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Testing the Antimicrobial Efficacy of Eco-Enzymes on *Staphylococcus aureus* and *Escherichia coli* as a Participatory Student Activity in Biotechnology for Food Waste Management

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ABSTRACT

This study investigates the antimicrobial efficacy of eco-enzymes derived from organic waste, specifically fruit and vegetable peels, against Escherichia coli and Staphylococcus aureus bacteria. Eco-enzymes were produced through a simple fermentation process involving kitchen scraps, molasses, and water. The results showed that the highest inhibition zones were observed at a concentration of 100%, followed by 75%, while 50% and 25% showed significantly reduced inhibition zones. This indicates that the antimicrobial properties of eco-enzymes are concentration-dependent, with higher concentrations exhibiting greater efficacy. Additionally, eco-enzymes contribute to sustainable organic waste management by converting waste into valuable bio-sanitizers, floor cleaners, bio-pesticides, and plant fertilizers. This research highlights the dual benefits of eco-enzymes in reducing environmental impact and supporting sustainable development goals, inspiring hope for a more eco-friendly future.
***Keywords**: antibacterial activity, biotechnology, eco enzyme, Escherichia coli, food waste, Staphylococcus aureus*

ABSTRAK

Penelitian ini menyelidiki efektivitas antimikroba eco-enzim yang berasal dari sampah organik, khususnya kulit buah dan sayuran, terhadap bakteri Escherichia coli dan Staphylococcus aureus. Eco-enzim diproduksi melalui proses fermentasi sederhana yang melibatkan sampah dapur, molase, dan air. Hasil penelitian menunjukkan bahwa zona hambatan tertinggi diamati pada konsentrasi 100%, diikuti oleh 75%, sementara konsentrasi 50% dan 25% menunjukkan zona hambatan yang jauh lebih kecil. Hal ini menunjukkan bahwa sifat antimikroba eco-enzim bergantung pada konsentrasi, dengan konsentrasi yang lebih tinggi menunjukkan efektivitas yang lebih besar. Selain itu, eco-enzim berkontribusi pada pengelolaan sampah organik yang berkelanjutan dengan mengubah sampah menjadi bio-sanitizer, pembersih lantai, bio-pestisida, dan pupuk tanaman yang bernilai. Penelitian ini menyoroti manfaat ganda dari eco-enzim dalam mengurangi dampak lingkungan dan mendukung tujuan pembangunan berkelanjutan, yang menginspirasi harapan untuk masa depan yang lebih ramah lingkungan.
***Kata Kunci:** aktivitas antibakteri, bioteknologi, eco-enzim, Escherichia coli, sampah makanan, Staphylococcus aureus*

INTRODUCTION

Increased human activities in producing waste and pollutants have caused severe environmental damage. (Ramadhan, 2023; Sompotan & Sinaga, 2022). Using environmentally friendly technology in waste processing is essential to reduce negative impacts on the ecosystem. (Tanveer et al., 2022). One promising approach is using eco-enzymes in environmental processing processes (Nurdin et al., 2022). In wastewater treatment, ecoenzymes can accelerate the decomposition of organic materials, remove harmful substances, and reduce pollutant levels. This research underscores eco-enzymes' potential in waste management, offering hope for a more sustainable future. Ecoenzymes can also decompose organic materials, such as agricultural and food industry waste composition. Research (Arwadani et al., 2024) shows that the advantages of ecoenzymes in environmental processing include flexibility in various ecological conditions, the potential for sustainable application, and high catalytic activity.

Environmental problems in Indonesia still need more intensive attention (Fatimah et al., 2020; Lestari and Trihadiningrum, 2019; Brotosusilo et al., 2020). Natural and human resources whose functions have not yet been effectively maximized will cause lasting damage if they are not immediately handled exclusively and sustainably. Household waste is continuously generated daily without considering the consequences of disposal and the increasing accumulation of waste, which will impact quality and even environmental damage (Hsiang et al., 2019; Sumargo and Fadlilah, 2019).

Eco-enzyme is an organic solution produced through fermentation using organic waste, such as vegetable and fruit scraps, with added sugar and water. The types of sugar used in eco-enzyme production vary, including brown sugar, white sugar, molasses, and jaggery, which serve as a carbon source for microbial activity during fermentation. This solution has various applications, including biosanitizer, floor cleaner, biopesticide, and plant fertilizer. (Primarista et al., 2022; Phanama et al., 2024; Sari et al., 2021). Ecoenzymes have an essential role in biochemical processes that occur in nature, including the decomposition of organic matter, nutrient cycling, and detoxification of dangerous substances (Kavitha & Ravikumar, 2020). In recent years, the use of ecoenzymes as products in the environmental processing of household waste has become a beneficial effort both in the fields of biopharmaceuticals, agronomy and households themselves because they contain compounds rich in benefits so that ecoenzymes are included in the implementation of Sustainable Development Programs (SDG's) at point number 13, is climate change in a based zero waste solution (Nurfajriah et al., 2021).

