

## **Effect of Types of Organic Materials and Microbial Enrichment on C/N Ratio, Nutrition of Compost, and Microbe Population with *Trichoderma* sp. Indigenous Activators**

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### **Abstract**

The use of organic matter as fertilizer for crop production has received great attention for sustainable crop productivity. This is because chemical fertilizers are expensive and have a negative impact on the environment. Compost is an excellent source of micro and macro nutrients, the nutrient content depends on the composition and nature of the waste. This study aims (1) to determine the effect of *Trichoderma* sp. indigenous activator on the C/N ratio of compost and (2) to obtain the best mix of organic matter to be used as compost. The research method used a completely randomized design with four treatments and three replications. The treatments used are: 1). K3BO : (Cow dung, straw, husk, bran, *Trichoderma* sp., *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Azotobacter*). 2). K1BO: (Cow dung, straw, *Trichoderma* sp., *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Azotobacter*. 3). K2BO: (Cow dung, husk, bran, *Trichoderma* sp., *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Azotobacter*. 4). K0BO. Cow dung, *Trichoderma* sp, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Azotobacter*. The results showed that the K3BO treatment consisting of cow dung, straw, husks, bran *Trichoderma* sp., *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Azotobacter* was the best compost mixture. Parameters observed on average temperature 37.34°C, pH 7.27,

C/N 11.59, water content 13.57 with nutrient content of P 2.33%, K 1.24%. Enrichment with microbes gave a bacterial population of  $530.67 \times 10^8$ .

**Keywords:** Organic Materials, Microbial, C/N Ratio, Nutrition, Compost, Microbe, *Trichoderma sp*

## 1. Introduction

The use of organic matter as fertilizer for crop production has received great attention for sustainable crop productivity (Arif *et al.*, 2014)[1]. Composting is an intensive microbial activity in the decomposition of organic matter, with aerobic, biochemical, and microbial processes involving hydrolysis of organic matter to become stable (Wei *et al.*, 2017)[2]. Compost is an excellent source of micro and macro nutrients, the nutrient content depends on the composition and nature of the waste (Morales-Corts *et al.*, 2018)[3]. Organic sources such as manure, poultry manure, forage, and compost as suppliers of organic matter also serve to improve soil fertility status (Mohammadi *et al.*, 2011)[4]. Sources of organic matter provide organic acids to dissolve soil nutrients so that they are available to plants (Husson, 2013)[5]. Attention to organic fertilizers is growing because of the high cost of inorganic fertilizers and their inability to provide health benefits (Oyededeji *et al.*, 2014) [6].

Compost is the final product of the fermentation process from organic materials, which have a lower C/N ratio than raw materials. D. Sulaiman (2006)[7] states that each organic material to be composted has certain characteristics that are useful to support the composting process, especially the content of carbon (C) and nitrogen (N), where the levels of C and N will determine the biological activity of microorganisms. According to Isroi (2004)[8], the ideal material for composting has C/N ratio of around 30 to 40, because in this ratio, microorganisms get enough carbon for energy and nitrogen for protein synthesis. If organic matter has C/N with a high ratio, the activity of microorganisms will be reduced and the decomposition process of organic matter will be slow. According to F. Nurainy and O. Nawansih (2008)[9], a high C/N ratio indicates that the organic matter is immature and will still undergo a decomposition process by microorganisms.

The process of decomposition of organic matter requires certain environmental conditions, especially temperature and humidity factors. The optimal temperature for composting ranges from 45-65°C, this temperature range will ripen the compost within 4-6 weeks. The rate of organic decomposition of the material also depends on the nature of the material to be composted. When organic matter comes from a younger plant, the decomposition process is faster. The reason is that the water content is still high. High nitrogen content, small C/N ratio, and low lignin (S. Simamora and Salundik, 2005)[10]. Plants can absorb nutrients from the reshuffled organic matter, if the compost is in a mature state, the C/N ratio indicator is between 5 and 20 (R. Sutanto, 2002)[11]; F. Nurainy and O. Nawansih (2008)[12]; and N. Djuarnani *et al.* (2008)[13], compost ready for use, has a C/N ratio equal to the soil C/N ratio of 10 to 12.

