Vermicast of Earthworm as Ecosystem Engineers within Different Vermireactor Shape

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ABSTRACT

Earthworms as ecosystem engineers can modify the quality of vermibed assembled from mixed soil with organic material in a vermireactor. Various in shapes of the vermireactor are used to produce a vermicast for biofertilizer in agriculture. The objective of this research was to study the vermicast production and its quality produced from a variety of vermireactor shapes. Earthworm (Pheretima sp.) which was applied to the vermireactor consists of: with-and without of earthworms. Three shapes of the vermireactor, namely cylinder, square, and rectangular has been tested. Compost of Chromolaena odorata pruning mixed with soil was used as vermibed. The vermicast process ran until 28 days. The results showed that vermireactor with earthworms which were successfully converted to vermicast was about 49.24% from the vermibed volume, however, no vermicast formed was found from vermireactor without earthworms. The difference in vermicast produced from different vermireactor shapes was not significant. The value of pH, total-N, and C/N ratio among the vermicast was different. The number of the morphological character of bacteria colonies in the vermicast produced from the square vermireactor was the highest, while from cylinder vermireactor was the lowest. We concluded that the quality of vermicast from earthworm (Pheretima sp.) activity was determined by the shape of the vermireactor.

Keywords: Ecosystem engineers, quality, vermibed, vermicast, vermireactor


Kata kunci: Kualitas, perekayasa ekosistem, vermibed, vermicast, vermireaktor

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INTRODUCTION

The earthworm has two functional roles, namely as decomposer and as soil ecosystem engineer (Jones et al. 1994, Pathma and Sakthivel 2012). The concept of its first role has been implemented in vermicomposting technology to process organic solid waste in an urban or agricultural area (Aalok et al. 2008, Singh et al. 2011). Many organic materials including solid organic waste, manure, feces, biochar, or compost can be used as vermicomposts and transformed into stable organic material through co-activity between earthworm and microorganism in a vermireactor (Tognetti et al. 2005; Nair et al. 2006; Lazcano et al. 2008; Hanc and Pliva 2013; Hanc and Vasak 2014). Such a product is known as vermicompost (Aira et al. 2011; Pathma and Sakthivel 2012). It contains a population of beneficial microbes, available nutrients as well as bio-stimulant promoting plant growth and suppressing the pathogen (Pathma and Sakthivel 2012; Rekha et al. 2018). Due to its rich content, vermicompost has been utilized as a biofertilizer to improve soil fertility, to amend soil quality, and to increase plant growth (Hanc and Pliva 2013).

The last role concept has been mostly applied to restore the soil quality and fertility (Wodika et al. 2014; Kilowasid et al. 2015; Singh et al. 2016). The earthworm is capable of mixing soil particles with organic material, as well as modifying soil environment, to create a new more appropriate environmental condition to modulate the growth of some useful microbes (Jouquet et al. 2006; Lavelle et al. 2016). Organic material is biochemically degraded by soil microorganisms mutualistic associating with earthworm guts (Edwards and Fletcher 1988). The mix of degraded soil particles and organic material in the intestines configures a biogenic structure (biostructure) in the form of vermicast, and it is released into the soil through the anus (Mora et al. 2006; Hoeffner et al. 2018). Vermicast contains some nutrients, vitamins, enzymes, and various advantageous microbes (Sim and Wu 2010). Some beneficial microbes found in vermicast are plant growth-promoting bacteria (such as Azosprillum, Azotobacter, Pseudomonas), and viable spores of arbuscular mycorrhizal fungi (AMF) (Reddell and Spain 1991, Pandya et al. 2014, Vijayabharathi and Sathyaa 2015). Thus, vermicast has a chance to be utilized as a carrier for the inoculum of beneficial soil bacteria and spore of AMF for agriculture, forestry, and recovery of degraded soil (Harinikumar and Bagyaraj 1994; Sekar and Karmegam 2010; Douds Jr et al. 2014).

