Peat Endogenous Lignocellulolytic Bacteria for Humic Waste Decomposition

F Solikhah1,*, W Assavalapsakul2, and E Zulaika1

1 Department of Biology, Institut Teknologi Sepuluh Nopember, Surabaya 60111, Indonesia
2 Department of Microbiology, Chulalongkorn University, Bangkok, Thailand

*Corresponding author: enny@bio.its.ac.id

Abstract. The low carrying capacity of the peat soil makes a consideration to accelerate peatland decomposition through the addition of endogenous lignocellulolytic bacteria. These bacteria can use nutrients found in the peat soil and secrete enzymes to accelerate peat decomposition. In this study, endogenous lignocellulolytic bacteria from Palangkaraya peat soil, Kalimantan was used in lab scale decomposition experiments. These bacteria were added into the peat soil and incubated for six weeks. Analysis of peat fiber decomposition was performed weekly and at the end of the incubation period, bacteria colony was counted using total plate count method. The results showed that all bacteria can decomposed peat soil with an average value of 86%. The number of bacterial colonies at the end of incubation ranges from $3.0 \times 10^3$ to $6.7 \times 10^4$ CFU/ml. This study can be used as a preliminary study of peat soil decomposition that subsequently can be developed through analyzing several factors to stabilize peats oil area.

1. Introduction
The increasing development causes many buildings to be built on poor soil conditions, such as on peatlands. One of the largest peatlands in Indonesia is Kalimantan which has 4.78 million hectares of peatland [1]. Peat soil is known as a soil that has high pores and water content so that the carrying capacity and compression ability are very low [2]. Most of the peat soils are formed from the incomplete decomposition of various plant materials in the marsh environment. In general, the constituent of plant cell walls on undecomposed peatland contains lignocellulosic complex, comprising cellulose, hemicelluloses, and lignin [3]. Various methods of peat decomposition to improve soil stability have been developed, including mechanical and stabilization methods through the addition of chemical substances [4]. However, the improvement of peat soils by these methods can have long-term environmental damage. The alternative way is using the environmental-friendly biological method, through the addition of endogenous lignocellulolytic bacteria. The capability of lignocellulolytic bacteria to decompose peat fiber can reduce the fiber content. Decomposition leads to changes in the structure, number and size of peat fiber [5]. Several lignocellulolytic bacteria have been identified are genus*Cellulomonas, Pseudomonas, Bacillus, Clostridium*, and *Fibrobacter* [6,7]. This study aims to determine the percentage of peat soil decomposition by lignocellulolytic bacteria and the viability of bacteria after decomposition.
2. Material and Methods

2.1 Experimental bacteria
The lignocellulolytic screening had been performed and the screened isolates were isolated D1, D2, D3, U2, U3, and U4. Furthermore, each isolate was applied to decompose peat soil.

2.2 Inoculating the bacteria culture
A loop of each lignocellulolytic bacteria respectively inoculated into 25 ml Nutrient Broth and incubated on a rotary shaker (100 rpm, 24 h) at room temperature. Each of 10 ml bacterial culture then inoculated into 90 ml of physiological saline solution (NaCl 0.85%) and performed serial dilutions up to 10\(^{-5}\). Two ml of lignocellulolytic starter bacteria were inoculated into 20 grams of peat soil homogeneously in a petri dish, closed and incubated at 30\(^{\circ}\)C for 6 weeks. This experiment was performed on 4 times replication.

2.3 Peat soil decomposition test
Twenty grams of treated peat soil were dried in the oven (105\(^{\circ}\)C, 5 h) and weighed as initial dry biomass. A total of 20 grams of treated peat soil was added by 100 ml of 5% sodium hexametaphosphate (NaPO\(_4\))\(_6\) solution as dispersing agent, stirred evenly, incubated for 3 hours and filtered (mesh size 0.15 mm). The soil was washed with tap water until the water passing through the sieve was clear. Then, the filtered soil was immersed in 2% HCl solution for 2 minutes to remove the remaining carbonate ions, washed again with tap water for 5 minutes to remove the remaining HCl. The remaining filtrate was placed on Whatman paper no. 4, washed through the funnel and dried by the oven at 105\(^{\circ}\)C for 5 h. The peat fiber biomass minus the mass of filter paper is recorded as the fiber dry biomass. The percentage of soil decomposition was calculated on the basis of the ratio of fiber dry biomass to the initial dry biomass of the soil, can be written as the following equation (1):

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\text{Percentage of decomposed soil and fiber} = \frac{\text{initial dry biomass} - \text{fiber dry biomass}}{\text{initial dry biomass}} \times 100\% \tag{1}
\]

2.4 Viability test
The viability test was used to determine the bacterial survival during the incubation period. The viabiliy test was performed during the sixth week of the incubation period. A total of 1 gram of soil was added into 9 ml of physiological saline solution (NaCl 0.85%) and performed serial dilutions up to 10\(^{-5}\). Each dilution was taken 0.1 ml and inoculated on Nutrient Agar by spread plate method, incubated for 24 hat 37\(^{\circ}\)C and counted the growing colonies [9].

