

## Comparison of the potential of local microorganisms several bio activators as biological agents for the production of organic fertilizers

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### Abstract

One way to reduce dependence on the use of inorganic fertilizers is to substitute organic fertilizers. Organic fertilizers come from the process of degradation of organic matter. To speed up the degradation process, a bio activator containing local microorganisms (LMO) was added as a starter. This study aims to compare bio activators from various sources of local microorganisms as a starter for organic fertilizer production. Media for microbial growth in LMO during fermentation (NA and MEA), media for testing the potential of bacterial isolates from each bio activator (modified GPA, SMA, CMC, SA, and YEMA). Experimental research methods, including; fermentation of organic matter making up the bio activator and testing the potential of LMO isolates for each bio activator. The number of bacteria respectively in BA-1, BA-2, BA-3 and Control;  $45 \times 10^5$ ;  $48.3 \times 10^5$ ;  $5.8 \times 10^5$  and  $1.09 \times 10^7$  CFU/ml, while total yeast respectively;  $13.3 \times 10^5$ ;  $16.4 \times 10^5$ ;  $5.3 \times 10^5$  and  $4.30 \times 10^7$  CFU/ml. The highest total microorganisms were found in BA-2. Proteolytic, amylolytic, cellulolytic, fermentative (LAB), and nitrate potency were positive for all treatments. BA-2 has the highest potency for proteolytic index 6.0, fermentative (LAB) 5.0, and nitrogen-fixing bacteria 4.0, the highest amylolytic and cellulolytic potentials are on BA-1 and BA-3 with indexes 11.0 and 5.0. The three types of LMO contain specific microorganisms and have catalytic potential so they can be used as bio activators for the manufacture of organic fertilizers. BA-2 is the best bio activator in terms of total microorganisms.

**Keywords:** Bacteria; Degradation; Organic Waste; Proteolytic

### 1. Introduction

Agriculture is one sector that plays an important role in national development. One of the efforts to increase agricultural productivity is fertilization. Generally, farmers use inorganic fertilizers (chemical fertilizers). However, inorganic fertilizers hurt the environment if their use in the long term causes a decrease in soil organic matter levels, damaged soil structure, few microorganisms in the soil, land degradation, and environmental pollution[1].

One way that can be done to reduce the use of inorganic fertilizers is to substitute the use of biological organic fertilizers. Organic materials that can be used as raw materials for making organic fertilizers can come from livestock waste. The degradation process of organic waste in nature can take place spontaneously and naturally, but it takes a long time and is influenced by radiation and weather factors[2]. One way to accelerate the rate of degradation of organic matter into POH is by adding a bio activator. Bio activators are materials that contain microorganisms that can work effectively and actively in the process of decomposing organic waste[3]. Several bio activators that can be used are from local microorganisms (MOL), EM4, bovine rumen, and other bio activators[4].

MOL solution is the result of fermentation that can be made from various materials available in the environment. This solution contains microorganisms that can break down organic matter and trigger plant growth[5]. The main component that must be met in the manufacture of LMO is carbohydrates. Cow rumen is a slaughterhouse waste. Beef

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rumen also contains nutrients that are used by microbes as a source of energy[6]. There are cellulolytic bacteria and ligninolytic bacteria in the rumen of cattle which are used to degrade cellulose and lignin[7]. Based on the above problems, it is necessary to develop optimal bio activator formulation biotechnology in the degradation process of organic matter to the formation of the best quality organic fertilizer, so that it can be used as a substitute for synthetic chemical fertilizers and is commercially feasible. This research aims to study the development of starter microorganisms as bio activator.

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## 2. Material and methods

### 2.1. Material

Organic material that makes up the bio activator, medium for the growth profile of local microorganisms during fermentation; Nutrient Agar (NA), Potato Dextrose Agar (PDA), and Malt Extract Agar (MEA), medium for testing the potential of isolates (Glucose Peptone Agar modification (GPA-CaCO<sub>3</sub>), Skim Milk Agar (SMA), Carboxymethylcellulose Agar (CMCA), Starch Agar (SA) and Yeast Extracts Mannitol Agar (YEMA).

