic that forms Y_{obs} slope of 0.0213/hour as seen in **Fig. 8**.

Based on **Fig. 9**, the maximum specific growth value is at 0.0261/hour and saturated concentration value (Ks) of 1.2 ppm. *Pseudomonas putida* and *Pseudomonas stutzeri* bacteria consortium growth happened on reaction order 1 with growth rate constant value of 0.169/hour (**Fig. 10**).

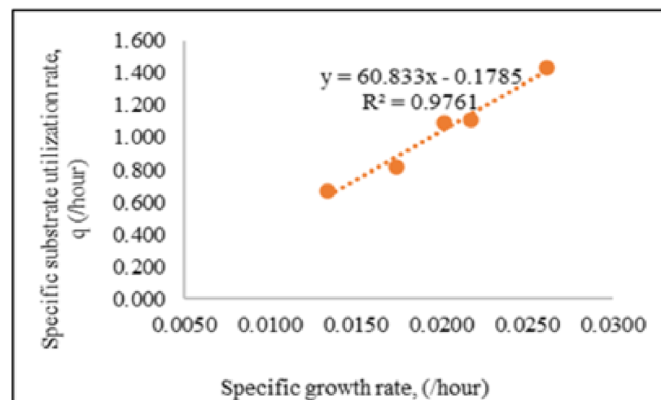

Fig. 7. Relationships between specific growth rate with specific substrate utilization rate

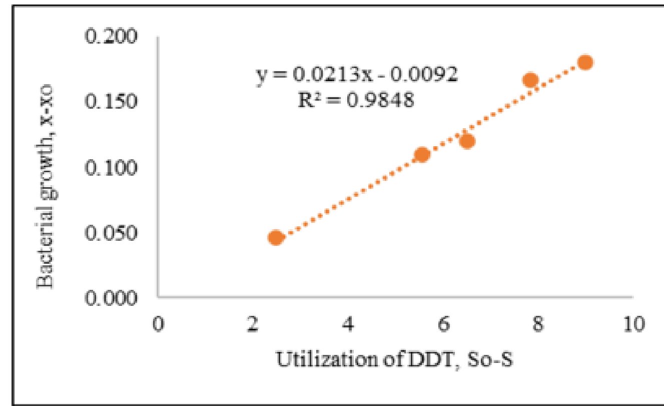

Fig. 8. The relationships of bacteria growth with DDT utilization

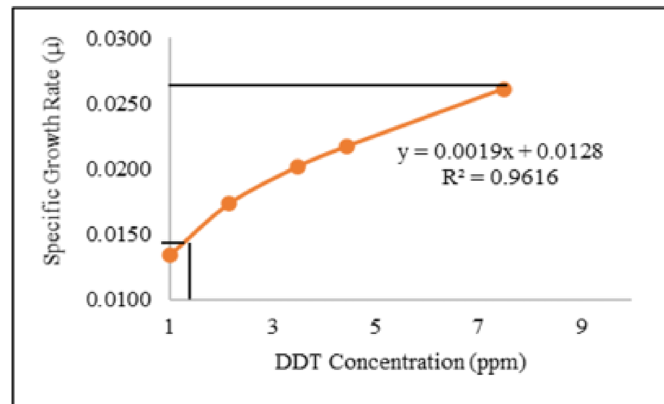

Fig. 9. The relationships between specific growth rate and substrate concentration

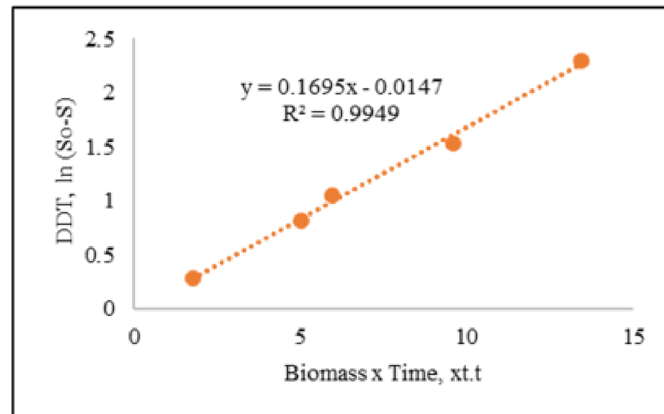

Fig. 10. Growth rate on order 1

3.3 Pilot scale implementation recommendation

Based on laboratory scale research result, we can recommend an implementation of DDT removal on pilot scale. 1% addition of yeast extract co-substrate produced the best result for bacteria to remove DDT. If there is 10 ppm DDT pollution on 100 m^3 of soil with specific weight of 1700 kg/m^3, the weight of the soil is at 170,000 kg. Polluted soil is stimulated by using 2 beds design as shown in **Fig. 11**.

