Natural Decomposer (MOL) developed from various banana waste and different storage times

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Abstract. MOL is a local microorganism or natural decomposer that is used as a starter culture in production of organic fertilizers which is known as bokashi. The advantage of using MOL is that it can be made from cheap materials or by utilizing agricultural waste so that it can increase the added value of waste as well as reduce the environmental pollution. The use of MOL as a biodecomposer in production of bokashi can accelerate the fermentation process. This study aimed to produce MOL where the characteristic is similar with commercial Effective Microorganism (EM4). This study used a factorial randomized block design (RBD) with 2 factors. First factor was the types of banana (J) consisted of three levels, namely awak banana (Musa paradisiacal var. Awak; J1), barangan banana (Musa acuminate Colla; J2), and kepok banana (Musa acuminate balbisiana Colla; J3). Second factor was the storage times consisted of three levels, namely 0 weeks after fermentation (L1), 2 weeks after fermentation (L2), and 4 weeks after fermentation (L3). The analysis carried out after fermentation included total cell counts (TCC), pH value, temperature, and organoleptic test in the form of hedonic test for color and description test for aroma. The best sample was determined based on the highest TCC, the lowest pH value and aroma using a ranking test. The best treatment was obtained from J1L2 treatment (awak banana and 2 weeks after fermentation) with 1.8 × 10⁵ CFU/ml of TCC, 3.17 of pH which is similar with the EM4 and medium tapai aroma.

Keywords: MOL, bananas, storage times, bokashi.

INTRODUCTION

Fertilizer is one of the necessities for plant growth. One of the organic fertilizers used in agriculture that can reduce the use of inorganic fertilizers is compost (1). The composting process takes a long time, which is about 5 weeks to 2 months. To accelerate the composting process, an activator can be added as an organic material remover during the process, so that the composting time can be shortened, which is about 2 weeks. The compost that is produced through the fermentation process with the addition of an activator to speed up the compost-making process is called bokashi (2).

One of the activators on the market is Effective Microorganism 4 (EM4). However, the use of EM4 is sometimes considered less effective for the community because not all people can buy it, so local microorganisms (MOL) can be an alternative to EM4 as an activator (3).

MOL is a microorganism that is used as a starter culture in production of organic fertilizers. The use of MOL as a biodecomposer in production of bokashi can accelerate the fermentation process (4). The advantage of using MOL is that it can be made from cheap materials or by utilizing agricultural waste with high fructose and glucose contents (5) so that it can increase the added value of waste and it can also reduce environmental pollution.

Based on previous unpublished work (6), the best fruits combination in MOL production were from peel and pulp of rotten papaya and bananas fruits which are no longer suitable for consumption made. The combination produced high quality bokashi fertilizer when the MOL was applied. When bananas and papayas are fully ripened, most of their starch content will break down into sucrose, glucose, fructose, and a small amount of maltose, so ripen fruits will taste sweeter than unripen fruits. The simple sugar and water content in these fruits are very important for microbial growth (7). To increase the carbohydrate content of the raw material of
MOL and utilize waste, rice washing water or coconut water can also be added (8).

MOL from papaya and banana has been made by (9). The TCC values ranged from $1.2 \times 3.5 \times 10^3$ CFU/ml to $7.7 \times 10^9$ CFU/ml. The results of ANOVA showed that the treatment of banana types (J) had a significant effect (P$\leq 0.05$) on TCC MOL. The effect of banana types on the TCC MOL value can be seen in Figure 1. The result of DMRT showed awak banana (J1) was different from barangan banana (J2) and kepok banana (J3). From Figure 1, awak banana (J1) produced the highest TCC value ($1.1 \times 10^9$ CFU/ml), while the lowest TCC value was obtained from kepok banana ($4.9 \times 10^8$ CFU/ml).

**RESULTS AND DISCUSSION**

**Total cell counts (TCC) of MOL**

The TCC MOL in this study ranged from $3.3 \times 10^4$ CFU/ml to $1.8 \times 10^9$ CFU/ml with a mean of $7.7 \times 10^6$ CFU/ml. The research data obtained were analyzed using Analysis of Variance (ANOVA). The results of the analysis showed a significant effect between treatments were further analysed using Duncan multiple range test (DMRT) by IBM SPSS Statistics version 22.

**Samples Ananlysis**

Samples after fermentation were analysed for TCC, pH value, temperature, and organoleptic tests in the form of a hedonic test for color and a description test for aroma. The organoleptic testing was carried out by 20 trained panelists with the criteria: recognizing the descriptive of MOL characteristics. The best sample was determined based on the highest TCC, the lowest pH value, and aroma using a ranking test. The best treatment was then compared with commercial EM4 in terms of TCC and pH parameter.