### 2.2. Methods

Enumeration of Local Microorganisms (Bacteria and Yeasts) in Bio activators: Microbial growth from bio activators was analyzed once every two days by counting the number of microorganisms that grew by dilution series technique and inoculated by pour plate technique on NA, PDA, and MEA medium. Count the number of colonies that grew[8].

$$\text{colony CFU/ml} = \frac{1}{\text{dilution}}$$

Testing the potential of bacteria on the bio activator: carried out qualitatively based on the formation of a clear zone around the colony. The diameter of the clear zone was measured and the respective index was calculated using the formula:[9].

$$I = \frac{\text{diameter of clear zone colony} - \text{colonies diameter}}{\text{Colony diameter}}$$

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## 3. Results and discussion

Bacteria and yeast growth can be seen in Figures 1 and 2. Bacterial growth is faster than yeast, where the number of bacteria reaches  $48.3 \times 10^5$  CFU/ml on day 12 while yeast is  $16.4 \times 10^5$  CFU/ml on day 6. The high number of bacteria compared to yeast This is because bacterial cell division takes 20 minutes while yeast cells take longer, which is 90 minutes. The nutrients contained in the bio activator are sufficient to support the growth of bacteria and yeast. In addition, microbial growth shows an increasing trend from day 2 to day 6, this is because on that day an exponential phase occurs where microbes divide rapidly and utilize nutrients optimally. also affect temperature and pH[10].

### 3.1. Enumeration of bacteria and yeast in bioactivator

The highest number of bacteria and yeasts was found in bio activator 2 (BA-2) because the organic matter of BA-2 consisted of sprouts containing phosphorus[11]. rice washing water, tape, and tape water as a carbohydrate source[12].brown sugar contains 75-90% sucrose [13]. tofu and tempeh dregs contain protein sources[14].

