THE ROLE OF GUT MICROORGANISMS FROM THE SUBTERRANEAN TERMITE *Macrotermes gilvus* Hagen DURING COMPOSTING PROCESS

by

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Abstract

The role of termites in the degradation of woody components is not accomplished by the termites alone but in association with microorganisms inside the termites' gut. Microbes in the digestive tract have the potential to be utilized as a starter for composting. In this study, an observation on the composting process employing the microorganisms from the guts of termites was performed. The study was conducted by using a complete random block design. *Macrotermes gilvus* Hagen (worker caste) were collected and guts were taken by centrifugation. A starter culture was prepared by inoculating the supernatant into NB medium. Inoculant was incubated at the temperature of 30 °C for 4 days. The compost formulation involved solutions of 0%, 10%, 20%, 30%, 40%, and 50% from the microbial starter culture and 1-5 weeks of composting. Composting was done using a modification of the Takakura technique using perforated composting bins. Each treatment was conducted in triplicate. The total number of microorganisms cells/g of compost and compost profiles were determined. The results indicated the total number of microorganisms reached 92.67x 10^7 cells/g in a compost system that was coupled with the 30% starter culture at 5 weeks of composting. The maturity of compost was determined by the characteristics of black color, crumb texture, and soil-odor. The chemical profiles of compost were a C/N ratio of 11.77, pH 7.0 and moisture content 60.80%.

Keywords: microorganisms, termites' guts, *Macrotermes gilvus* Hagen, organic material degradation, compost

Introduction

Insects that live in woody environments have adjusted their physiological and biochemical pathways in a fascinating way that provides an efficient degradation of plant polymers, breakdown of lignocellulose, detoxification of plant secondary metabolites and enzyme inhibitors (Holt and Lepage, 2000). There are several orders of insects that have the ability to digest woody components, such as cellulose, lignocellulose and hemicellulose, i.e. Thysanura, Plecoptera, Orthoptera, Isoptera, Coleoptera, Trichoptera, Hymenoptera, Phasmida, Blattodea and Diptera (Scharf and Boucias 2010).

Termites (Blattodea: *Macrotermes gilvus* Hagen) are essential detrivores, feeding on a wide range of dead plant material at various stages of decomposition and are considered the best-known and most successful wood-degraders on earth. In termites a two-enzyme system plays an important role on the decomposition of plant polymers. Those systems are the endogenous insect enzymes and enzymes secreted by a variety of gut microorganisms, including protozoa and bacteria (Inward et al. 2007, Ni and Tokuda 2013). In fact, there are three stages involved in termite digestion of lignocellulosic materials. The first stage occurs in the insect guts as hydrolysis, followed by oxidation and/or fermentation, and finally acetogenesis and/or methanogenesis involving the participation of the Archaea (Watanabe and Tokuda 2010). The populations and diversity of microorganisms are influenced by characteristics of the termite gut, and immune system, including the gut pH, intestinal structures, and diet (Noirot and Darlington 2000).

In fact, the association of mutual symbiosis, i.e. termites together with the microorganisms in both their guts and the environment allow utilization of a wide array of food resources. Termites activities such as nest construction involves processes like carbon mineralization and nutrient recycling, especially in tropical areas (Noirot and Darlington 2010). On the other way, the nest of termites, i.e. mounds building oby *Macrotermes gilvus* Hagen provides suitable climatic conditions for the colony and its symbionts. It protects them from predators and may promote a strong influence on soil profile development. Through the activity of mound construction, soil translocation and soil repacking results in increased soil porosity and soil water content (Bignell & Eggleton, 2000). The capacity of termite gut microorganisms to produce enzymes for organic material degradation, support the ability of termites to translocate soil and highlights their potential for application in organic waste decomposition or composting.

In Indonesia, waste, especially organic waste is a growing problem magnified by the growth of Indonesians human population. Open dumping and burning of waste are traditional forms of waste management. Other constraints faced by the government are the lack of landfills, limited transportation capacity, and the duration of the composting process. The management of waste in this country has been improving with the help of innovative methods of composting. Commonly, a traditional composting process requires 3-4 months, whereas drum rotation, Takakura, and closure methods take only 1 month. There are biological agents, such as worms, bacteria, fungi and insects employed to produce organic fertilizer by the composting method (Simanungkalit et al. 2009). Some termites are able to modify the chemical properties of soil by secreting saliva and fecal material that enrich surface soils with nutrients useful to plants (Lisa & Conacher 2000). Therefore, it is important to explore the microorganisms in termite

guts and their application in the composting process. In this study, the role of termite guts microorganisms used as a starter for composting, composting duration and compost profiles is described.

Materials and Methods

Collection of termites' guts

The termites used were *Macrotermes gilvus* Hagen, workers collected from Universitas Negeri Semarang. After collection in a clean bottle, termites were treated using 70% ethanol and washed using sterilized distilled water. Termite guts were taken aseptically using micro-tweezers and macro-tweezers were used to hold the termites. The guts were placed in sterilized microtubes.