**Data analysis**

The research data obtained were analyzed using Analysis of Variance (ANOVA). The results of the analysis showed a significant effect between treatments were further analysed using Duncan multiple range test (DMRT) by IBM SPSS Statistics version 22.

**METHODOLOGY**

**Experimental design**

This study used a factorial randomized block design (RBD) with 2 factors. First factor was the types of banana (J) consisted of three levels, namely awak banana (Musa paradisiaca var. Awak; J1), barangan banana (Musa acuminata Colla; J2), and kepok banana (Musa acuminata balbisiana Colla; J3). Second factor was the storage times consisted of three levels, namely 0 weeks after fermentation (L1), 2 weeks after fermentation (L2), and 4 weeks after fermentation (L3). Each treatment was repeated 2 times to obtain 18 experimental units.

**MOL production (9)**

The waste of papaya and banana (awak banana, barangan banana and kepok banana) was cut into small pieces. Each fruit waste was weighed as much as 250 g. The fruits (500g) were crushed in a blender. Fruits that have been crushed in a blender were then added with 0.5 liter of rice washing water (obtained from 250 g of rice washed in 0.5 liters of water) and 20 g of granulated sugar while continuing to stir until homogeneous. The homogeneous material was put in a fermentation container (plastic bottle) and then closed tightly. The ingredients in the container were fermented at room temperature (26-30°C) for 7 days. After fermentation, the mixtures were then stored according to the length of treatment (0, 2, and 4 weeks after fermentation) at room temperature (26-30°C).

Figure 1. The effect of banana types (J) on TCC of MOL in the DMRT with CV = 42.15% (the value followed by the same letter does not show a significant difference).

The difference in TCC that develops in each type of banana was due to differences in the nutritional content of each types of banana. According to (10), the calories, protein, fat, and...
carbohydrates in awak bananas are higher than barangan and kepok bananas. Therefore, the higher the nutritional value of bananas, the more nutrients the microorganisms can use for their growth.

The results of ANOVA also showed that the treatment of storage times (L) had a very significant effect (P≤0.01) on TCC MOL. The effect of storage times on the TCC MOL value can be seen in Figure 2. The result of DMRT_L0.05 showed the TCC at the L3 was different from the L1 and L2. From Figure 2, the TCC value was still high until 2 weeks of storage before it decreased dramatically. The highest TCC value was achieved at 0 week after fermentation (L1; 1.2 × 10^9 CFU/ml).

The pH value of MOL, which was still high until 2 weeks of storage before it decreased dramatically. The highest TCC value showed that the type (J) and storage times (L) had a very significant effect (P<0.01) on TCC MOL. The interaction between the two treatments (JL) had no significant effect (P> 0.05).

The TCC MOL value tends to decrease with the longer the MOL was stored. This is presumably because the longer the MOL is stored, the less nutrients that can be used by microorganisms for their growth.

**pH Value of MOL**

The pH value of MOL ranged from 3.17 to 3.40 with an average of 3.24. The results of ANOVA showed that the types of banana (J) and storage times (L) had a very significant effect (P<0.01) on the pH of MOL, while the interaction between the two treatments (JL) had no significant effect (P> 0.05).

The effect of banana types (J) on the pH value of MOL is presented in Figure 3. The graph shows that the awak banana (J1) produced the lowest pH value (3.20), while the highest pH value is in the kepok banana (J3; 3.32). The DMRT_L0.05 showed that the pH value of the awak banana (J1) was not different from the barangan banana (J2).

The pH value of MOL, which was high in kepok banana, was thought to be influenced by low nutrients (calories, protein, fat, carbohydrates, calcium, phosphorus, Fe, vitamin C and potassium) in kepok banana compared with awak banana and barangan bananas (10). Carbohydrates will undergo a hydrolysis process by cellulosic microbes with the help of cellulolytic enzyme which can convert cellulose into D-glucose and finally fermented into acid, ethanol, CO₂, and H₂O. Amylolytic microbes will produce amylase enzymes which play a role in converting starch into glucose. Water in the fermentation process functions as a medium for bacterial growth in addition to functioning as a solvent (12). According to (13), the carbohydrate content in the fermentation material determines the amount of alcohol and acidic compounds formed in the fermentation process. The process of lowering the pH can occur at the beginning of fermentation due to the formation of acids (acetic acid, pyruvic acid, and lactic acid).
According to (15), usually the pH will decrease at the beginning of fermentation due to the activity of bacteria that produce acids, as seen in L1. After a few days, the pH will rise which is thought to be due to the appearance of other microorganisms from the decomposed material.

