Bacillus stratosphericus from Oryctes rhinoceros larvae as decomposer of oil palm empty fruit bunches

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Abstract. The use of oil palm empty fruit bunches (OPEFB) as a source of organic fertilizer in oil palm plantations copes with obstacles. The application of the OPEFB for breeding medium of Oryctes rhinoceros pests from egg stage to pupa and the composting period of organic material reach 12-16 months. In this study, it was found that the utilization of bacterial symbionts of O. rhinoceros larvae namely Bacillus stratosphericus, mixed with Trichoderma sp. And yiest was able to decompose OPEFB for 10 weeks. The resulting compost indicated a pH of 8.46 - 8.76 and a moisture content of 79.39 - 81.74%. The C/N ratio for the treatment of B. stratosphericus + Yeast, the treatment of B. stratosphericus + Trichoderma sp. And the treatment of B. stratosphericus + Yeast + Trichoderma sp. is 17.5, 13.3 and 14.7, respectively. The chemical compounds composition of OPEFB decomposed for 10 weeks with the addition of B. stratosphericus and Trichoderma sp as well as yeast ranged from 25.6 to 30.46% C-org, 1.81-2.07% N, 0.1-0.22% P, 2.54 - 3.68% K and 0.43 - 0.50% Mg.

1. Introduction

Oil palm empty fruit bunches are the main waste of the palm oil processing industry into palm oil. The percentage of the empty fruit bunches waste is 23% of fresh fruit bunches, while the percentage of fibre and seed shells is 13% and 5.5%, respectively [1]. The empty fruit bunches (OPEFB) are composed of 45.95% cellulose, 22.85% hemicelluloses and 16.49% lignin. Lignin contained in the OPEFB is complex and difficultly degradable [2]. The empty palm oil bunches are stacked and left to rot into a source of organic fertilizer in the palm oil plantation areas. The chemical properties of OPEFB comprise 50.87% C, 1.1% N, 0.17% Ca, 0.13% Mg, 2.06% K and 0.11% P with the C/N ratio of 46.25 [3].

The existence of OPEFB affects the arrival of the main pest of Oryctes rhinoceros in the oil palm plantation areas. In the sites of palm oil plantations, it is found that the oil palm empty fruit bunches encompass the density of O. rhinoceros throughout the year [4]. OPEFB and piles of oil palm stems are appropriate for breeding media of O. rhinoceros [5].

Intracellular symbionts of insects are classified as primary or secondary endosymbionts. The symbiotic relationship between bacteria and insects varies from mutuality and commensalism. The 3rd instar O. rhinoceros larvae from the OPEB were isolated and identified molecularly and found Bacillus stratosphericus species [6]. The bacteria can be used to speed up the process of composting litter and cow dung from 30 days to 18 days. The research result of [7] revealed the endurance of B.
Stratosphericus in poor conditions including resistance to UV radiation and the tolerance to halo containing up to 17.5% NaCl and remained undamaged in the cell. B. Stratosphericus can grow at temperatures between 8° C and 37° C and at pH of 6-10. Furthermore, the bacterium is very tolerant of Fe, Co, Ni and Cu ions and is quite tolerant of Zn.

The amount and type of microorganisms determine the success of the decomposition or composting process. The decomposition process of organic matter in nature is not only carried out by one monocolonial microorganism but also accomplished by the consortia of microorganisms such as Trichoderma, Bacillus spp, Pseudomonas spp, Clostridium spp and Streptomyces spp.

Microorganisms of the organic matter decomposers consist of fungi and bacteria. The decomposing fungi (cellulolytic) of organic matter generally have a better capability than bacteria in breaking down plant residues (hemicellulose, cellulose and lignin). According to [8], the decomposing fungi are the best decomposers which can quickly break down the organic matter into simple organic compounds and also inhibit the growth of plant pathogenic organisms. The decomposers applied are in the form of cellulolytic fungi Trichoderma with high cellulolytic activity. The fungi possess cellulase enzymes which consist of exoglucanase enzymes (β-1,4glukanhidrolase) and cellulase (β-glicosidase). Trichoderma sp. is one of the fungi capable of producing cellulase enzyme components [9].

