Endosulfan insecticide removal planning with bioaugmentation-landfarming bioremediation method

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Abstract. Endosulfan is a toxic organochlorine insecticide and is persistent in the environment. Endosulfan residue can be accumulated underground and lower soil quality, pollute water sources, and create bioaugmentation. This research aims to gather required information and study the potential of bacteria consortium consists of Bordetella sp., Bordetella petrii, and Achromobacter xylosoxidans to remediate endosulfan polluted soil. Bioremediation on laboratory scale conducted in a soil reactor, the pH level of 7, 20% humidity, and adjusted temperature to field temperature. Endosulfan was added into a reactor with a concentration of 2mg/g. The bacteria consortium utilized endosulfan as a nutrient source to decently grow up until this research was finished on the 30th day. Maximum removal occurred on upper layer soil with 99% of alpha-endosulfan and beta-endosulfan removal rates. Pilot-scale removal can be implemented with landfarming bioremediation. Two (2) processing beds were prepared with 15m of length, 7.5m of width, and 0.5m of height. This method was able to remove 99% of endosulfan in just 457.75 hours. This research can be implemented to remediate endosulfan polluted soil through the bioremediation method by utilizing bacteria consortium.

1. Introduction

The increasing population growth has driven the agricultural sector to be able to ensure food supply fulfillment. The success of the agricultural sector requires various supporting factors such as the availability of fertilizer, farming tools, and chemical materials such as insecticide. The insecticide is widely used as a pest-controlling agent to prevent crop damages [1, 2]. The organochlorine insecticide is an environmental contaminant because it is persistent and cannot be naturally degraded [3]. The insecticide is categorized as a manufactured xenobiotic compound that can pollute the environment and is difficult to degrade, accumulating and bringing negative impacts to the environment [4].

Endosulfan is a toxic insecticide but is globally used as a pest-controlling agent to increase agricultural productivity. The chemical composition of endosulfan is C₉H₆Cl₆O₃S [5]. Technically, endosulfan is synthesized of two stereoisomers which are 70% alpha-endosulfan and 30% beta-endosulfan [6]. Chronic toxicity can be found in neural and behavioral disorders (neurotoxin) [7]. Environmental pollution due to excessive insecticide utilization has drawn a large number of attention [8]. Excessive insecticide application through spraying activity can lead to direct exposure in the land. As a result, endosulfan biodegradation by bacteria, chemical hydrolysis, photolysis, and vaporization will occur, but endosulfan can spread through surface water flow [10]. Insecticide residue
left on soil can be dangerous for the environment because it will affect land fertility and be highly toxic for living things [11, 12]. Insecticide can enter the food chain and will reach humans through biomagnification along the food chain [13].

There are several applicable technologies to solve insecticide exposure in the environment, one of which is through a physic-chemical method such as coconut shell active charcoal that can lower underground endosulfan residue concentration up to 80.36%-95% [14]. Besides that, researcher [15] has proven that photocatalytic degradation can remove up to 95% of endosulfan. However, this method has weaknesses with its high cost and inability to degrade the recalcitrance compound properly, so it needs additional treatment [16].

Besides the physic-chemical method, the insecticide pollution problem can be solved with the biotechnology approach through bioremediation. Bioremediation is one of the technologies that can be used to overcome environmental pollution through indigenous bacteria utilization. One way to conduct bioremediation is through bioaugmentation, which is implemented by adding bacteria into a polluted environment to degrade insecticide [17]. The advantages of bioremediation are that it comes at a low cost than any other technology. It can transform insecticide into CO₂, H₂O, and biomass. A more straightforward explanation can perfectly and permanently remove pollution and be safe for the environment [18, 19]. Both external and internal factors influence the effectiveness of bioremediation. External factors include physical, chemical, and environmental aspects such as temperature, pH, humidity, soil type, oxygen availability, and chemical structure. Internal factors consist of bacteria activity utilized in the bioremediation process. Bacteria will produce a suitable enzyme to degrade insecticide compounds as substrate [21]. Examples of bacteria able to degrade endosulfan in soil are *Bordetella* sp. [22], *Bordetella petrii* [23], and *Achromobacter xylosoxidans* [24, 25]. Several pieces of research on endosulfan biodegradation in liquid media only focused on biodegradation in culture media. There are only a few studies that research on how endosulfan degradation in various layers of soil. This research aims to analyze endosulfan insecticide remediation through the enzymatic way by bacteria activity on various soil layers in a controlled environment can be a basis to conduct endosulfan bioremediation on a pilot scale.

2. Methodology
Endosulfan removal data on soil layers act as secondary data. The primary literature utilized in this research refers to [26]. We will calculate the implementation of research results on a pilot scale through landfarming bioremediation based on the secondary data.