In practice, there are three common-used two-dimensional figures of vermireactor to produce vermicompost or vermicast, i.e. cylinder, square, and rectangular for creating cylindrical, cube, and beam vermireactors, respectively (Subramanian et al. 2010; Raphael and Velmourougane 2011; Huang et al. 2014; Nigussie et al. 2016). The understanding of production and quality of vermicast produced from various two-dimensional figures of vermireactors can assist in choosing the proper shape of vermireactor to generate vermicast on-field or farm level. The productivity of vermicast from vermireactor is strongly determined by surface area and volume (ratio of surface area and volume) of those vermireactors (Ganesh et al. 2009). Meanwhile, the information regarding the production and quality of produced vermicast from various shapes of vermireactors with similar areas of two-dimensional and vermicast volume is rarely reported. Theoretically, the minimum surface area of each two-dimensional figures of those vermireactors is reached in the same volume and at stationary state. On the minimum condition, the surface area of the cylindrical is the smallest; while the surface area of the beam is the largest. Consequently, under such conditions, a ratio of the surface area of the cylindrical on volume is smaller compared to the ratio of the surface area of a cube on volume, and the ratio of the surface area of a cube on volume is smaller than a ratio of the surface area of a beam on volume.

This research was conducted to study the variation of production and quality of vermicast from three different shapes of vermireactors possessing the similarity in surface area of two-dimensional figures and volume of vermicast.

MATERIALS AND METHODS

Simple Reactor of Cardboard

Three reactors with different two-dimen- sional figures were made from cardboards, such as cylinder, square, and rectangular for cylindrical, cube, and beam reactors, respectively. To generate a relatively similar area in each two-dimen- sional figure, i.e. approximately 144 cm², the re- actor was built from the two-dimensional figure of cylinder, square, and rectangular with 23.7 cm in diameter, 21 cm × 21 cm and 25 cm × 17.64 cm in dimensions, respectively. Different
vermireactor from each shape was formed with a height dimension of 21 cm. The interior of each vermireactor was covered with transparent duct tape to protect the vermireactor from damage during the vermicast process.

**Compost Production**

Vermibed was comprised of soil spiked with compost from pruned *C. odorata*. Composting of pruned *C. odorata* was processed using a suspension of fermented microorganisms following the procedure of Formowitz *et al.* (2007). Total of 3 kg of sliced (± 1 cm in size) banana corm, 650 g of brown sugar, 2 liters of starch water, and 1 liter of water was put into 10 liter-volume of a plastic bucket and stirred using a wood stirrer. The top surface of the bucket was covered with black plastic, and a small hole was provided on the cover for hose connecting the solution in the bucket with water in 1 liter-volume plastic bottle which was placed around the bucket. After 10 days of fermentation, the solution was filtered using a sieve with 0.2 mm per hole in size, then put into a plastic bottle and kept at room temperature until ready for use. A total of 100 kg of chopped fresh pruned *C. odorata* (± 3 cm per slice) and 50 kg of fine sawdust were manually mixed using shovel and hoe on the surface of a tarp with 4 m × 6 m in dimension. Those mixtures were moisturized with the diluted stock solution using tap water up to a concentration of 75% (75cc/liter of tap water). All of the moist materials stirred until relatively homogenous, and the material wrapped in the tarp. The compost material at any time stirred during the composting period. The composting process was conducted over 30 days, and the mature compost is ready for use.

**Experiment Design**

The experiment was performed following the split-plot design in the Laboratory of Biodiversity, Faculty of Agriculture, University of Halu Oleo, Kendari (Indonesia). The main plot consisted of without and with earthworms (*Pheretima* sp.), and sub-plots were three shapes of vermireactors, i.e. cylindrical, cube, and beam. Each treatment was repeated three times following a randomized completed block design. Compost made from pruned *C. odorata* was put into each vermireactor up to a height of 3 cm, then the soil from vegetation area of *C. odorata* was placed on the surface of compost layer of 3 cm in height. This procedure produced total volume filled with vermicast for each vermireactor around 2.646 cm³. The material in each vermireactor was mixed until homogenous. Five starved-earthworms (*Pheretima* sp.) with clitellum character and body length of 6 cm – 9 cm in range were released on the surface of vermibed in each vermireactor. After all parts of earthworm were penetrated vermicast, the surface of each reactor was covered with a plastic screen (size of 2 mm per hole). The moisture of vermicast in each vermireactor was kept by spraying the water every two days. Besides, the dead earthworm and cocoon were also checked. Vermicasting process was conducted for 28 days. Vermibed was taken out of each vermireactor and placed into different plastic buckets. The earthworm and cocoon were sorted out from vermicast using a hand sorting technique. Furthermore, vermicast was also sorted using the same technique to separate vermicast from non-vermicast, and each weight was measured.

