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MICROBIAL POPULATION DYNAMICS AND FIBER REDUCTION IN THE INITIAL DECOMPOSITION OF BEEF CATTLE WASTE COMPOSTING

(Dinamika Populasi Mikroba dan Reduksi Serat Kasar pada Dekomposisi Awal Pengomposan Limbah Sapi Potong)

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ABSTRAK

Dekomposisi awal memiliki peran penting dalam proses pengomposan. Dalam dekomposisi awal, ada perubahan signifikan dalam suhu dan pH dalam komposisi mikroba dan kandungan serat dalam substrat. Penelitian ini bertujuan untuk menentukan dinamika populasi mikroba dan degradasi lignin selama proses dekomposisi awal. Limbah ternak pada umumnya memiliki rasio karbon dan nitrogen yang rendah sehingga diperlukan penambahan jerami padi sebagai sumber karbon. Penelitian ini menggunakan tiga perlakuan rasio C / N: 20, 25, dan 30. Perubahan suhu, pH, jumlah total bakteri, jumlah aktinomiset, jumlah cetakan, dan kadar lignin diamati selama 7 hari dari proses dekomposisi awal. Hasil penelitian menunjukkan bahwa rasio C / N 30 menghasilkan proses dekomposisi tertinggi, dengan fase termofilik terjadi pada hari 2 dengan suhu tertinggi 57oC dan pH 8,79, masing-masing. Temperatur mencerminkan proses dekomposisi yang tinggi melalui aktivitas mikroba dalam mendekomposisi bahan organik. Total jumlah bakteri dan aktinomiset yang dicapai pada fase termofilik adalah 179 x 1011 cfu / g dan 87 x 105 cfu / g. Tunas berkembang pada suhu mesofilik pada hari ke 5 dan volume total tertinggi mencapai 48 x 1011 cfu / g pada hari ke 6. Proses dekomposisi awal mampu mengurangi lignin sebesar 30,57%. Hasil penelitian menunjukkan bahwa dinamika populasi mikroba dipengaruhi oleh ketersediaan nutrisi dalam substrat yang dijelaskan oleh rasio C / N. Pertumbuhan bakteri dan aktinomisetes tertinggi terjadi pada suhu termofilik sementara kapang berkembang pada suhu mesofilik. Kandungan lignin dan hemiselulosa pada substrat menurun.

Kata kunci : *limbah sapi potong, degradasi serat kasar, dekomposis awal, dinamika mikroba*

INTRODUCTION

Composting is a method of waste processing that is commonly applied in the management of beef cattle waste. Composting typically reduces manure volume by 30% to 50%, which makes the material significantly more affordable and provides many other benefits. Organic materials in beef cattle waste will be decomposed biologically into simpler

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compounds. In the composting process there is an interaction between organic material and microbes as well as among the microbes involved, namely bacteria, fungi, and actinomycetes.

The three phases in composting include: the mesophilic phase, the thermophilic phase, the cooling phase. During the first phase there is an increase in the temperature. The substrate is reduced due to the degradation of organic matters (carbohydrates and proteins) by the action of mesophilic organisms (Zeng et al., 2011; Pan et al., 2012). The mesophiles are replaced by thermophiles in the second phase, an increase in the temperature in the compost piles from 45 °C to 70 °C and (Kumar, 2011; Aziz et al., 2018), and the third phase begins with the decrease of temperature of the compost pile, reduced nutrition available in the substrate for microbial activity causes microbial death in this phase (Worrel and Vesilind, 2012; Pan et al., 2012).

Most studies on the structure of the microbial community in composting have specifically focused on the early stages of the process because of under aerobic condition, temperature is the biggest selective factor of microbial populations (Erickson et al., 2009; Fourti et al., 2011; Villar et al., 2016). In the initial phase of composting or initial decomposition there is an active change in organic matter by microorganisms, one of which is lignin content.

Lignin is a complex polymer of phenylpropane units, which are cross-linked to each other with a variety of different chemical bonds. This complexity has thus far proven as resistant to detailed biochemical characterization as it is to microbial degradation, which greatly impedes our understanding of its effects. Nonetheless, some organisms, particularly fungi, have developed the necessary enzymes to break lignin apart. The initial reactions are mediated by extracellular lignin and manganese peroxidases, primarily produced by white-rot fungi (Huang et al., 2013; Janusz et al., 2017). Actinomycetes can also decompose lignin, but typically degrade less than 20 percent of the total lignin present (Ruiz-Duenas and Martinez, 2009; Priyanga and Kannahi, 2018).

