ISSN: 1410-8917
Jurnal Kimia Sains & Aplikasi
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 28 (10) (2025): 553-559

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Chemical Quality Analysis and Antibacterial Properties of Eco-enzyme Derivative Products as Liquid Organic Fertilizers and Natural Disinfectants

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https://doi.org/10.14710/jksa.28.10.553-559

Article Info

Article history:

Received: 25th May 2025
Revised: 20th December 2025
Accepted: 22nd December 2025
Online: 31st December 2025

Keywords:
eco-enzyme; liquid fertilizer; antibacterial; natural disinfectant

Abstract

Bioz Nutrition is the product of second-stage fermentation of eco-enzyme (EE) or Garbage Enzyme (GE) for 21 days with the addition of specific organic materials. This study aimed to evaluate the potential of Bioz in enhancing plant nutrient content and exhibiting antibacterial activity as a natural disinfectant. Two types of Bioz, coded Bioz1-n and Bioz2-n, were produced through a process involving four main stages: the production of EE from pineapple and orange waste via anaerobic fermentation for 100 days; the production of Bioz through further fermentation of EE for 21 days with added organic materials; analysis of the chemical composition (nitrogen, phosphorus, potassium, and pH) and evaluation of antibacterial and antifungal activities; and testing against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) using the disc diffusion method. Chemical analyses were conducted using the Kjeldahl method (N), UV-Vis spectrophotometry (P and organic carbon), atomic absorption spectrophotometry (K), and a pH meter. Antibacterial and antifungal activities were tested at various dilution ratios of EE to water (1:0, 1:10, 1:50, and 1:100). The results showed that second-stage fermentation improved the nutritional content of Bioz and that a 1:10 dilution (Bioz1-1 and Bioz2-1) was the most effective in inhibiting bacterial growth, indicating its potential as a natural disinfectant. This process provides a sustainable approach to utilizing agricultural waste, adding economic value while supporting environmentally friendly agricultural and household applications.

1. Introduction

Organic fertilizers can generally be categorized into two forms: liquid organic fertilizers (POC) and solid organic fertilizers (POP). POC is produced through the fermentation of organic materials such as agricultural and food waste, typically under anaerobic conditions. POC offers several advantages over other fertilizer types due to its liquid form, including ease of application, the ability to efficiently address nutrient deficiencies, resistance to nutrient leaching, and a high microbial content. According to research, the optimal liquid organic fertilizer consists of a mixture of banana, mango, and pineapple peels fermented for 7–14 days, resulting in C-organic, N-total, K_2O, and P_2O_5 contents of 17.4%, 6.05%, 2.50%, and 0.15%, respectively. This composition meets the quality standards stipulated by the Indonesian Minister of Agriculture Regulation Number 261 of 2019, except for the P_2O_5 content. Nonetheless, this POC formulation surpasses the quality of several commercially available fertilizers. The production cost of this POC is estimated at IDR 770,554, with a break-even point (BEP) of 10 liters [1].

Disinfection refers to the process of removing or inactivating microorganisms, such as bacteria, fungi, and viruses, from inanimate surfaces using disinfectants. Disinfectants are specifically intended for non-living objects and must not be applied to humans or other living organisms, unlike antiseptics, which are designed for such use [2]. According to the World Health Organization (WHO), disinfectant solutions can effectively kill bacteria and viruses within 10 to 60 minutes post-application, primarily due to the chlorine content in substances like bleach. Although the WHO's disinfectant formula is widely adopted by the public for sanitizing household environments, misuse persists. Many people mistakenly apply disinfectants directly to the body, despite the WHO's warning that doing so is ineffective against viruses like the coronavirus, which reside inside the body. Furthermore, such practices pose health risks as chemical exposure to skin, clothing, or mucous membranes (e.g., eyes, mouth) can be harmful. Therefore, there is an increasing interest in developing natural, environmentally friendly disinfectants and antiseptics that exhibit antibacterial and antifungal activity, such as eco-enzymes.