Beside bacteria and fungi as decomposers, it can also be applied local microorganisms which are available in the environment and have the ability to decompose organic matter due to their decomposing microorganism content. One of the local microorganisms which can be utilized is yeast because of its microorganism content, especially containing bacteria and fungi capable in breaking down organic matter [10].

The aim of this study is to determine the effect of giving B. Stratosphericus and several bioactivators to accelerate the composting process of oil palm empty fruit bunches.

2. Materials and Methods

This research was conducted at Pest Laboratory, Faculty of Agriculture, Universitas Sumatera Utara, Medan from March 2018 to completion. The materials used were 1-week-old oil palm empty fruit bunches in the field, Trichoderma sp, yeast, B. Stratosphericus isolated from O. Rhinoceros larvae and propagated while Tririchoderma sp was gained from the collection of Plant Disease Lab. in the Faculty of Agriculture, University of Sumatera Utara, Medan.

The research was determined using descriptive observation method by the way of visual approach and analysing chemical compounds to determine changes in the OPEFB applied by B. Stratosphericus bacteria and the role of bioactivator in related to making compost. In completing the research, two stages were involved. The first step was about isolating and multiplying B. Stratosphericus and preparing bioactivator. In the second stage, the OPEFB were taken from the field and then chopped become smaller size. Before being mixed, the chopped OPEFB were dried first. The study utilized 1 kg empty palm oil empty fruit bunches, 30 ml B. Stratosphericus with 10^9 density, 50 g yeast, 30 ml Trichoderma sp and 250 ml water. All the materials were mixed in a bucket and then covered with black plastic and placed in a glass house. The temperature measurement using a thermometer was first done after the stack was 1 day old. Afterward, the temperature was measured every week. The temperature measurement in the compost stack was executed by inserting the thermometer on the stack for 5 minutes at a depth of 25 cm. The reversal was performed once a week in order to get rid of excessive heat, allow fresh air into the pile of materials, flatten the decaying process in each part of the stack, equalize the water supply and help the breakdown of the materials into small particles. Watering was carried out if the compost material piles were too dry and should be accomplished before the reversal in order that the water would be mixed itself when being reversed.

The observed parameters for composting embraced (1) the temperature of compost; (2) C/N Ratio; (3) pH level; (4) chemical compounds of C, N, P, K, Mg; (4) changes in the colour of compost material which would be compared with compost standards based on SNI 19-7030-2004.
3. Results and Discussions
A bioactivator is a combination of several microorganisms including bacteria and fungi as well as additional materials, so that the optimum combination of bacteria and fungi together with Local Microorganism (MOL) can be beneficial to determine the time used for composting.

The continuity of composting was indicated by the size of the heat and cold of a material applied to show maturity of compost. The increase of the temperature was due to the metabolic process of microorganisms producing heat in the form of decomposition.

The highest temperature measurement with the addition of \textit{B. stratosphericus} + Yeast + \textit{Trichoderma sp.} in the OPEFB for 4 weeks reached 45$^\circ$C. The highest temperature recorded was 47$^\circ$C for the treatment of \textit{B. stratosphericus} + Yeast and 49$^\circ$C for the treatment of \textit{B. stratosphericus} + \textit{Trichoderma sp} in the fifth week. This showed that \textit{B. stratosphericus} was able to speed up the decomposition of the OPEFB and proved thermophilic (figure 1).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Temperature of composting oil palm empty fruit bunches}
\end{figure}

In the 5th to 8th week there were a drastic temperature decreased indicating a lessening of \textit{B. stratosphericus} activity. \textit{B. stratosphericus} produced lipase enzymes, combined proteins and remained stable at a temperature range of 35$^\circ$ - 55$^\circ$C [11].

The composting of the OPEFB in the treatment of \textit{B. stratosphericus} + Yeast + \textit{Trichoderma sp} and \textit{B. stratosphericus} + \textit{Trichoderma sp.} revealed stabilization of decreases and temperature starting from the 8th to 10th weeks. This demonstrated synergistic microorganisms of \textit{B. stratosphericus} and \textit{Trichoderma sp.} in the decomposition process. \textit{Bacillus} produces hydrolase enzymes such as amylase, protease, lipase, gelatinase and cellulase which are able to catalyse the subsequent lignin \textit{Trichoderma sp.} is one of the fungi capable of producing cellulase enzyme components [9].