Composting is a microbial-driven process. Like other living creatures, microbes need the right environment to survive and thrive. For successful composting, microbes need nutritious "food"; suitable moisture, pH, and temperature; and oxygen. During composting, microbes break down organic compounds to obtain energy to carry on their life processes and acquire nutrients (N, phosphorous, potassium) to sustain their populations. Of the many elements required for microbial decomposition, C and N are the most critical. The ideal C/N ratio for composting is generally considered to be around 30:1, or 30 parts C for each part N by weight (Chen et al., 2011), but this ideal may vary depending on the bioavailability of the carbon and nitrogen (Richard and Trautmann, 1996). Beef cattle waste has a low C/N ratio, which is approximately 18. The pattern of feeding in beef cattle greatly influences the C/N ratio in the waste produced.

This study aims to study microbial population dynamics and changes in lignin content that occur during the initial decomposition of composting beef cattle waste by adding rice straw as a carbon source.

MATERIALS AND METHODS

Sample Preparation. The substrate used in this study was beef cattle waste. The substrate was enhanced with rice straw to

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give C/N ratios of 20: 1, 25:1, and 30: 1, and adequate sterile distilled water was added by spraying to give moisture contents as much as 60%. Rice straw was chopped into 2 cm to expand the surface. The carbon and nitrogen content of each material was measured to determine the ratio of material mixture. Calculation of C/N ratio was based on the formulation by Richard and Trautmann (1996) as follows:

$$Q_2 = \frac{Q_1 \times N_1 \times \left(R - \frac{C_1}{N_1}\right) \times (10}{N_2 \times \left(\frac{C_2}{} - R\right) \times (100 -}$$

Composting System. Composting was carried out in a laboratory scale, with a model developed at Laboratory of Microbiology and Livestock Waste Management, Faculty of Animal Husbandry, Universitas Padjadjaran, Indonesia. Compost materials were stacked evenly in a pored sack with a diameter of 50 cm and a height of approximately 1 m. The initial decomposition was taken place for 7 days and temperature as well as pH was measured daily.

Microbial Analysis. Bacterial, actynomycetes, and fungi were counted daily with random sampling in each part of compost pile. Raw compost (1 g wet weight, screened to 4 mm) was placed in 25 ml glass test tubes containing 10 ml of sterile 0.9% (w/v) sodium chloride solution (adjusted to pH 7.0). Each dilution mixture of compost and sodium chloride solution was shaken for 1 minute at 200 rev/min and allowed to sit for 5 min to dissolve aggregates. Serial dilutions of 10^{-2} to 10^{-11} were prepared by sequentially transferring 1 ml samples onto test tubes containing 9 ml of sterile 0.0% sodium chloride. Subsamples at selected dilutions (10^{-11}) were pipetted onto two plates each of nutrient agar for total bacteria plate count, potatoes dextrose agar for total fungi plate count, and Actinomycete isolation agar for Actinomycetes (Dehydrated, Difco^{TM} Lab.). Each plate was incubated at 37°C for 24-48 hours.

Lignin Analyses. The procedure was in 2 stages. A ground, air-dried raw compost harvested at 7 days as much as 2 g was refluxed gently with 100 ml of acid-detergent solution (20 g CTAB per liter N H_2SO_4) and 2 ml of decalin for 60 min, filtered and washed well with water at 90ºC to 100°C until a wash with acetone failed to remove any color. Acetone was removed and the fiber was dried at 100ºC for 8 h or overnight, cooled in a desiccator and weighed. For the second stage asbestos approximately equal in volume to that of the fiber was added to the crucible unless asbestos had been added in the first stage. The crucible, supported on a 50-ml beaker, was half filled with 72% H_2SO_4 to cover the fiber and asbestos which were mixed to a smooth paste. The crucible, maintained at 23ºC, was refilled with 72% H_3SO_4 and stirred at hourly intervals as the acid drained away. After 3 h the acid was removed under vacuum, the contents of the crucible were washed with hot water until free from acid and dried at 100ºC. After cooling in a desiccator and weighing the crucible was ignited at 500ºC for 2 h and then transferred whilst hot to a desiccator, cooled and weighed. The acid-detergent lignin was calculated from the loss in weight on ignition. (Sluiter et al., 2010).