Eco-enzyme (EE) is a multipurpose liquid derived from the fermentation of organic materials, including fruit and vegetable waste, sugar, and water. The type of raw materials used greatly influences the success of the fermentation process. After at least 100 days of fermentation, a high-quality eco-enzyme typically has a pH below 4 and a distinctive, fresh, sour aroma, similar to other fermented products. Various plant-based wastes, such as orange, pineapple, and papaya peels, are suitable for EE production due to their high content of bioactive compounds. For example, orange peels contain flavonoids that exhibit antimicrobial properties by inhibiting nucleic acid synthesis, disrupting cytoplasmic membranes, and interfering with biofilm formation, porin function, permeability, and bacterial enzyme interactions. EE is recognized for its versatility, serving as an antibacterial, antiseptic, and anti-parasitic agent that reduces contamination by worm eggs on fresh produce. The enzymes naturally present in EE, including lipase, protease, and amylase, contribute to the disruption of microbial cell structure, making it an effective and eco-friendly wash for fruits and vegetables.

In addition to its disinfectant properties, eco-enzyme also functions as a natural fertilizer, enhancing soil fertility. Research on EE made from cassava waste revealed nutritional contents of 6.96% crude fiber, 7.538% crude protein, and 0.321% crude fat [3]. When applied as a fertilizer, EE improves the growth of seasonal crops, such as shallots and soybeans, by increasing soil fertility and plant productivity [4, 5]. Furthermore, the integration of EE with 75% topsoil and 25% compost has been shown to positively influence the growth parameters of shallot plants, including height and leaf number [6]. Community-based EE production using household organic waste supports sustainable agriculture practices, as demonstrated in Suka Damai Village, Langkat Regency [7], highlighting its potential as a liquid organic fertilizer [8].

As a natural disinfectant, EE exhibits significant antibacterial activity against various bacterial species. Studies have shown its effectiveness in inhibiting bacterial and fungal growth, suggesting its suitability as a natural disinfectant and hand sanitizer [9]. Phenolic compounds present in EE are responsible for its antimicrobial activity, enabling it to suppress the growth of bacteria and fungi such as those found in pig pens [10]. EE has demonstrated antibacterial effects against pathogenic microbes, such as *S. aureus* and *E. coli*. For instance, EE diluted to 1:100 effectively limited *S. aureus* growth [11]. Moreover, EE derived from papaya, pineapple, and orange exhibited notable antibacterial activity against *Enterococcus faecalis* at concentrations exceeding 50%, supporting its potential use as a substitute for sodium hypochlorite (NaOCl) in endodontic treatments [12]. Other studies reported EE's ability to inhibit bacteria such as *S. aureus* and *Propionibacterium acnes*, with clear zone diameters of 13.1 mm around EE-treated discs prepared from pineapple and orange peels [13].

Quality evaluations of EE-based disinfectants have confirmed their compliance with SNI 06-1842-1995 standards, specifically in terms of pH (6–11) and stable emulsification in hard water. EE samples diluted at various concentrations (1:10 to 1:50) maintained acceptable pH levels (6–7) and exhibited stable emulsification without sediment or phase separation. This stability underscores EE's suitability for environmental applications, including organic degradation, composting, wastewater treatment, leachate processing, and disinfection [14]. For certain microbial groups, eco-enzymes represent a feasible, cost-effective, and sustainable biological sanitation alternative [15].

This study investigates Bioz, a derivative of eco-enzyme, as a liquid organic fertilizer and its antibacterial activity against *S. aureus* and *E. coli* at various dilution concentrations derived from different fruit and vegetable waste materials. While existing research confirms that the composition and concentration of EE influence its antibacterial efficacy, limited information is available regarding the second-stage fermentation of eco-enzymes. Therefore, this study focuses on classifying organic waste by type for initial EE fermentation, followed by a second-stage fermentation incorporating additional organic materials such as banana stumps and noni. The objective is to identify which organic material compositions optimize nutrient content to meet liquid organic fertilizer standards and determine the most effective dilution concentration for antibacterial and antifungal activity against *S. aureus* and *E. coli*.