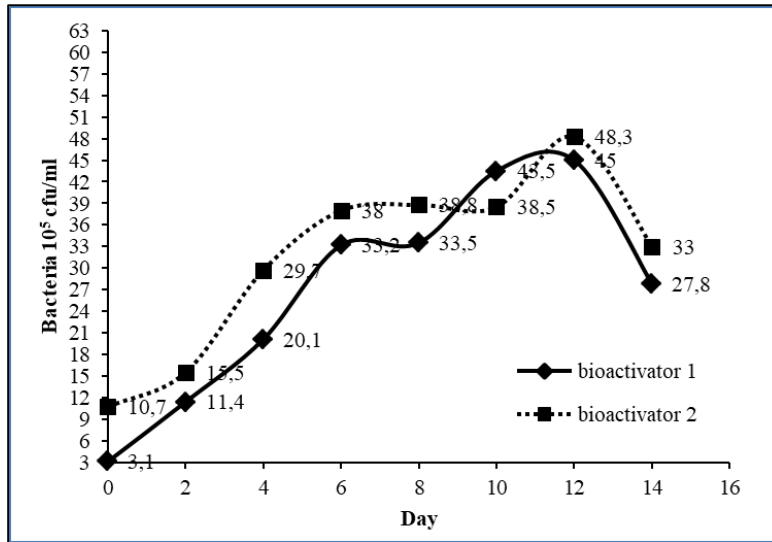


Figure 1 Bacterial Growth Profile in Bioactivator

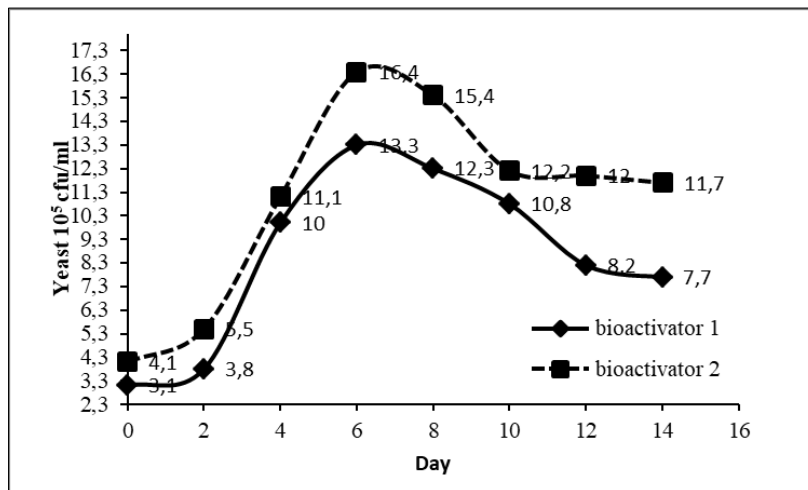


Figure 2 Yeast Growth Profile in Bioactivator

### 3.2. pH and Temperature in bioactivator

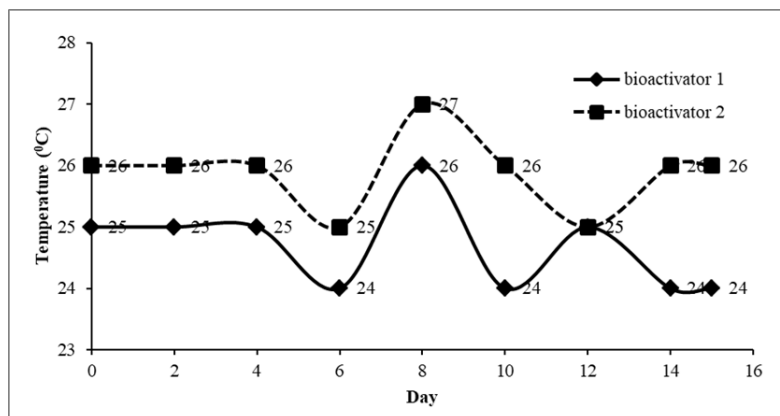


Figure 3 Graph of temperature Bioactivator

The temperature of the three bio activators ranged from 26-36°C. The increase in temperature occurred on day 8 in the stationary phase because in this phase there was a microbial metabolic process that produced heat energy as a result of the anaerobic fermentation process so the temperature increased.

The pH of the bioactivator showed a decreasing trend of 3.4-5.2 during the fermentation process. The decrease in pH in the stationary phase where in this phase there is a process of formation of secondary metabolites in the form of lactic acid, resulting in an acidic pH. Acidity caused by microbial activity in decomposing organic matter will also produce CO<sub>2</sub> gas. This CO<sub>2</sub> gas will form carbonic acid (H<sub>2</sub>CO<sub>3</sub>) which is easily decomposed into H<sup>+</sup> and HCO<sub>3</sub> ions. These H<sup>+</sup> ions will affect the acidity so that the pH of the LMO solution decreases (acidity increases)[15].

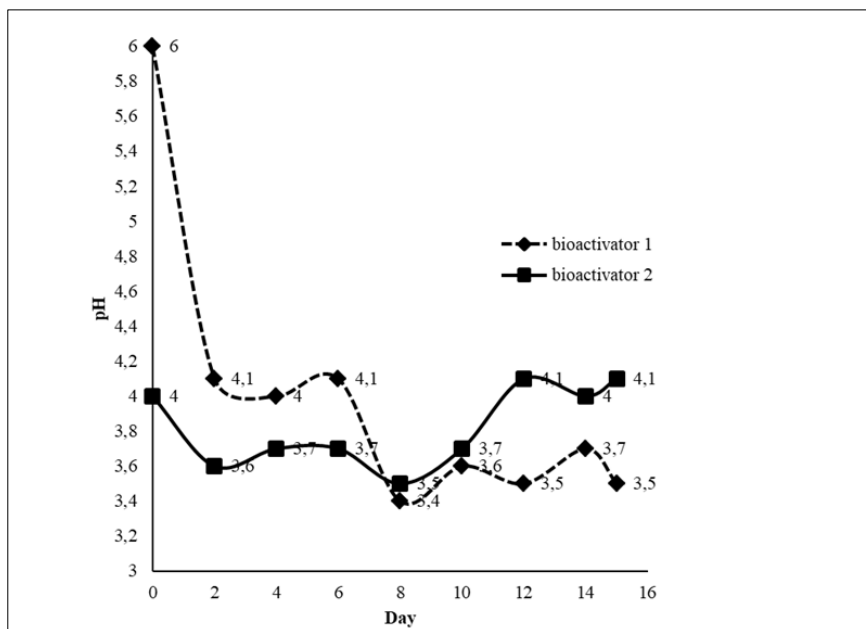


Figure 4 Graph of pH Bioactivator

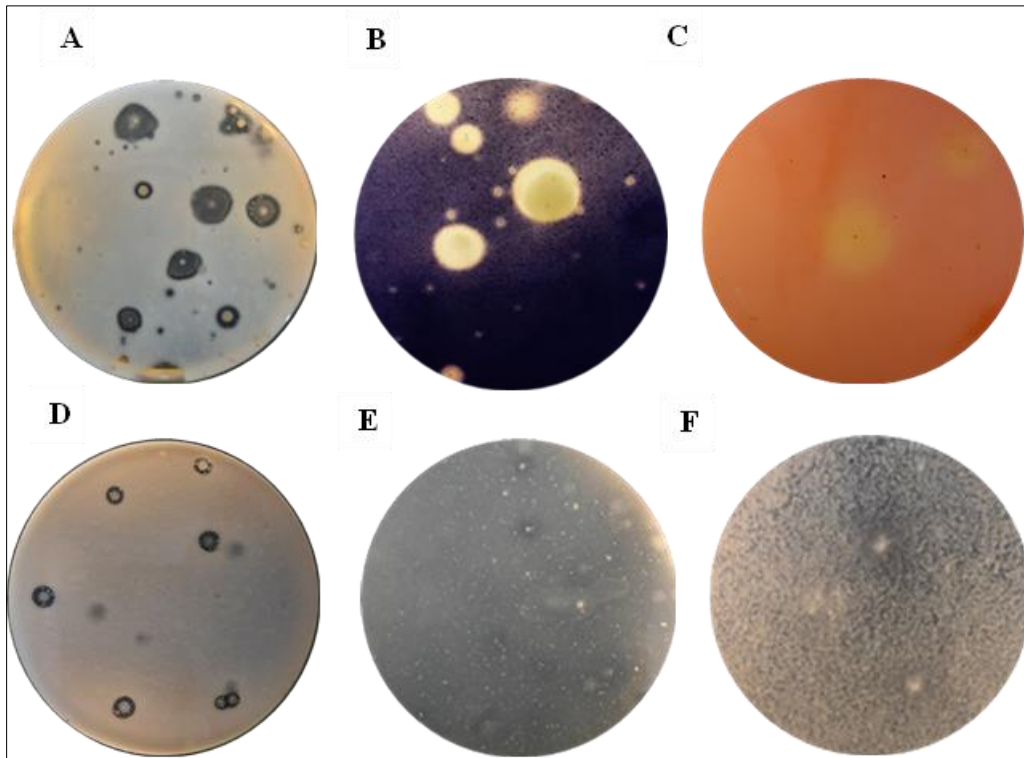
### 3.3. Potential of Proteolytic, Amylolytic, Cellulolytic, Fermentative and nitrate Bacteria on Bioactivator

