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The Roles Of Bacteria In The Guts Of Subterranean Termites *Macrotermes gilvus* Hagen As The Biological Agents Of Organic Material Degradation

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ABSTRACT

Termites are insects that have role as the primary degradator in nature. Termites have lignocellulosic microbes in its digestive tract that help in the degradation process of organic materials such as wood and leaf litter. Microbes in the digestive tract have the potential to be utilized as a starter for composting. The purpose of this study was to identify the total number of bacterial cells (CFU/g) on the compost which has been added concentration bacteria starter of 0%, 10%, 20%, 30%, 40%, 50%, and the composting duration were 1, 2, 3 , 4, 5 weeks, to determine the maturity scale of the compost in the addition of concentration bacteria starter of 0%, 10%, 20%, 30%, 40%, 50% and the composting duration 1, 2, 3, 4, 5 weeks, the compost profile on the degradation products of mature leaf litter bins, and the concentration of starter bacteria from the guts of subterranean termites *Macrotermes gilvus* Hagen and the optimal duration to compost the leaf litter bins. The study was conducted by using a Complete Random Block Design. Two hundred of termites *Macrotermes gilvus* Hagen in the worker caste were grabbed its guts. The guts of the termites crushed and added 1 ml of sterile distilled water and centrifuged. 0.3 ml of aliquots were taken with a pipette and put into the medium of Nutrient Agar (NA). Inoculant was incubated at the temperature of 300 C in the incubator for 4 days. Furthermore, the inoculant which has grown was transferred into medium of Nutrient Broth (NB). Composting test was done by using a modified takakura technique using perforated bucket composting. The variations in the concentration of microbes starter from the guts of termites which have been tested on composting were 0%, 10%, 20%, 30%, 40% and 50% and observation block on the composting duration were 1 week, 2 weeks, 3 weeks, 4 weeks and 5 weeks. Each treatment was repeated for 3 times repetition. The collecting data included physical data, profiles and biological compost. The biological data was the total number of bacteria (CFU/g) from compost during the composting process. The physical data were the color of compost, the texture of compost and the smell of compost. Data compost profile was the ratio of C/N, humidity and pH. Compost research results indicated the total number of bacteria reaching 275 million cells on the compost that has been coupled with the 50% of starter bacteria and the composting duration was 5 weeks. The maturity of compost was able to achieve the maximal scale that were black compost, crumb texture and smell like soil. The chemical content of compost are C / N ratio of 1.81, pH 7 and moisture 60.80%. The optimum starter concentration affecting composting was 50% with a 3-week composting duration.

Key Word: bacteria, *Macrotermes gilvus* Hagen, degradation, compost

INTRODUCTION

Waste is a global problem that occurs in many countries one of which is in Indonesia. The data of Ministry of Environment (MOE) concerning garbage volume in 2014 reached an average of 130 thousand tons per day with the most percentage is organic waste. The amount of this waste will be growing along with the growth of Indonesian population. Waste management in Indonesia until now is still being improved by innovating the methods of composting. In accordance with Law number 18/2008, waste management is not only the responsibility of the governments, but also the responsibility of the public and businesses as the waste generator to create a clean and healthy environment. Some of the constraints faced by the government in waste management are the lack of public awareness to manage waste from its sources, waste management technologies which are still traditional (open dumping and burning), the lack of landfill, the limited transportation of garbage and duration of the composting process.

The duration of composting using the traditional method takes 3-4 months. The development of further composting methods directed

at the composting process in a faster time and can degrade the organic components more leverage. Currently, waste treatment methods which widely applied by the community are drum rotates, takakura, and method of closure. Composting with that method takes an average of one month. One way of producing organic fertilizer is using trash or waste which is very abundant. The composting process can not be separated from the roles of biological agents. Biological agents often used in the composting process are worms, bacteria, fungi and insects. Composting duration is affected by manufacture of the compost (Simanungkalit et al. 2009).

Termites are insects that have role as the primary degradator in nature (Subekti, 2011). Generally, termites do not attack tree or live plants, except most of the genus Coptotermes. The process of degradation done by termites can degrade lignocellulose more optimal than degradation process done by other organisms. Termites are the first organisms that digest lignocellulose into smaller molecules before other organisms such as worms and soil bacteria play a role in maximizing the degradation process of organic material into the soil constituent elements. Lignocellulose consists of 20-50% of cellulose, 15-35% of hemicellulose, and 18-35% of lignin. degradation activity of cellulose by termites roled by enzymes produced by the termites themselves and the microorganisms in its gut (Ni and Tokuda, 2013). Cellulose is a linear polymer of glucose molecules linked to each other with the type of ß1,4 glycosidic bond. The bond is hydrolyzed by cellulase enzyme which also plays a role in the recycling of polysaccharides. According to Prins and Kreulen (1991), termites have the ability to digest lignocellulosic up to 74-99%. This potential can be used to degrade organic waste optimally by isolating and multiplying microorganisms in the digestive tract of termites. Potential bacteria in the gut of termites need to be tested as a stareter of litter waste composting.

