Antibacterial Activity of Ecoenzymes with Various Dilutions Against *Escherichia Coli*

Najla Lubis
najlalubis@dosen.pancabudi.ac.id
Rizki Damayanti
damay.vie@gmail.com
Sheila Wardani
shelawardani257@gmail.com

Agrotechnology Study Program
Universitas Pembangunan Panca Budi, Indonesia

Abstract

Garbage enzyme (GE), also known as ecoenzyme (EE), is a biotechnology product made by fermenting water, organic components from waste and agricultural products, and carbohydrates. Finding the ideal concentration of ecoenzyme as an antibacterial agent is the goal of this study. Organic components from waste pineapple, orange, papaya, star fruit, kuni, and mango peels (Bioz1) as well as waste pineapple, orange, banana tubers, noni, and kuni (Bioz2) are used to make ecoenzymes. *Escherichia coli* bacteria that are gram-negative are cultured using the disc method. Four different dilution ratios are used: 0; 1:10; 1:50; and 1:100. The best dilution variation to inhibit *Escherichia coli* bacteria, according to the data, was a 1:10 ratio.

***Keywords**:* Antibacterial, Ecoenzymes, Escherichia Coli, Dilution

Introduction

Ecoenzyme (EE) or garbage enzyme (GE) is a biotechnology product derived from the fermentation of carbohydrates, organic agricultural waste, and water. As a multifunctional solution, EE offers numerous advantages, including a 20 ml eco-enzyme treatment with a nutritional value of 6.960% crude fiber, 7.538% crude protein, and 0.321% crude fat. (RB Ginting et al., 2023), in the cultivation of annual crops [1], increases the fertility of shallot plants with an EE: water ratio of 1: 100 and soybean plants soil treatment of 75% topsoil + 25% compost with the provision of ecoenzymes can respond togrowth such as plant height and number of shallot leaves [4], making EE using household organic waste supports sustainable agriculture in Suka Damai Village, Langkat [5], andfunctions as liquid organic fertilizer [6]. Providing Ecoenzyme with a dose of 20 mL in cassavawaste fermentation can increase crude protein levels, but reduce crude fiber and crude fat in animal feed [7].

Plant waste like oranges, pineapples, and papayas, as well as other organic waste, can be utilized to make EE. Citrus waste can be used because it includes bioactive components suchas flavonoids, which have antibacterial properties. Flavonoids' inhibitory mechanism as antibacterial agents includes blocking nucleic acid synthesis, disturbing cytoplasmic membrane function, and influencing biofilm development, porins, permeability, and bacterial enzyme interaction. Ecoenzymes can be synthesized and employed as antibacterials, antiseptics, and agents to prevent worm egg contamination in fresh fruits and vegetables. Ecoenzymes comprise lipase, protease, and amylase enzymes, which can cause damage to microbial cell structures and are used as an environmentally friendly washing liquid for fruits and vegetables. Waste enzyme production via fermentation is an efficient pathway for converting organic waste into value-added products in environmental matrices such as organic degradation, composting, wastewater, leachate treatment, and disinfection

Ecoenzyme has been shown to have high antibacterial activity against a variety of microorganisms. Several studies have employed eco enzymes to prevent the growth of bacteria such as *Staphylococcus aureus* and *Propionibacterium acnes* [8]. The results showed that pineapple (*Ananas comasus* L) and orange (Citrus 13.1 mm) had formed a clean zone around the disc. Ecoenzymes have also been discovered to have potential as antibacterials since they have a low pH, which can harm the flexibility of bacteria. In addition, eco enzymes can also be used as antiparasitic agents, such as in inhibiting the growth of *Ascaris lumbricoides*, with high concentrations of acetic acid effectively damaging the walls of parasite egg cells.

Ecoenzymes have been investigated for their efficacy as disinfectants against harmful microorganisms such as Escherichia coli. The study found that eco-enzymes can significantly suppress the growth of E. coli bacteria. According to research, eco-enzymes can limit E. coli development in a variety of situations. Research shows that coffee skin ecoenzymes fermented for 2 months had antibacterial activity of 1.423 ± 0.214 cm against E. coli bacteria. [11], and14.43 $\pm$ 2.71 mm in EE from coffee and papaya waste [12] Other studies found that eco-enzymes exhibit antibacterial action against *Escherichia coli*, as demonstrated by the creation of a clean zone surrounding the paper disc.

