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# Effect of raw materials C/N ratio on the quality of Bokashi

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ORIGINAL RESEARCH

## Abstract:

**Purpose**: To assess the effect of C/N ratio in organic raw materials on the quality of bokashi and to obtain the proper C/N formulation of raw materials and fermentation time for bokashi production.

**Method**: The bokashi was made using a Completely Randomized Design (CRD) with two factors: the C/N ratio in raw materials (R; 30, 70, 110) and the fermentation time (L; 10, 17, 24 days). Then, the bokashi was evaluated for microbial and chemical properties. In the second stage, the bokashi was made using raw materials with C/N of 30 and 150 and fermentation time (0, 10 days) with 5 replications for analyzing the C/N. The pH and temperature were also observed every day during 10 days.

**Results**: The highest TCC  $(1.44 \times 10^{11} \text{ CFU/g})$  was in the bokashi which produced from raw materials with C/N of 70. In general, C/N of bokashi was lower than that of initial raw materials; however, 10 days fermentation was not sufficient to decompose organic matter in raw materials with a C/N of 150 perfectly. Daily observations of the pH and temperature during 10 days fermentation showed a continuously increasing of pH, while temperature first increased and began to experience a constant decrease starting on the 8<sup>th</sup> day.

**Conclusion**: A good quality bokashi which match the soil C/N ratio range of 10 - 20 can be produced from organic raw materials with C/N of 30 to 70 within 10 days fermentation as well as from raw materials with C/N of 110 after being fermented for 24 days.

Keywords: Compost; Organic waste; MOL (Local Microorganisms); Biodecomposer

# 1. Introduction

Organic matter naturally takes a few months to fully decompose into compost, depending on the material and the environment. The use of bio-activators, either EM4 (Effective Microorganism 4) or Local Microorganisms (MOL) can accelerate the organic matter decomposition process into a product known as bokashi. The use of the consortium of microorganisms in decomposition process is more effective than any individual isolate (Pan et al. 2012). The decomposition or fermentation process in the production of bokashi can take up to 10 days (Irfan et al. 2017; Maridhi et al. 2020), 15 days (Irfan et al. 2020), 24 days (Cortes-Tello and Lopez 2020), 25 days (Oliveira et al. 2022) or 42 days (Kamolmanit and Reungsang 2006).

The use of bokashi can improve the physical and biological structure of the soil as well as the content of nitrogen and other nutrients so that the characteristics of the soil become looser (Lasmini et al. 2018). Some of materials or organic wastes suitable to make bokashi are animal manure, bran, sawdust and husk ash (Irfan et al. 2017). Other wastes can be added to the material in certain proportions such as 30% of sago pulp (Maridhi et al. 2020) and 35% of paper wastes (Irfan et al. 2020). Sayği (2021) used 20.12% of green manure (*Soja hispida* and *Vigna sinensis* L.) and poultry manure (Sayği 2021).

One of the factors that greatly determine the success of producing bokashi is the carbon/nitrogen (C/N) of the raw

material mixture. Organic matter that has not been decomposed cannot be used directly by plants because the C/N in the material does not match the C/N found in the soil. Based on Indonesian Standard, the C/N ratio in bokashi or compost (SNI-19-7030-2004) ranges from 10-20. According to the previous findings (Yang et al. 2021; Kumar et al. 2010), the C/N of the initial raw material ranges between 25 to 30 which is good to stimulate proper fermentation process. However organic waste around the community in Indonesia possess a very high C/N ratio (Fauzan et al. 2022). A high initial C/N in raw material will lead to a slower start of the composting process and longer composting time (mrini et al. 2022). In contrary if the C/N ratio of the raw material is low, then nitrogen will be released, converted into ammonia (NH<sub>3</sub> emission), causing a foul odor (Kuroda et al. 2022). The right formulation of C/N of raw materials is required to correctly mixe organic waste with various C/N for bokashi production. Most studies still utilize organic waste in a certain proportion. Unfortunately, they were not based on the value of the C/N ratio of raw materials. Results from a previous study (Fauzan et al. 2022) showed that bokashi made from the C/N ratio up to 110 could be applied as a fertilizer based on the sensory characteristics of texture, aroma and color only. However, the effect of the C/N of raw materials on biochemistry characteristics was not examined yet. Therefore, the aim of this research was to analyze the effect of the C/N of raw materials on total colony counts (TCC), pH, water content and the C/N of bokashi, as well as to obtain the proper C/N formulation of raw materials for bokashi production. Also, this research was conducted to examine the pH and temperature changes during 10 days fermentation process.