Compost plays a role in improving the physical, chemical, and biological properties of the soil. The role of physical compost is to increase porosity and aeration; save water and reduce erosion (as mulch). Chemically, compost (1) provides macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (Zn, Cu, CO, Mo, Mn, and Fe); (2) increases soil CEC; and (3) compounds can form complexes with metal ions (Al and Fe) that are toxic to plants. Biology acts as a source of energy and food for soil organisms so that it can increase the activity of soil

microorganisms. Microorganisms make nutrients in mineralized soils available to plants (M.C. Brady, 1990[14]; Rusastra *et al.*, 2005[15]).

Some of the organic materials that can be composted in this study include straw, husks, and bran. According to F. Nurainy and O. Nawansih (2008), straw gave 20 t ha<sup>-1</sup> equivalents to 112 kg of urea, 203 kg of TSP, and 162 kg of KCl. The activator used in making this compost is *Trichoderma sp.* from the rhizosphere of the Junjuang rice variety. This *Trichoderma* has known molecular characteristics (Elita, N., *et al.*, 2022)[16]. Application of *Trichoderma sp.* indigenous Junjuang variety, can increase rice yield with SRI method 68.15% (Elita, N., *et al.*, 2021)[17].

Based on this, research has been carried out on the use of various organic materials in composting with *Trichoderma sp.*, an indigenous activator from the Junjuang variety, which is new information. The aims of this study were (1) to determine the effect of *Trichoderma sp.*, an indigenous activator on the C/N ratio of compost, and (2) to obtain the best mix of organic matter to be used as compost.

## 2. Research Method

The research was conducted in a composting house and soil laboratory from June-September 2022. The materials consisted of straw, rice husks, bran, cow dung, *Trichoderma sp.* activator, *Pseudomonas fluorescens* microbes, *Bacillus thuringiensis*, *Azotobacter*, and chemical analysis of materials. The tools used are scales, ovens, thermometers, laboratory instruments, basins, and plastic buckets. This study used experimental compost raw materials, microbial decomposers, and microbial enrichment arranged in a completely randomized design with 4 treatments and three replications.

Treatment is: K3BO: Cow dung, straw, husk, bran, *Trichoderma sp.*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Azotobacter*. K1BO: Cow dung, straw, *Trichoderma sp.*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Azotobacter*. K2BO: Cow dung, husk, bran, *Trichoderma sp.*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Azotobacter*. K0BO: Cow dung, *Trichoderma sp.*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Azotobacter*.

### Work Procedures

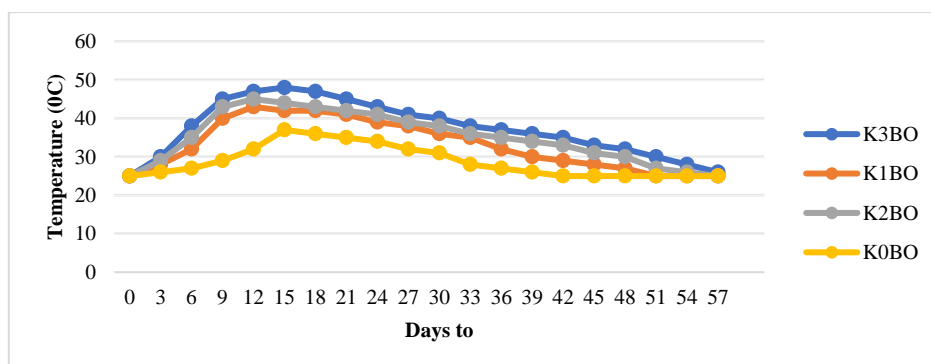
Cow dung with organic matter in a ratio of 7:3 (based on dry weight of the material) and a total of 100 kg of mixture. Each treatment of organic matter was composted with *Trichoderma sp.* An indigenous activator first with cow dung. The humidity of cultivated compost ranges from 40–60%. Compost turning is done once a week until the compost has matured for eight weeks. Microbial enrichment was given at 2 weeks of age by adding *Pseudomonas fluorescens*, *Bacillus thuringiensis*, and *Azotobacter* microbes that had been mass propagated in coconut water by pre culture. A microbial application was given with a spore density of 103/ml. For 100 kg of compost mixture, microbes are given a volume of 100 ml. The parameters measured were: composting temperature, pH, water content, nutrient content of N, P, and K, C-org and N, in order to obtain the ratio of C/N, the number of microbes after harvest.

