



# Biological Quality of Horse Manure Compost with the Addition of *Azotobacter* Culture Based on the Vegetative Growth of *Pennisetum purpureum* and *Glycine max* (L) Merrill

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**Abstract.** As pseudoruminant animals, horse manure is the most ideal compost material among animal feces due to its high C/N ratio of 25 or more. The addition of decomposers such as *Azotobacter* microbial culture will accelerate the process of decomposition. The aim of the study was to analyse the biological quality of horse manure compost with the addition of *Azotobacter* culture based on the vegetative growth of *Pennisetum purpureum* and *Glycine max* (L) Merrill. The materials used were horse manure, *Azotobacter* culture, *Pennisetum purpureum* and *Glycine max* (L) Merrill seeds. The research was done experimentally with 4 treatments (addition of culture *Azotobacter* of T<sub>1</sub> (0 ml), T<sub>2</sub> (150 ml), T<sub>3</sub> (250 ml) and T<sub>4</sub> (350 ml/100 kg of mixed compost materials wet based) and 6 replications. The variables observed were number of leaves, plant height and number of tillers of *Pennisetum purpureum*; and number of leaves, plant height and number of plant branches of *Glycine max* (L) Merrill. The result showed that the treatments have highly significant effect ( $P < 0.01$ ) to all variables observed. The best treatment was the highest addition of *Azotobacter* culture (350 ml). The conclusion is that the addition of *Azotobacter* culture to horse manure compost had positive effect to the vegetative growth of *Pennisetum purpureum* and *Glycine max* (L) Merrill.

**Keywords:** Animal Waste Management · Decomposition · N-fixing Microbes From The Air

## 1 Introduction

Faeces are the result of residual digestive processes in the body of animals. Horse faeces contain hemicellulose of 23.5%, cellulose 27.5%, lignin 14.2%, nitrogen 2.29%, phosphate 1.25% and potassium 1.38% [1]. Manure from horse faeces ferments and heats up faster than cow manure and pig manure [2]. Horse faeces have a higher carbon and nitrogen content than cow faeces. In addition, it also contains cellulose, hemicellulose, phosphate and potassium which is higher than cow faeces, except for the lignin content. Cow faeces has a higher lignin content than horse faeces [3]. The association between

non-symbiotic N fixation with plants is the contribution of N to plants. The average N fixation ability of *Azotobacter* sp. is 10 mg N/g sugar in pure culture in N-free medium and the maximum value is 30 mg N/g sugar [4].

*Azotobacter* sp. is a gram negative bacteria, aerobic, polymorphic and has various shapes and sizes. These bacteria produce polysaccharides. *Azotobacter* sp. is sensitive to acids, high salt concentrations and temperatures above 35 °C [5]. *Azotobacter* sp. is a mesophyll soil bacterium with an optimum temperature of around 35 °C. EPS production of *Azotobacter* sp. was higher at a temperature of 30 °C than at room temperature for either 24, 36 or 48 h of incubation. *Azotobacter* sp. lives optimally at a neutral pH [6]. The aim of the study was to analyse the biological quality of horse manure compost with the addition of *Azotobacter* culture based on the vegetative growth of *Pennisetum purpureum* and *Glycine max* (L) Merrill.

## 2 Materials and Methods

The research was carried out using an experimental method, with completely randomized design of 4 treatments and 6 replications. Each replication consisted of 3 plants of *Pennisetum purpureum* or *Glycine max* (L) Merrill so that 72 plants were obtained of each plant. The treatment given were:

- T0: 100 kg of horse feces (without *Azotobacter* culture)
- T1: 100 kg of horse feces + 150 ml of *Azotobacter* culture
- T2: 100 kg of horse feces + 250 ml of *Azotobacter* culture
- T3: 100 kg of horse feces + 350 ml of *Azotobacter* culture (Cholis *et al.*, 2016)

The research begins with the preparation of compost raw materials, including:

1. Collected fresh horse faeces and dried in the sun to a moisture content < 10%.
2. Large-sized stools are pressed by hand so that they are relatively the same size so that it is easier to homogenize.
3. Uniformity of particle size is done by sieving.
4. Horse faeces were weighed as much as 100 kg for each treatment.