# 2. Material and methods

#### Materials

# The materials used for bokashi production were cow manure, husk ash, bran, sawdust and MOL which was made using papaya and banana wastes (Yunita et al. 2021). The manure was obtained from cattle farms, and the husk ash and the bran from rice mills. All were located in Aceh Besar District, Aceh Province, Indonesia.

#### Experimental design

This research was conducted in two stages using a Completely Randomized Design with two factors. In the first stage, the experimental design was performed according the method described by Fauzan et al. where the first factor was the C/N ratio of raw materials (R; 30, 70, 110) and the second factor was fermentation time (L; 10, 17, 24 days) (Fauzan et al. 2022). Then, in the second stage, the bokashi was made using 30 and 150 of the C/N ratios of raw materials and fermentation time (0 and 10 days) with five replications.

#### MOL production (Yunita et al. 2021)

Papaya and banana waste were cut into small pieces. The portion of waste (each 250 g), along with rice washing water (0.5 liters) and sugar (20 g) were crushed in a blender until homogeneous. The mixture of ingredients was put in a closed container and fermented at room temperature for 7 days. The liquid of MOL (+ 1 liter) was used as bio-

## activator in bokashi production.

## **Raw materials preparation** (Fauzan et al. 2022)

Cow manure, bran, sawdust and husk ash were analyzed for C and N so that the C/N ratio of each raw material could be calculated. The amount of each material used was then calculated according to the C/N ratio treatment at each stage. All raw materials were mixed based on the formulation shown in Table 1. MOL (0.5%) was then added to the mixture and mixed evenly. Water adequacy is known from a handheld test. The mixture was covered by a thick black plastic according to the treatment. Turning was performed every 3 days so that the fermentation process was properly distributed.

#### Sample analysis

The analysis carried out on bokashi included microbial properties (total colony counts; TCC) and chemical properties (pH value, moisture content, and C/N ratio).

#### Total colony counts (TCC)

Prior to measurement, 2.73 grams of nutrient agar (NA; Oxoid CMO003) was dissolved and sterilized at 121°C for 15 minutes. A sample of 10 grams was diluted in 90 ml of peptone. The dilution was conducted until  $10^{-10}$  by pour plate method. The sample and media were then incubated at 47°C for 12 hours. Calculation of the total bacteria was done by using a colony counter. The number of colonies was counted using the following formula:

$$TCC(cfu/g) = \frac{\text{Number of cells}}{\text{Volume}} \times \frac{1}{\text{Dilution factor}} \quad (1)$$

#### pH value

The pH of bokashi was analyzed using a soil tester. Before analysis, the points where the pH was measured must be selected to represent the whole area of bokashi. The soil tester was inserted into the bokashi until 12 - 15 cm of depth. If the bokashi is too dry, distilled water should be added before inserting the soil tester. Also, the needle must be at pH 7 before used. Before analyzing at another point, the soil tester must be washed with distilled water.

#### Moisture content

Oven method was used to analyze the moisture content of bokashi. The cup must be dried in the oven at 100°C for 30 minutes, then it was cooled in a desiccator and weighed before used. Homogeneous bokashi samples (5 g) were placed in an empty and dried cup for 5 hours. The cup was then cooled in a desiccator for 15 minutes and was cooled in a desiccator and weighed again. The sample was reheated in the oven for 30 minutes, cooled in a desiccator and then weighed again. These procedures were repeated until the weight was constant.

