Application of Fermented Maja Fruit (*Aegle marmelos*) and Bamboo Shoot (*Bambusa oldhamii*) in Rabbit Manure Composting

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Abstract. This research aims to know the effect of indigenous microorganisms (MOL) from fermentation products of the maja fruit and bamboo shoot mixture on the characteristics and quality of rabbit manure composting. Physical parameters from fermented maja fruit and the bamboo shoots, the observation of growth MOL on the solid and liquid medium, gaseous NH_3 reduction measurement, colony calculation of MOL, and quality of the physical, chemical, and biological were observed. The NH_3 gas reduction was determined upon adding different levels of MOL in rabbit manure 0% (blank), 1%, 3%, and 5% of the total weight of rabbit manure. This research showed that adding fermented products from the mixture of maja fruit and bamboo shoots at 5% gave the best results in decreasing ammonia levels after 14 days of composting. The chemical quality observation result showed that the addition of 5% has a significant effect on the increases in total N compost. The compost product had moisture content at 4.88%, organic matter at 53.03%, C-organic at 30.75%, P-total at 0.38%, K-total at 0.59%, N-total at 4.48%, and C:N Ratio at 6.9%. This research concludes that adding 5% showed the best result in decreasing the ammonia emission levels and increasing the quality of the total nitrogen during rabbit manure composting.

Keywords: Ammonia emission · bamboo shoots · compost · maja fruit · rabbit manure

1 Introduction

Rabbit manure can be used as an excellent organic compost and organic fertilizer. A rabbit can produce manure of about 28 g per day [1]. Rabbit manure contains nutrients N (2.44%), Mg (2.03%), S (0.58%), P (2.20–2.76%), K (1.86%), (C:N: (10–12%), and Ca (2.08%) [2]. Based on these nutritional content, rabbit manure compost is one of the primadonnas as organic fertilizer used in agriculture [3]. During the composting process, ammonia and sulfur are mainly released when organic matter is actively decomposed [4].

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The concentration of these compounds is closely related to this phenomenon. The emergence of unpleasant odors from composted feces, one of these compounds is ammonia (NH_3), which at high enough concentrations can interfere with human health and can reduce a large amount of nitrogen content during processing. This incident led to a decrease in the value of compost as fertilizer and complaints about odors and pollutants that may cause social conflicts. Based on this phenomenon, it is necessary to develop a potential bio-starter in the composting process of rabbit manure so that pollutants odors can be minimized and accelerate the composting process. For example, one alternative bio-starter can use local microorganisms (MOL) obtained from the fermentation of several local materials containing bioactive compounds to inhibit the formation of ammonia gas emissions. In addition, the fermentation process can be carried out to make functional microorganism bio-activators [5]. The materials potentially used as raw materials for MOL production are bamboo shoots [6] and maja fruit [7].

Bamboo (*Bambusa oldhamii*) is a plant that can be used as a vegetable in the form of bamboo shoots. It is further known that the product's fermentation of bamboo shoots contains a lot of lactic acid bacteria, including *Lactobacillus brevis*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Pediococcus acidilactici,* and *Leuconostoc fallax*. These strains showed antimicrobial activity against pathogenic Gram-negative and Gram-positive bacteria [8, 9]. Maja fruit (*Aegle marmelos*) is commonly used as an herb for several diseases in humans, and it has also been found that contains antibacterial compounds. Maja fruit contains several bioactive compounds, such as marmelosin, psoralin, luvangetin, aurapten, and marmelide [10]. Recent studies found that marmelosin has good potential as an antibacterial [11, 12] and urease inhibitor [13]. Based on the explanation above, Local Microorganisms (MOL) fermented products of maja fruit and bamboo shoots are thought to have the ability to inhibit the release of ammonia gas by microorganisms. Therefore, it is necessary to research the effect of adding fermented products of maja fruit and bamboo shoots fermented products to reduce ammonia gas levels and the quality of rabbit feces compost.