The next stage is the preparation of the *Azotobacter* culture decomposer solution which will be given to the compost material, including:

1. Prepared equipment and materials to be used, including measuring glass and buckets, *Azotobacter* culture, water and molasses.
2. Prepare *Azotobacter* culture using a measuring glass according to the treatment used.
3. *Azotobacter* culture was mixed with 25 L of water and 5 L of molasses.
4. All ingredients are homogenized until evenly mixed, then put into a sprayer.
5. The decomposer is ready to use.

The process of making horse manure compost with the addition of *Azotobacter* culture can be seen in the following Fig. 1.

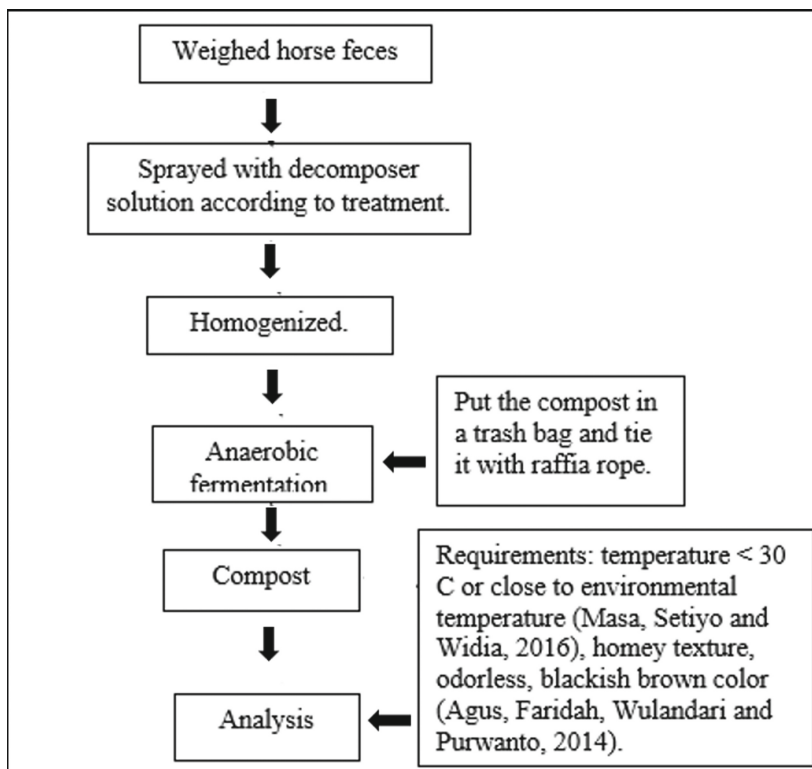


Fig. 1. Diagram of horse manure composting

The implementation of the trial plant planting begins with the preparation of planting media in poly bags with a ratio of top soil and compost 3:1. Before being used as a mixture of planting media, the fermented compost is aerated for one day to reduce heat and gas. Elephant grass stems which will be used as seed cuttings are separated from the leaves and cut to a length of approximately 20–30 cm (contains at least 2 nodes). At the time of cutting, the stem must not be broken, the tip of the cutting must be pointed at an angle of 45 degrees [7], at least 1 node must be above/below the soil surface. The selection of the best quality *Glycine max* (L) Merrill starts from seeds that are whole, not hollow or cracked and of uniform size. With a spacing of 20 × 20 cm, then thinned at the age of one week after planting, leaving one plant per polybag. Maintenance activities include watering activities carried out every morning and evening except when it rains, cleaning from weeds and controlling pests and diseases using insecticides.

Observation variables on *Pennisetum purpureum* were plant height, number of leaves and number of tillers which were carried out on week 1, 2, 3, 4, 5, 6, 7 and 8 after planting. Variables observed in *Glycine max* (L) Merrill were plant height, number of leaves and number of branches carried out in the 8th week after planting. The data obtained was then analysed using analysis of variance.

### 3 Results and Discussion

#### 3.1 Temperature and pH During Composting

Temperature and pH are important indicators that determine the success of the composting process. Temperature and pH can indicate the level of activity of microorganisms that decompose organic matter into compost. The results of the observation that the compost temperature and pH were presented in Tables 1 and 2. Temperature measurements during the composting process took place every day for 6 days, in the morning. Turning of compost is done every two days to increase aeration in the compost heap.