## 3. Result And Discussion

### Temperature

The temperature during composting with *Trichoderma sp.* activator was as shown in

Figure 1.

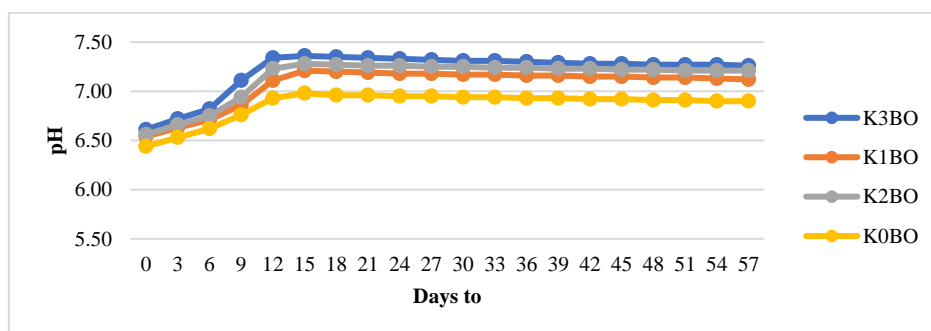


**Figure 1.** Temperature during the composting process with *Trichoderma sp.*, an indigenous activator

In Figure 1, it can be seen that there is a change in the temperature of the pile of organic matter during the composting process. The initial temperature of the pile of organic matter was around 25°C, and the ambient temperature was 24°C.

### Degree of Acidity (pH)

The results of pH measurements during the composting process are presented in Figure 2.



**Figure 2.** pH during the composting process with *Trichoderma sp.* indigenous activator

At the beginning of the composting process, the pH value ranged from 6.44 to 6.61. This shows the condition of organic matter that is composted in an acidic state, due to the activity of *Trichoderma sp.* an indigenous as a decomposer that causes the formation of organic acids. The pH value of compost is increasing due to *Trichoderma sp.* which decomposes nitrogen in organic matter in compost into ammonia, causing alkaline conditions. At the end of the composting process, the pH values in all treatments decreased to near neutral, with a range of 6.9–7.26. The lower pH value at the end of the composting process indicates that nitrogen decomposition has decreased.

The results of the BNT test analysis on the temperature and pH values in the composting process are presented in Table 1. The temperature in the composting process shows significantly different values for the four treatments. The highest pH value obtained at K3BO was not significantly different from K1BO and K2BO, but significantly different from K0BO. The temperature and pH values of the composting process with *Trichoderma sp.*, an indigenous activator, after statistical analysis are presented in Table 1.

**Table 1.** Average temperature and PH values during the composting process treatment

Treatment	Temperature	pH
K3BO	37.34 A	7.27 A
K1BO	33.29 C	7.13 AB
K2BO	35.30 B	7.21 A
K0BO	28.87 D	6.94 B

The same letter behind the mean value indicates a value that is not significantly different ( $p > 0.05$ )

The temperature values for all treatments were significantly different, the maximum value was between K3BO, K1BO, K2BO, and K0BO (37.34 – 28.87°C). Based on the results of the BNT test analysis on the composting process of organic matter with *Trichoderma sp.* as an indigenous activator for C-organic, N-organic and C/N as presented in Table 2.

**Table 2.** Provision of *Trichoderma sp* indigenous activator in the composting process according to C organic, N-organic, and C/N ratio.