**Production and Quality of Vermicast**

Production of vermicast from each shape of vermireactor was determined according to the percentage of successfully converted vermicast into vermicast. Such percentage of vermicast (% w/w) was calculated using formula:

\[
\% \text{ vermicast} = \frac{\text{vermicast weight}}{\text{vermicast weight} + \text{non-vermicast weight}} \times 100\%
\]

The quality of vermicast was measured through some parameters such as pH, organic-C, total-N, C/N ratio, P₂O₅, and the number of morphological characters of bacterial colonies. pH vermicast was determined according to the concentration of dissolved H⁺ in water and measured using pH meter. Organic-C content was determined according to measurement on the intensity of the formed green color of Cr³⁺ due to orange Cr⁶⁺ was reduced by organic carbon under acid condition. The intensity of that green color was measured using a spectrophotometer at 561 nm wavelength. Total-N was determined using the Kjeldahl method and the intensity of formed indophenol blue color was measured using a spectrophotometer at 636 nm wavelength. Content of total-P (P₂O₅) was determined using an extract of HCl 25 N, and the intensity of appeared blue color due to the application of staining P reagent was measured using a spectrophotometer at 889 nm wavelength. All measurement methods for those chemical parameters followed the technical guidance of chemical analysis for soil, plant, water, and fertilizer published by Soil Research Institute (Eviati and Sulaeman 2009).

Morphological characters of the bacterial colony were identified by characterizing the bacterial culture on an agar plate (Pollack *et al.* 2009). A total of 10 grams of a composite sample from each
vermicast put in different Scoot bottle 250 ml, then added 100 ml of sterile distilled water, and it was shaken using a reciprocal shaker for 24 hours (Germida and de Freites 2008). A total of 50 îL of each diluted suspension (10^{-8} and 10^{-9}) was spread on the culture medium Tryptic Soy Broth (TSA) in a different petri dish labeled with K01, K02, K03,…, and K16. Each petri dish was incubated at room temperature for 48 hours. To obtain single colonies, the colonies formed on the TSA media were taken using the different needle and then transferred onto the TSA media surface in the other petri dish, and incubated for 48 hours under room temperature. The morphological characters of each colony formed were identified following the guidelines of Pollack et al. (2009).

### Statistical Analysis

Variations in production and chemical quality of vermicast between main plots, subplots, and interactions were analyzed using analysis of variance (ANOVA) at the p < 0.05 level. The homogeneity of the variance of each data was tested using the Levene Statistic at the p > 0.05 level. The data did not meet the homogeneity of variance was transformed by \( \sqrt{x + 0.5} \), and still did not meet the data could be analyzed using the Kruskal-Wallis H test at the p < 0.05 level. The difference among the treatments was used Least Significant Difference (LSD) test at the p < 0.05 level. Variations in the number of forms of bacterial colony among different vermicast-shape were analyzed using the descriptive approach.

### RESULTS AND DISCUSSION

#### Vermicast product

Analysis of variance showed that the application of earthworm (Pheretima sp.) significantly affected (p < 0.05) the percentage of successful vermicast converted into vermicast, whereas the shape of vermicast and their interaction were not significant (p > 0.05). The result of the t-test (at p = 0.02 level) revealed that the percentage of formed vermicast in vermicast with the application of Pheretima sp. was higher than without Pheretima sp. for 28 days of vermicast process period (Figure 1).

Figure 1 illustrated that there was no vermicast formed in vermicast without any application of Pheretima sp. (0% vermicast), while 49.24% of vermicast was produced with the application of Pheretima sp for 28 days of vermicasting process. In addition to composition and quality, moisture and aeration of vermicast from a vermicast reactor was also highly determined by the ratio of surface area and volume of vermicast (Jain et al. 2003).

