



## CAPABILITY OF CELLULOLYTIC BACTERIA ISOLATES IN REMOVING CELLULOSE-BASED MATERIALS INTO COMPOST

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### ABSTRACT

There is a dispersed presence of microorganisms exploiting polysaccharides from decaying plant biomass such as compost. To determine the ability of cellulolytic bacteria in the degradation process, the addition of 3 combinations of isolates, namely, I1 (inoculum of all isolates of positive cellulolytic bacteria), I2 (inoculum of 3 isolates with the highest enzyme activity), I3 (inoculum of isolates with the highest enzyme activity). The average physical condition of the compost in the three treatments was relatively similar to each other so that the difference in compost quality was not very distinguishable from the physical observation of this compost. On average, the color of the compost has a blackish color, although in the combination of adding 1 isolate with the highest enzyme activity, the brown litter color is still slightly visible. For the aroma produced by the compost, most of it smelled like soil in general, but a few still had an unpleasant smell, the average temperature for each replication and treatment was the same, which was 26°C, while the pH in each compost treatment was quite different in the range of 6.81 - 7.65 on each replicate. The average percentage of the highest N and K elements was found in the addition of 3 isolates with the highest enzyme activity, namely the percentage of N elements 11.03% and K element values 1.434%.

**KEYWORDS:** cellulases, cellulose, compost, degradation, litter.

### INTRODUCTION

In complex biological systems, such as decaying trees or plant debris in the soil, cellulose can decompose on a monthly time scale (Schwarz, 2001). There is a widespread presence of microorganisms exploiting polysaccharides from decomposed plant biomass such as compost and waste. Aerobic microbes usually produce enzymes in large enough quantities such as cellulases and hemicellulases (Bayer et al., 2004). Bacteria and fungi are key agents in the decomposition of plant litter in ecosystems (Romani et al., 2006). Several types of bacteria that have been known to have cellulolytic abilities are *Trichonympha*, *Clostridium*, *Actinomycetes*, *Bacteroides succinogenes*, *Butyrivibrio fibrisolvens*, *Ruminococcus albus*, and *Methanobrevibacter ruminantium* (Schwarz, 2001).

The degradation of lignocellulosic biomass is carried out by the collaboration of many microorganisms, including various genera of fungi and bacteria that can produce various cellulolytic enzymes under aerobic or anaerobic conditions. Cellulolytic microorganisms are very important in the biosphere in hydrolyzing cellulose

where cellulose is the most abundant carbohydrate produced by plants. Cellulolytic microorganisms can degrade cellulose-based plant litter (Soares et al., 2013). Cellulose degradation occurs through a biological process controlled by a group of cellulase enzymes (Gupta et al., 2012).

Compost is a product resulting from the process of weathering or decomposition of the remains of organic matter biologically under controlled conditions. The main function of compost is to help improve the physical, chemical and biological properties of the soil. Physically, compost can loosen the soil, because the application of compost to the soil will increase the number of voids in the soil. The advantage of compost is the complete content of macro and micronutrients. The macronutrients contained in compost include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S), while the content of microelements includes chlorine (Cl), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), and molybdenum (Mo) (Stoffella and Kahn, 2001).

## RESEARCH METHODOLOGY

The research was conducted from May-July 2021 at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, and Lab. Biotechnology, Laboratory of Sumatran Biota, Andalas University, Padang. 10 isolates of cellulolytic bacteria isolated from the Biological Education and Research Forest floor of Andalas University.

For procedures for isolation, screening, characterization, and testing of enzyme activity on 10 isolates obtained are attached to previous research journals (Febria et al, 2021).

### Making Cultures on Liquid Medium

Cellulolytic bacterial isolates were propagated in liquid media in the form of 10 g of granulated sugar and 10 g of shrimp paste and made up to 500 ml with a mixture of rice washing water and coconut water in a ratio of 1:1. The mixture of ingredients was put into a bottle and sterilized then the cellulolytic bacterial isolate was inoculated on the medium and incubated and shaken for 7 days.

### Test of the Degradation Ability of Bacterial Isolates Applied to Litter

To determine the ability of cellulolytic bacteria in the degradation process, 3 combinations of isolates were added, namely:

I1: Inoculum of all cellulolytic positive bacterial isolates.

I2: Inoculum 3 isolates with the highest enzyme activity.

I3: Inoculum isolate with the highest enzyme activity.