2.1. Soil media preparation
Endosulfan insecticide contaminated soil was gathered from some locations, namely insecticide producer locations in Ambatur, farming land in Thiruvallur District in Tamilnadu, and insecticide contaminated land in Kasargod District in Kerala. These soil samples were taken from different levels of depth. Soil samples were inserted into a reactor with a 15 x 10 x 40 cm dimension and then divided into upper layer soil, middle layer soil, and lower layer soil.

2.2. Endosulfan removal on soil media
2 mg/g of endosulfan concentration and bacteria consortium consisting of *Bordetella* sp., *Bordetella petrii*, and *Achromobacter xylosoxidans* were put into contact with soil. Biodegradation was observed on the pH level of 7, 20% humidity, and adjusted temperature with field temperature for 30 days and five days of sample taking intervals.

2.3. Endosulfan removal efficiency
This method is used to measure degraded insecticide levels. The removal calculation is as follows:

\[
\text{Endosulfan Insecticide Removal (\%) = \frac{C_O - C_a}{C_O} \times 100}
\]

\[(1)\]

Co represents initial insecticide concentration; Ca represents final insecticide concentration
3. Result and discussion

3.1. Endosulfan bioremediation on soil layers

Endosulfan bioremediation on various soil layers with a concentration level of 2 mg/g was conducted on the pH level of 7, 20% of humidity, and adjusted temperature with field temperature. Bioremediation was conducted for 30 days [26]. Figure 1 shows endosulfan removal graphics on various layers of soil.

![Endosulfan removal graphics on various layers of soil](image)

**Figure 1.** Endosulfan removal graphics on various layers of soil [26] (A) upper layer soil, (B) middle layer soil, (C) lower layer soil.

On the 30th day, up to 99% of alpha-endosulfan and beta-endosulfan removals occurred on lower layer soil. Endosulfan removal increased on deeper soil layer was caused by low organic carbon availability. On a lower layer of soil, endosulfan provides more organic carbon than those available in the soil. This situation drives the bacteria consortium to utilize endosulfan as its nutrient source and accelerate degradation [26]. Endosulfan sulfate and endosulfan diol are the most common metabolites in endosulfan biodegradation process [27]. Endosulfan diol can be mineralized into a more degradable compound and will eventually become CO$_2$ [28].

3.2. Bacteria consortium growth rate in soil that contains endosulfan

The specific substrate ($q$) utilization rate on the lower soil layer is at 0.0003 to 0.0009 hour$^{-1}$. Meanwhile, the specific growth ($\mu$) rate is at 0.00030 to 0.00154 hour$^{-1}$. Based on Figure 2, the relationships between the values of $q$ and $\mu$ will produce a total growth rate value ($Y_T$) of 2.70 hour$^{-1}$ with a bacteria death constant value (Kd) of 0.000541 hour$^{-1}$. The maximum substrate utilization rate calculation and the relationship between specific growth rate and alpha-endosulfan substrate concentration are shown in Figure 3. The maximum substrate utilization rate value ($q_{max}$) is at 0.00154, and with ($\frac{q_{max}}{2}$) value of 0.00077, the saturated constant value (Ks) is at 0.02 hour$^{-1}$.
Based on the calculation, we obtained a result of $L$ and the volume is at 0.5 m. The bed is planned to be rectangle-shaped with a length-to-width ratio of 2:1. One bed unit is expected to cover 50% of 100 m$^3$, which means that implementation on a larger scale by utilizing landfarming bioremediation should be available. Based on the literature study results, the bacteria consortium conducted in the laboratory scale, which means that implementation on a larger scale by utilizing landfarming bioremediation should be available. Based on the literature study results, the bacteria consortium is proven to remove 99% of 2 mg/g endosulfan in 30 days or 720 hours. Based on these results, the bacteria consortium growth rate on the lower soil layer that contains alpha-endosulfan occurred on order 1 with an $R^2$ value of 0.9578 and a growth rate constant of 0.0013 hour$^{-1}$.

**Figure 2.** The relationships of specific growth rate and specific substrate utilization rate.

$Y_{obs}$ value of 0.7067 hour$^{-1}$ was obtained from the bacteria growth relationship graphic by utilizing endosulfan, as shown in Figure 4. Reaction order determination was conducted by creating a growth rate graphic. The reaction order selected based on the closest $R^2$ value is order reaction 1, as shown in Figure 5. Based on that, the bacteria consortium growth rate on the lower soil layer that contains alpha-endosulfan occurred on order 1 with an $R^2$ value of 0.9578 and a growth rate constant of 0.0013 hour$^{-1}$.