2 Materials and Methods

2.1 The Preparation of the Materials

2.1.1 Preparation of Local Microorganism (MOL)

This study used materials to produce MOL: unripe maja fruit from the local district (Turi) and bamboo shoots from the local market (Kranggan) in Yogyakarta. The composition material to produce MOL was conducted from 1 kg of the flush of maja fruit and 1 kg of bamboo shoots chopped, crushed, and put in the chamber. Then 300 g of molasses was dissolved in 2 L of water and put in a fermenter (Fig. 1). The mixture of these ingredients is fermented for 16 days and then filtered. MOL harvesting is done by filtering the fermentation products in the digester. The filtrate is a MOL solution used as a bio-starter agent in composting rabbit feces.

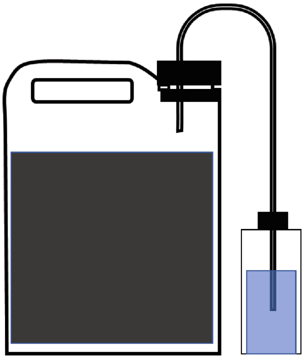


Fig. 1. Fermentor design

2.1.2 Preparation of Mediums

Stock solution (100 mL) contained 1 g of meat extract, 1 g of biological peptone, 0.5 mL of NaCl, and 70 mL of distilled water. The medium was set at pH 7.2 and then distilled water was added until it reached a volume of 100 mL. The medium was poured into a 100 mL Duncan tube and stored in the freezer. Agar medium (100 mL) contained 1 mL of stock solution, and 1.5 g of agar powder were put in an Erlenmeyer containing 99 mL of distilled water. The agar medium is stirred and heated until it boils. The agar medium was covered with cotton plaque and aluminum foil and then sterilized by autoclaving at 121 °C for 15 min. The liquid medium containing 1 mL of stock solution was put in an Erlenmeyer containing 99 mL of distilled water. The medium was covered with cotton plaque and aluminum foil, then sterilized by autoclaving at 121 °C for 15 min.

2.2 Measurements and Observation Methods

2.2.1 The Growth Observation of MOL Product

A total of 1 mL of MOL solution was put into a sterile bottle. The samples were serially diluted 10^{-1} to 10^{-4} using sterile distilled water. The results of the 10^{-2}, 10^{-3}, and 10^{-4} dilutions were taken at 100 μL using a micropipette, grown on agar medium using the pour plate method, and incubated at room temperature for 3 days. Furthermore, observations and calculations of the number of colonies that grew were carried out. Observation of MOL Growth in liquid medium. Measurement of optical density (OD). A total of 1 mL of MOL was added to 100 mL of stock liquid medium, then shaken at a speed of 120 rpm. Every 2 days, 1 mL was taken to measure bacterial growth on a spectrophotometer with a wavelength of 600 nm.

2.2.2 Composting Rabbit Manure

As much as 500 g of rabbit manure was put into a plastic bottle (1.5 L). Then the MOL solution was added according to the treatment (0% (blank/control), 1%, 3%, and 5%). The manure was fermented for 14 days. To determine the emission of ammonia gas by MOL. The gas formed was flowed with an aerator through a plastic hose into an

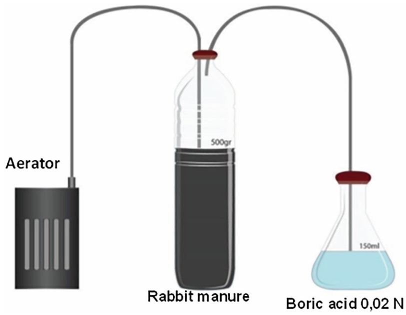


Fig. 2. Ammonia (NH_3) trapping design during rabbit manure composting

Erlenmeyer (250 mL) containing 200 mL of 0.02 N boric acids to capture NH_3 gas [14]. Equipment for capturing NH_3 gas in a series is shown in Fig. 2. NH_3 gas trapped in 0.02 N boric acid was analyzed by the Nessler method. As much as 1 mL of sample (taken from a tube containing boric acid and NH_3 mixed with 0.2 mL of Nessler's solution) will produce a yellow-to-brown color. The color formed is measured by absorption with a spectrophotometer at a wavelength of 425 nm.