Statistical Analyses. To campare the difference in microbial counts (total bacteria, total fungi, total actinomycetes) of different treatments (C/N rasio), data were analized by analysis of variance with a test criterion (F statistic). The Tukey multiple-comparison procedure of the Statistical Analysis System SPSS 23.0.0 was used.

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RESULTS AND DISCUSSION

Heat was release as a result of microbial activity during organic matter decomposition. At day 2, the substrate temperature reached its highest point, except at C/N ratio 20 (Fig. 1)

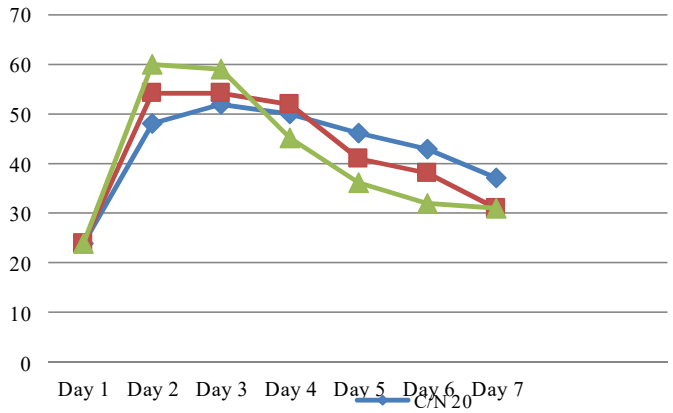

Fig. 1. Temperature during initial decomposition

Temperature was the main factor affecting the activity of microorganisms in overhauling organic matter (Sihag et al., 2014; Li et al., 2018; Moxley et al., 2019). However, the temperature also illustrated the occurrence of microorganism activity in remodeling organic matter. Temperature control during the decomposition process needed to be done to optimize microbial growth and function in reducing pathogenic bacteria (Hidayati et al., 2015; Marlina et al., 2016). The optimal initial decomposition resulted in a reformation of the substrate organic material into a simple compound and would be converted into minerals at the maturation stage. The ideal temperature in composting was a thermophilic temperature because at this temperature there was a decomposition of highly active organic matter (Marlina et al., 2016). PH measurement was important because microorganisms in composting performed in pH range 6.0 to 8.8 (Fig. 2).

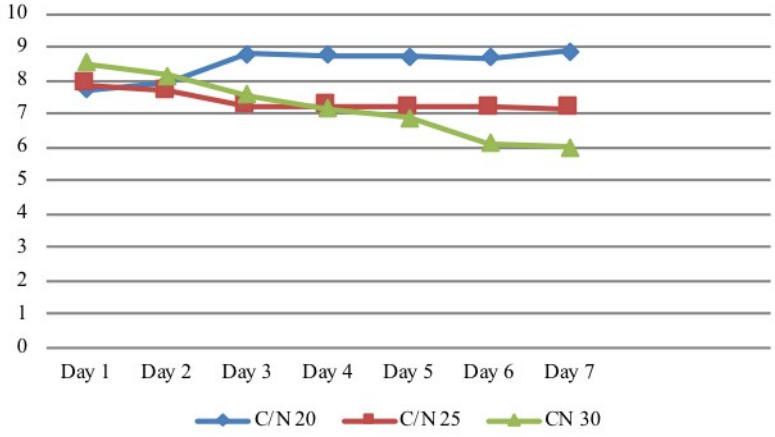

Fig. 2. pH during initian decomposition

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During the initial decomposition phase organic acids would be formed. The acid condition during composting was a suitable condition for mold growth and degraded lignin and cellulose (Sluiter, 2010; Madadi and Abbas, 2017). At this pH, molds were still in the stage of multiplying cells, had not yet produced an enzyme that was able to degrade lignin and cellulose completely. The mold would start working at the maturation stage. The growth of microorganisms in the initial decomposition was an indicator of the success of the decomposition process. Microorganisms grown namely bacteria, actinomycetes, and mold (Fig 3, 4, 5)