Microorganisms are found everywhere and occupy a variety of habitats. They are found in the soil, water, and air in our planet's atmosphere, including around humans, especially where there is a source of nutrition for them. Microorganisms are also found on the surfaces of the objects we use, in the air we breathe, and even on our bodies, such as the skin's surface. Water is an essential source of life for humans and other living creatures. However, water can be contaminated by various harmful bacteria that can cause disease. Preventing water bacteria is crucial to maintaining public health and protecting water resources. Antibacterial substances inhibit the growth of bacteria and can kill pathogenic bacteria. Antibacterial agents can be divided into two categories: bacteriostatic, which prevents the growth of bacteria, and bactericidal, which can kill bacteria (Magani, Talei, & Kolondam, 2020). Methane gas is the main component of natural gas, and its most straightforward chemical composition consists of a tetrahedral molecule with four equal C-H bonds, first discovered and isolated by Alessandro Volta (1778) di Danau Maggiore, Italia (Kampono et al., 2021).

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Bacterial-contaminated water contains dangerous microorganisms, such as bacteria, viruses, and parasites. This contamination can come from various sources, such as wastewater from households that are not adequately treated and can contaminate groundwater and other water sources with coliform, Salmonella, and *Shigella bacteria*. Waste from livestock and agricultural activities can contaminate water with *E. coli, Campylobacter,* and *Leptospira bacteria*. Garbage thrown away carelessly can pollute water with Staphylococcus aureus and Streptococcus bacteria. However, some strains of *E. coli* can cause infections in humans, including urinary tract infections, gastroenteritis, and severe systemic infections (Singha et al., 2023). According to (Rind, 2023), one of the best-known strains is Escherichia coli O157:H7, which can cause serious diseases such as hemolytic uremic syndrome (SHU), which can be fatal, especially in children. Transmission of *E. coli* generally occurs through consuming food or drinks contaminated by human or animal feces containing this bacteria.

The *Staphylococcus aureus* bacteria is part of the normal flora of human skin, but conditions that allow it to infect human skin and cause acne and swelling. *Staphylococcus aureus* can also infect wounds, then enter the bloodstream and spread to other organs, causing pneumonia, inflammation of the heart valves, which can lead to heart failure, bone inflammation, and even shock, which can lead to death. (Huda, 2018).

Using contaminated water may increase the risk of exposure to bacteria. Building and maintaining an effective wastewater treatment system is critical to neutralize harmful bacteria and pathogens before they are released into the environment. One of the benefits of coenzyme for aquaculture is that it helps purify polluted water by decomposing organic matter and pollutants, reducing odors and turbidity of the water, increasing dissolved oxygen levels in the water, and helping to decompose waste and fat in the wastewater. Therefore, this practicum design tries to provide alternative solutions related to the challenges mentioned previously in the practicum design entitled "*Testing the Antimicrobial Efficacy of Eco-Enzymes on Staphylococcus aureus and Escherichia coli as a Participatory Student Activity in Biotechnology Material for Food Waste Management*."

METHODS

Two eco-enzyme samples were used: the first sample, made from organic waste in the form of green mustard greens and fruit peels with a pH of 4.17 and dark in color, was tested twice. The second eco-enzyme sample, made from fruit peels such as papaya, orange, cucumber, and watermelon with a pH of 3.20 and light in color, was tested once by the students. The samples used in this study consisted of organic waste from fruit and vegetable peels, molasses, and water in a 1:3:10 ratio for making eco-enzymes. The method of making eco-enzymes in this study refers to the research conducted by Irianto et al. (2023) and has been modified according to the needs of this research. For testing antibacterial activity, a suspension of Staphylococcus aureus and Escherichia coli bacteria, incubated for 24 hours before treatment, was used. Additionally, eco-enzyme fermentation samples aged for 3 months with concentrations of 100%, 75%, 50%, and 25% were diluted with distilled water.