2. Experimental

2.1. Materials and Tools

The materials used for eco-enzyme production consisted of organic matter derived from agricultural products and waste, carbohydrates, and mineral water. Microbial isolates of *Staphylococcus aureus* and *Escherichia coli* were obtained from the Microbiology Laboratory, Universitas Sumatera Utara (USU). The equipment employed in this study included knives, analytical balances, measuring cups, nutrient agar, Mueller-Hinton agar (MHA), Mannitol Salt Agar (MSA), Mannitol Salt Broth (MSB), Petri dishes, inoculating needles, an autoclave, a pHS-3C pH meter, and paper discs. Nutrient analysis was performed using a Kjeldahl apparatus (KjelTRON™ Rapid Automatic Nitrogen Estimation System) for nitrogen determination, a 721G visible spectrophotometer for phosphorus analysis [16], and a PerkinElmer PinAAcle 900T Atomic Absorption Spectrophotometer for potassium measurement.

2.2. Research Procedures

2.2.1. Eco-enzymes Production

The eco-enzyme was prepared using a 3:1:10 ratio, consisting of 3 kg of organic materials from agricultural products and waste, 1 kg of carbohydrates (molasses), and 10 L of mineral water. In the first stage, organic waste from pineapples, oranges, and papayas was washed, cleaned, cut into small pieces, and weighed to obtain a total of 3 kg. A plastic container was filled with 10 L of mineral water, into which molasses was added and stirred until fully dissolved. After all components were homogenized, the container was tightly sealed and left to ferment under anaerobic conditions at room temperature for 100 days (Figure 1). Upon completion of the fermentation period, the container was opened, and the eco-enzyme solution was filtered to separate the liquid from the residue. The filtrate was then transferred into a clean, dry plastic container for storage.

2.2.2. Production of Bioz with Added Organic Materials

Bioz was an eco-enzyme (EE) product that underwent further fermentation with the addition of organic materials for 21 days. The EE was divided into two containers: Bioz-1 and Bioz-2. Bioz-1 was made by adding eggshells, spinach, and long beans to the EE, while Bioz-2 contained banana stems, noni, and kuini fruit peels. Production began by placing 2 L of filtered pure EE (separated from the residue) into a clean, dry plastic container, and then adding organic materials at a ratio of 4:1 EE to organic material. The same procedure was applied for Bioz-2. The production process is illustrated in Figure 2.