**Table 1: Physical Analysis of Compost.**

Inoculum Combination	Color	Scent	Temperature	pH
I1	3	3	26°C	7,36
I2	3	3	26°C	7,31
I3	2	3	26°C	7,41

#### Note

Color: 1=Brown, 2=Dark brown, 3=Black

Scent: 1=Odorless, 2=Slight odor, 3=Odorless (soil smell)

Inoculum Combination : I1>All isolates of cellulolytic bacteria

Composting litter using selected cellulolytic bacterial inoculum is carried out in the following steps: The litter is collected and then added 10% fine bran, 1% molasses, 0.5% urea, and 10% cellulolytic bacterial inoculum then stirred until evenly distributed and put 100 g into a plastic box and the composting process was carried out for 35 days.

### Compost Physical and Chemical Analysis

Physical observations were made in the form of color, aroma, temperature, and pH of the compost, chemical analysis in the form of measuring levels of nitrogen, phosphorus, and potassium in compost-based on (Eviati and Sulaeman, 2009), and observations were made on the 5th week of composting.

## RESULT

From previous studies, 10 isolates of cellulolytic bacteria with different enzyme activities were obtained from the biological education and research forest floor of andalas university as follows: FFB 2 (0,166 U/ml), FFB 4 (0,091 U/ml) , FFB 7 (0,124 U/ml), FFB 12 (0,104 U/ml), FFB 16 (0,099 U/ml) with the highest enzyme activities in 72 hours and FFB 3 (0,109 U/ml), FFB 6 (0,120 U/ml), FFB 8 (0,157 U/ml), FFB 10 (0,107 U/ml), FFB 14 (0,146 U/ml) with the highest enzyme activities in 96 hours.

### Compost Physical Analysis

The results of physical observations of compost can be seen in Table 1.

I2=Isolate with 3 highest enzyme activities

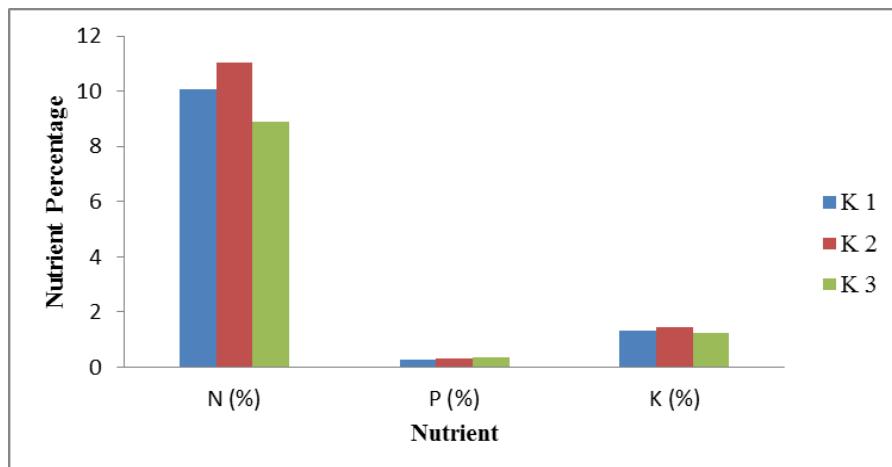
I3=Isolate with highest enzyme activity

### Compost Chemical Element Analysis

The average percentage values of N, P, and K elements of the compost can be seen in Table 2.

**Table 2: Compost Chemical Analysis Results.**

Inoculum Combination	N (%)	P (%)	K (%)
I1	10,07	0,267	1,322
I2	11,03	0,322	1,434
I3	8,88	0,355	1,223



**Figure 1: Percentage of Compost Nutrients in the Three Combinations of Cellulolytic Bacterial Inoculum.**

## DISCUSSION

Table 1 shows that the average physical condition of the compost in the three treatments was relatively similar to one another so that the difference in compost quality was not very distinguishable from the physical observation of this compost. On average, the color of the compost has a blackish color, although, in the combination of adding 1 isolate with the highest enzyme activity, the brown litter color is still slightly visible. Most of the aromas produced by the compost smell like soil in general, but some still have an unpleasant smell, this may be because the bacterial isolates in the compost are still working or the composting process has not been fully completed. The average temperature in each replication and treatment was the same ( $26^{\circ}\text{C}$ ), while the pH in each compost treatment was quite different in the range of 6.81-7.65 in each replication.