The area of two-dimensional figures of each vermicast shape and tested height of vermicast in this experiment indicated that the ratio of surface area and vermicast volume in vermicast reactor made from two-dimensional figures of cylinder, square and rectangular were 0.50, 0.52, and 1.09, respectively. By comparing those three ratios, it was recognized that ratios of surface area and vermicast volume in rectangular vermicast reactor were 2.10 and 2.18 times higher than those of square and cylinder vermicast reactor, respectively. Figure 2 showed that the

![Figure 1. Proportion of vermicast from vermicast reactor tested with and without earthworm applied. /moment]
production of vermicast tended to increase in the vermireactor cylinder, followed by square and rectangular. However, the difference in the proportion of successful vermicast converted into vermicast among those three vermireactors was not significant (at p > 0.05 level). This implied that the increment up to 2.18 times the ratio of surface area and vermibed volume in vermireactor could not significantly increase the vermicast production of *Pheretima* sp. yet. This fact indicated that the performance of *Pheretima* sp in producing vermicast in established vermireactors from three different two-dimensional figures (cylinder, square, and rectangular) with relatively same area was similar.

**Chemical Quality of the Vermicast**

Chemical indicators of the vermicast quality were measured through parameters of pH, organic-C, total-N, C/N ratio, and P$_2$O$_5$ (extracted with HCl 25 N). Effects of different vermireactor shapes on pH (ANOVA at the p = 0.04), total-N (Kruskal-Wallis at the p = 0.12 level; df = 2, $\chi^2 = 8.828$), and C/N ratio (Kruskal Wallis at the p = 0.001 level; df = 2, $\chi^2 = 13.011$) were significant, while organic-C (ANOVA at the p = 0.101 level) and P$_2$O$_5$ (Kruskal-Wallis at the p = 0.067 level; df = 2, $\chi^2 = 5.396$) were not significant. Comparison of pH, organic-C, total-N, C/N ratio, and P$_2$O$_5$ in vermicast from different vermireactor shapes with the application of *Pheretima* sp was presented in Table 1.

The conversion process of vermicast by earthworms was always followed by the decomposition process of organic material. The release of CO$_2$ to atmosphere occurred during the decomposition process of vermicast and also followed by the release of some organic acids into the soil environment (Amouei et al. 2017). These released organic acids were extremely potential to alternate pH substrate (Subramanian et al. 2010). Table 1 showed that pH vermicast produced from three vermireactors was 6.08 - 6.60 in range. pH vermicast of cylinder vermireactor was the significant lowest among the other two shapes of vermireactor (at p < 0.05 level). This low pH value
was assumed correlating with higher produced organic acids during the engineering process of vermicast by earthworms in-cylinder vermireactor than those of other shapes.

During the vermicast process in vermireactor, the activity of earthworm contributed to alteration on contents of organic-C, total-N, as well as C/N ratio in vermicast (Pramanik 2010; Huang et al. 2014). This research found that the difference in the number of organic-C of vermicast produced from three shapes of vermireactor was not significant at p > 0.05 level (Table 1). In contrast, the content of total-N of produced vermicast from the shape of the vermireactor was significantly different (at p<0.05 level). Though, the difference in content of total-N vermicast from cylinder and square vermireactors was not significant (at p > 0.05 level). Total-N of vermicast from each vermireactor compared to total-N vermicast from square vermireactor was significantly different at p < 0.05 level (Table 1). The difference in content of total-N vermicast among shapes of vermireactor might due to the difference in the content of N-excreta (mucus and urine) produced by earthworm (Needham 1957; Salmon 2001). Value of C/N ratio in vermicast from rectangular vermireactor was the highest and significantly different compared to other two vermireactors (at p < 0.05 level), while the lowest C/N ratio was from cylinder vermireactor, and this value was also significantly different (at p < 0.05 level) compared to the value of C/N ratio in vermicast from square vermireactor. These C/N ratio values described that quality of vermicast produced from three shapes of vermireactor varied. The difference in content of P2O5 in produced vermicast from each shape of the vermireactor was not significant at p < 0.05 level (Table 1). Many other researchers reported that the content of total-P in vermicompost produced from rectangular vermireactor increased in comparison with the initial content of total-P in vermicast (Pramanik et al. 2007; Sharma and Garg 2018). This increment was possibly related to enzymatic activities (acid and alkaline phosphatase enzymes) from phosphate dissolving bacteria in gut and cast of earthworms which were capable of releasing phosphorus (Jones and Oburger 2011; Soobhany et al. 2015). In this research, the content of P from vermicast was extracted using strong acid (HCl 25 N), and this result showed that difference in the content of P2O5 in vermicast from three shapes of vermireactor was not significant at p > 0.05 level (Table 1). This fact explained the possibility that the performance of Pheretima sp. to stimulate the activity of acid and alkaline phosphatase enzymes producing microbial population was similar in three shapes of vermireactor.