Treatment	C	N	C/N
K3BO	28.91 A	2.62 A	11.59 A
K1BO	26.42 B	2.35 AB	11.23 AB
K2BO	25.31 C	2.18 B	11.05 B
K0BO	19.64 D	2.15 B	9.12 C

The same letter behind the mean value indicates a value that is not significantly different ( $p > 0.05$ )

### **Moisture Content, P, and K**

Based on the results of the BNT test analysis of the organic matter composting process with *Trichoderma sp.* as an indigenous activator, on the compost moisture content, P, K, and organic matter nutrients are presented in Table 4.

**Table 4.** Provision of *Trichoderma sp.* indigenous activator in the composting process on water, phosphate, and potassium content

Treatment	Moisture content	Phosphor	Potassium
K3BO	13.57 A	2.33 A	1.24 A
K1BO	13.36 AB	2.22 AB	0.92 B
K2BO	13.26 BC	2.14 B	0.77 C
K0BO	12.37 C	1.19 C	0.54 D

The same letter behind the mean value indicates a value that is not significantly different ( $p > 0.05$ )

### **Moisture content**

The highest water content of compost obtained in K3BO treatment was not significantly different from K1BO but significantly different from K2BO and K0BO treatments. The K1BO treatment was not significantly different from the K2BO treatment.



### Phosphate

The results of the BNT test analysis on the highest compost P nutrients were obtained in the K3BO treatment and were not significantly different from K1BO, but significantly different from K2BO and K0BO. The treatment of K1BO was not significantly different from that of K2BO but significantly different from that of K0BO. The lowest compost P nutrient content was found in the K0BO treatment.

### Potassium

The results of the BNT test analysis of the highest compost K nutrients in the K3BO treatment were significantly different from the other three treatments.

### Microbial Enrichment

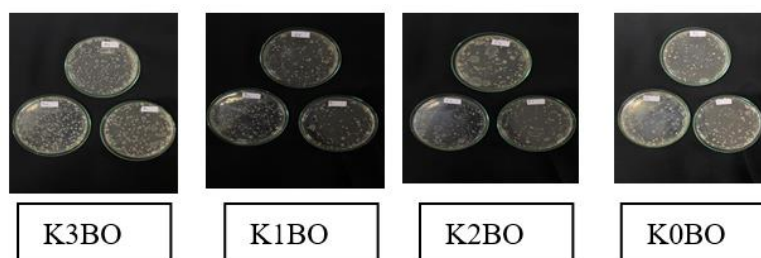
Bioorganic compost enriched with *Pseudomonas fluorescens*, *Bacillus thuringiensis*, and *Azotobacter microbes*. The addition of these microbes will accelerate the degradation process of organic matter. Based on the results of the BNT test analysis of the data on the number of microbes in the compost material with *Trichoderma sp.* indigenous activator, the results of the K3BO and K2BO treatments were not significantly different, but significantly different from the K1BO and K0BO treatments. The lowest number of microbial populations was found in the K0BO treatment, as presented in Table 5.

**Table 5.** Microbial population in bioorganic compost

Treatment	Microorganism Population
K3BO	530.67 x 10 <sup>8</sup> A
K1BO	432.00 x 10 <sup>8</sup> B
K2BO	511.33 x 10 <sup>8</sup> A
K0BO	279.33 x 10 <sup>8</sup> C

The same letter behind the mean value indicates a value that is not significantly different ( $p > 0.05$ )

The highest microbial population was found in the K3BO and K2BO treatments, the high number of microbes was due to this treatment having organic matter consisting of straw, husks, and bran, as opposed to K3BO and K2BO treatments consisting of husks and bran. In treatment B, the microbial population was 432 x 10<sup>8</sup> lower than in K3BO and K2BO treatments, K1BO treatment with organic matter from straw. The K2BO treatment with organic matter of husk and bran increased the number of microbes to 508 x 10<sup>8</sup>. The lowest number of microbes in the K0BO treatment was 279.33 x 10<sup>8</sup>, the K0BO treatment without organic matter was only an activator of *Trichoderma sp.* This result is in line with the amount of organic matter present in the compost (Table 4). The description of the number of microbial populations in Figure 3.