![Figure 4](image-url)  
**Figure 4.** Effect of storage time (L) on the pH value of MOL in the DMRT<sub>0.05</sub> with CV = 0.90% (the value followed by the same letter does not show a significant difference).

![Figure 5](image-url)  
**Figure 5.** Effect of banana types (J) on the temperature of MOL in the DMRT<sub>0.05</sub> with CV = 1.80% (the value followed by the same letter does not show a significant difference).

**Temperature of MOL**

The MOL temperature ranged from 27°C to 29°C with an average of 27.7°C. The results of ANOVA showed that the types of banana (J) and storage times (L) had a significant effect (P<0.05) on the MOL temperature. The interaction between the two treatments (JL) had no effect (P> 0.05).

The effect of banana types (J) on temperature is presented in Figure 5. The DMRT<sub>0.05</sub> showed that the MOL temperature made from awak banana (J1) was different from the MOL temperature made from kepok banana (J3). The awak banana (J1) produced the highest temperature (28.2°C), while the lowest temperature is obtained from kepok banana (J3; 27.3°C).

The higher temperature in awak banana was related to the activity of microorganisms in decomposing organic matter that produces energy in the form of heat, CO₂, and water steam. From TCC results, the microorganism activity was also supported by the nutrients in the awak banana. The activity of these microorganisms could increase the temperature.

The effect of storage time (L) on temperature is presented in Figure 6. In the graph, the temperature tends to increase from 0 weeks of storage time after fermentation (L1) to 2 weeks after fermentation (L2), but the DMRT<sub>0.05</sub> indicated that the temperature at the L1 treatment did not differ from the L2 treatment. Furthermore, the temperature decreased slightly at 4 weeks after fermentation (L3). The temperature of the L3 treatment was different from the L1 and L2 treatments. However, based on (16), the temperature increased convectively during storage occurs due to the heat that arises in the alcoholic fermentation of the sugar substrate.

The heat was controlled by replacing the MOL in a room temperature. The MOL temperature during fermentation must be no higher than 70°C to avoid the mortality of the microorganisms. The release of energy through anaerobic respiration activity by yeast during the fermentation process caused a change in temperature. In short, glucose (C₆H₁₂O₆) which is the simplest sugar, through fermentation will produce ethanol (C₂H₅OH). When it reached the peak, the MOL temperature decreased again, presumably because the activity of microorganisms has decreased. The results of this study are in line with the results of research by (9), where the MOL temperature decreased after 4 weeks fermentation.
Hedonic Test of MOL Color
The results of the panelists' preference for the MOL color ranged from 3 (slightly like) to 5 (very like) with an average of 4 (like). The results of ANOVA showed that the types of banana (J) and storage times (L) had a very significant effect (P<0.01) on MOL color, while the interaction between the two treatments (JL) had no significant effect (P>0.05).

The effect of banana types (J) on MOL color is presented in Figure 7. Based on the results of the DMRT, the MOL color made from awak banana (J1) was not different from the J2 treatment (barangan banana). The graph shows that the panelist values tend to prefer awak banana (J1) and barangan banana (J2) with a value of 4 (like), followed by kepok banana 3 (somewhat like).

![Hedonic Test of MOL Color](image)

**Figure 7.** The effect of banana types (J) on hedonic test of MOL color in the DMRT with CV = 7.76% (the value followed by the same letter does not show a significant difference). Hedonic scale 1 = very dislikes, 2 = dislikes, 3 = slightly like, 4 = like, and 5 = really like).

Microorganisms that grow and develop on a raw material can cause various physical changes such as color changes (17). Some microbes cause food coloring because they form pigments or form certain polysaccharides. Degraded sucrose will produce carbon dioxide and water and then produce a brown color in the product (Suroyya, 2016). Kepok bananas are pale yellow in color because they have the lowest sugar content (18).

The effect of storage times (L) on MOL color is presented in Figure 8. Based on the DMRT, the MOL color at 0 week after fermentation (L1) was different from 2 weeks (L2) and 4 weeks (L3) after fermentation. The graph shows that panelists tend to prefer MOL stored 0 weeks after fermentation (L1) with a value of 5 (really like). The longer MOL was stored, the panelists' preference for MOL colors also tended to decline.

![Hedonic Test of MOL Color](image)

**Figure 8.** The effect of storage times (J) on hedonic test of MOL color in the DMRT with CV = 7.76% (the value followed by the same letter does not show a significant difference). Hedonic scale 1 = very dislikes, 2 = dislikes, 3 = slightly like, 4 = like, and 5 = really like).