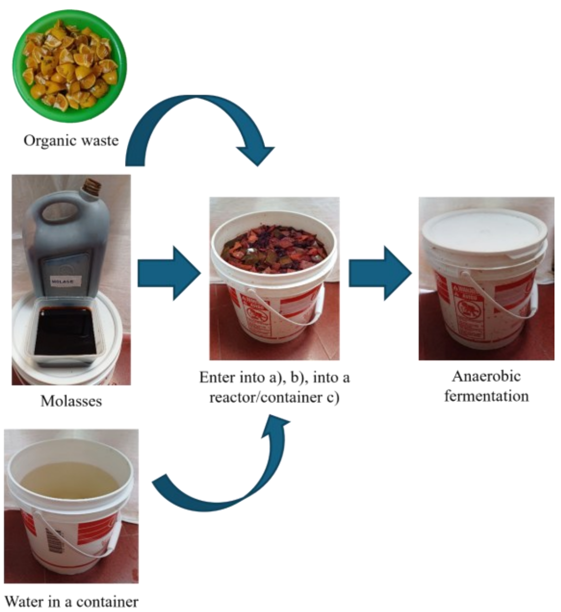

Figure 1. Eco-enzyme production

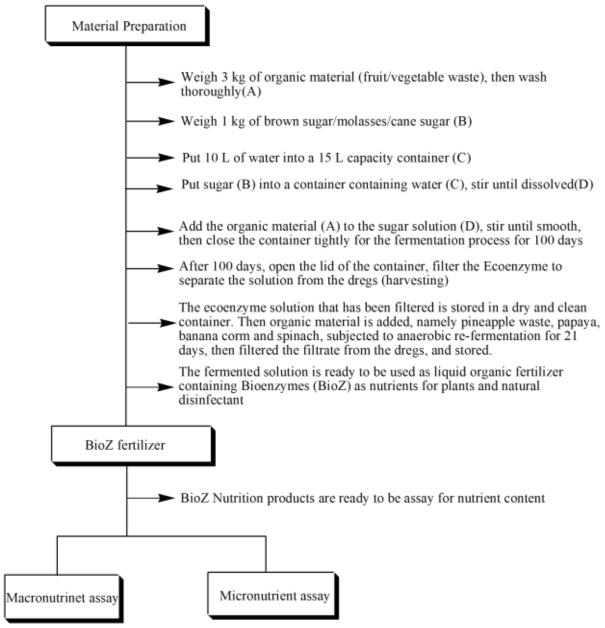

Figure 2. Flow diagram of the Bioz production process

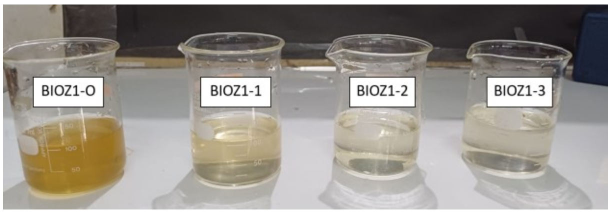

Figure 3. Variation of the eco-enzyme dilution

2.2.3. Antibacterial Activity Test

The antibacterial activity of Bioz was tested on pure EE (Bioz-0) and on Bioz-1 and Bioz-2 at various dilutions. Pure eco-enzyme (Bioz1-0) was diluted at ratios of 1:10 (10 mL EE in 100 mL distilled water, Bioz1-1), 1:50 (2 mL EE in 100 mL distilled water, Bioz1-2), and 1:100 (1 mL EE in 100 mL distilled water, Bioz1-3). Variation of the eco-enzyme dilution is presented in Figure 3.

2.2.4. Preparation of Standard Solution

McFarland standard solutions were used to standardize bacterial counts in suspension fluids. The McFarland turbidity standards served as surrogates for bacterial cell density estimation in antimicrobial testing. A 0.5 McFarland standard corresponded to approximately 1.5×10^8 bacterial cells. The procedure began by preparing labeled test tubes with distilled water. Bacterial samples were transferred into their respective tubes and homogenized. The turbidity of each bacterial suspension was then adjusted to match the 0.5 McFarland standard before further testing.

2.2.5. Antibacterial Testing of Lactic Acid Bacteria Against Pathogens

MHA media was prepared by dissolving 7.2 g of MHA powder in 250 mL of distilled water, then heating and homogenizing the solution until it was clear. The media was poured into 12 petri dishes and left to solidify. Chloramphenicol antibiotic discs (positive control) and blank discs were prepared for antibacterial testing. Each pathogenic bacterial colony was diluted to match the 0.5

McFarland standard. Then, 0.1 mL (10 µL) of each bacterial suspension was spread evenly on the MHA plates using an L-shaped rod. Two holes were punched into each plate using a sterile perforator. Chloramphenicol discs were placed in the center of one hole as positive controls. Plates were incubated inverted at 37°C for 24 hours. After incubation, inhibition zones were measured with calipers at 24 and 48 hours. Each test was performed in duplicate.

3. Results and Discussion

3.1. Eco-enzyme Production

The resulting eco-enzyme was dark brown with a pH of 3.7, consistent with research indicating that effective eco-enzymes have a pH range of 3.5 to 4. The color of the eco-enzyme varied depending on the type of sugar and organic materials used. The observation results for Bioz are presented in Table 1.