2.2.3 Measurement of the Compost Quality

After 14 days, the compost samples were taken, and the physico-chemical and microbiological quality were measured. Determining the physical quality of compost included observing the color, odor, texture, and temperature. The chemical quality of compost was determined by the moisture, pH, organic compound, total Nitrogen, Phosphor, Kalium, Carbon, and C:N ratio [15]. For the microbiological test, each compost sample will be seen the number of microbial colonies contained in it by using the total plate count (TPC) method with the planting method (spread plate). This method uses 1 g of compost sample added with 9 ml of distilled water. The extracts were then vortexed and planted on nutrient agar with dilutions of extracts 10^{-3}, 10^{-4}, and 10^{-5}. One day later, colonies were observed on the nutrient agar in the 30 to 300 colonies range. The bio-starter medium was also carried out by a spread plate method with dilutions of 10^{-3}, 10^{-4}, and 10^{-5} on nutrient agar.

2.3 Data Analysis

The obtained data of fermented maja fruit and bamboo shoot products, observations of MOL growth on agar and liquid medium, and the calculation of MOL colonies were analyzed descriptively. The data on the ammonia gas emission and compost quality (physico-chemical and microbiological) were analyzed using a *Completely Randomized Design* (One-Way ANOVA). If it showed significant results, it was followed by the analysis of *Duncan's New Multiple Range Test* (DMRT) [16].

3 Result and Discussion

3.1 The Characteristic of Maja Fruit and Bamboo Shoots Fermented Products

The results of MOL liquid from the fermentation of maja fruit and bamboo shoots were carried out for 16 days in the digester and then separated from the solids presented in Fig. 3. The result of observations on the pH value after 16 days was obtained at 3.27. This value indicates that the fermentation is in an acidic medium. The results on the pH value of fermented maja fruit and bamboo shoots may indicate that the bacteria found in the solution are a group of lactic acid bacteria. The results obtained at the time of measurement on the results of fermentation of maja fruit and bamboo shoots were at a temperature of 25 °C. The color of fermentation results obtained a brownish-dark color and had a strong sour aroma. Based on the calculation of the number of colonies that have been carried out, the data obtained from the calculation of the number of colonies obtained an average number of colonies at 2.02×10^5 CFU/mL. Microbial growth during fermentation is influenced by several factors, including C-sources, N-sources, mineral sources, pH, and environmental temperature. In addition, the fermentation may cause pH to decrease into firm acidity because of the growth of lactic acid bacteria [17].

Based on the observations made by measuring the Optical Density (OD) of fermented maja fruit and bamboo shoots, the growth character of MOL was presented in Fig. 4. Based on the graph of bacterial growth of MOL, positive trend results were obtained until the 48^{th} hour and then stable in the next hour. The presence of microbial growth in the liquid medium affects the rate of motion of the graph in the Optical Density test observation. Microbial growth consists of lag, log, stationary, and death phases. The lag phase experienced by microbes is the adaptation phase to adapt to the substrate and its environment to survive and grow on the medium. The length of the adaptation phase varies depending on the speed of adjustment to the surrounding environment. When the lag phase ends, it will be followed by a log phase that the cell microbial begins to grow and divide rapidly. In this phase, the growth rate is strongly influenced by the medium in which it grows, such as pH and nutrient content, temperature, and humidity. The stationary phase occurs after the log phase ends. In this phase, there is an increase

Fig. 3. The solution of maja fruit and bamboo shoots at initial observation (left) and after 16 days of fermentation (right)