METHOD

The Taking of Terminate's Gut

The termites were collected from the observation around campus of Semarang State University. The termites used as research objects are subterranean termites *Macrotermes gilvus* Hagen worker caste. The termites which have been collected, its body surface sterilized using ethanol 70% and washed using sterile distilled water. The termites' guts taken aseptically using tweezers micro and tweezers macro to hold the body of termites. The guts that have been taken are collected in microtub.

Microbial culture of termites *Macrotermes gilvus* Hagen

Microbial cultures of subterranean termites *Macrotermes gilvus* Hagen performed according to the method of Tay et al (2010). Digestive tract that has been taken in microtub mixed with 1 ml of distilled water and centrifuged at 1000 rpm for 1 minute to separate the debris with the liquid. Subsequently, 0.3 ml aliquots were suspended on the NA medium and cultured at 30 ° C for 4 days then propagated in the NB medium.

Starter bacteria manufacture of Termite's Guts *Macrotermes gilvus* Hagen

Starter stock solution is made by planting bacteria that grow on the NA medium into NB medium 9 ml. The bacteria grown in NB medium were incubated for 2 times 24 hours at temperature of 30^0C. Furthermore, the starter stock solution is stored in the freezer.

The Determination of Starter Microbe Concentration of the Termite's Guts

Starter stock solution is done through dilution in various concentrations of 0%, 10%, 20%, 30%, 40% and 50%. Concentration of 10% made by 1 ml of starter stock solution dissolved in 9 mL of NB. Concentration of 20% made by 2 ml of starter stock solution dissolved in 8 ml of NB. Concentration of 30% made by 3 ml of starter stock solution dissolved in 7 ml of NB. Concentration of 40% made by 4 ml of starter stock solution dissolved in 6 ml of NB. Concentration of 50% is made by 5 ml of starter stock solution dissolved in 5 ml of NB. Concentration of 0% only consists of NB 10 ml to be used as a control.

Composting

Small Scale Composting Test

Small scale composting test is done in the laboratory by using a bottle jar. Garbage litter enumerated with ± 2 cm diameter mixed with compost in ratio 40%: 60% and starter bacteria of termites *Macrotermes gilvus* Hagen. Starter concentration variation used is 0%, 10%, 20%, 30%, 40% and 50% and repetitions for three times. Starter dissolved into molasses and water with ratio 1:1:50. Small-scale composting test conducted in a bottle jar with the composting composition of 4 grams of chopped litter garbage mixed with 6 grams of compost then added with the starter. Starter solution on a small scale composting test is 100 ml of starter added 100 ml of molasses and dissolved into 5000 ml of sterile distilled water. The starter solution is mixed with litter bins and compost. The humidity of compost is conditioned to reach 60%. The duration of composting are 1 week, 2 weeks, 3 weeks, 4 weeks and 5 weeks. Parameters measured are temperature, pH, and moisture which measured every 2 days. Another measured parameter are the smell of compost, the color of compost, the texture of compost, the C/N levels of compost and the number of bacterial

cells per 10 gram of compost. During the composting process, performed stirring process twice a day.

Large Scale Composting Test

Large-scale composting test conducted in the composting room by using a modified takakura method uses perforated bins. Inside of the trash lined with cardboard to prevent insects enter the trash. Inside of the base bins lined with husk pillows which has function to absorb leachet of the compost. Over the pillow, put 300 grams of compost mixture and 200 grams of litter bins which were already chopped. The comparison between compost and litter bins is adjusted by the comparison to the Takakura method which is the ratio of compost: litter bins 60%: 40%. Compost and litter bins are then mixed with a solution of starter, sugar and water. Composting compositions are 800 grams of litter bins that has been chopped mixed with 1200 grams of compost then added to a solution of 200 ml of starter until the compost humidity reaches 60%. Starter variations tested in this study were 0%, 10%, 20%, 30%, 40% and 50%. Starter concentration variations is performed by using a solution of Nutrient Broth. Starter dissolved into molasses and water at ratio of 1:1:50. Starter solution on a large scale composting test is 2 ml of starter added 2 ml of molasses and dissolved in 100 ml of sterile distilled water. Starter solution is mixed with litter bins and compost. The humidity of compost is conditioned up to 60%. The duration of composting is 1 week, 2 weeks, 3 weeks, 4 weeks and 5 weeks. Parameters measured are temperature, pH, and moisture measured every 2 days. Another measured parameter are the smell of compost, the color of compost, the texture of compost, the C/N levels of compost and the number of bacterial cells per 1 gram of compost. During the composting process, the stirring process is carried out once every two days. Furthermore, the top of the compost coated with husk pillows, black cloth, and last closed with a lid bucket (Nurulita and Budiyono, 2012; Ying and Ibrahim 2013).