## C/N ratio

The C/N ratio was calculated by dividing %C and %N. Determination of Carbon content (%C) followed the SOP for soil organic carbon which was analyzed using Walkley and Black method (GLOSOLAN 2019). The compost samples were first oxidized with potassium bichromate, then destructed with concentrated sulfuric acid and phosphoric acid. The amount of C lost due to oxidation is the C content in the bokashi. The bokashi sample (1 g) and 10 ml of 1 N  $K_2Cr_2O_7$  solution were put into a 500 ml Erlenmeyer flask. Concentrated H<sub>2</sub>SO<sub>4</sub> (20 ml) was added using a measur-

Table 1. Formulation of raw material mixture.

Research Stage	C/N Ratio of Raw Material Mixture	Proportion of Raw Materials (%)			
		Cow Manure	Bran	Sawdust	Husk Ash
1 <sup>st</sup> Stage	30 (R1)	85	5	5	5
	70 (R2)	55	15	15	15
	110 (R3)	25	25	25	25
2 <sup>st</sup> Stage	150 (R4)	5	25	35	35

ing cup and shaken slowly. Distilled water (200 ml), 85% H<sub>3</sub>PO<sub>3</sub> (10 ml) and 3 – 4 drops of barium diphenylamine sulfonate indicator were added. This solution was then titrated with 0.5 M FeSO<sub>4</sub> until the initial color change from dark green to cloudy blue, and to bright green at the end point of the titration.

Carbon Content(%C) = 
$$\frac{\mathbf{v}_1 - \mathbf{v}_2}{s} \times \mathbf{N} \times 0.39\%$$
 (2)

Where:

 $V_1$  = volume of FeSO<sub>4</sub> used for blank titration (ml)

 $V_2$  = volume of FeSO<sub>4</sub> used for sample titration (ml)

S = weight of 105°C oven dried sample (gram)

 $N = FeSO_4$  normality (0.5 N)

 $0.39\% = 3 \times 10^{-3} \times 100\% \times 1.3$  (3 = carbon equivalent weight)

Nitrogen content (%N) was determined using Kjeldahl Method (Irfan et al. 2020). Bokashi sample (0.2 g) was put into a 30 ml Kjehdal flask.  $K_2SO_4$  (2 g), HgO (50 mg) and HCl (2 ml) were added and boiled for 1 – 1.5 hours until the liquid became clear. The mixture was then cooled and the distilled water was added slowly. The mixture was transferred to the distillation apparatus and 8 – 10 ml of NaOH.NaS<sub>2</sub>O was added and distilled. The distillate (15 ml) was collected in 125 ml Erlenmeyer which already contained 5 ml of H<sub>3</sub>BO<sub>3</sub>. Two drops of indicator were added. The distillate was diluted to at about 50 ml and titrated with 0.2 N HCl until the color changes to gray. A blank solution is made by replacing the material with distilled water.

Nitrogen Content(%N) = 
$$\frac{a-b}{s} \times N \times 14 \times 100$$
 (3)



Where:

a = volume of H<sub>2</sub>SO<sub>4</sub> used for sample titration (ml) b = volume of H<sub>2</sub>SO<sub>4</sub> used for blank titration (ml) s = weight of 105°C oven dry sample (gram) N = normality of H<sub>2</sub>SO<sub>4</sub> (0.05 N) 14 = (molecular weight of N) ×10<sup>-3</sup> × 100% 100 = dilution factor

Data analysis

All research data were analyzed by Analysis of Variance (ANOVA), Duncan Multiple Range Test (DMRT) was carried out using SPSS (IBM Software version 22) to demonstrate if there was an effect on the treatment.