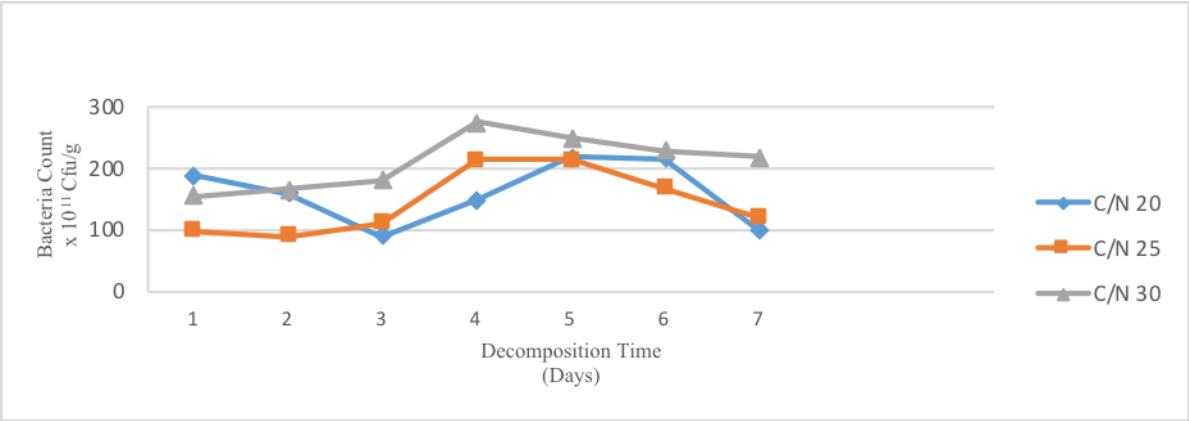

Fig 3. Bacteria growth during initial decomposition

At the beginning of initial decomposition, the number of bacteria decreased (Fig 3). This was estimated to be related to the increase in temperature that occurred on day 2 which reached 48 to 60ºC (Fig.1).

The availability of nutrients for the growth of microorganisms was very important in composting. The ideal carbon and nitrogen balance (30:1) in this study resulted in a high bacterial growth. In multiplying microbial cells required carbon as a source of energy and nitrogen was an important component of proteins, nucleic acids, enzymes and co-enzymes that had an important role in cell growth and function. The population of decomposer bacteria which was high on the substrate during initial decomposition, allowed the organic material to be maximized to modify. Several studies have shown that low carbon and nitrogen balance resulted in inefficient decomposition processes (Chen et al., 2011; Neugebauer et al., 2017) because excessive nitrogen supply tended to be overhauled into ammonia gas.

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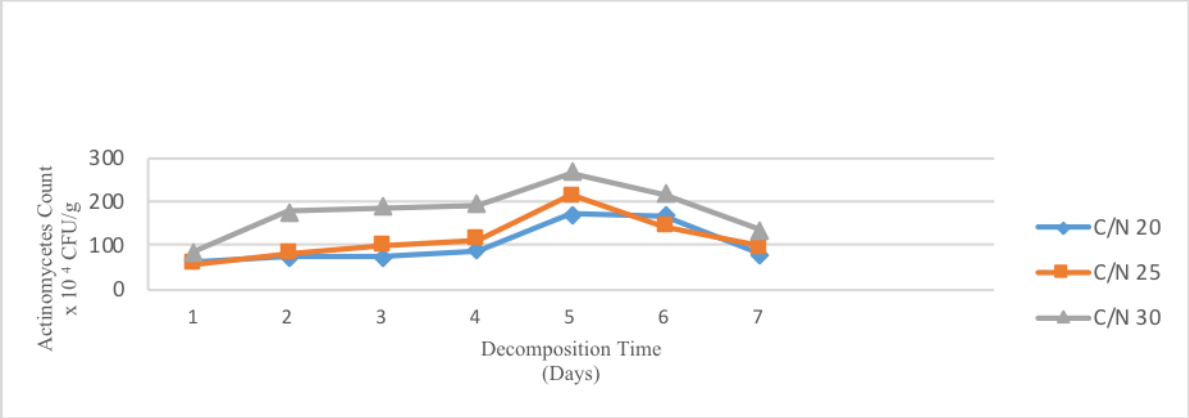

Fig 4. Actinomycetes growth during initial decomposition

Growth of actinomycetes was directly proportion with total bacterial growth, although the number of actinomycetes was less (Fig. 4). The actinomycetes were a large group of aerobics, gram-positive bacteria that form branching filaments or hyphae and asexual spores (Sharma et al., 2014). The bacterial morphology was more like a fungus, therefore in the compost raw pile hyphae covered the substrate. The growth of actinomycetes in the initial decomposition was expected to work synergistically along with cellulolytic bacteria that grew well in the thermophilic phase to overhaul the fibers in the substrate. High fiber content in beef cattle waste would be remodeled efficiently in the initial decomposition process.