In Table 1, it can be seen that the average initial temperature for all treatments was 27 °C at the beginning of composting (Day 1). The temperature quickly increased on the second day and then decreased on the third day to the sixth day, returning to the same as the beginning of composting. The composting process at T<sub>0</sub> is less than optimal. At T<sub>0</sub> and T<sub>1</sub>, the temperature began to rise on the third day. The highest temperature increased only reached 29 °C on the second day, but of T<sub>2</sub> and T<sub>3</sub> experienced a temperature increase of up to 33 °C. After reaching its peak, the temperature then decreases until the end of composting to be as it was at the beginning, which is 27 °C. According to Widiarti [8] an increase in temperature can occur quickly in the compost heap. Temperatures of 30–60 °C indicate composting activity or rapid decomposition of organic matter.

As pseudo ruminants, horses produce faeces that also carry decomposing bacteria from the caecum. The addition of *Azotobacter* microbial culture makes the number of bacteria that decompose organic matter increase, and the decomposition process runs faster. Decomposition of organic matter, in addition to producing NH<sub>3</sub>, CO<sub>2</sub> and water vapor is also heat. This heat production causes an increase in the temperature of the compost. Suwatanti and Widiyaningrum [9] said that the increase in temperature occurred due to the activity of bacteria in decomposing organic matter. The decrease in the temperature of the compost after reaching its peak is caused by the reduced amount of organic matter that can be decomposed.

Turning treatment every two days also plays a role in reducing the temperature of the compost. In this study, on the sixth day the temperature of the compost was the same as the initial temperature of the compost. The compost height <30 cm in this study caused the high temperature achieved to be lost more quickly. Based on the available temperature data for all treatments, it can be concluded that the composting process takes place at a mesophilic temperature, which is below 45 °C. In the early stages of the composting

**Table 1.** Average compost temperature (°C)

T	Days to					
	1	2	3	4	5	6
T <sub>0</sub>	27	29	28	28	28	28
T <sub>1</sub>	27	30	28	28	28	28
T <sub>2</sub>	27	33	30	29	28	27
T <sub>3</sub>	27	33	30	29	28	27

**Table 2.** Average compost pH.

T	Days to					
	1	2	3	4	5	6
T <sub>0</sub>	6.5	6.5	6.5	7	7	7
T <sub>1</sub>	6.5	5	5	5.5	6	6.5
T <sub>2</sub>	5	5	5.5	6	7	7
T <sub>3</sub>	5	5	5.5	6	7	7

process the role is mesophilic bacteria that live at a temperature of 20–40 °C. According to Suwatanti and Widiyaningrum [9] mesophilic conditions are more effective because the activity of microorganisms is dominated by fungi and proteobacteria.

Compost is considered mature if the temperature is close to the ambient temperature. The final composting temperature on the sixth day for all treatments, namely T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were 28 °C, 28 °C, 27 °C and 27 °C, respectively. These results were in accordance with the provisions of the National Standardization Agency 19-7030-2004, which must be in accordance with groundwater temperature or a maximum of 30 °C. The pH measurement was carried out every day along with the temperature measurement. As shown in Table 2, during the composting process, all treatments tended to be acidic with a pH ranging from 5–6.5 on the first to third day. This possibility is caused by the decomposition of organic matter which produces simple organic acids, but over time the pH has increased due to the decomposition of protein elements and the release of ammonia [10]. pH at the end of composting for all treatments reached 7 (neutral) except for T<sub>1</sub> which was still close to neutral or 6.5. This is in accordance with the compost pH requirements at the National Standardization Agency 19-10030-2004 which has a value of 6.80–7.49. According to Widarti et al. [11], the final pH that is close to neutral is caused by the decomposition process of resistant materials such as lignin, hemicellulose and cellulose by fungi and *Actionmycetes*.

### 3.2 Planting Media Quality

Good quality compost is one that contains the nutrients that plants need. Table 3 shows the results of the laboratory analysis of the nutrient content of compost, fresh horse faeces and soil in the study. It was seen from Table 3 that the addition of *Azotobacter* culture was able to reduce the organic C of the compost, but in this study with an irregular pattern. T<sub>1</sub> has almost the same organic C as T<sub>0</sub> but T<sub>2</sub> should have higher organic C than T<sub>3</sub> but in this study the value was much lower (5.3 vs 26.4%). It is possible for errors to occur during analysis in the laboratory either due to human or equipment errors.

T<sub>1</sub> had a highest value of C/N (52). The cause of this phenomena was not known, maybe there was some mistaken in laboratory analysis because logically it should be that T<sub>1</sub> had C/N lower than T<sub>0</sub> because of the presence if addition microbe population of *Azotobacter* culture. The same phenomena also happened to percentage of Organic Matter. In this study, percentage of total N of T<sub>0</sub> was the highest i.e. 1.6% vs. 1.0–1.1 of other treatments. The highest percentage of P in this study was reached by T<sub>0</sub>.