**Figure 3.** The relationships of specific growth rate ($\mu$) and endosulfan substrate concentration.

**Figure 4.** The relationships of bacteria growth and endosulfan utilization.

**Figure 5.** The growth rate in order 1

### 3.3. Pilot Scale Endosulfan Removal Simulation

The research on this literature study is an initial stage of endosulfan bioremediation by bacteria consortium conducted on the laboratory scale, which means that implementation on a larger scale by utilizing landfarming bioremediation should be available. Based on the literature study results, the bacteria consortium is proven to remove 99% of 2 mg/g endosulfan in 30 days or 720 hours. Based on these results, the initial endosulfan concentration is at 2 mg/g pollutes 100 m$^3$ of clay with a density level of 1700 kg/m$^3$, we can calculate that the weight of the soil is at 170,000 kg. Contaminated soil processing is planned to be conducted in 2 processing beds.

The bed is planned to be rectangle-shaped with a length-to-width ratio of 2:1. One bed unit is expected to cover 50% of 100 m$^3$ soil volume or 50 m$^3$. With assumption that the soil height is at 0.5 m, the volume is at 0.5 m, and the height is 0.5 m, it means that 50 m$^3$ = length x width x height = 2L x L x 0.5 m = 2L$^2$ x 0.5 m. Based on the calculation, we obtained a result of $L^2$ = 50, which means that $L = 7.071$ m rounded.
into 7.5 m, the length is at \( P = 2 \times 7.5 \text{ m} = 15 \text{ m} \). The volume becomes: \( P \times L \times T = 15 \text{ m} \times 7.5 \text{ m} \times 0.5 \text{ m} = 56.25 \text{ m}^3 \). Processing bed design can be seen in Figure 6, with the following calculations:

**Figure 6.** Processing bed design. (A) front view (B) top view.

On the landfarming method with a volume of 56.25 m\(^3\), we can process 95,625 kg of endosulfan polluted soil. In this design, on processing bed unit will be processing 85,000 kg of soil. Based on that, two beds should process 170,000 kg of soil so all polluted soil amounts can be processed.

This literature study informs that 7650 grams of polluted soil with 2mg/g endosulfan can be degraded by 1500 ml of bacteria consortium in 30 days of 720 hours with a maximum level of removal at 99%. If this result is considered at pilot scale, and similar condition should occur, soil polluted by 2 mg/g of endosulfan concentration with soil weight of 85,000 kg in one bed, endosulfan degrading bacteria consortium required should be at:

\[
\frac{\text{amount of soil (gr)(research)}}{\text{amount of bacteria consortium (ml)(research)}} = \frac{\text{amount of soil (gr)}}{\text{amount of bacteria consortium (ml)}}
\]

\[
7650 \text{ gr} = 85,000 \times 10^3 \text{ gr} \quad \frac{1500 \text{ ml}}{x}
\]

Based on the calculation above, the required bacteria consortium is at 16,666.667 L/bed for each bed. If the two beds, bacteria consortium requirement is at 33,333.333 L. Based on this calculation result, the formulation of order 1 reaction rates \( Y = 0.0013 x + 0.4719 \) and growth curve formula on exponential phase is at \( Y = 0.0071 x + 6.08 \). To acknowledge the time (t) factor on order 1 in removing endosulfan, we can utilize the following calculation:

\[
t = \frac{\ln \frac{S_0}{S} + 0.4719}{0.0013} = 769.23 x \ln \frac{S_0}{S} + 368
\]

The maximum detention time required by bacteria consortium to degrade endosulfan is as follows:

\[
T = \frac{769.23 x \ln \frac{1.6}{0.001} + 368}{0.0071 t + 6.08}
\]

\[0.0071 t^2 + 6.08 t - 4271.84 = 0\]

\[
t = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} = \frac{-6.08 + \sqrt{6.08^2 - 4 \times 0.0071 \times (-4271.84)}}{2 \times 0.0071} = 457.75 \text{ hours} = 20 \text{ days}
\]

Bacteria consortium requires 457.75 hours or 20 days to degrade 99% of endosulfan.

**4. Conclusion**

Based on this research, it is proven that *Bordetella* sp., *Bordetella petrii*, and *Achromobacter xylosoxidans* bacteria consortium can survive on 2 mg/g endosulfan polluted soil by utilizing the pollutant as a nutrient source. On a laboratory scale, bacteria consortium requires 30 days to reach up to
99% removal efficiency. The laboratory-scale research result is recommended to be utilized as a basis to conduct pilot-scale endosulfan removal. To obtain exact endosulfan removal rates using two processing beds, we required 33,333 L of bacteria consortium and 20 days of detention time.

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