## 3. Results and discussions

## 3.1 Total microorganism of Bokashi

The results of ANOVA showed that the treatment of the C/N ratio of R and L had a significant effect ( $P \le 0.05$ ) on TCC of bokashi. Based on the R treatment, the highest TCC was obtained in R2 bokashi which produced from raw materials with C/N of 70 ( $1.4 \times 10^{11}$  CFU/g). Further test showed that the TCC of R2 bokashi was different from the TCC of other two levels (Fig. 1). The nutrient content in the mixture of raw materials and the pile conditions of R2 bokashi were suitable for the growth of microorganisms in MOL. The addition of the MOL has an important role as a decomposer of organic materials in bokashi production. MOL contains photosynthetic microbes, actinomycetes, lactic acid bacteria, fungi and phosphate solubilizing microbes (Ramli 2020). In R1 bokashi (raw materials C/N of 30), the



**Figure 1.** Effect of C/N ratio of raw materials (R) on total colony counts of bokashi.

Value followed by unequal letter indicate a significant difference at  $P \le 0.05$ , DMRT<sub>0.05</sub> = 6.75, coefficient of diversity = 2.9%.

**Figure 2.** Effect of fermentation time (L) on total colony counts of bokashi.

Value followed by unequal letter indicate a significant difference at  $P \le 0.05$ , DMRT<sub>0.05</sub> = 6.75, coefficient of diversity = 2.9%.



Figure 3. Effect of interaction between C/N ratio of raw materials and fermentation time (RL) on the pH of bokashi. Value followed by unequal letter indicate a significant difference at  $P \le 0.05$ , DMRT<sub>0.05</sub> = 0.38, coefficient of diversity = 6.7%.

pile condition became denser due to a lack of structure materials, so that the availability of oxygen was very limited and suboptimal for microbial growth. Limited oxygen supply in composting materials can reduce microbial activities (Kurniati et al. 2022). Also, in R3 bokashi (raw materials C/N of 110), microbial growth was limited due to an increasing of a harder texture of raw material such as sawdust which has an individual C/N of 283 (Fauzan et al. 2022).

Based on the L treatment, the lowest TCC was recorded with L2 bokashi which was fermented for 17 days  $(1.7 \times 10^{11} \text{ CFU/g})$ . Further test showed that the TCC of L2 bokashi was different from that of others (Fig. 2). This indicates fluctuation in the development of microorganisms during fermentation. Microbial community succession normally occurs during the composting process under natural conditions (Meng et al. 2019). The rise and fall of TCC during bokashi fermentation are related to the availability and suitability of nutrients for the type of microorganisms that are developing (Irfan et al. 2017).

## 3.2 pH value of Bokashi

The results of the variance showed that the interaction between the C/N ratio of RL had a significant effect ( $P \le 0.05$ )



**Figure 4.** Effect of C/N ratio of raw materials (R) on water content of bokashi.

Value followed by unequal letter indicate a significant difference at  $P \le 0.01$ , DMRT<sub>0.05</sub> = 15.91, coefficient of diversity = 15.25%.



**Figure 5.** The effect of the interaction of raw material C/N and fermentation time (RL) on C/N of bokashi.

Value followed by unequal letter indicate a significant difference at  $P \le 0.01$ , DMRT<sub>0.05</sub> = 3.33, coefficient of diversity = 9.49%.

on the pH of the bokashi. The highest pH value was found in the R1L3 bokashi (raw material C/N of 30, fermentation time of 24 days) with a value of 7.8 which based on the DMRT<sub>0.05</sub> test was different from the pH of others (Fig. 3). The pH value of the study ranged from 6.1 - 7.8 which was wider than the Indonesian Standards requirements (SNI 19-7030-2004) namely 6.8 - 7.5 (Sidabutar 2012).

At the beginning of the compost process, bokashi had an acidic pH because organic matter generally contains organic acids (Hutagalung and Rinaldi 2019). During fermentation, the pH of the bokashi generally increased due to the activity of microorganisms in decomposing organic matter (Oliveira et al. 2022). According to Tognetti et al., the pH became more alkaline due to protein decomposition which could produce ammonia with the release of -OH ions (Tognetti et al. 2007).