The C/N ratio in the 10th week exhibited that the oil palm empty fruit bunches were completely decomposed. The lowest C/N ratio was 13.3 in treatment of \textit{B. stratosphericus} + \textit{Trichoderma sp}, while the highest was 17.5 in the treatment of \textit{B. stratosphericus} + Yeast as shown in table 1. The used materials accelerated the OPEFB decomposed to obtain a good compost. Khalid \textit{et. al.} (2000) reported that the decomposition process of oil palm empty fruit bunches was influenced by macro climate, microclimate, material quality and microorganism activity in the areas of oil palm plantation in order to obtain 12-18 months of composting time and good compost with the ratio of C/N $\leq$ 20.
Table 1. The chemical composition of the decomposed OPEFB for 10 weeks

| Treatment                          | pH | Water Content | C-Org  | N    | P   | K  | Mg | C/N |
|------------------------------------|----|---------------|--------|------|-----|----|----|-----|
| B. stratosphericus + Yeast         | 8.76 | 79.39        | 30.46  | 2.07 | 0.22 | 3.68 | 0.43 | 17.5 |
| B. stratosphericus + Trichoderma sp. | 8.46 | 81.74        | 31.67  | 1.81 | 0.17 | 2.73 | 0.50 | 13.3 |
| B. stratosphericus + Yeast + Trichoderma sp. | 8.52 | 80.16        | 25.61  | 1.92 | 0.20 | 2.54 | 0.47 | 14.7 |

Result Analysis at PT. Socfin Indonesia

The measuring results of the acidity level of the OPEFB with the administration of *B. stratosphericus* and *Trichoderma sp.* with or without yeast revealed a pH of 8.46 - 8.76 or an alkaline property. At thermophilic temperature, the composting process mostly occurs anaerobically. This is consistent with the results obtained from the measurement of water content of 79.39 - 81.74%. Anaerobic composting occurs at a temperature of ≥ 40% and is referred to as thermophilic temperature [12]. Compost with thermophilic temperature is dominated by bacteria with a characteristic of an acid intolerance. In addition, the mineralization process of base cations, such as K+, Mg2+ and Ca2+ also affects the pH increase [13].

Availability of chemical compounds of the compost in the treatment of *B. stratosphericus* + Yeast was compared to the treatment of *B. stratosphericus + Trichoderma sp.* + Yeast. However, the results of the composting process of the OPEFB by utilizing *B. stratosphericus* and *Trichoderma sp.* with or without yeast exhibited the increase of the availability of chemical compounds in the OPEFB as shown in table 1. The chemical composition of oil palm empty fruit bunches which had been decomposed for 12 weeks contained 1.48% N; 0.428% N; 2.11% K; 0.32% Ca; 0.26% Mg and C / N of 32.6 [3].

The texture of the oil palm empty bunches at the beginning of composting seemed unchanged or was still roughly light brown. After experiencing composting, the texture of the organic material gradually changed to more fibrous crumbs because the decaying process had begun. Consequently, the colour became dark brown and gradually turned into black brown. The polyphenol compounds released in the composting process was lignocellulose compounds which developed into quinones. Subsequently, the quinones reacted with amino compounds to form dark or black fulvic acid. Polyphenols, as part of lignin monomers, are derived from three phenylpropanoid monomers (coniferyl, p-coumaryl and synapil alcohol) which undergo dehydrogenation and polymerization processes with enzymes [14].

Another indication of the compost maturity level was the change in colour to blackish brown resembling soil. This change was led to by the formation of humic compounds in the composting process (figure 2). Visually, the fibres were smoother with a blackish brown colour and characterized by smell of soil. The composting treatment with the addition of *B. stratosphericus + Trichoderma sp.* + Yeast demonstrated more significant results compared to the addition of *B. stratosphericus + Trichoderma sp.* and *B. stratosphericus + Yeast* (table 1). The results of visual observations of the texture changes in the decomposed OPEFB for two weeks (a), five weeks (b) and 10 weeks (c) can be seen in figure 2.
Note: the colour and texture changes of the OPEFB composting; treatment of bacteria + yeast

Note: the colour and texture changes of the OPEFB composting; treatment of bacteria + yeast + *Trichoderma sp.*

Note: The colour and texture changes of the OPEFB composting; treatment of bacteria + *Trichoderma sp.*