**Table 3.** The nutrient content of compost, fresh horse faeces and soil used in the study

Parameter	Fresh Horse Feces	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	Soil
Water (%)	9.6	10.0	9.5	9.2	9.3	3.3
Org.C (%)	56.3	54.1	53.4	5.3	26.4	1.1
Tot N (%)	0.9	1.6	1.0	1.1	1.1	0.07
P2O5 (%)	1.4	3.1	1.9	2.1	2.0	135 ppm
K2O (%)	2.7	1.6	1.7	1.7	1.8	–
C/N	59.3	33.6	52.3	23.7	23.0	15.8
OM (%)	97.2	93.3	92.1	43.7	45.5	1.9
Form	Crumb	crumb	crumb	crumb	crumb	crumb
Color	light brown	brown	dark brown	dark brown	dark brown	black
Smell	horse faeces smell	a little rotten	sour	smells like earth	smells like earth	earthy smell

The K percentage of all treatments were between 1.6–1,8%. In general, compost of T<sub>3</sub> was the best planting media followed by T<sub>2</sub> based on requirements at the National Standardization Agency 19-10030-2004.

### 3.3 Effect of Treatment on Plant Height, Number of Leaves (*Pennisetum purpureum* and *Glycine max* (L) Merrill), Number of Tillers (*Pennisetum purpureum*) and Number of Branches (*Glycine max* (L) Merrill)

Biological test or planting is the best test tool to determine the quality of compost. In the Table 4, the average plant height (cm) and number of leaves of *Pennisetum purpureum* and e *Glycine max* (L) Merrill; number of tillers (*Pennisetum purpureum*) and number of *Glycine max* (L) Merrill branches is presented. [12] stated that the increase in vegetative characters such as plant height, number of leaves and number of branches was caused by the role of the element Nitrogen (N). The main role of Nitrogen for plants is to stimulate overall growth, especially stems, branches and leaves. In this study, percentage of total N of T<sub>0</sub> was the highest i.e. 1.6% vs. 1.0–1.1 of other treatments but because the value of C/N of T<sub>0</sub> also high, 33,6. T<sub>1</sub> had a highest value of C/N (52).

The cause of this phenomena was not known, maybe there was some mistaken in laboratory analysis because logically it should be had C/N lower than T<sub>0</sub> because of the presence if addition microbe population of *Azotobacter* culture. Putro et al. [13] said that high C/N (>30) causes slow decomposition and inhibits plant growth. Nitrogen in plants plays an important role in the production of chlorophyll and protein synthesis [14]. It was further stated that when there is a shortage of N, plants will grow stunted. Root growth is stunted and leaf color turns yellow so that overall plant growth will be stunted.

**Table 4.** The average plant height (cm) and number of leaves of *Pennisetum purpureum* and *Glycine max* (L) Merrill; number of tillers (*Pennisetum purpureum*) and number of *Glycine max* (L) Merrill branches.

Variables	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Plant Height (cm)				
– <i>Pennisetum purpureum</i>	146.7 <sup>a</sup> ± 4.9	143.2 <sup>a</sup> ± 5.6	156.0 <sup>b</sup> ± 4.3	166.0 <sup>c</sup> ± 3.6
– <i>Glycine max</i> (L) Merrill	37.6 <sup>a</sup> ± 3.4	36.4 <sup>a</sup> ± 2.6	43.2 <sup>b</sup> ± 1.0	43.6 <sup>b</sup> ± 2.2
Number of leaves				
– <i>Pennisetum purpureum</i>	27.7 <sup>a</sup> ± 2.5	26.8 <sup>a</sup> ± 1.9	32.2 <sup>b</sup> ± 1.0	33.8 <sup>b</sup> ± 1.8
– <i>Glycine max</i> (L) Merrill	37.4 <sup>a</sup> ± 2.4	35.7 <sup>a</sup> ± 4.2	44.7 <sup>b</sup> ± 2.1	48.2 <sup>b</sup> ± 4.4
Number of tillers				
– <i>Pennisetum purpureum</i>	2.3 ± 0.6	2.6 ± 0.5	2.9 ± 0.5	3.1 ± 0.4
Number of branches				
– <i>Glycine max</i> (L) Merrill	4.8 <sup>a</sup> ± 0.5	4.4 <sup>a</sup> ± 0.4	6.0 <sup>b</sup> ± 0.6	6.6 <sup>c</sup> ± 0.56