Ecoenzymes diluted at a 1:30 ratio can effectively prevent *E. coli* development in pig breeding pens [13]. Other research has shown that low pH eco-enzymes can serve asinhibitors, reducing bacterial community growth and variety, making them useful disinfectants and cleaners. Furthermore, eco-enzyme has been tested against *E. coli* in the context of hand sanitizer, with the results indicating that eco-enzyme hand sanitizer successfully inhibits E. coligrowth. These findings imply that eco enzymes may be an alternative to standard disinfectantsin limiting the spread of *E. coli* and other pathogenic bacteria.

However, multiple studies have failed to determine the concentration or dilution ratio of eco enzymes derived from organic agricultural waste to water that is efficient in reducing theactivity of *E. coli* bacteria. The purpose of this investigation was to investigate which concentration changes of eco enzyme dilution would be most efficient in preventing *E. coli* bacteria growth.

Literature Review

Eco Enzyme, derived from pineapple (*Ananas comosus*), banana (*Musa paradisiaca*), and papaya (*Carica papaya*) fruit waste, inhibits *Staphylococcus aureus* at doses of 1:100, 1:200, or 1:300. It has the highest inhibitory power when diluted 1:100 [9]. The bacteria S. aureus is known to cause skin infections and a variety of other disorders. The study's findings indicate that this ecoenzyme has the potential to be used as a natural antibacterial in food medicine and preservation, as well as in other applications requiring microbial control. Horticultural plant waste, such as fruit and vegetable peels, can be utilized to create environmental enzymes that function as antibacterials. Other studies have indicated that ecoenzymes made from a combination of fruit and vegetable peel waste, water, and molasses can be used as an efficient antibiotic against *Staphylococcus aureus* and *Staphylococcus* epidermidis, both of which cause skin infections. Eco enzymes' capacity to suppress germs means they can work as natural disinfectants and hand sanitisers. [10].

Escherichia coli, sometimes known as *E. coli*, is a species of bacteria that is commonlyfound in the environment, food, and the intestines of humans and animals. Most strains of E. coli are harmless, but some can cause sickness in people. Diarrhea, stomach discomfort, vomiting, and fever are common symptoms of an *E. coli* infection, which can affect people of all ages and cause a variety of disorders including gastroenteritis, urinary tract infections, and pneumonia. E. coli infections can spread through contaminated food and beverages, as well asdirect contact with bacteria that have not been adequately preserved. Age is a risk factor for *E. coli* infection, as children, pregnant women, and the elderly are more sensitive to *E. coli* bacteria-related disorders.

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Research Methodology

This work was carried out in vitro at the Agrotechnology work Program Laboratory at Universitas Pembangunan Panca Budi and the Microbiology Laboratory at the Faculty of Mathematics and Natural Sciences at Universitas Sumatera Utara.

Mineral water, carbohydrates, and organic components from agricultural waste are the resources utilized to make ecoenzymes. bacteria identified as *Escherichia coli* that were taken out of the USU Microbiology Lab. A knife, scales, measuring cup, nutritive agar, MHA, MRSA, MRSB, petridish, ose needle, autoclave, pH meter, and paper disc were among the instruments utilized.

Eco-enzyme Production

The ratio of 3:1:10, or 3 kg of organic material from garbage and agricultural products, 1 kg of carbohydrates (molasses), and 10 L of mineral water, is used to produce ecoenzymes. Organic waste from pineapple, orange, papaya, star fruit, kuini, and mango peel (example code Bioz1), as well as garbage from noni, kuini, banana corm, and pineapple (sample code Bioz2), are used to make ecoenzymes. After the organic material has been cleansed or washed, it is weighed and broken up into little pieces, providing a total weight of 3 kg. Ten liters of water should be ready in a plastic container. Add the molasses and swirl until it dissolves in the water. Following the homogenization of all the ingredients, the container is properly shut and let to ferment for 100 days. After the fermentation period is over, the container is opened, and the ecoenzyme solution is filtered to separate the eco enzyme filtrate from the dregs (residue). The filtered enzyme is placed in a dry and clean plastic container.

Ecoenzyme dilution

Pure eco enzyme (Bioz-0) is diluted with different dilution ratios, such as 1:10 by dissolving 10 mL of eco enzyme in 100 mL of distilled water (Bioz1-1), 1:50 by dissolving 2 mL of eco enzyme in 100 mL of distilled water (Bioz1-2), and 1:100 by dissolving 1 mL of eco enzyme in 100 mL of distilled water (Bioz1-3).