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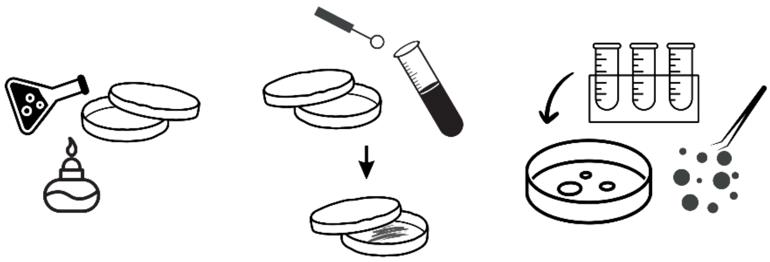
Table 1. Percentage of eco-enzyme and equate content used

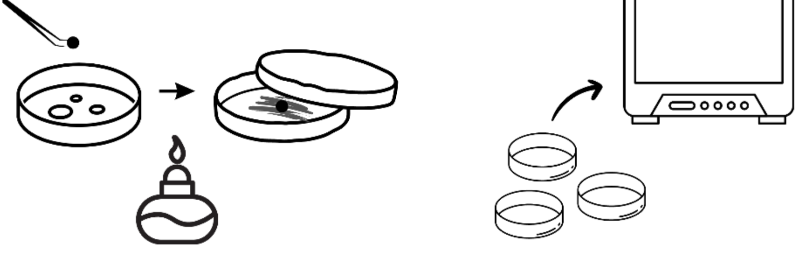
Variable	Eco-enzyme	Aquades
I	100%	0%
II	75%	25%
III	50%	50%
IV	25%	75%
Control	0%	0%

The bacterial media incubated for 24 hours was removed, and 4 ml of NaCl was added to each bacterial suspension in the test tubes. Adding NaCl to the microbial suspension helps maintain microbial stability, and then the mixture was shaken until evenly mixed. Next, antimicrobial test media using MCA were prepared in two Petri dishes: the first petri dish tested the effectiveness of eco-enzyme as an antimicrobial agent against Staphylococcus aureus, and the second petri dish tested the efficacy of eco-enzyme as an antimicrobial agent against *Escherichia coli*.



Picture 1. experimental design for making media and treatments

The media were allowed to solidify. Bacterial isolates were then taken using a cotton swab and streaked evenly on each medium. Each eco-enzyme with different 100%, 75%, 50%, and 25% concentrations was poured into each petri dish. A paper disk containing eco-enzymes of various concentrations was dipped into each petri dish. The paper disks soaked in eco-enzyme were placed on the media containing bacterial isolates. The dishes were incubated for 24 hours. After incubation, the inhibition zones on the growth media were observed using a ruler.



Picture 2. Treatment bacterial incubation experimental design

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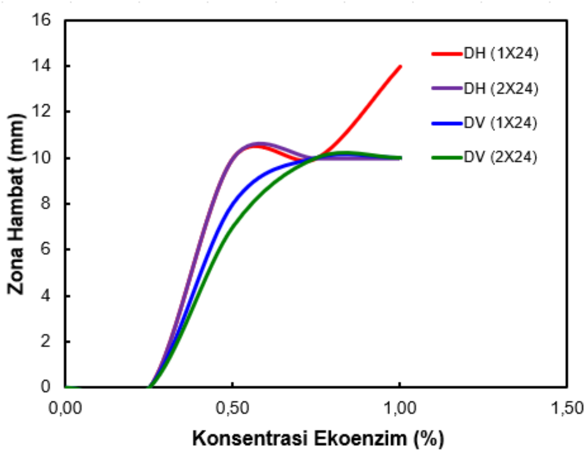
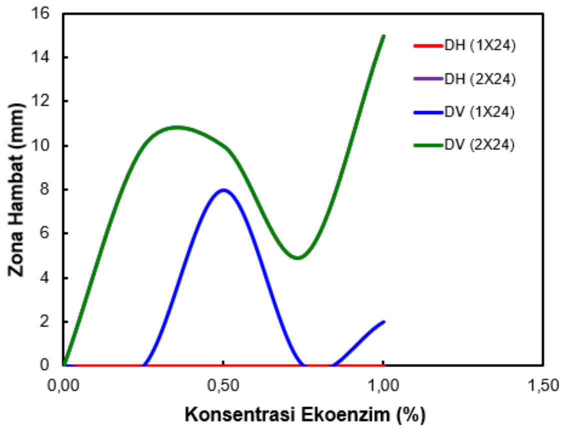
RESULTS AND DISCUSSION

The Role of Antimicrobial Compounds in Eco-Enzyme Content

In this study, researchers discovered something interesting: inhibition zones against Escherichia coli and Staphylococcus aureus growth were observed due to the discs soaked in different concentrations of Eco-enzyme solutions. The researchers conducted the study in two repetitions to observe the inhibitory effects of each concentration of Eco-enzyme in the Petri dishes. Below is the graph showing the rate of inhibition zones in the experiment conducted by the researchers on *Staphylococcus aureus* and *Escherichia coli* bacteria.