According to (19), the difference in color changes that occurs in the fermentation process is the result of the large or small activity of microorganisms. The color-forming components come from fungi that produce enzymes. This enzyme acts as a starch remover in the material used to become sugar. The enzyme produced by MOL converts sugar into alcohol which then becomes esters. Esters are part of the formation of the MOL color change during fermentation. In addition, the color change is also due to a browning reaction.

Descriptive test of MOL aroma
The results of the descriptive test of MOL aroma ranged from 3 (weak tapai aroma) to 5 (strong tapai aroma) with an average of 4 (medium tapai aroma). The results of ANOVA showed that the storage time (L) had a significant effect (P<0.05) on the descriptive test of MOL aroma. While the type of banana (J) and the interaction between the two treatments (JL) had no significant effect (P>0.05).

The effect of storage times (L) on the descriptive test of MOL aroma is presented in Figure 9. The DMRT showed that the MOL aroma at 4 weeks after fermentation (L3) was different from 0 week (L1) and 2 weeks (L2) after fermentation. The graph shows that the highest MOL aroma is at 0 weeks after fermentation (L1) with a value of 4 (medium tapai aroma) which is no different with 2 weeks after fermentation (L2). At 4 weeks after fermentation (L3), the MOL aroma became weak tapai aroma.
Quality assessment of Local Microorganisms (MOL) Made from Various Types of Banana

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Figure 9. The effect of storage time (L) on descriptive test of MOL aroma in the DMRT$_{0.05}$ with CV = 14.02% (the value followed by the same letter does not show a significant difference). Descriptive scale 1 = bad smell, 2 = no tapai aroma, 3 = weak tapai aroma, 4 = medium tapai aroma, and 5 = strong tapai aroma).

The presence of tapai aroma (alcohol) in MOL is due to the fermentation process that forms acid and alcohol (20). In the hydrolysis of glucose to ethanol, pyruvic acid is formed. Pyruvic acid can turn into ethanol and lactic acid. Organic acids from alcohol form aromatic esters so that MOL has a distinctive aroma like the aroma of tapai (21). At the storage time of 4 weeks after fermentation, it was suspected that MOL had undergone a breakdown reaction of protein into amino acids which became ammonia gas which resulted in the appearance of a rotten aroma.

Best treatment and comparison with EM4
Ranking test is used to obtain the best treatment results from all treatments. The best treatment was determined based on the results of three parameters (TCC, pH and descriptive aroma). The ranking of each treatment of each tested parameter can be seen in Table 1. The highest score is chosen as the best treatment which is J1L2 (MOL was made from awak banana and was stored until 2 weeks after fermentation). This treatment was then compared with commercial EM4 (Table 2). Based on Table 2, the best MOL in this research has similar quality parameters (TCC and pH) with commercial EM4.

CONCLUSION
MOL from awak banana was better than others banana in terms of higher TCC and lower pH MOL. However, MOL quality remains good until 2 weeks storage after fermentation. The TCC and pH characteristics of MOL made from awak banana and stored for up to 2 weeks after fermentation (J1L2) were similar with the commercial characteristics of EM4. The J1L2 treatment had the following characteristics: TCC of $1.8 \times 10^9$ CFU/ml, pH of 3.17, temperature of 29°C, a MOL color that was very liked by the panelists, and a medium tapai aroma.

Table 1. Determination of the best treatment by ranking method.

| Treatments | Parameter | TCC | pH | Descriptive Test of Aroma | Total | Average | Ranking |
|------------|-----------|-----|----|---------------------------|-------|---------|---------|
| J1L1       |           | 7   | 6  | 5                         | 18    | 6       | 3       |
| J1L2       |           | 9   | 7  | 5                         | 21    | 7       | 1       |
| J1L3       |           | 3   | 3  | 3                         | 9     | 3       | 7       |
| J2L1       |           | 8   | 6  | 5                         | 19    | 6       | 2       |
| J2L2       |           | 5   | 5  | 2                         | 12    | 4       | 5       |
| J2L3       |           | 2   | 2  | 1                         | 5     | 2       | 8       |
| J3L1       |           | 4   | 4  | 2                         | 10    | 3       | 6       |
| J3L2       |           | 6   | 5  | 4                         | 15    | 5       | 4       |
| J3L3       |           | 1   | 1  | 1                         | 3     | 1       | 9       |

Table 2. Comparison between best treatment and commercial EM4

| Parameter | Best Treatment (J1L2) | Commercial EM4 |
|-----------|-----------------------|----------------|
| TCC       | $1.8 \times 10^9$ CFU/ml | $1.8 \times 10^9$ CFU/ml |
| pH        | 3.17                  | 3.58           |

J1L2: MOL was made from awak banana and stored until 2 weeks after fermentation.
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