**Figure 3.** Total microbial population in bioorganic compost

In Figure 3, it can be seen that compost, which contains a lot of organic matter, has more microbial growth due to the availability of a lot of food.

## 4. Discussion

In Figure 1, it can be seen that there is a change in the temperature of the pile of organic matter during the composting process. The activity of *Trichoderma sp* in the process of decomposition of organic matter increases with the increasing amount of organic matter. At the beginning of the composting of organic matter, the compost undergoes an acclimation process, namely the process of adjusting the temperature of the organic material in the compost, where the activity of *Trichoderma sp.* functions to remodel the organic compost mixture to adapt to mesophilic conditions (Madrini, 2016)[18]. The most important factors affecting compost quality are temperature, moisture content, and aeration. Temperatures in anaerobic systems range from 15 to 45°C, while some systems produce temperatures from 30 to 55°C. Organisms that carry out the decomposition process in an anaerobic mass are called mesophilic micro-organisms (Poincelot, 1977)[19].

At the beginning of the composting process, the pH value ranged from 6.44 to 6.61. This shows the condition of organic matter that is composted in an acidic state, due to the activity of *Trichoderma sp.* an indigenous decomposer that causes the formation of organic acids. The pH value of compost is increasing due to *Trichoderma sp.* which decomposes nitrogen in organic matter in compost into ammonia, causing alkaline conditions. At the end of the composting process, the pH values in all treatments decreased to near neutral, with a range of 6.9 – 7.26. The lower pH value at the end of the composting process indicates that the nitrogen decomposition has decreased. The results of the BNT test analysis on the temperature and pH values in the composting process are as presented in Table 1.

The temperature values for all treatments were significantly different, the maximum value was between K3BO, K1BO, K2BO, and K0BO 37.34 – 28.87°C. The significant difference in temperature was due to the type of organic matter used in the K3BO treatment, which consisted of straw, husks, and bran; in the K0BO treatment, there was no organic matter, only cow dung with *Trichoderma sp.* activator. The organic matter that is the source of the *Trichoderma sp.* fungus is a microbial nutrient, and the more types of organic matter that are mixed in the composting process, the higher the temperature of the compost pile will be. According to Chinakwe *et al.* (2019)[20] compost made from cow dung, the composting temperature can increase to 46°C after 2 weeks. The highest temperature in cow dung reached 62°C on day 29. The temperature of the compost heap dropped on day 49 to 34–40°C.

The results of the C-organic test showed that there were significant differences for all treatments. The highest organic C was obtained in the K3BO treatment, and the lowest was obtained in the K0BO treatment. The K3BO treatment consisted of three types of organic matter, namely straw, husk, and bran, which were sources for microbes as an energy source, while in the K0BO treatment there was no organic matter. The large amount of organic matter in the K3BO treatment causes the water content of the compost to be sufficient for the activity of microbial decomposers.

The results of the N-organic test in the K3BO treatment obtained the highest value, which was not significantly different from the K1BO treatment, but significantly different from

the K2BO and K0BO treatments. The K1BO treatment was not significantly different from the K2BO and K0BO treatments. The high value of N-organic in K3BO was caused by the treatment that organic matter consisted of three types, namely straw, husk, and bran, which had a lot of organic matter, so that the decomposition resulted in a high value of N-organic.

The C/N value in K3BO (husk, bran, and straw) treatment was not significantly different from K1BO (straw), but significantly different from K2BO and K0BO (without organic matter). The lowest C/N value was obtained in the K0BO treatment. The high value of C/N in K3BO and K1BO treatments was because both treatments contained straw organic matter. Straw is an organic material that contains a lot of lignin and nutrients. According to Chivenge *et al.* (2020)[21], rice straw contains 80, 40, and 30% of the potassium (K), nitrogen (N), and phosphorus (P) available for rice plants, respectively.