Fungi had an important role in decomposing complex compounds such as cellulose, hemicellulose, and lignin. Fungi could live in the mesophilic and thermophilic phases, however the growth of fungi decreased when the temperature increased (Fig 4). In composting process, fungi tended to grow dominant in the maturation phase. In the thermophilic phase the growth of fungi tended to be restrained. The growth of fungi would increase again as the temperature declined. The growth of fungi on the substrate was clearly seen through the formation of filaments throughout the surface of the substrate. Filaments appeared could be white or gray arising from the formed spores. It was estimated that in the initial decomposition phase the fungi still multiply cells but had not produced enough enzymes to degrade the fiber.

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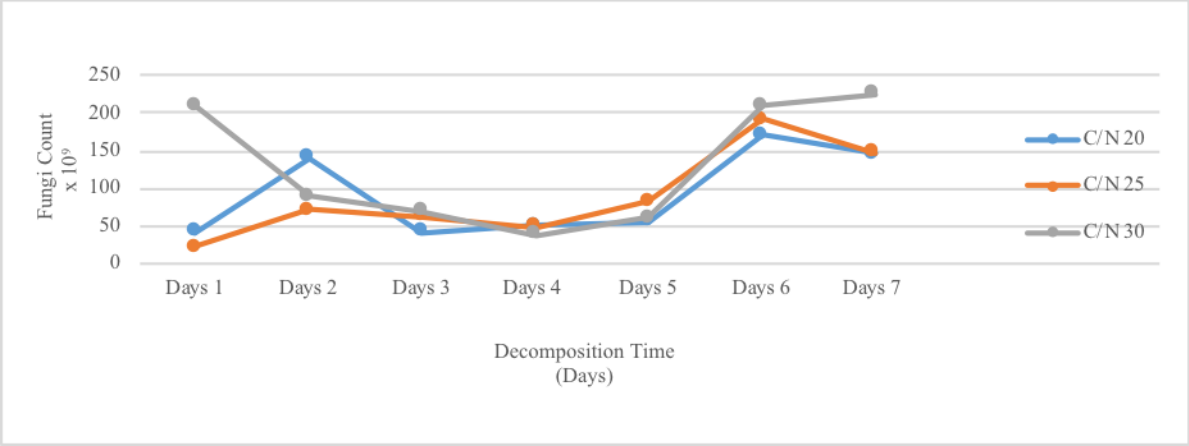

Fig. 5. Fungi growth during initial decomposition

The nutrient content of beef cattle waste was lignin, cellulose, and hemicellulose. The level of this fiber would increase with the addition of rice straw to the compost substrate. The effectiveness of initial decomposition in degrading crude fiber was strongly influenced by C/N substrate ratio (Table 1).

Table 1. Fiber reduction in the initial decomposition

Treatment	Lignin			Selulosa			Hemiselulosa		
	Before (%)	After (%)	Reduct -ion (%)	Before (%)	After (%)	Reduct -ion (%)	Before (%)	After (%)	Reduct -ion (%)
C/N 20	8.90	7.83±0.58	12.06	39.80	36.80±0.67	7.54	24.17	21.98±1.12	9.05
C/N 25	9.24	7.25±0.51	21.52	41.22	35.99±1.25	12.69	27.85	25.01±0.62	10.21
C/N 30	9.78	5.48±0.54	43.98	43.12	31.53±1.18	26.87	29.11	21.83±0.32	25.03

This was closely related to microbial activity, namely bacteria, actinomycetes, and fungi during initial decomposition. Although the decomposition of lignin in aerobic conditions was still debated, some researchers claimed that lignin degradation occurred during the thermophilic phase with a time variation of composting (Chatterjee et al., 2013; Priyanga and Kannahi, 2018). Adequate air flow on the substrate provided sufficient oxygen for the oxidation process carried out by aerobic microorganisms. The most active microbes in the thermophilic phase were bacteria and actinomycetes, while fungi were more active in the mesophilic phase.

CONCLUSION

The microbial population dynamics in the initial decomposition process of beef cattle waste was in principle similar as the composting process in other materials. Crude fiber that dominated the nutrient content in beef cattle waste had begun to be degraded in the thermophilic phase.

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