#### 3.3 Water content of Bokashi

The results of the variance showed that only the C/N ratio in the R showed a very significant effect (P  $\leq$  0.01) on the water content of the bokashi. The highest water content (55.40%) was produced by the treatment level of R1 level (raw material C/N of 30) which based on the DMRT<sub>0.05</sub> test, was significantly different from that of others (Fig. 4). As the C/N of raw materials increased, the water content of the resulting bokashi tended to decrease. Raw materials with higher C/N ratio generally had coarser texture, so they easily released water. Water was evaporated because during the fermentation process heat was generated by mi-



**Figure 6.** Daily observation of pH of bokashi made from raw material with C/N ratio of 30 and 150 for 10 days fermentation.



Figure 7. Daily observation of temperature of bokashi made from raw material with C/N ratio of 30 and 150 for 10 days fermentation.

crobial activity which decomposed organic matter (Irfan et al. 2017).

## 3.4 C/N ratio of Bokashi

Organic waste could give the best quality of compost if the optimal process conditions were met, including the maintenance of C/N ratio (Zohra et al. 2022). The C element in the raw material was used as energy source by the microorganisms during the composting process whereas nitrogen was an important component of cell structure (Kurniati et al. 2022). A good C/N ratio for composting raw materials differed between 22 (mrini et al. 2022), 25 - 30 (Kumar et al. 2010) and 30 (Kamolmanit and Reungsang 2006). However, the results of the first stage in this study indicated that the C/N of raw materials up to 110 showed no effect on the C/N of bokashi, and the C/N of resulting bokashi were all within the range of C/N of soil (10-20) (Goyal et al. 2005) or complied the C/N of bokashi according to Indonesian Standards. Although the C/N of the raw materials used changed greatly from 30 to 110, the C/N ratio of the resulting bokashi had dropped to the range of 11.7 - 14.7. In the second stage of this study, the bokashi was made using R with C/N of 30 and 150 as well as L of 0 day (without fermentation) and 10 days. The results showed that the treatment of R, L, and the interaction of RL (Fig. 5) had a very degree of significant effect ( $P \le 0.01$ ) on the C/N of bokashi. The lowest C/N was gained at the interaction of R1L1 bokashi (raw material with C/N of 30 and after 10 days fermentation), which further tests showed different from the C/N of other interaction levels. The C/N of R1L1 (19.9) was considerably good since it met the range of C/N of soil. According to El mrini et al., raw material with high C/N such as R4L1 (37.0) required longer fermentation time since the C/N should be decreased to that of soil (10-20) (mrini et al. 2022). However, a study of urban organic solid waste in Paipa, Boyacá, Colombia conducted by Molano et al. showed the C/N ratio was 21.4 after 65 days of composting (Molano et al. 2021). Sadeli et al. made bokashi using the microbe enrichment from two different bio-activators (stardec and EM4) and 7 days fermentation (Sadeli et al. 2022). The results showed that the average value of the obtained C/N ratio ranged from 19 to 28 for stardec and 17 to 23 for EM4.

The decrease in the C/N ratio during the manufacture of bokashi was due to the release of C element into the air as result of the decomposition of organic matters (Natalina et al. 2017). C element is used by microorganisms as a source of energy in the formation of new cells. This process produced carbon dioxide (CO<sub>2</sub>) under aerobic conditions or methane gas (CH<sub>4</sub>) under anaerobic conditions (Palaniveloo et al. 2020), so the amount of C during fermentation tended to decrease. On the other hand, during fermentation, the N element in bokashi could increase as the number of microorganisms increased. By decreasing C element and increasing N element during fermentation, resulting in lowering of C/N ratio (Goyal et al. 2005).