Therefore, using rice straw compost as a component fertilizer for the rice planting system can reduce the need for fertilizer for the next crop. Furthermore, composting rice straw in the soil reduces the impact of heavy metal contaminants on plants. On the other hand, rice straw compost can significantly increase crop yields in fields contaminated with mine waste compared to plots without compost (Cuevas *et al.*, 2019)[22].

A C/N ratio that is too high ( $> 35$ ) delays the process, as microorganisms must oxidize excess carbon until a C/N ratio comfortable for their metabolism is reached. A balance of nutrients in the form of an optimal C/N ratio is essential for formulating an efficient compost mix. As composting time progressed, the C/N variation projected the rate of organic degradation as regulated by the rate at which carbon was converted to CO<sub>2</sub>. Ideally, the required C/N ratio lies in the range of 25–35; it is stated that microorganisms require 30 parts C per unit part N (Kutsanedzie *et al.*, 2015)[23].

*Trichoderma sp.* functions as a decomposer of organic matter for composting, which can reduce the C:N ratio (Nghie *et al.* 2020)[24]. *Trichoderma sp.* also functions as a biocontrol agent, natural decomposition, bioremediation, stress tolerance, and biofertilizer by helping rice plants take up nutrients from the soil (Debnath *et al.* 2020[25]; Zin and Badaluddin 2020[26]). *Trichoderma sp.* in combination with several microbes was used to determine the different functional groups associated with the production of secondary metabolites in compost (Zeilinger *et al.*, 2016)[27]. A higher C/N ratio (compared to the recommended one) slows down the composting rate and reports nutrient deficiencies in the microbiota, due to substrate accumulation. Meanwhile, lower C/N results in increased N content per C (degradable) and inorganic nitrogen, most likely to be lost as ammonia through evaporation or leaching (Zhang *et al.*, 2016)[28].

The highest water content of compost obtained in K3BO treatment was not significantly different from K1BO, but significantly different from K2BO and K0BO treatments. The K1BO treatment was not significantly different from the K2BO treatment. The high water content of the compost in the K3BO treatment was because the organic matter in this treatment consisted of three types, so its ability to store water was higher. Straw and husks have the ability to store enough water so that the compost's moisture content increases.

Moisture conditions affect microbial activity, oxygen uptake rate, temperature, and porosity in composting (Petric *et al.*, 2015)[29]. Effective composting requires about 50–60% (v/w) moisture content according to the composition of the raw material (Bernal *et al.*, 2009)[30]. There is an inverse relationship between water content and temperature, showing an increase in temperature when the water content decreases (Varma and Kalamdhad,



2015)[31]. High temperatures result in higher evaporation, resulting in a decrease in the rate of decomposition of organic matter. Discussions need to be had about maintaining humidity for the functioning of waste microbes. On the other hand, higher than required humidity during composting can result in anaerobic conditions that might stop the composting process (Makan *et al.*, 2013)[32]. In lignocellulose composting with rice straw as raw material, a higher water content is required to soften the strong fibrous material, which implies a positive effect on the process (Kadar *et al.*, 2012)[33].

The results of the BNT test analysis on the highest compost P nutrients were obtained in the K3BO treatment and were not significantly different from K1BO, but significantly different from K2BO and K0BO. The treatment of K1BO was not significantly different from that of K2BO but significantly different from that of K0BO. The lowest compost P nutrient content was found in the K0BO treatment. The P nutrient content of compost is largely determined by the raw materials of compost and decomposing microorganisms. In this treatment, the compost contains microbes such as *Trichoderma sp.*, *Pseudomonas fluorescens*, and *Bacillus thuringiensis*, which are classified as phosphate solubilizing microorganisms. These three microbes decompose organic matter consisting of straw, husks, and bran in the K3BO treatment so that the availability of P increases. Phosphorus solubilizing microorganisms produce organic acids to solubilize unavailable P to become available (Krishnaraj and Dahale, 2014)[34]. The addition of a combination of phosphate solubilizing microbes to compost increases the efficiency of P nutrient use, increases growth, yield of cotton seeds, and results in a very significant improvement in the morphology of cotton compared to the use of inorganic single fertilizers (Arief *et al.*, 2018)[35].