Based on the National Standardization Agency (2004), the standard for good compost quality is black, has an aroma like the smell of soil in general, has a pH in the range of 6.80-7.49, and also has a temperature such as the temperature of groundwater which is based on government regulation No. 82 2001 concerning water quality management and water pollution control stated that good groundwater has the same temperature as air temperature ( $20\text{-}30^{\circ}\text{C}$ ). based on these criteria, most of the compost produced from this study met these criteria. According to Nurtjahyani et al (2020), at the beginning of the process, compost has a brownish color with a sweet smell (sugar), but over time it smells sour and then smells like soil with a blackish color. This character indicates that the compost can be harvested or used.

In addition, at the time of composting, the pH value tends to increase where the initial pH value of the litter is 6.35 this is because at the time of composting the organic matter releases the compounds contained in it so that it can result in changes in the pH value. Following Siregar et al (2017) and Hamed (2014), which said that the increase in compost pH caused by organic matter in the decomposition process would release organic

compounds, either in the form of organic acids or base cations, which will increase in pH.

Table 2 shows the average percentage of the highest N and K elements found in the addition of 3 isolates with the highest enzyme activity, with the percentage of N elements being 11.03% and K element values being 1.434%. This shows that the three isolates were able to work synergistically in degrading compost and most likely the three isolates used were not antagonistic to each other. According to the National Standardization Agency (2004), the minimum standard of N in compost is 0.40%, this shows that the compost produced from the three treatments tested meets the requirements for use because the N content in the three treatments tested is greater than 0.40%. In addition, the minimum standard P-value is 0.10%, this also shows that the compost produced in the three treatments meets the minimum P-value for compost because the P-value in the three treatments is greater than 0.10%. Likewise for the minimum level of element K at 0.20% were the three treatments also met the requirements for the minimum value of K element content for compost because the K element content in the three treatments was greater than 0.20%.

The availability of nitrogen in the compost is due to the decomposition process of organic matter carried out by microorganisms. This nitrogen is obtained through three stages of the reaction, namely amination reaction, ammonification reaction, and nitrification reaction. The amination reaction is a decomposition reaction of proteins contained in organic materials into amino acids, the ammonification reaction is the change of amino acids into ammonia compounds ( $\text{NH}_3$ ) and ammonium ( $\text{NH}_4^+$ ), and the nitrification reaction is the conversion of ammonia compounds into nitrates by involving the bacteria *Nitrosomonas* and *Nitrosococcus*. The decomposition of nitrogen-containing organic compounds is carried out mainly by ammonifying bacteria, using enzymes released into the substrate, converting proteins into forms available for plant nutrition. Immobilization of mineral nitrogen is carried

out by microorganisms capable of assimilating ammonia, nitrogen nitrate. The nitrogen content available in the substrate depends on the degree of immobilization because 25 to 35% of the total nitrogen of the substrate can be included in the microbial biomass. Mineral nitrogen content is an important indicator of the value of compost fertilizer. This work aims to assess the compost quality of leaf litter as a potential fertilizer (Kornievskaya et al, 2020). The value of N has increased and decreased during the composting process, this happens because nitrogen (N) is fluctuating. At the time of composting, there is usually an increase in nitrogen levels. Nitrogen is needed by microorganisms to maintain and form body cells. The more nitrogen content, the faster the organic matter decomposes, because microorganisms that decompose compost require nitrogen for their development.

In the composting process, microorganisms have an important role in the formation of phosphorus. Based on the nature of element P as organic matter, element P has a very essential role in fertility where nutrient intake from organic matter helps increase nutrient levels in achieving optimal fertility intensity. Phosphorus is needed to make up 0.1 – 0.4% of plant dry matter. This element is very important in the process of photosynthesis, and the chemical physiology of plants. Phosphorus is also needed in cell division, tissue development and plant growth points (Widarti et al., 2015).

Phosphorus is bound in the form of  $P_2O_5$  at the end of the decomposition process. Phosphorus exists in two forms, namely inorganic and organic such as nucleic acids, pectin, and lecithin. With the available carbon and nitrogen sources, bacteria can break down lecithin and nucleic acids and release phosphorus as phosphate. In addition, the presence of potassium in compost occurs because a lot of potassium comes from organic matter. Organic matter can increase the cation exchange capacity, this is related to the negative charges originating from the  $-COOH$  and  $OH$  groups which dissociate into  $COO^-$  and  $H^+$  and  $O^- + H^+$ . This negative charge has the potential for humus to adsorb cations such as Ca, Mg, and K which are bound with moderate strength, so that they are easily exchanged or undergo a cation exchange process. Potassium is used by microorganisms in the substrate material as a catalyst, with the presence of bacteria and their activities will greatly affect the increase in potassium content (Hidayati et al., 2011).

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