3.2. Quality Analysis of Nutrient Levels from Bioz

The results of the quality analysis of Bioz nutrients are presented in Table 2. From Table 1 and Figure 4, it is evident that the nutrient content of Bioz liquid organic fertilizer, derived from EE fermentation, remains lower overall compared to the standards for nitrogen (N), phosphorus (P), and potassium (K). However, the phosphorus and potassium levels in Bioz met the SNI 19-7030-2004 quality standards for liquid organic fertilizers after the second fermentation stage (F2) for 21 days. Among the samples, Bioz-1 (prepared with banana stem organic material) showed nitrogen, phosphorus, and potassium values closest to the SNI standards, with P = 0.10 and K = 0.83, meeting the minimum SNI requirements of 0.10 (P) and 0.2 (K).

These findings align with research by Hakim *et al.* [8], which reported that liquid organic fertilizers from various agricultural wastes, including banana stems, significantly improved plant growth and yield in onions. The extended fermentation in the second stage likely increased the nutrient content, as enzymes such as protease, amylase, and lipase break down proteins into amino acids rich in nitrogen, thereby raising the N levels over time. Additionally, the increase in nitrogen in Bioz1 is partly due to the addition of eggshells, which are known for enhancing tomato growth due to their high nitrogen content [17].

Fermentation transforms chemical compounds into organic compounds through the action of aerobic and anaerobic microorganisms. Since organic compounds contain carbon (C), hydrogen (H), and oxygen (O), longer fermentation times increase the carbon content, which explains why Bioz from the second fermentation stage has a higher organic carbon content than pure EE.

The higher phosphorus content in Bioz-2 compared to Bioz-0 (pure EE) is attributed to the banana stems, which are rich in N, P, and K, with potassium known to increase sugar levels in sweet corn [18]. The phosphorus increase in Bioz-1 is linked to spinach addition, which has high P levels [19].

Phosphorus is vital for energy production, flowering, fruiting, root growth, seed formation, and cell division in plants. Potassium regulates photosynthesis, carbohydrate transport, stomatal function, and water movement in tissues. This aligns with Efendi [20], who found that Gamal leaf fertilizer enhanced the chlorophyll content of mustard leaves, reflecting improved photosynthesis regulated by potassium. Potassium deficiency causes leaf burn and drop.

Bioz may function more effectively as a biofertilizer than just a liquid organic fertilizer, due to its rich microbial content that aids in root decomposition and nutrient uptake of macro- (N, P, K) and micronutrients (Fe, Mn, Cu), thereby enhancing plant productivity. Biofertilizers made from industrial waste or by-products provide a low-cost source of beneficial microbes; however, further research is required [21].

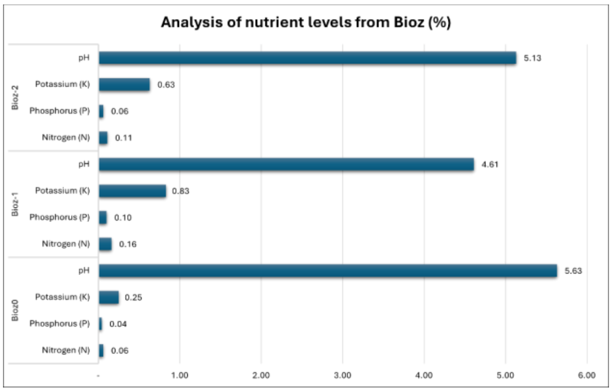

Figure 4. Results of Bioz nutrient content analysis

Table 1. Eco-enzyme parameters

Indicator	Color	pH
Bioz-1	Dark brown	3.8
Bioz-2	Dark brown	3.6

Table 2. Quality analysis of Bioz liquid organic fertilizer

Parameters	Unit	Pure EE (Bioz-0)	Bioz-1	Bioz-2	National quality standard
C-Organic	%	1.08	0.70	1.90	Min 10
Nitrogen (N)	%	0.06	0.11	0.16	Min 2
Phosphorus (P)	%	0.04	0.06	0.10	Min 2
Potassium (K)	%	0.25	0.63	0.83	Min 2
pH		5.63	5.13	4.61	4-9