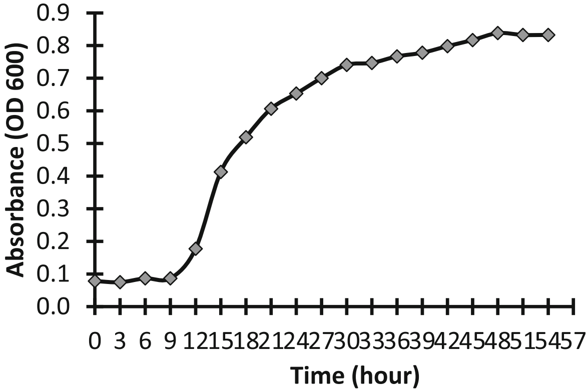


Fig. 4. Graph of microbial growth of MOL from fermented maja fruit and bamboo shoots

in the cell population. In the stationary phase, the number of cell populations remains because the number of cells that grow is the same as the number of cells that die [17]. In this study, it can be seen from the graphical growth (Fig. 4) that the microbes from MOL experienced a lag phase at initial 9 h. Finally, after the 9th hour, there was a drastic increase, and at the 51st hour, they reached the stationary phase.

3.2 Observation of Ammonia Gas Emission During Composting of Rabbit Manure

Based on the observation of ammonia gas emission in rabbit manure compost after 14 days of treatment, data on the concentration of ammonia gas from the compost was divided into 3 times (0, day 7, and 14 days). The analysis of ammonia gas emission observation during composting of rabbit manure is presented in Table 1. Based on the statistical analysis results of measurements of compost ammonia gas emissions with the addition of MOL in several different levels. It shows differences in ammonia concentrations in each treatment. Ammonia comes from the degradation of protein compounds into polypeptides, which are then reformed into amino acids and converted into ammonia through the ammonification process. One of the nitrogen compounds resulting from the transformation of organic N through the ammonification process is ammonia (NH_3) [18].

In addition, the accumulation of ammonia concentration per day shows that the most significant accumulation was in the control treatment (P0), and the smallest was at the level addition of 5% MOL (P3). The additional level of MOL can influence the fluctuations in ammonia accumulation. Fluctuations in ammonia gas emissions in rabbit manure compost during 14 days of treatment are presented in Fig. 5. Based on the graph of the measurement results of ammonia gas released by rabbit manure composting per day, it can be seen that there are fluctuations in ammonia gas emissions for each day. P3 treatment used the most significant addition of MOL, resulting in the lowest ammonia gas emissions from other treatments. The observations in Fig. 5 show that the higher the

Table 1. Analysis of ammonia gas emission from rabbit manure composting

Treatment	Observation (Days)		
	0^{ns}	7	14^{ns}
P0 (blank/control)	3.05 ± 0.78	32.30 ± 8.10^{b}	71.03 ± 25.45
P1 (1% Mol addition)	1.68 ± 0.46	14.20 ± 0.80^{a}	68.90 ± 14.90
P2 (3% Mol addition)	2.07 ± 1.28	15.60 ± 2.75^{a}	39.23 ± 25.80
P3 (5% Mol addition)	2.20 ± 0.94	11.20 ± 1.60^{a}	32.94 ± 6.30

Notes:
^{ns}There were no different significant effects (P > 0.05)
^{a,b}Different superscripts in the same column showed significant differences in effect (P < 0.05)