**Figure 2.** The color and texture changes of the OPEFB composting

### 4. Conclusion

This study found that the best compost as fertilizer is *B. stratosphericus* + Yeast with a C / N value of 17.5 and the administration of a mixture of *B. stratosphericus, Trichoderma sp* with or without yeast was able to decompose the OPEFB for 10 weeks. The quality of the produced compost contained the C/N ratio of 13.3 - 17.5, pH of 8.46 - 8.76, water content of 79.39% - 81.74%, The composition of the compost chemical compounds ranged from 25.61 to 31.67% C-organic, 1.81-2.07% N, 0.17-0.22% P, 2.54-3.68% K and Mg 0.43-0.50%.

**References**

[1] Fuadi, Ahmad and Pranoto H 2016 Pemanfaatan limbah tandan kosong kelapa sawit sebagai bahan baku pembuatan glukosa [Utilization of waste of oil palm empty fruit bunches as glucose making material] *CHEMICA Jurnal Teknik Kimia* 3 1 pp 1-5

[2] Ibrahim M N M and Chuah S B 2004 Characterization of lignin precipitated from the soda black liquor of oil palm empty fruit bunch empty by various mineral acid. *Asian Journal of S & T for Development* 21 pp 57-67
[3] Kalla D R, Rosenani A B, Fauziah C I and Thohirah L A 2009 Composting Oil Palm Waste and Sewage Sludge for Use in Potting Media of Ornamental Plants *Malaysia Journal of Soil Science* **13** pp 77-9

[4] Marheni 2012 *Bioecology Characteristics of Oryctes rhinoceros (L.) on Palm Oil Land.* [Disertation] (Yogyakarta: Gadjah Mada University) p 143

[5] Prawirosukarta, S, Susanto A, Harahap Z A and Purnomo E 2009 Control of Oryctes rhinoceros horn beetle in oil palm empty fruit bunches *Proc. Conf. on The Coconut Technical Meeting III* (Medan: Palm Oil Research Center) pp 51-61

[6] Sijabat, Octanina, Marheni and Bakti D 2017 The identification of bacterial symbionts of the larvae Oryctes rhinoceros L and the role of the bacteria in composting process *Journal of Community Service and Research* **1** pp 43-8

[7] Shivaji S, Chaturvedi P, Suresh K, Reddy G S, Dutt CB, Wainwright M, Narlikar J V and Bhargava PM 2006 *Bacillus aerius sp*.** nov. Bacillus aerophilus sp. Bacillus stratosphericus sp.*** nov.** and *Bacillus allititudinis sp.*** nov. isolated from cryogenic tubes used for collecting air sample from high altitudes *Int. J. Syst. Evol. Micr* **56** pp 1465-73

[8] Mitchell R 1992 *Environmental Microbiology* (New York: Wisley-Liss A. John Wisley and Sons.Inc. Publication)

[9] Salma and Gunarto 1998 Enzim selulase dari Trichoderma spp [Cellulase enzyme from Trichoderma spp] *Journal mikrobiologi indonesia* **2** 2

[10] Firdaus F, Purwanto B P and Salundik 2014 Dosage for the use of local microorganisms (MOL) rage tempe dan isi rumen untuk pengomposan [Dosage for the use of local microorganisms (MOL) tempe yeast and fill the rumen for composting] *Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan* **2** 1 pp 257-61

[11] Gricajeva A, Bendikiene V and Kalediene L 2016 Lipase of *Bacillus stratosphericus* L1: cloning, expression and characterization *International Journal of Biological Macromolecules* **92** pp 96-104

[12] Braun R 2007 *Anaerobic Digestion Amulti Faceted Process for Energy, Environmental Management and Rural Development. Improvement of crop Plants for Industrial end Uses*, ed P. Ranalli (Netherlands: Springer)

[13] Yuwono T 2006 Yuwono T 2006 Kecepatan dekomposisi dan kualitas kompos sampah organik [Decomposition speed and quality of organic waste compost] *Jurnal inovasi pertanian* **4** 2 pp 116-23

[14] Hendrik A T W M and Zoeman G 2009 Pretratman to enhance the digestibility of lignocellulose biomassa *Journal Bioresource Technology* **100** pp 10-8