3. Results and Discussions

3.1 Decomposition of peat soil by lignocellulolytic bacteria
The ability of each lignocellulolytic bacteria to decompose peat soil is shown in Table 1 and Figure 1.

| Isolate code | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|--------------|--------|--------|--------|--------|--------|--------|
| D1           | 83.24  | 81.07  | 85.37  | 86.87  | 86.94  | 89.25  |
| D2           | 86.46  | 84.04  | 84.66  | 88.64  | 89.54  | 88.90  |
| D3           | 86.4   | 82.2   | 88.81  | 85.47  | 88.86  | 88.65  |
| U2           | 79.11  | 88.88  | 85.19  | 84.38  | 85.91  | 89.56  |
| U3           | 84.48  | 86.78  | 87.25  | 88.41  | 88.71  | 87.97  |
| U4           | 81.43  | 88.86  | 87.03  | 87.30  | 87.82  | 88.23  |
| Control      | 73.04  | 74.29  | 83.21  | 80.23  | 83.41  | 84.65  |
Figure 1. Percentage of Decomposed Peat Soil And Fiber on Each Lignocellulolytic Bacteria for 6 Weeks Incubation Period

The result showed that all the bacteria are able to decompose peat soil faster than the control. However, the soil decomposition charts tend to fluctuate for six weeks of incubation. During six weeks of incubation, the percentage of soil and fiber degradation increased, ranging from 2-10%, depending on each bacteria. The highest decomposition percentage was achieved by isolate U2 that can decomposed 89.56% of peat soil on the sixth week, followed by isolate D1 that showed 89.25% decomposition rate. Control indicated a slight decomposition activity caused by endogenous bacteria in the peat soil without any addition.

Decomposition of plant material is an on-going process, driven by microorganisms that use the decaying organic matter as an energy source and building material [10,11]. The degree of peat decomposition showed the quantitative ratio of dark amorphous matter, consisting of humic compounds and the other products of the plant. However, in general, vegetative plant organs, especially leaves and stems, decompose rapidly, forming an amorphous peat mass [12]. In this study, high levels of decomposition were affected by experimental peat soil conditions that comprise of undecomposed plant material. The rate of decomposition of dry peat soils is relatively higher than wet peat [13]. The decomposition rate of fibrous peat can be accelerated by altering the carbon: nitrogen (C:N) ratio, pH, oxygen supply and temperature for optimum conditions [5].

3.2 Viability of lignocellulolytic bacteria
On the sixth week of the incubation period, the bacteria still remain in the peat soil, although the bacteria density decreased from the initial number (Table 2 and Figure 2).
Table 2. Number of Bacteria on The Early and End of Incubation Period

| Decomposition medium | Number of bacteria colony (CFU/ml) | Early of incubation | End of incubation |
|----------------------|------------------------------------|---------------------|------------------|
| 1                    | 61.8 × 10^7                       | 2.9 × 10^4          |
| 2                    | 58.1 × 10^7                       | 1.63 × 10^4         |
| 3                    | 64.7 × 10^7                       | 2.87 × 10^4         |
| 4                    | 46.5 × 10^7                       | 6.7 × 10^4          |
| 5                    | 46.8 × 10^7                       | 6.2 × 10^4          |
| 6                    | 58.4 × 10^7                       | 4.24 × 10^4         |
| Control              | N/A                               | 0.3 × 10^4          |

Figure 2. The viability of Each Bacteria on The Early and End of Incubation Period

The number of bacteria on the treated soil was higher than the control. The number of peat soil bacteria on the end of the incubation period ranging from 3.0 × 10^3 to 6.7 × 10^4 CFU/ml. At the end of incubation, the highest number of bacteria was found in medium 4 i.e, peat soil added by isolate culture U2, having 6.7 × 10^4 CFU/ml, followed by medium 5 i.e, peat soil added by isolate culture U3, showed 6.2 × 10^4 CFU/ml. The endogenous bacteria of control also showed the viability until the end of incubation, although the number of bacteria at the early of incubation was not calculated. Another research reported the mean microorganism counted for moderately decomposed Sphagnum peat by dry mass basis was 10^5 CFU/g after 10 days of incubation [14]. Similar findings have reported that the mean of microorganism measured for the natural peat are consistent with the value of 2.6 × 10^5 CFU/g dry peat [15]. The fewer number of bacteria on this research presumably because the available nutrients on the treated soil had already been exhausted during the incubation.
4. Conclusion
Peat undergoes continuous biological decomposition. This decomposition process is performed by lignocellulolytic bacteria that have been screened. All bacteria have the ability to decompose peat soils with an average decomposition value of 86%. After six weeks of the incubation period, the bacteria still viable, ranging from $3.0 \times 10^3$ to $6.7 \times 10^4$ CFU/ml, includes the control. However, the application of these experimental findings in the field may not be straightforward and will require further studies in the field.

5. References
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