Figure 1. Dilution of eco-enzymes with numerous variations

Results

The results of the Ecoenzyme antibacterial activity test against *Escherichia coli* bacteria are shown in Table 1.

Table 1. *Escherichia coli* antibacterial activity test results from ecoenzyme

No	**Sample code**	**Inhibition zone diameter (mm)**
	BIOZ1	
1	BIOZ1-0	0
2	BIOZ1-1	7.9
3	BIOZ1-2	0
4	BIOZ1-3	0
	BIOZ2	
1	BIOZ2-0	0
2	BIOZ2-1	7.2
3	BIOZ2-2	0
4	BIOZ2-3	0

The results of Table 1 demonstrate that microorganisms are inhibited by the EE of Bioz-1 (waste of pineapple peel, orange, papaya, starfruit, kuini, and mango) and Bioz-2 (waste of pineapple, orange, banana tuber, noni, and kuini). E. coli measuring 7.9 mm and 7.2 mm, respectively, are classified as being in the middle range by David and Stout. Given that Bioz-1samples outnumber Bioz-2 samples in EE with respect to the kind of organic material, Bioz-1 has a somewhat stronger bacterial inhibitory power than Bioz-2. The development of a clean zone around the well serves as evidence of this.

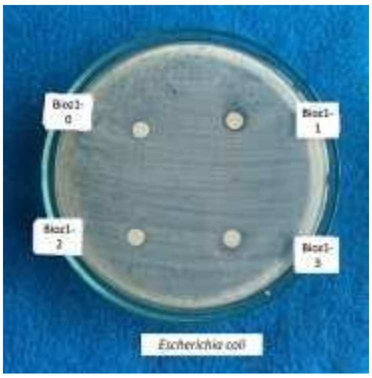

Figure 2. The antibacterial activity test of EE against *E. coli* bacteria

Neither pure EE (1:0) nor any other dilution exhibits any inhibitory effect against the E. coli bacteria. Ecoenzymes (Bioz-1 and Bioz-2) with a solution dilution ratio of 1:10 are the best and most efficient (optimal) dilutions; even though they fall into the medium category, their 5-110 mm zone contains an inhibitory zone [14]. This is most likely owing to the fact that the EE material contained fruit peels rather than veggies. This is consistent with prior study, which shows that EE from orange peel, pineapple, and papaya contains phenols that are included in eco-enzyme fluid and is helpful in decreasing bacterial development, thus it can be used as a natural disinfectant. In the antibacterial test, only a few bacteria or microbes developed when the material was diluted 1:10. [15] as illustrated in Fig.2.

Ecoenzymes have no inhibitory activity against *E. coli* bacteria; however, it is likely thatthe beneficial bacteria that make organic acids are not yet active since they have not been mixedwith distilled water. This differs slightly from the results of previous research [9], which confirmed that Eco Enzyme diluted in a concentration of 1:100 has strong antimicrobial activityagainst *Staphylococcus aureus*, possibly due to differences in the cell walls of gram-positive bacteria from *S. aereus* and gram-negative bacteria from *E. coli*.

Gram-negative bacteria have a cell wall composed of an outer membrane and many layers of peptidoglycan. The outer membrane is composed of lipoproteins, phospholipids, and LPS. Peptidoglycan remains intact on the lipoproteins of the outer membrane, which are found in the periplasmic fluid that exists between the plasma and outer membranes. The periplasm contains proteins and degrading enzymes that aid in the movement of molecules.

Because EE has a very high distilled water percentage, good bacteria are less active at 1:50 and 1:100 dilutions; as a result, the dilution at 1:10 varies. Alcohol, phenolics, organic acids, and hydrogen peroxide (H_2O_2) are all present in EE. Because EE contains several active compounds, it has an antibacterial effect and can be used as a natural disinfectant that is safe to use on skin, safe to use in the environment, and made of easily found organic ingredients in the home. For this reason, using eco-enzymes at home is highly advised.

Conclusion

Ecoenzymes with varying concentrations of 1:10 effectively inhibit the growth of Escherichia coli bacteria using the disc method and can be used as a natural disinfectant that is environmentally friendly, safe on the skin, and uses organic ingredients that are readily available in the household environment.

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