

# The potential of indigenous microbes from beef cattle waste to convert organic materials into macronutrients in liquid organic fertilizer

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**Abstract.** Implementing effective waste management practices involves using an aerobic fermentation system to produce liquid organic fertilizer. The study investigates the chemical and microbiological properties of liquid organic fertilizer (LOF) made from beef cattle waste, which is extracted and filtered using rice straw as a carbon source for decomposer microbe growth. The research experimented with three C/N ratio treatments (P1= C/N 22.5, P2= C/N 25, and P3= C/N 27.5). The parameters tested include the dynamics of bacterial and mold populations in the initial decomposition process, macronutrients and nitrogen-fixing bacteria, and phosphate-solubilizing bacteria in the LOF. Data were analyzed using ANOVA and Tukey's Test. The study found that a C/N ratio of 25 produced the highest average total bacteria on day 5 and the highest mold population on day 7 of initial decomposition. The macronutrient content of the LOF was N: 1.00 - 2.13%, P<sub>2</sub>O<sub>5</sub>: 0.46 - 0.53%, and K<sub>2</sub>O: 1.42 - 1.68%. The highest population of nitrogen-fixing bacteria was found at a C/N ratio of 27.5, while the population of phosphate-solubilizing bacteria did not differ in all treatments. The LOF meets Minister of Agriculture Regulation No. 01/2019's quality requirements for liquid organic fertilizers, with a content of N+P+K >2%.

## 1 Introduction

Beef cattle waste includes feces, blood, and other organic remains. Beef cattle feces are rich in various microbes. Some microbes in beef cattle waste play a role in decomposing organic materials into simpler compounds, including *Bacillus*, *Clostridium*, *Pseudomonas*, *Aspergillus*, and *Saccharomyces* [1, 2]. However, several pathogenic microbes harm the environment [3]. Careful management of beef cattle waste is essential to minimize the spread of pathogens and environmental impacts. Aerobic fermentation can be used to produce liquid organic fertilizer from waste effectively. The composition and activity of microbes in these processes are critical to ensure efficient and safe decomposition of organic waste.

Liquid organic fertilizer is fertilizer made from organic materials and in liquid form. This fertilizer provides plant nutrients by spraying it directly onto the leaves or pouring it into the

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soil around the roots. Liquid organic fertilizer has many benefits, including improving soil structure, increasing fertility, and supporting plant health [4, 5]. Beef cattle waste can be transformed into liquid organic fertilizer through aerobic fermentation with the help of indigenous microbes found in the waste. Regulating the oxygen supply during fermentation is essential to ensure optimal microbial activity and nutrient production [6].

In general, liquid organic fertilizer contains macronutrients and micronutrients that are important for plant growth and functional bacteria in the form of nitrogen-fixing bacteria and phosphate-solubilizing bacteria, which will determine the quality of liquid organic fertilizer [7, 8]. The nutrient content of liquid organic fertilizer varies depending on the raw materials used and the manufacturing process. This research studied the quality of liquid fertilizer from beef cattle waste through an aerobic fermentation process using indigenous microbes.

## 2 Materials and Methods

### 2.1 Initial decomposition

Prepare the materials for the initial decomposition, particularly beef cattle waste and rice straw. Weigh each mixed ingredient proportionally to achieve the necessary C/N ratio for the treatment (22.5, 25.0, and 27.5), and place it in a compost bag with a volume of 50 liters for the solid fermentation process. Then, incubate for seven days aerobically.

### 2.2 Liquid organic fertilizer production

The substrate resulting from the initial decomposition process is aired until it reaches a 20-30% water content. Extraction was carried out using hot water at 80-85 °C with a ratio of 1 kg substrate: 4 L of water and continued with a filtration process to separate the filtrate from the residue. The filtrate was fermented aerobically for 30 days.

### 2.3 Medium preparation

Weigh the NA (bacteria) and MEA (yeast) according to the label, then dissolve each in distilled water up to 1 liter and sterilize using an autoclave at 121°C for 15 minutes. After they cool, put 10-15 ml of each medium into separate petri dishes.