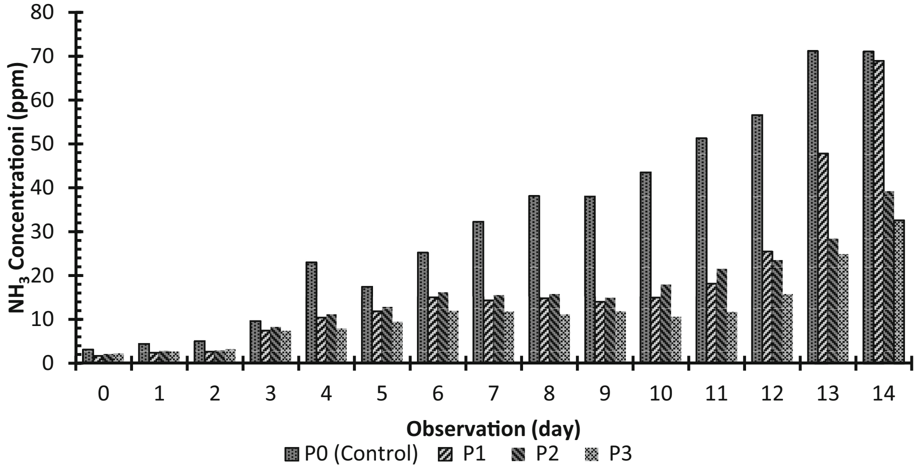


Fig. 5. The fluctuation of ammonia emission during 14 days of rabbit manure composting

concentration of fermented maja fruit and bamboo shoots added, the lower the emission of ammonia gas produced from the rabbit feces compost.

3.3 The Quality of Rabbit Manure Compost

Compost maturity was involved by composting time. Therefore it is necessary to analyze the parameters of physical, chemical, and biological quality, including color, odor, texture, pH, temperature, Moisture, Organic matter, Total C organic, Total N, Total P, Total K, C:N Ratio, and total colony microbial. The observations on the physical quality of rabbit manure compost after 14 days composted can be seen in Table 2. Quality measurement is done by testing the physical quality of rabbit manure compost harvested from 14 days of composting. The measurement of the physical condition includes pH, color, odor, and texture tests. The average results of the physical parameters of the compost showed that the results were brownish-black in color, smelled like soil, and had a loose

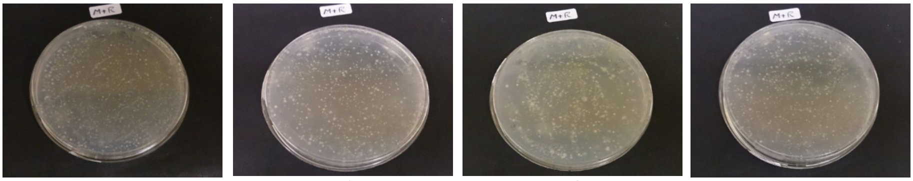
Table 2. The quality of rabbit manure compost after 14 days of fermentation

Composition	Treatments			
	P0 (control)	P1	P2	P3
Color	Brownish-black	Brownish-black	Brownish-black	Brownish-black
Aroma	Like soil	Like soil	Like soil	Like soil
Texture	Loose like soil	Loose like soil	Loose like soil	Loose like soil
pH^{ns}	7.30 ± 0.49	6.90 ± 0.25	7.30 ± 0.36	7.40 ± 0.36
Temperature (°C)^{ns}	27.83 ± 0.29	28.00 ± 0.00	27.83 ± 0.29	27.60 ± 0.52
Moisture (%)^{ns}	5.31 ± 0.28	5.50 ± 0.62	8.12 ± 4.04	6.11 ± 0.41
Organic matter (%)^{ns}	52.93 ± 5.02	55.69 ± 2.26	54.49 ± 1.55	53.02 ± 3.19
Total C organic (%)^{ns}	30.70 ± 2.92	32.30 ± 1.31	31.60 ± 0.90	30.75 ± 1.85
Total N (%)	3.33 ± 0.07^{a}	2.90 ± 0.56^{a}	3.05 ± 0.63^{a}	4.48 ± 0.33^{b}
Total P (%)^{ns}	0.48 ± 0.11	0.40 ± 0.10	0.38 ± 0.06	0.38 ± 0.06
Total K (%)^{ns}	0.51 ± 0.06	0.53 ± 0.12	0.48 ± 0.08	0.58 ± 0.05
C:N Ratio	9.22 ± 1.05^{ab}	11.45 ± 2.55^{b}	10.72 ± 2.59^{b}	6.89 ± 0.85^{a}
Total colony microbial (TPC/mL)	1.03×10^5	1.39×10^5	1.78×10^5	2.43×10^5