Propagation of guts' microorganisms

A culture of microorganisms from the gut of *M. gilvus* was performed according to Tay et al (2010). The digestive tracts collected in microtubes were mixed with 1-ml of sterilized distilled water, and centrifuged at 1,000 rpm for 1 min to separate the supernatant from the debris. Subsequently, 0.3 ml aliquots of supernatant were suspended in NB medium and observed in NA medium. Finally, the microbial suspension was maintained at 30 °C for 4 days.

Preparation of microbial starter culture for stock solution and working solution

A starter culture preparation of the gut microorganisms was obtained by taking 1 ml of the aforementioned microbial suspension added to 9 ml NB medium. The mixture was incubated at 30 °C for 48 h and a working solution of the starter culture prepared by dilution. Various concentrations of 0%, 10%, 20%, 30%, 40% and 50% were obtained by mixing the stock solution with NB medium. The 0% dilution consisted of 10 ml NB medium that acted as a control.

Large scale composting

Large scale composting was conducted in a composting room using perforated bins in a modification of the Takakura method. The bin was lined with cardboard to prevent insect escape. All experiments were done in triplicate. Each of the working solutions of the microbial culture (0%, 10%, 20%, 30%, 40% and 50%) were dissolved in molasses and sterilized distilled water at the ratio of 1:1:50, respectively. Organic litter was chopped into the particle size ± 2-cm in diameter. The base-line part of each bin was lined with husk paddings to absorb the compost leachate. Over this padding 200 g compost mixture and 300 g chopped organic litter was spread. Compost and litterbins were then mixed with 2.6-l of the microbial solutions at the ratio of 1:1:50 molasses, microbial stock solution and water, respectively. The duration of composting was 1-5 weeks. The parameters temperature, pH, and moisture were measured every 2 days. Other subjective parameters were also measured including the smell, color, texture of the compost. Lastly, we measured the C/N ratio and the number of bacterial cells per 1 gram of compost. During the composting process, stirring was carried out once every two days. Furthermore, the top of the compost was covered with husk pillows, a black cloth, and a bucket lid (Nurulita and Budiyono, 2012; Ying and Ibrahim 2013).

Results and Discussion

Microbial numbers and compost maturity

Microbial growth as measured by cell numbers was variable depending on the concentration of the starter culture working solution and composting duration (Figure 1). The most abundant number of microorganisms was found in compost containing starter culture solution compared to the controls. The addition of higher concentrations of starter culture also increased the total number of microbial population in the compost.

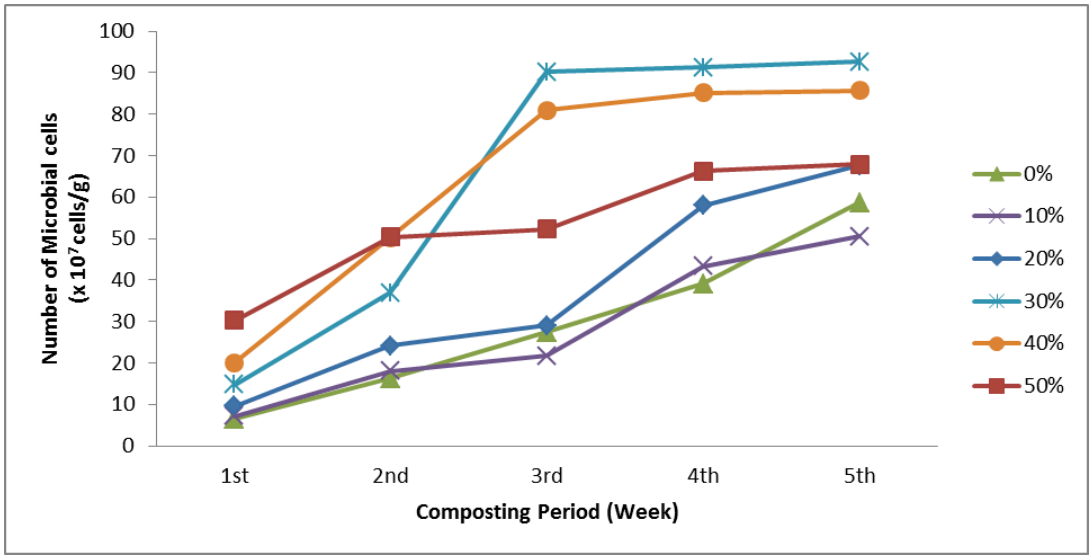

Figure 1. Number of microbial cells (x 10^7 cells/g) during composting were affected by the concentration of starter culture and composting period

An increased number of microbial cells was found at all starter culture concentrations during the 5 weeks of the composting process. A slow growth rate was found at the first and second week of the composting period. This phenomenon illustrated that the microorganisms were in an adaptation phase or lag phase where their metabolic pathways had adjusting to the environmental nutritive elements. After the second week of composting, the total number of microorganisms at 3rd, 4th, and 5th week showed significant growth. This stage represented the exponential growth or log phase where the mass and volume of the cells increased in accordance to nutrition and environment conditions. The speed of cell growth was up to 2-5 times higher than the initial cells number. At this phase, cells reproduced and the enzymes for lignocellulosic degradation process were secreted. In fact, the environmental conditions including temperature during this composting period averaged 32-34 °C. The exponential growth phase might also have occurred during days 6-15 depending on the availability of nutrients and environmental conditions (Firman et al. 2013)