### 2.4 Total bacteria and mold population count

Collect a 10-gram sample from the compost bag and grind it using a mortar. Then, place the ground sample in an Erlenmeyer flask and add 90 ml of NaCl solution. Homogenize the mixture and dilute the bacterial suspension to achieve bacterial growth in the petri dish within 30-300 colonies. The dilution should be carried out to 10<sup>-10</sup>. Next, plant the bacterial suspension in a Petri dish using the pour plate method. Pour Nutrient Agar (NA) media for bacterial growth and Potato Dextrose Agar (PDA) media for mold growth. Homogenize for a few moments, wait for the media to solidify, and then incubate at 37°C for 24 hours. After incubation, count the number of bacterial colonies growing in the Petri dish. The number of bacterial colonies counted was between 30-300 colonies. Next, the bacteria that grow in the media are calculated using the following formula [9]:

$$\frac{\text{CFU}}{\text{g}} = \sum \text{coloni} \times \frac{1}{\text{dilution factor}} \quad (1)$$

N : Number of bacterial/mold colonies per gram (CFU/gram)  
 $\Sigma$  Coloni : Number of bacterial/mold colonies on the plate (30-300)

## 2.5 Macronutrient analyses

The vermicompost's total nitrogen (N) content was determined using the Kjeldahl method (SNI 7763:2018 point 6.6.1). This method involves treating the sample with sulfuric acid to transform all nitrogen into ammonium sulfate. The amount of nitrogen present is then determined by titrating the resulting solution with a standard sodium hydroxide solution. The quantity of  $P_2O_5$  was determined using the wet destruction method (SNI 7763:2018 point 6.7.4.2.1). This method involved digesting the sample with nitric and perchloric acid. The resultant solution was then heated until it dried and dissolved in water to determine the amount of  $P_2O_5$  present. The flame photometer method (SNI 7763:2018 point 6.7.4.2.2) was used to analyze the amount of  $K_2O$ . In this analytical technique, the specimen is introduced into a flame, which subsequently energizes the atoms, leading to the emission of light. Subsequently, the intensity of the emitted light is measured to quantify the presence of  $K_2O$ .

## 2.6 The functional bacteria count

Nitrogen-fixing bacteria (NFB) and Phosphates-solubilizing bacteria (PSB) populations used the total plate count method with serial dilutions, carried out up to 6 x dilution ( $10^{-6}$ ). NFB was grown on the Jensens Medium and PSB on the Pikovskaya medium [10]. Using a sterile pipette tip, drop the tip into the Petri dish add the medium, and swirl. Incubate the bacterial culture at 37 °C for 24 hr incubator. Calculating the NFB and PSB population uses the same formula as calculating the total bacterial population.

## 2.7 Data analysis

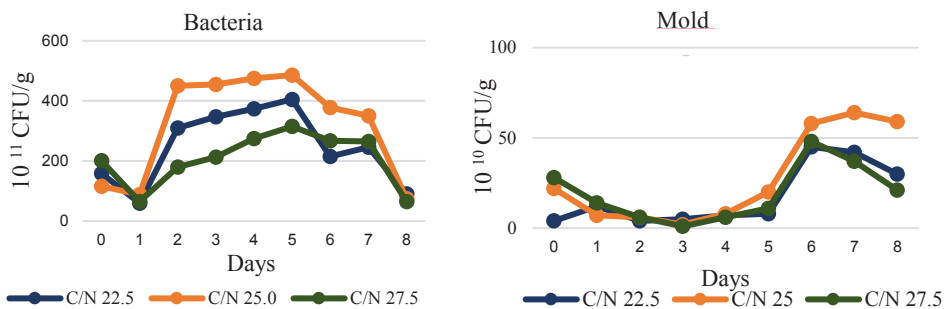
The parameters were analyzed using ANOVA and Tukey's advanced test with a confidence level of 95% SPSS 26 (2022).