Source: Results of Laboratory Test Analysis of BSIP North Sumatra, Medan, 2024

3.3. Antibacterial Activity

The antibacterial activity results of eco-enzyme against *E. coli* and *S. aureus* are presented in Table 3. EE from Bioz-1 and Bioz-2 showed inhibition zones of 7.9 mm (Bioz1-1) and 7.2 mm (Bioz2-1) against *E. coli*, and 6.3 mm (Bioz1-1) and 6.5 mm (Bioz2-1) against *S. aureus*, as illustrated in Figures 5 and 6. According to Davis and Stout [22], the antibacterial activity falls into the moderate category. The inhibitory effect of Bioz1-1 was slightly higher than Bioz1-2, as evidenced by a larger clear zone around the well. Additionally, Bioz-1 showed a greater inhibition zone than Bioz-2 against both bacteria at the same concentration, likely due to the presence of papaya in Bioz-1. Papaya contains various phytochemical compounds, including phytosterols, tocopherols, flavonoids, alkaloids, and carotenoids [23].

Pure eco-enzyme (1:0) and higher dilutions showed no inhibitory effect against *E. coli* bacteria. The optimal dilution was 1:10 (Bioz-1 and Bioz-2), which demonstrated moderate antibacterial activity with inhibition zones ranging from 5 to 10 mm [22]. This is likely because the eco-enzyme contains fruit peels rather than vegetables, consistent with previous studies showing that eco-enzymes derived from orange, pineapple, and papaya peels contain phenolic compounds effective at suppressing bacterial growth and acting as natural disinfectants. At the 1:10 dilution, bacterial growth was minimal [24], as seen in Figure 5.

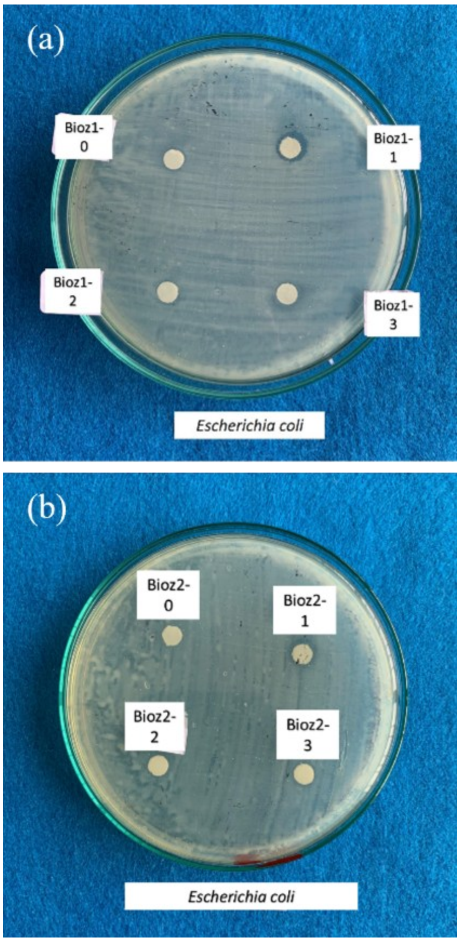

Figure 5. Antibacterial activity test of EE against *E. coli*: (a) Bioz-1 and (b) Bioz-2

Table 3. Antibacterial activity test of econzymes

Code		Diameter of the inhibition zone (unit of measurement)	
		E. coli	*S. aureus*
Bioz1	Bioz1-0	0	0
	Bioz1-1	7.9	6.3
	Bioz1-2	0	0
	Bioz1-3	0	0
Bioz2	Bioz2-0	0	0
	Bioz2-1	7.2	6.5
	Bioz2-2	0	0
	Bioz2-3	0	0
Chloramphenicol (control)		21.8	20.5

The pure eco-enzyme showed no inhibition against *E. coli* and *S. aureus*, possibly because the beneficial bacteria producing organic acids were inactive before dilution with distilled water. At higher dilutions (1:50 and 1:100), the antibacterial activity decreased due to the high proportion of water, which reduced the concentration of active compounds. Eco-enzyme contains antibacterial agents such as alcohols, phenolics, organic acids, and hydrogen peroxide (H_2O_2), making it a safe, eco-friendly, and effective natural disinfectant suitable for home use.