Picture 3. Graph of the inhibition zone rate of Ecoenzyme on the growth of *Escherichia coli* bacteria in the first experiment with pH 3.20.



Picture 4. Graph of the inhibition zone rate of Ecoenzyme against the growth of *Staphylococcus aureus* bacteria in the first experiment with pH 3.20.

The research results showed a very high inhibition zone rate, indicating that *Escherichia coli* and *Staphylococcus aureus* bacteria were effectively eliminated by eco-enzyme. The highest rate was observed in the 1×24 hour incubation treatment, reaching up to 14 mm in horizontal diameter and 10 mm in vertical diameter in the growth plates of *Escherichia coli*, eliminated by eco-enzyme at the highest concentration of 100%. The highest rate in the 2×24 hour incubation treatment reached 13 mm in horizontal diameter and

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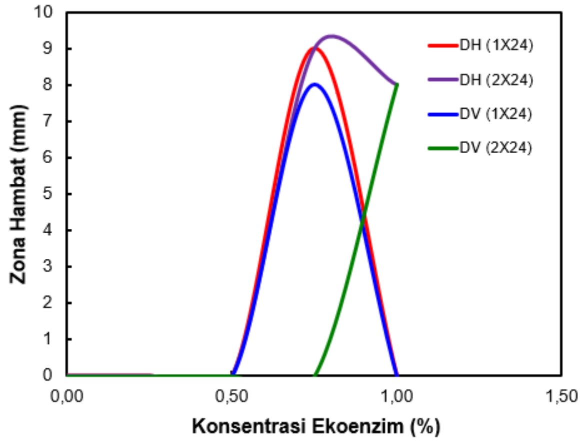
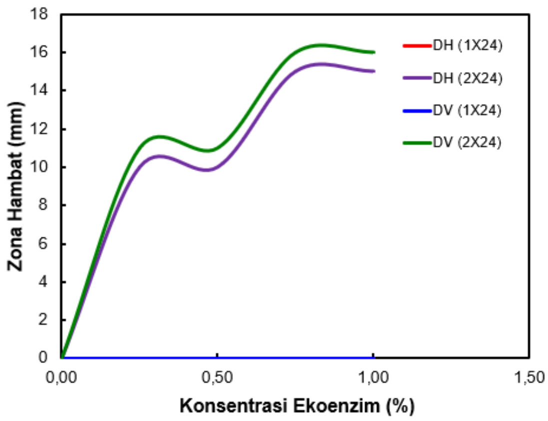
15 mm in vertical diameter in the growth plates of *Staphylococcus aureus*, also eliminated by eco-enzyme at the highest concentration of 100%. The weak antimicrobial growth rate was observed with lower concentrations of eco-enzyme, precisely 50%, showing 10 mm horizontal diameter (HD) and 8 mm vertical diameter (VD), and 25%, showing 0 mm in the 1×24 hour incubation treatment. This was due to more distilled water mixed in the paper disc in the petri dish. The study by (Wang et al., 2021) showed a clear correlation between increasing concentrations of ecological enzymes and inhibition of Escherichia coli and Staphylococcus aureus growth, influenced by concentrated concentrations.



Picture 5. Graph of the inhibition zone rate of Ecoenzyme on the growth of *Escherichia coli* bacteria in the second experiment with pH 4.17.



Picture 6. Graph of the inhibition zone rate of Ecoenzyme against the growth of *Staphylococcus aureus* bacteria in the second experiment with pH 4.17.