### Number of Bacterial Colonies

A total of sixteen morphological types of the bacterial colony was identified in vermicast produced from three different shapes of vermireactors at the 28 days after the vermicast engineered by earthworm (Pheretima sp.) activity. The various morphological types of the bacterial colony were presented in Table 2.

Many heterotrophic bacteria in vermicast could be cultured under laboratory conditions (Olsen and Bakken 1987). Morphological characteristics of an established bacterial colony in a cultural medium might be used to represent their environmental condition and biochemical history (Cooper et al. 1968). Such characteristics could be utilized to explain the relationship between physicochemical conditions and bacterial diversity on the substrate, especially vermicast (Lebaron et al. 1998; Franklin et al. 2001). The richness in the type of morphological colony was also frequently used to represent the alteration on bacterial diversity due to the change of physicochemical conditions in vermicast (Müller et al. 2002). Physicochemical factors, such as pH, organic-C, and C/N ratio of substrate were positively correlated with the richness of bacteria (Ge et al. 2010).

Table 2 showed that there were three-, eleven-, and six-morphological types of bacterial colonies found in vermicast produced from a cylinder, square, and rectangular vermireactors, respectively. Morphological colony of K09 and K10 types were only observed in vermicast produced from cylinder vermireactor, K14, K15, and K16 types were solely found in vermicast of rectangular vermireactor, and morphological types noticed with K01, K02, K03, K04, K05, K06, K07, and K08 were merely found in vermicast of square vermireactor. The morphological type of K13 was found in vermicast produced from all shapes of the vermireactor. Wu et al. (2017) reported that pH substrate was an important factor in establishing the richness of bacteria. Thus, it could be explained that variation in the morphological colony of bacteria occurring among shapes of vermireactor was strongly related to a significant difference in pH of vermicast among vermireactors. The richness of bacterial species was the highest at near-neutral pH and slightly decreased from near-neutral pH to alkaline pH (Zhang et al. 2017). The value of the C/N ratio was one of the indicators for the quality of substrate associating with the availability of carbon and nitrogen for the growth of various bacteria (de Menezes et al. 2015). A low value of the C/N ratio enabled the substrate to produce higher ammonia restricting the growth of various bacterial populations, whereas high ratio
caused the shortage of available nitrogen for supporting the growth of various bacteria (Egli and Quayle 1986). Based on C/N ratio in those vermicasts (Table 1), it could be expressed that the lowest number of morphological types in the bacterial colony in vermicast of cylinder vermireactor might be caused by high production of ammonia, which might inhibit the bacterial growth (Leejeerajumnean et al. 2000). Number in types of morphological colony in vermicast from rectangular vermireactor was more than vermicast from cylinder vermireactor, and less than that in vermicast from square vermireactor. Such findings were probably due to the availability of nitrogen limiting the growth of various bacteria. The number of colony morphotypes in vermicast from square vermireactor might be highly associated with the balanced availability of carbon and nitrogen in vermicast enabling the growth of various bacterial populations (Mooshammer et al. 2014). It indicated that the shape of the vermireactor had an implication on the diversity of bacterial morphotypes in vermicast.

Table 2. Morphological type of bacterial colony identified in vermicast during 28 days of vermibed modified in the different of vermireactor shape.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Morphological characteristics of the bacterial colony</th>
<th>Presence of the bacterial colony type from cast produced by different vermireactor shape</th>
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<tr>
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<tr>
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<tr>
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Note: 1 is indicated present; 0 is indicated absent; K is symbol of petridish where bacteria grown on TSA medium.

CONCLUSIONS

Production of vermicast from earthworm (Pheretima sp.) using a mixture of soil from land with C. odorata vegetation and compost of pruned C. odorata as vermibed was similar between reactor in forms of cylinder, square and rectangular. The difference in chemical quality of produced vermicast from various shapes of vermireactor can be found on pH, total-N, and C/N ratio. Variation on morphological characters of bacteria in vermicast from Pheretima sp. produced by square vermireactor was more than the cylinder and rectangular vermireactors.

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REFERENCES