## 3 Results

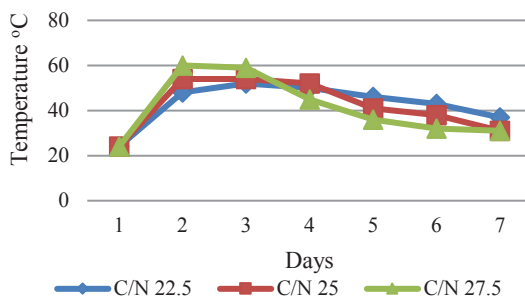
Initial decomposition is a crucial process in the production of liquid organic fertilizer (LOF). The growth of microorganisms at the beginning of decomposition indicates that the fermentation process is going well. The dynamics of microbial activity (bacteria and mold) are presented in Fig. 1. During the initial stage of the process, the bacteria count decreased. This decrease is believed to be linked to the rise in temperature on day 2, which peaked at 50-60 °C (Fig. 2).

The growth of bacteria and mold during the initial decomposition was markedly higher in the C/N ratio 25 treatment than in other treatments (refer to Fig. 1). Apart from bacteria, fungi also play an important role in decomposing organic materials. Fungi are generally more tolerant of high C/N ratios and can help decompose more carbon-rich materials, thereby helping to balance the C/N ratio for decomposing bacteria.

Macronutrients in organic fertilizers are essential in supporting plant growth and development. The phenomenon is strongly associated with better microbial activity at a C/N ratio 25 (Fig. 1).



**Fig. 1.** Total bacteria and mold in initial decomposition.



**Fig. 2.** Temperature during the initial decomposition.

Functional bacteria in organic fertilizers are essential in increasing soil fertility and supporting plant growth, including nitrogen-fixing and phosphate-fixing bacteria [18, 19]. A C/N ratio of 25 is sufficient to provide the nutritional needs for decomposer microbes in breaking down complex compounds into simpler compounds, and the mineralization process produces macronutrients in organic fertilizer (Table 2). Moreover, A C/N ratio of 22.5 - 25 produced a higher nitrogen-fixing bacteria population than the C/N 27.5 treatment (Table 3).

**Table 2.** Average of macronutrient content of liquid organic fertilizer

C/N Ratio	N total (%)	P <sub>2</sub> O <sub>5</sub> (%)	K <sub>2</sub> O (%)
C/N 22.5	1.19 ±0.08 a	0.46 ±0.21 a	1.42 ± 0.18 a
C/N 25.0	1.00 ±0.05 a	0.52 ±0.11 a	1.47 ± 0.17 a
C/N 27.5	2.13 ±0.29 b	0.53 ±0.15 a	1.68 ± 0.07 b

Note: a, b Means with the same letter were not significantly different at 0.05.

**Table 3.** Average of nitrogen-fixing bacteria and phosphate-solubilizing bacteria on liquid organic fertilizer

C/N ratio	Nitrogen-fixing bacteria (CFU/ml)	Phosphate-solubilizing bacteria (CFU/ml)
C/N 22.5	1.1±0.82 x 10 <sup>6</sup> a	3.6 ±1.84 x 10 <sup>5</sup> a
C/N 25.0	1.4±0.50 x 10 <sup>6</sup> a	5.7 ±2.14x 10 <sup>5</sup> a
C/N 27.5	3.0±1.55 x 10 <sup>4</sup> b	1.0 ±0.21x 10 <sup>5</sup> a

Note: a, b Means with the same letter were not significantly different at 0.05.