## 3.5 pH and temperature changes during fermentation

Observation of the pH and temperature in the process of bokashi production was carried out every day for 10 days. Fig. 6 shows that the pH value experienced an increasing trend in both types of samples (raw materials C/N of 30 and 150). It is interesting to note that pH of bokashi which was made from raw materials with C/N ratio of 30 was higher than that of 150. Raw materials with C/N ratio of 30 contained more N element than that of 150 and could give more OH ions which results in a higher pH value. The recommended pH of bokashi to be used as fertilizer is that in the range of 6.5 - 7.8. Ripe bokashi has a pH that is close to neutral (Hutagalung and Rinaldi 2019; Pan et al. 2012). Fig. 7 shows the measured temperature development during 10 days of fermentation. The temperature of the bokashi made from raw materials with C/N ratio of 30 was 31°C on 0 day fermentation, reached the peak  $(38.6^{\circ}C)$  on the 6th day and decreased to 35.2°C on the 10<sup>th</sup> day. The temperature of the bokashi made from raw materials with C/N ratio of 150 reached the peak on the  $2^{nd}$  and  $3^{rd}$  day fermentation (42.8°C) and decreased to 33.6°C on the  $10^{th}$  day.

Based on these observations, even though at the beginning of the fermentation an increase in temperature occurred, starting on the  $8^{th}$  day, the fermentation temperature began to experience a constant decrease. Research conducted by De Oliveira et al., Yang et al. also showed an increase in temperature at the beginning of the bokashi fermentation process (Oliveira et al. 2022; Yang et al. 2021). The conversion of nitrogen and degradation of cellulose mainly occurred in the early stages of composting process (Shi et al. 2021). After microorganisms grow, the temperature increases further due to the byproduct heat released in the ongoing decomposition of organic complex degradable solid matters into organic compost by the presence of microorganisms (Azim et al. 2018). There are two types of microorganisms which are responsible during composting process, the mesophilic at temperature of  $25^{\circ}C-45^{\circ}C$  and thermophilic microorganisms at  $45^{\circ}C-85^{\circ}C$  (Zohra et al. 2022; Biyada et al. 2021; Maccari et al. 2020). The role of these two types of microorganisms however depends on the composting condition and turning frequency. Turning is important not only for oxygen supply, but also to reduce overheating. Turning frequency of twice a week can produce a final compost of better quality (mrini et al. 2022; Boyle 2015).

Lignin and hemicellulose were mainly degraded in thermophilic phase (Shi et al. 2021). According to Yang et al., lignin and cellulose degradation occurred mainly at the thermophilic and late mesophilic phases of composting (Yang et al. 2021), while hemicellulose at the maturation phase. Some enzyme activities such as manganese peroxidase, lignin peroxidase, polyphenol oxidase and peroxidase stimulate lignocellulose degradation. Thermophilic microorganisms such as Proteobacteria and Actinobacteria play key roles at high temperature, while the mesophilic ones become dominant at heating, cooling and maturity stages (Óscar et al. 2017). After the decomposition is complete, the temperature drops again. Maintaining a stable temperature of 40°C-50°C during the fermentation process is very important; too low temperature can inhibit the growth of microorganisms to work normally, while too high temperatures can kill not only pathogens and weed seed (USEPA 2002; Monson and Murugappan 2009) but also decomposing bacteria (Makaruku and Wattimena 2022).

# 4. Conclusion

So far, it was believed that a good C/N ratio of raw organic material mixtures for bokashi production was 30. This research showed that even C/N ratio of initial raw materials above 30 up to a certain extent could still be used for bokashi production, however the fermentation time should be adjusted and the quality of the bokashi produced would vary. A good quality bokashi which match the C/N ratio of soil could be produced from organic raw materials with C/N of 30 to 70 within 10 days fermentation, while for raw materials with C/N of 110 the fermentation time should be prolonged to 24 days. This result is very important to motivate farmers in Indonesia to process organic wastes into bokashi because most organic wastes in Indonesia contain a C/N ratio above 30. There are still many aspects that required to be considered in future research in order to get better optimization for the utilization of organic wastes which have a much larger C/N to become bokashi in a shorter time.

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## Author Contributions:

The authors confirm that the study conception and design: I., analysis and interpretation of the data: D. Y., data collection: M. F., and draft manuscript preparation: F. The results were evaluated by all authors, and the final version of the manuscript was approved.

## Compliance with ethical standards:

Conflict of interest: The authors declare that there are no conflicts of interest associated with this study.

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