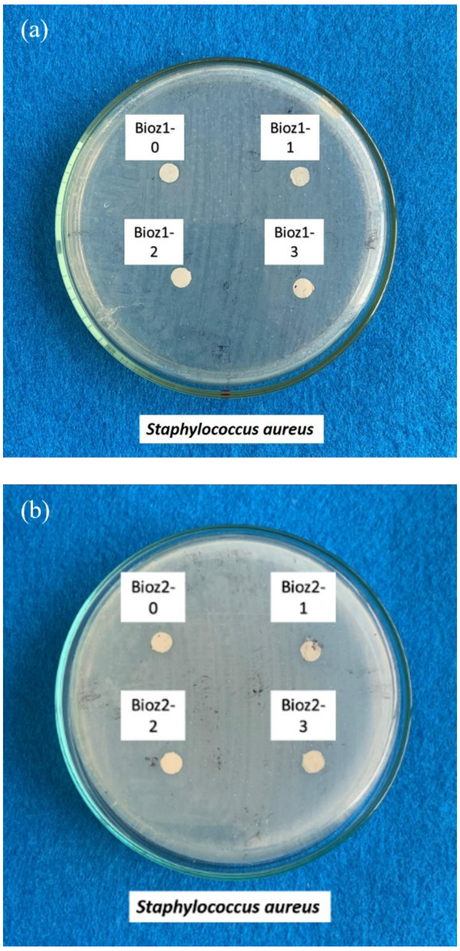

Figure 6. Antibacterial activity test of EE against *S. aureus*: a) Bioz-1 and b) Bioz-2

This differs slightly from previous studies, which reported that eco-enzymes derived from pineapple skin contain tannins and saponins with antibacterial activity, inhibiting *P. acnes* and *S. aureus* at minimum inhibitory concentrations (MICs) of 50% and 100% (v/v), respectively. However, no effective concentration was found against *P. acnes* [25]. The difference may be due to our study using eco-enzymes made from five different organic materials, while prior studies used only one type of waste. A greater diversity of organic waste in eco-enzyme production enriches the variety of organic and phytochemical compounds, enhancing their ability to inhibit pathogenic bacteria. Supporting this, Rijal [26] reported that eco-enzymes from eucalyptus leaves showed inhibition zones of 14.42 mm for *E. coli* and 12.58 mm for *S. aureus*.

The antimicrobial effectiveness of eco-enzymes is attributed to their composition, which includes various fruits and vegetables such as papaya, soursop, banana, orange, and pineapple, all of which contain bioactive compounds like flavonoids, tannins, saponins, lactic acid, and a low pH. These compounds inhibit nucleic acid synthesis, disrupt bacterial cell membranes, interfere with energy metabolism, and alter membrane function due to pH differences, collectively disrupting bacterial metabolic processes. Consequently, eco-enzymes are recognized as natural antibacterial agents suitable for use as environmental disinfectants [27].

4. Conclusion

This study shows that eco-enzymes (EE) produced from fermented organic waste have promising potential as both liquid organic fertilizers and antimicrobial agents. The eco-enzyme demonstrated suitable pH and color, indicating successful fermentation, and its derivative product, Bioz, exhibited improved nutrient content, especially with the addition of banana stems and noni peels; however, nitrogen, phosphorus, and potassium levels remained below national standards. At a 1:10 dilution, Bioz effectively inhibited *E. coli* and *S. aureus*, supporting its use as a natural, environmentally friendly, and skin-safe disinfectant made from common household organic materials. Further studies are recommended to evaluate antifungal activity, optimize concentrations, and test combinations of fruit waste with other plant parts for enhanced antimicrobial effects.

Acknowledgement

We thank the Rector of Universitas Pembangunan Panca Budi for providing internal research grant funding through the Institute for Research and Community Service (LPPM), contract number 333/8/F/17/2023.

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