## 4 Discussion

Complex organic materials will be converted into simple compounds with the help of decomposer microbes during the initial decomposition process. Organic material broken down by a decomposer is needed to produce good quality organic fertilizer, which will then be processed further in liquid fermentation. In addition, the initial decomposition process produces high temperatures, allowing pathogens to be maximally reduced in the LOF. Pathogenic bacteria, such as *Salmonella* sp. and *E. coli* found in beef cattle feces, will be reduced optimally in this temperature range. Pathogenic bacteria will generally die at thermophilic temperatures, and the death of *Salmonella* spp bacteria will reach ten times at temperatures  $>55$  °C [11]. One way to process livestock waste through aerobic fermentation is to reduce pathogenic bacteria so that the organic fertilizer produced does not pollute groundwater when applied to plants [12, 13]. Microbes in compost use oxygen to break down organic material into CO<sub>2</sub>, water vapor, and heat. Bacteria that cannot tolerate heat, including pathogenic bacteria, will die. After the second day, bacteria increased until the fifth day and decreased until the eighth day. Once the substrate temperature reaches a high level, the bacteria that grow are thermophilic; they can grow at temperatures  $>45$  °C. Furthermore, when the temperature dropped again to 31 (days 6-7) (Fig. 2), the bacteria that grew were mesophilic; that is, they grew optimally at 25-37 °C.

Mold is crucial in breaking down complex compounds like lignin and cellulose. This breakdown allows bacteria to decompose further cellulose previously broken down by mold [14, 15]. Mold can thrive in both mesophilic and thermophilic phases. However, mold growth decreases at thermophilic temperatures on days 2-5 (see Fig. 1). In composting, mold becomes visible through the development of hyphae or filaments all over the substrate's surface. These visible filaments can be either white or gray.

The C/N ratio is a critical indicator of nutrient availability for bacterial and mold growth. Additionally, the carbon-to-nitrogen ratio (C/N ratio) significantly impacts decomposing bacteria's initial decomposition and growth in the organic material decomposition process. Decomposing bacteria need carbon as an energy source and nitrogen to synthesize proteins and other cellular materials. An optimal C/N ratio will provide ideal conditions for the growth and activity of these bacteria. A C/N ratio that is too high (e.g.,  $>30:1$ ) indicates more carbon relative to nitrogen. Under these conditions, the bacteria may experience a nitrogen deficiency, inhibiting their growth and activity. Conversely, a C/N ratio that is too low (e.g.,  $<20:1$ ) indicates that there is more nitrogen relative to carbon. Under these conditions, excess nitrogen can cause nitrogen loss in gaseous form (e.g., ammonia), which is also unfavorable for decomposing bacteria.

The macronutrient content of organic fertilizer is influenced by several factors, including the raw materials used, the decomposition process, environmental conditions, and processing methods [16, 17]. Beef cattle waste provides nitrogen content in organic fertilizer. Likewise, the C/N ratio, which influences the decomposition process, will influence the nitrogen content in organic fertilizer. A C/N ratio of 25 is sufficient to provide the nutritional needs for decomposer microbes in breaking down complex compounds into simpler compounds, and the mineralization process produces macronutrients in organic fertilizer (Table 2).

A good decomposition process influences the availability of functional bacteria in organic fertilizer. Functional microorganisms in organic fertilizer play a role in the decomposition process of organic material and increase the availability of nutrients for plants. Meanwhile, inorganic fertilizers generally do not contain functional microorganisms because they are produced synthetically. Long-term use of inorganic fertilizers can reduce soil microbial populations due to the lack of organic material that supports microbial life [20, 21].

## 5 Conclusions

Indigenous microbes in beef cattle feces play a role in decomposing complex compounds in organic materials into simple compounds and continue with the mineralization process in fermented liquid substrates to produce nutrients that can support plant growth. The high content of functional bacteria in the form of nitrogen-fixing and phosphate-solvent bacteria also enriches the benefits of liquid organic fertilizer. The appropriate C/N ratio represents the nutritional requirements for developing spoilage and functional bacteria. Liquid organic fertilizer from livestock waste can provide the nutritional needs of plants, so it can be an essential support in developing organic agriculture. Organic fertilizer from livestock waste can fulfill plants' nutrient requirements, making it crucial for advancing organic agriculture.

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