The collecting data includes data through physical, chemical and biological. The physical data are pH, moisture, temperature, the smell of compost, the color of compost and the texture of compost. The chemical data is the content of the C / N compost which is tested at the end of composting. The biological data is the number of bacterial cells in 1 gram of compost during the composting process.

Data Measurement

Measurement of PH, Humidity and Temperature

Measurement of pH, humidity and temperature done every day during the composting process are 1 week, 2 weeks, 3 weeks, 4 weeks and 5 weeks. pH and humidity are measured using a pH measurer / moisture at the root level, the temperature is measured using a thermometer.

The Colour of Compost, the Texture of Compost, and the Smell of Compost

Smell, color and texture of the compost compared with the maturity parameter of compost according to SNI No. 19-7030-2004 (2004), namely:

1) The color of compost: blackish like soil
2) The texture of compost: crumbs, such as land and no longer texture prior to composting
3) The smell of compost: as soil

Testing of C/N Ratio Levels

Ratio levels testing of compost C / N conducted at the Laboratory of Soil BPTP Ungaran. Compost samples tested moisture as humidity factor (HF), the levels of Carbon and the levels of Total Nitrogen. C / N ratio of compost is obtained by dividing the levels of carbon to total nitrogen in the compost.

RESULTS AND DISCUSSION

Based on the research results, the growth of bacteria in the compost with the starter concentration addition of 0%, 10%, 20%, 30%, 40%, 50% and the composting duration of 1, 2, 3, 4, 5 weeks showed that the number of bacteria varied. The number of bacteria was more in the compost added with starter when compared with the controls so the addition of starter on the leaf litter bins gave the effect than the one which was not added with starter bacteria from the guts of subterranean termites *Macrotermes gilvus* Hagen. The addition of starter concentration on the leaf litter bins with higher concentrations, it also increased the total number of bacteria in the compost. It was also the duration of composting affected on the total number of compost bacteria. The total number of bacteria on the composting duration of 3, 4 and 5 weeks showed that the number cell has been more and more.

The composting duration 1 and 2 weeks showed that the amount of bacteria decreased. This stage illustrated that the bacteria through adaptation phase resulting in slow growth even many bacterial cells die. Bacterial cell death shown on the composting duration in the second week that the total number of bacteria less than in the first week. According to Kusumaningati et al., 2013, the growth of cells number has slowed down or reduced because the process of adaptation and an increase in cell size. Lag phase was an important stage of adjustment to add metabolites on the bacterial cells for achieving maximum cell synthesis.

Furthermore, on the composting duration of 3, 4 and 5 weeks, the number of bacteria was constantly increasing. This stage was a stage of exponential growth or log phase. During this phase, mass and volume of the cells was increased by the same factors included nutrition and environment. The increase speed in cell number could be designated with the growth of bacterial cells on the compost up to two times from the cells number. Bacterial cells divided at this phase with constant speed determined by the intrinsic properties of

bacteria and environmental conditions. Composting temperature at this stage reached an average of 32-34^0 C. The material volume for composting effect on the temperature achieved during the composting process. Litters bins and materials used in research consecutive 200 grams and 300 grams so that the compost temperature was more influenced by the temperature of environment around. The exponential growth phase might also occured in the composting duration of day 6-15 depending on the availability of nutrients and environmental conditions (Firman, et al, 2013)

The total number of bacteria which was added to the leaf litter also affected the maturity level of the compost. Leaf litter bins coupled with starter 30%, 40% and 50% reached maturity at 3 weeks old. Whereas the leaf litter which added starter 10% and 20% reached maturity at 4 weeks old. Based on the observation, the higher starter concentration is added to the leaf litter, then the faster the compost maturity. The maturity level of compost done by adding bacterial starer proportional to the total number of bacteria. The maturity of compost reached maximum maturity when it was in accordance with ISO 2004 that is the color of compost is black, the texture is crumb, and the smell is like soil. The scale of maximum maturity assessed with scale 4 in this study to quantitatively compare the quality of compost between treatments.

The quality of mature compost was reinforced with the results of compost profiles test that included the levels of organic-C, the levels of N-total, C / N ratio, pH and the moisture of compost. The results showed that compost profile qualified compost that has reached maturity. Organic-C compost 27.41%, the levels of N-total compost 1.8%, the ratio of C / N 15,11, pH 7 and humidity 60.80%.