Note: <sup>a-c</sup> Different superscripts on the same line were highly significant different ( $P < 0.01$ )

Leaves are the most important plant organs because all the nutrients obtained will be processed by the leaves through the process of photosynthesis for plant growth and development. Nurrohman et al. [15] stated that the development of plant tissue is strongly influenced by macro and micro nutrients and planting media. Parnata [16] states that nitrogen is very useful for stimulating leaf growth, while phosphorus and potassium function to stimulate fruit formation.

The average number of leaves of *Pennisetum purpureum* and *Glycine max* (L) Merrill treated with compost with the addition of *Azotobacter* culture obtained higher yields ( $P < 0.01$ ) started from T<sub>2</sub> treatment. Overall in this study, smallest addition of *Azotobacter* culture gave the same performance of vegetative growth with T<sub>0</sub>. In the plant *Pennisetum purpureum*, T<sub>3</sub> produce the highest number of leaves, followed by T<sub>2</sub>. They have same percentage of total N but T<sub>3</sub> has lower of C/N compared to T<sub>2</sub>.

Riyantini et al. [17] stated that the results of the photosynthesis process in the form of carbohydrates will be used as food reserves, then will be accumulated in young growing tissues, so that it greatly affects plant height, and the number of branches becomes optimal. This is supported by the statement of Rahman et al. [14] that plants that have a higher average number of leaves at the beginning of their growth will grow faster because of their ability to produce photosynthate which is higher than plants that have a lower average number of leaves.

Furthermore, it is said that the number of plant leaves will affect the growth of other plant tissues. Rahman et al. [14] stated that N has an important role in chlorophyll production and protein synthesis. When there is a shortage of nitrogen, plant leaves will grow stunted, root growth is stunted and leaves turn yellow so plant growth is stunted. This is in line with the results of a study conducted by Khaerunnisa et al. [18] that nitrogen fertilization has a significant effect on the number of leaves leaf width and leaf

area, although the number of leaves and leaf size are also influenced by genotype and environment. Agustina [19] also stated that nitrogen is useful for plants to accelerate plant growth in general, especially in the vegetative phase, plays a role in the formation of chlorophyll, forming fats, proteins and other compounds. Furthermore, it is said that the macro nutrients P and K contained in the compost also play a role in the growth and development of plant organs. P nutrients play a role in photosynthesis, while K plays a role in the formation of starch, translocation of photosynthetic products and helps the formation of chlorophyll.

The number of tillers is one of the variables used in the planting test, which can show growth and development, especially in the vegetative phase. The number of tillers in question are all young individuals that emerge from the base of the plant in a clump of plants [20]. In Table 4 it can be seen that the application of horse compost fertilizer given *Azotobacter* culture had no significant effect ( $P > 0.05$ ) on the number of tillers of *Pennisetum purpureum*, but it is highly significant effect to the number of tillers of *Glycine max* (L) Meril ( $P < 0.01$ ). There is a tendency of increasing number of tillers of *Pennisetum purpureum* along with the higher dose of *Azotobacter* culture addition. The average number of tillers of *Pennisetum purpureum* were between 2.3–3.1. The number of tillers of *Glycine max* (L) Meril T<sub>3</sub> showed the best treatment, with an average result of  $4.2^b \pm 0.6$  unit, followed by T<sub>2</sub>, T<sub>0</sub> and T<sub>1</sub> which were  $2.2^a \pm 0.2$ ,  $2.3^a \pm 0.3$  and  $2.2^a \pm 0.2$ , respectively.

In general, the number of grass tillers that grow is determined by how the plants are cared for, including the presence or absence of weeding. It aims to accelerate the formation of tillers. The incoming sunlight also affects the growth of new tillers. Sulaiman et al. [21] stated that sunlight entering the land greatly affects plant tillers, the higher the sunlight, the more the number of tillers.

## 4 Conclusions

Based on the result of the study, it was concluded that the addition of *Azotobacter* culture to horse manure compost had positive effect to the vegetative growth of *Pennisetum purpureum* cv. Mott and *Glycine max* (L) Merill. The best treatment is the addition of 350 ml/100 kg compost material of *Azotobacter* culture.

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