According to the results, the optimum concentration of microbial starter culture concentration was 30%. This concentration gave the highest number of microbial populations in the compost, and it promoted faster organic material degradation to reach compost maturity. The concentration of microbial starter culture added to the compost

and organic litter affected the compost maturity level. Organic litter coupled with microbial starter at 30%, 40% and 50% reached maturity at 3 weeks old; whereas the organic litter, at starter concentrations of 10% and 20%, reached maturity at 4 weeks. We conclude that a three-week composting duration is optimal for the composting process with the addition of 30% microbial starter from the gut of termites *M. gilvus* . The composting duration at 5 weeks showed the best composting results compared to the composting durations of less than 5 weeks. It can be concluded that based on our observations, the starter culture concentration and the duration of composting were in line with the maturity of compost. The higher starter concentration added to the process and the longer the duration resulted in faster compost maturity. In accordance with SNI (2004), the maturity of compost reached a maximum level when its color is black, the texture is crumb, and the smell is similar to soil. The scale of maximum maturity was assessed at the scale 4 in this study to quantitatively compare the quality of compost among treatments. Based on the assessment of compost quality and maturity, a four scale was found at compost treatment with 30% microbial starter culture solution.

Compost profiles

The quality of mature compost was reinforced with the results of compost profile assays that included the level of organic-C, N-total, P-total, C/N ratio, pH, and the moisture. The results of the compost profiles verified that the product qualified as mature compost (Table 1).

Table 1. Compost profiles obtained from compost using 30% microbial starter culture solution

Compost profiles	Value
Organic-C	48.87 ± 1.23 %
N-total	4.15 ± 0.11 %
P-total	4.21 ± 0.08 %
C/N ratio	11.77 ± 0.67
pH	7.0 ± 1.17
Moisture content	60.8 ± 1.81 %

The compost humidity reached 60.80%, higher than the standard of compost released by the Ministry of Agriculture, which states that the moisture content of compost should be between 15-25%. High moisture was observed due to the damp condition of the compost before treatment. Drying the compost is best accomplished in direct sunlight and it was difficult to conduct the drying process since this study was conducted during the monsoon period. Drying with oven or direct sunlight exposure could trigger the release of carbon in the organic materials. Some microorganisms that play an important role in the degradation of organic material are sensitive to heat and direct sunlight. However, a moisture content during the composting process of 60.80% is suitable for microorganisms according to Saithep et al. (2009)..

Compost maturity is determined by carbon content and our results showed that the carbon content was quite high, almost half of the compost by weight. In the process of composting, the degradation of organic compounds produces carbon dioxide released to the atmosphere. Thus, it would decrease the total carbon content gradually until it reached a static value, which is the indication of compost maturity (Hendri et al. 2009).

The nitrogen content is also an indicator of compost maturity. Based on the results obtained, the total nitrogen of our compost was in accordance with the standard

level of NPK ratio, around 4%. A C/N ratio of compost that has a value less than 20 indicates the occurrence of mineralization on the organic compounds. The results showed that the C/N ratio was 11.77 ± 0.67. Microbes use the nitrogen available in compost for cell reproduction. An increased number of microorganisms would increase the decomposition activity of organic materials by decreasing the organic material reserves, one might improve the availability of N and other nutrients in the final compost. Besides that, evaporation of N could lead to lower N content and increased C/N ratio. Therefore, the composting system must be completed in a closed place to prevent the immobilization of nitrogen (Illiyin et al. 2012).

Testing of C/N ratio in this study was done from compost that was in the excellent maturity category. Compost at 5-weeks and the addition of 30% starter concentration showed the most excellent compost maturity. The longer the composting time, the degradation process of organic materials is optimized. Based on the observation (data not shown) a composting process of more than 5 weeks resulted in a decreased microbial population. A good quality compost is not only indicated by its physical form, but also the growth of microorganisms. At the moment when the microbial population decreases the degradation process has been stopped.

Microorganisms associated with composting were mainly bacteria, yeast, fungi, as well as protozoa. Microorganisms at the surface of the composting bin were confronted with fluctuating microclimatic conditions, and they may be exposed to stress in particular with regard to desiccation and ultraviolet radiation. Therefore, we performed frequentl stirring in order to overcome this problem (Nurulita and Budiyono 2012). Anaerobic and facultative anaerobic bacteria consume a portion of the acetate, and the other organic acids, that have been released by the microorganisms. However, anaerobic bacteria use oxygen diffusing into the bin for oxidation processes maintaining the anoxic status of the internal composting regions.

Conclusion

Microorganisms obtained from guts of *Macrotermes gilvus* Hagen play an important role in the composting process together with the microorganisms indigenous to compost and litter. The optimum composting process was obtained in 5 weeks of with the addition of 30% microbial starter culture. The optimum maturity level of compost was observed through the physical form, chemical and biological characteristics including organic-C, N-total, P-total, C/N ratio, pH, and moisture content.

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