Based on the test results of moisture in the compost at 60.80%, the moisture was higher than the standard of compost in Permentan in 2011 which amounted to 15-25%. High moisture was due to the compost before being tested was not dried beforehand so that the compost was still in damp conditions. Drying on the compost enough by drying it and must not be affected to direct sunlight. Compost that was exposed to direct sunlight could affect on the carbon content in the compost. Some microorganisms that play role in the degradation process of organic material were sensitive to direct sunlight. So that when the compost exposed to direct sunlight, the bacteria could not perform the degradation process of organic matter maximally and resulted carbon in oganic material is not degraded. The moisture of 60.80% suitable with the compost moisture levels which required by the bacteria in the degradation process of organic materials. The range of bacteria needed moisture during the composting process was 60-63%. (Saithep et al., 2009).

The carbon content was nutrients as the determinants of compost maturity. On the results, it was obtained the carbon content in the amount of 27.41% showed that carbon content was enough when compared to standard of Permentan 2011 that the carbon content is at least 15. In the composting process occured degradation of organic compounds that one product is CO2 gas to be released into the atmosphere. Thus, in the composting process, it would decrease the total carbon content gradually until it reached a fixed price which became the indication of compost maturity (Hendri et al., 2009).

The nitrogen content was also the indicator of compost maturity. On the results obtained that the nirogen levels in the compost is 1.8% showed that the nitrogen levels are fairly compliant to the Permentan 2011 with a total percentage ratio of NPK on the compost is 4%. C/N ratio of compost has value less than 20 indicated the occurrence of mineralization on the organic matter. The nitrogen in the compost was originally used by microbes to reproduce themselves. Increasing the number of bacteria would increase the decomposition activity of organic material so that N mineralization increased. By shrinking organic matter reserves will improve the availability of N and other nutrients, causing C/N ratio of compost be lower. C/N ratio which was high above 30 indicates a lack of bacteria that degrade organic. Besides that, evaporation of N nutrients could lead to lower N content and increased C / N ratio. So that the high of C / N ratio more than 30 due to the immobilization of nitrogen (Iliyin et al., 2012).

The combination of composting duration and the addition of most optimum concentration starter on the composting process is 3-week composting duration and 50% of starter concentration addition. The process of composting for 3 weeks with the 50% of bacterial starter concentration addition showed the results of mature compost. Maximum compost maturation achieved on the scale of 4 with the quality of dark black compost resemble soil, the texture was very devastated and the compost smell was like the smell of soil.

3-week composting duration is the optimum time of composting process with the addition of 50% starter bacteria from the gut of termites *Macrotermes gilvus* Hagen because in each week of composting duration gave influence on the compost maturity. The composting duration at 5 weeks showed the best composting results than the composting old less than 5 weeks.

Testing of C/N ratio in this study was done from the compost that has the most excellent maturity. Compost which has 5 week composting duration and the addition of 50% starter concentration showed having the most excellent compost maturity. 50% starter treatment compost, 3-week composting time has reached maturity. Hence, in the composting duration continues until week 5, the maturity of compost became better. The longer of the composting time, the process of degradation is maximum. The compost has the highest number of bacteria that is 275 million bacteria

cells/ 1 gram of compost. The growth of bacteria in compost as much as 275 million cells / 1 gram of compost directly proportional to good composting results so that the bacteria act as the biological agents in the composting process. Based on the observational data Figure 1, the number of bacteria in composting more than 5 weeks, the amount of bacteria decreased. Good compost, not only indicated by the physical form of its compost mature but also could be seen from the growth of bacteria which decreases. At the moment the number of bacteria decreases, it has meaning that the degradation process of the compost material has been stopped. In the fifth week, compost with the addition of 50% starter concentration has better compost maturity. Maturity of compost in the treatment of 50% starter concentration indicated by the C/N ratio of compost at 15.11.

Based on the observation, it could be known that the minimum composting duration was 2 weeks with the starter concentration addition in the amount of 40%. While, the optimum time to reach faster compost maturity is composting with a long period of 3 weeks and the starter concentration addition in the amount of 50%. Maximum composting was possible based on the observation at 5-week composting duration and the starter concentration addition in the amount of 50%.

CONCLUSION

The use of starter bacteria from the gut of subterranean termites Macrotermes gilvus Hagen gives the results of compost with the number of bacteria $2{,}75x10^8$ cells / g. The profile of compost product with starter concentration 50% and 5-weeks composting duration has black color, crumb texture, aroma like soil, C / N ratio 15.11, pH 7.0 and moisture 60.80%.

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