The results of the second experiment conducted by the students showed a very high inhibition zone rate, indicating that *Escherichia coli* and *Staphylococcus aureus* bacteria were effectively eliminated by eco-enzyme. The highest rate was observed in the 2×24 hour incubation treatment, reaching up to 8 mm in both horizontal and vertical diameters in the growth plates of *Escherichia coli*, eliminated by eco-enzyme at the highest concentration of 100%. The highest rate in the 2×24 hour incubation treatment reached 15 mm in horizontal diameter and 16 mm in vertical diameter in the growth plates of *Staphylococcus aureus*, also eliminated by eco-enzyme at the highest concentration of 100%. The weak antimicrobial growth rate was observed with lower concentrations of eco-enzyme, precisely 50% and 25%, which did not affect inhibiting microbial growth. No inhibition zone was observed in the 1×24 hour incubation treatment in this experiment. This is because, during the increased incubation time, bacteria experience an increase in cell biomass, which leads to an increase in cellulase enzyme production (Martsiningsih et al., 2023). The optimum output of bacteriocin by these bacteria was detected during the stationary phase, which occurs at 48 hours of incubation (Kurniawati et al., 2019). Eco-enzyme exhibit antimicrobial efficacy due to bioactive compounds such as flavonoids, tannins, saponins, and organic acids produced during fermentation. These compounds disrupt bacterial cell walls, inhibit nucleic acid synthesis, and interfere with bacterial metabolism, leading to bacterial growth inhibition and cell death (Irianto et al., 2023; Permatananda et al., 2023). The acidic environment created by eco-enzyme fermentation further enhances its antibacterial properties, making it effective against Staphylococcus aureus and Escherichia coli (Kavitha & Ravikumar, 2020).

The Effect of Ecoenzyme Production on Organic Waste Management

Eco-enzymes are products that can protect the earth from damage caused by methane gas produced from the decomposition of organic materials in vegetables and fruits. Eco-enzymes have many advantages and can be used in everyday life. According to (Novantri, 2021), methane gas is highly flammable and, when it reacts with air at certain concentrations, can cause respiratory problems or explosions. This poses a significant threat to all of us. Therefore, there is a need for organic waste management efforts.

Organic waste management is a necessary process with two main goals: to convert waste into economically valuable materials and to process waste into materials that are not harmful to the environment and the surrounding community. The organic waste management process is divided into four stages: on-site handling, collection, transportation, and processing. The concept of waste management can be effectively implemented to raise awareness in the community that waste can bring economic value by exchanging waste. In the future, training programs for the community will be needed to increase production (collection of economically valuable waste) and efficient and socially beneficial sales.

Regarding organic waste management, eco-enzymes can accelerate natural biochemical reactions and produce beneficial enzymes from fruit or vegetable waste. Enzymes derived from organic waste are a method of waste management that uses kitchen scraps, such as fruit and vegetable peels, for beneficial purposes. This liquid can be used as a household cleaner and an effective natural fertilizer and pesticide.

Student Participation in Food Waste Management Efforts through Ecoenzymes

Eco-enzymes are products that can help mitigate environmental damage caused by methane gas emissions. Methane gas is highly flammable and, when mixed with air at specific concentrations, can cause respiratory problems or even explosions. This poses a significant threat, particularly in Indonesia, the second-largest producer of food waste globally. Therefore, early organic waste management initiatives are crucial. This research explores an alternative approach by utilizing organic waste for sustainable

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environmental management and aims to engage students actively in community-based food waste management.

In this study, fourth-semester students participated actively in collecting and preparing eco-enzymes. Over two weeks, they gathered organic waste, such as fruit and vegetable peels, from their daily consumption. Additionally, they collected food waste from their surroundings, including campus cafeterias and local markets, to ensure a sufficient supply of raw materials. The fermentation process was conducted in the university's Microbiology Laboratory, where conditions were standardized to maintain controlled fermentation.

Although eco-enzymes contain various microorganisms contributing to their enzymatic activity, this study did not specifically identify or control the microbial composition of fermentation. Instead, the research focused on evaluating the antimicrobial potential of the produced eco-enzyme against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria. Future studies could include microbial analysis further to understand the role of specific microorganisms in eco-enzyme effectiveness.

CONCLUSION

Several key findings and implications can be drawn based on the comprehensive study on the antimicrobial efficacy of eco-enzymes against Escherichia coli and Staphylococcus aureus. Firstly, the research demonstrated that eco-enzymes derived from organic waste, specifically fruit and vegetable peels, exhibit significant antimicrobial activity against *E. coli* and *S. aureus*. This antimicrobial activity was observed by forming inhibition zones in agar plates, where higher concentrations of eco-enzymes correlated with larger inhibition zones. This indicates that eco-enzymes have the potential to be effective alternatives in mitigating bacterial contamination in various settings, including food processing, sanitation, and wastewater treatment.

Moreover, the study underscores the importance of eco-enzymes in organic waste management. By converting kitchen scraps into eco-enzymes through a simple fermentation process, not only can organic waste be effectively repurposed, but the resulting eco-enzymes can also serve as versatile bio-sanitizers, floor cleaners, bio-pesticides, and plant fertilizers. This dual benefit of waste reduction and resource creation aligns with sustainable development goals (SDGs), particularly in addressing climate change and promoting zero-waste solutions.

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