Notes:
^{ns}There were no different significant effects ($P > 0.05$)
^{a,b}Different superscripts in the same column showed significant differences in effect ($P < 0.05$)

texture like soil. According to *SNI-19-7030-2004*, the mature compost has the same temperature as the groundwater, is brownish-black, has a texture, smells like soil, has a pH level of 6.5–8.0, and the final temperature has similar to the temperature of groundwater value (<30 °C) [19]. In the other reference, the mature compost no longer releases CO_2 and heat. Compost smells and has an earthy texture, dark brown, or tends to be black [20]. Given the addition of MOL, Rabbit manure requires a relatively faster composting time and needs less than one month to get the physical condition of the crumbs and loose-like soil. Based on the observation data, the average pH value of compost in this study was 6.90–7.40, and the average temperature was around 27.60–28.00 °C. These pH and temperature compost met the requirement according to standard compost as fertilizer *SNI-19-7030-2004.* According to the other study, acidity or pH in compost affects microbial activity. The pH range of a good compost material for composting is around 6.5–7.5 [21].

The rabbit manure compost harvested on the 14th day has shown the physical characteristics of the mature compost. Chemical parameter tests were then carried out to determine the quality of rabbit manure compost. The chemical parameter test of rabbit manure compost showed in Table 2. Table 2 shows that the chemical composition has no different effect ($P > 0.05$) in the rabbit manure compost in all treatments except in the N-total and C:N ratio. The percentage of N-total in Table 2 shows that the levels of organic matter composted with the addition of MOL fermented at several levels at



P0 P1 P2 P3

Fig. 6. Growth of microbial colonies from composting rabbit feces after 14 days

P0 (3.33%), P1 (2.90%), P2 (3.05%), and P3 (4.48%). The highest N-total content of compost fertilizer was found at the addition level of 5% of fermentation products (P3). The high nitrogen content was assumed due to the presence of N as a product of protein decomposition from the decomposition process during composting. The N-total content showed that adding MOL with acidic product conditions caused a significant difference in the N-total content. The acidic conditions of manure will form nitrogen into NH^{4+}, and the bioactive compound from maja fruit plays a role in the inhibition of ureolytic bacteria so that NH_3 evaporation can be minimized [12, 13].

The chemical composition of Rabbit manure compost using MOL from maja fruit and bamboo shot fermentation as a bio-starter decomposer has met the requirements of the Indonesian Standard for compost product SNI: 19–7030-2004. The standard requirement at the moisture content (max 50%), organic matter (27–58), total C-organic (9.8–32), N-total (>0.4%), P-total (>0.1%), K-total (>0.2%), and C:N ratio (10–20) [19]. From the rabbit manure compost number of colonies observations, it can be seen that the most significant number of colonies was found in fertilizers with the addition of 5% (P3) MOL from fermented maja fruit and bamboo shoots which observed 2.43×10^5 CFU/mL. The lowest number of colonies was found in fertilizer with a control treatment (P0) of 1.03×10^5 CFU/ml. According to [17], microbial growth is influenced by several factors: energy, protein, mineral, pH, and temperature. The colony growth of each compost in each treatment can be seen in Fig. 6.

4 Conclusion

Based on the research, it can be concluded that adding the MOL products from fermented maja fruit and bamboo shoots can reduce the ammonia gas released by rabbit manure compost. However, the addition of MOL at different levels obtained different results. The best results in reducing ammonia gas levels were obtained in the treatment of adding MOL at an additional level of 5% (P3) of the weight of the compost. The addition of MOL at different levels in rabbit manure compost did not increase the chemical quality of water content, organic matter, C-organic, P-total, and K-total but affected the quality of the total N content and the ratio C/N.

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