The results of the BNT test analysis of the highest compost K nutrients in the K3BO treatment were significantly different from the other three treatments. Application of compost containing high level of K increases the growth and percentage of sucrose in sugar beets significantly and can replace chemical fertilizers with potassium. It will naturally help reduce environmental pollution (Hellal *et al.*, 2013)[36].

Bioorganic compost enriched with *Pseudomonas fluorecens*, *Bacillus thuringiensis*, and *Azotobacter* microbes. The addition of these microbes will accelerate the degradation process of organic matter. The highest microbial population was found in the K3BO and K2BO treatments, the high number of microbes was due to this treatment having organic matter consisting of straw, husks, and bran, as opposed to the K3BO and K2BO treatments consisting of husks and bran. In treatment B, the microbial population was  $432 \times 10^8$  lower than in K3BO and K2BO treatments, and lower than in the K1BO treatment with organic matter from straw. The K2BO treatment with organic matter of husk and bran increased the number of microbes to  $508 \times 10^8$ . The lowest number of microbes in the K0BO treatment was  $279.33 \times 10^8$ . the K0BO treatment without organic matter was only an activator of *Trichoderma sp.*

This result is in line with the amount of organic matter present in the compost (Table 4). The amount and type of organic matter contained in the compost affect the number of microbes present in the compost. It seems that compost containing bran has a very high microbial count; bran has a carbohydrate content.

According to Marlina *et al.* (2020)[37] the dynamics of the microbial population in compost is influenced by the availability of nutrients in the substrate, followed by the value of the C/N ratio. Compost is a medium for bacteria, so it supports the highest bacterial population, reaching 78% (Boulter *et al.*, 2010)[38]. Furthermore, in composting, microbes require C, N, P, and K as the main nutritional ingredients, namely the decomposition of C-organic for energy

supplements and the development of microbial population activity (Iqbal *et al.*, 2015)[39].

Some of the known strong cellulose-producing bacteria include; *Cellulomonas*, *Pseudomonas*, *Bacillus spp.*, and *Thermoactionmycetetes*. Also, fungal species such as *Aspergillus*, *Trichoderma*, *Sclerotium*, and white rot fungi, produce extracellular enzymes that are responsible for degrading cellulose and lignin during composting (Awasthi *et al.*, 2015)[40]. Excellent compost quality by adding microbes (Rastogi *et al.* 2022)[41].

Composting with different microbial combinations can have a contrasting effect, depending on the substrate (Voberkov *et al.*, 2017)[42]. The effect of bioactivators on the composting process is different in the stages of decomposition of lignin and cellulose, the maximum degradation of lignin occurs in the ripening phase for all treatments (Fersi *et al.*, 2019)[43].

In Figure 3, it can be seen that compost, which contains a lot of organic matter, has more microbial growth due to the availability of a lot of food. The addition of microbes to the compost mixture, affects the temperature profile and ammonia emission due to mesophilic and thermophilic bacterial populations (Barthod *et al.*, 2018)[44]. In addition, increasing the enzymatic activity and minimizing the initial blank time of the biological process, make this microbial accelerated composting more effective (Saad *et al.*, 2013)[45].

## 5. Conclusion

This study showed that compost treated with K3BO, consisting of cow dung, straw, husks, bran, *Trichoderma sp.*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, and *Azotobacter*, was the best compost mixture. Parameters observed at an average temperature of 37.34°C, pH 7.27, C/N 11.59, and water content of 13.57 had a P nutrient content of 2.33%, and a K nutrient content of 1.24%, which were better than other treatments. Enrichment with microbes gave a bacterial population of 530.67 x 10<sup>8</sup>, also more than with other treatments.

## 6. Acknowledgments

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