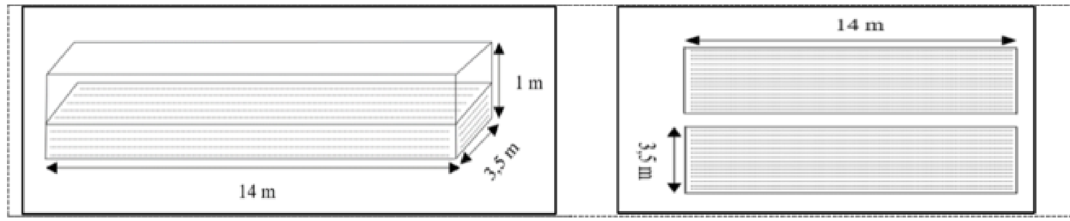

Fig. 11. Pilot Scale Processing Bed Design

The bed shape is planned to have a rectangle shape with length: width ratio of 4 to 1. The weight is assumed to be at 0.5m, based on that, with design volume of $50m^3$, the calculation of L would be length x width x height = 4L x L x 0.5. L^2 value is at $25m^3$, which makes L at 5m. The length measurement is at 20m, so the volume would be at $50m^3$. 85.000kg of soil can be inserted in a $50m^3$ bed. Based on that, with the utilization of 2 beds, we can process 170,000 kg of soil.

Based on laboratory research, 10 gr of 10 ppm DDT polluted can be degraded up to 90% by 25 ml of bacteria consortium with 5% co-substrate addition on 1% concentration for 96 hours. On similar condition, when a land is polluted by 10 ppm DDT with 85,000 kg of weight, the required bacteria consortium and co-substrate needed to degrade the soil are respectively at:

a. Bacteria consortium requirement

$$\frac{\text{amount of research soil(gr)}}{\text{research mix culture(ml)}} = \frac{\text{amount of soil (gr)}}{\text{amount of mix culture (ml)}} \quad (1)$$

$$\frac{10\text{ gr}}{25\text{ ml}} = \frac{170.000.000\text{ gr}}{\text{mix culture (ml)}}$$

Based on that, the requirement is at 425,000 L of bacteria consortium

b. Yeast extract co-substrate requirement

$$\frac{\Sigma\text{ research soil (gr)}}{\Sigma\text{ research yeast extract co-substrate (ml)}} = \frac{\Sigma\text{ soil (gr)}}{\Sigma\text{ yeast extract co-substrate(ml)}} \quad (2)$$

$$\frac{10\text{ gr}}{1{,}25\text{ ml}} = \frac{170.000.000\text{ gr}}{\text{mix culture (ml)}}$$

Based on that, the requirement of yeast extract co-substrate is at 21,250 L

Based on these calculations, the required mixed culture for both beds is 425000 L, so the required co-substrate is 21250 L. Based on the reaction rate equation, it is known that the first-order reaction rate has an R2 value that is closest to 1, so that the value of Y = 0.1695x - 0.0147 and the growth curve equation in the exponential phase is Y = 0.0025x + 0.0361. By using order 1, the detention time (t) for degrading DDT can be calculated as follows:

$$t = \frac{\ln\frac{So}{S} - \text{intersep}}{\text{slope}} \quad (3)$$

The So value comes from the initial concentration of DDT, which is 10 ppm, the S value comes from the DDT removal value for 96 hours to 90% is 1 ppm, the intercept and slope values are obtained from the first order graph equation, so that the following equation is obtained:

$$t = \frac{5{,}89\ x\ \ln\frac{10}{1} + 0{,}087}{0{,}0025t + 0{,}0361} \tag{4}$$

$$0{,}0025\ t^2 + 0{,}0361\ t - 13{,}65 \quad = 0 \tag{5}$$

$$t\ = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} = \frac{-0{,}0361 + \sqrt{0{,}0361^2 - 4\ x\ 0{,}0025\ x\ (-13{,}65)}}{2\ x\ 0{,}0025} = \frac{0{,}335}{0{,}005} = 67{,}02 \text{ jam} \tag{6}$$

$$t = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} = \frac{-0{,}0361 - \sqrt{0{,}0361^2 - 4\ x\ 0{,}0025\ x\ (-11{,}22)}}{2\ x\ 0{,}0025} = -\frac{0{,}407}{0{,}005} = -\ 81{,}46 \text{ jam} \tag{7}$$

Thus, the detention time required by the mixed culture to set aside 90% of DDT is 67 hours.

4 Conclusion

Based on this research, it was proven that the consortium of *Pseudomonas putida* and *Pseudomonas stutzeri* bacteria was able to degrade 10 ppm of DDT. The increase in bacterial growth and the degradation rate of DDT indicated that the presence of co-substrate had a positive effect on DDT degradation and could be utilized properly by the bacterial consortium as a source of additional nutrients. From the calculation results, it is known that the detention time required by the bacterial consortium to remove 90% of DDT is 67 hours.

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