The potential of bacteria on three bio activators using selective medium showed positive results for proteolytic, amylyolytic, cellulolytic, fermentative, and nitrate. The formation of a clear zone on the selective medium indicates that the bacteria can remodel the organic materials that make up the bio activator.

Table 1 Potential of Proteolytic, Amylolytic, Cellulolytic, Fermentative and nitrate Bacteria on Bioactivator

Number	Bioactivator	Potency	Number of colony	Index value
1	BA-1	Amylolytic	4	11
2	BA-2	Proteolytic	33	6,0
		Fermentative (LAB)	33	5,0
		Nitrate	6	4,0
3	BA-3	Cellulolytic	17	5,0

The measurement results of the proteolytic, amylyolytic, cellulolytic, fermentative, and nitrate-fixing index have a high index value (>3.1), this is following [16]. where the index value is a low category (<2.1), moderate (2.1-3.1) and high (>3.1). The highest proteolytic, fermentative, and nitrate index values were found in BA-2, this indicates that BA-2 contains organic protein, acid, and nitrate producers. The highest amylyolytic index value was found in BA-1, this indicates that BA-1 contains high organic starch. The highest cellulolytic index value was found in BA-3, this indicates that BA-3 contains high cellulose organic matter. Stated that there are cellulolytic bacteria in various organic materials used to degrade cellulose[7]. Cellulolytic bacteria are a group of bacteria that can digest (remodel) cellulose.



**Figure 5** (A) Potential of Proteolytic Bacteria (B) Potential of Amylolytic Bacteria (C) Potential of Cellulolytic Bacteria (D) Potential of Bioactivator LAB Bacteria (E) potential of bioactivator nitrate bacteria

enzymes are hydrolyze peptide bonds in protein molecules to produce peptides or amino acids[17]. Amylolytic bacteria produce amylase enzymes which function to hydrolyze starch in starch to form a clear zone in the media, while cellulolytic bacteria produce cellulase enzymes. Cellulase enzyme production uses Carboxymethyl Cellulose (CMC) media because this medium contains cellulose which is used as a substrate for enzymatic reactions[18]. The carbon source that functions as a source of cell energy and the main element in cell formation is fulfilled by the presence of CMC[19]. Cellulase enzymes will hydrolyze cellulose from organic materials as the basic ingredients for making bio activators. In a fermentative medium, see the ability of bacteria to hydrolyze acid in the medium (GPA) added with (CaCO<sub>3</sub>). Calcium Carbonate serves to neutralize the lime in the colony area so that a clear zone is formed. Medium Glucose Peptone Agar + Calcium Carbonate (GPA + CaCO<sub>3</sub>) was used to see fermenting bacteria that have the potential as lactic acid bacteria[20].

#### 4. Conclusion

The three types of LMO contain specific microorganisms and have catalytic potential (proteolytic, amylolytic, cellulolytic fermentative (BAL), and nitrate so that they can be used as bio activators to produce organic fertilizers. BA-2 is the best bio activator in terms of total microorganisms and catalytic potential. Meanwhile, amylolytic and cellulolytic potentials highest in BA-1 and BA-3 bio activators.

#### Compliance with ethical standards

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##### *Conflict of interest statement*

The authors declare that there is no conflict of interest.

*Statement of ethical approval*

The article does not contain any studies with human or animal participants performed by any of the authors.

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