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Effect of Eco-Enzyme's Storage Duration on Hydrogen Peroxide Level and Its Antibacterial Activity

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Abstract

Eco-enzyme is a filtrate produced by fermenting sugar and a mixture of organic matter for 3 months. Eco-enzymes have been widely used as cleaning fluids and disinfectants. This study aims to investigate the levels of hydrogen peroxide and the antibacterial activity of eco-enzymes stored at different times. Hydrogen peroxide level was analyzed by redox titration and the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* was analyzed by paper disc method. This study shows that there were significant differences in the levels of hydrogen peroxide and the antibacterial activity of the eco-enzymes stored for a certain time. *Hydrogen peroxide* levels increased from 0 months of storage to reach the highest point at 10 months of storage with 2664.9 ppm and then it began to decrease to the lowest point at 114 months of storage. The highest antibacterial activity against Escherichia coli and Staphylococcus aureus was shown by eco-enzyme stored for 0 months, with inhibitory zones of 6.37 mm and 6.59 mm, respectively. Eco-enzyme no longer showed antibacterial activity after 15 months of storage. Overall, the storage duration of the eco-enzyme affects the hydrogen peroxide level and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* due to the amount of lactic acid bacteria in the eco-enzyme.

Keywords: antibacterial activity, eco enzyme, hydrogen peroxide, storage duration

INTRODUCTION

One of the problems that the world, including Indonesia, is currently facing is food waste. Based on data released by the Ministry of Environment and Forestry in 2022, a total of 23,260,640.04 tons of waste arose in Indonesia with 41.71% of the waste being food waste and 38.55% coming from household waste [1]. The high percentage of food waste generated by households is indirectly due to a lack of public education about waste management, both organic and inorganic waste. The increase in organic waste can be prevented, especially from households, by processing food waste that does not decompose into eco-enzymes. Since the pandemic in 2020, information regarding the management of organic waste into eco-enzymes has begun to be widely introduced in society, especially because of its function as a disinfectant. Eco-enzyme is a fermented filtrate from a mixture of organic waste and sugar for 3 months [2]. The fermented filtrate, which is called eco-enzyme, has been widely used, as a disinfectant [3-4], treating household wastewater [5-6], and increasing the nutrition of agricultural soil [7-9].

The concept of eco-enzyme fermentation, initiated by Dr. Rosukon, is a fermentation that during the process relies on local microorganisms from organic materials. Several previous studies suggested that local microorganisms that play a role during the fermentation process are mold and bacteria, such as *Aspergillus niger*, *Saccharomyces cerevisiae*, *Leuconostoc, Acetobacter*, *Lactobacillus plantarum*, *Pseudomonas*, and *Yersinia* [10-11]. Most of the bacteria that play a role in the fermentation process are lactic acid bacteria consortiums. Two types of lactic acid fermentation take place, according to the type of lactic acid bacteria involved. The first fermentation is homofermentative lactic acid fermentation which takes place due to the presence of homofermentative lactic acid bacteria, where the product of this fermentation is lactic acid. In addition, there is a heterofermentative fermentation that takes place due to the presence of heterofermentative and facultative bacteria, where the products produced from this fermentation are lactic acid, acetic acid, and/or ethanol [12]. In addition to organic acids and ethanol which are formed during the

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fermentation process, microbes in eco-enzymes also excrete enzymes, such as amylase, lipase, and papain [13].

The more variety of organic waste used in the eco-enzyme fermentation, the more organic acids, ethanol, and even enzymes contained in the filtrate. In addition to the previously mentioned compounds, the lactic acid bacteria consortium also produces hydrogen peroxide as a by-product during fermentation [14]. Previous studies have found that hydrogen peroxide can act as a bactericidal due to its ability to be an important mediator in inhibiting bacterial growth [15]. Although hydrogen peroxide is also produced during the eco-enzyme fermentation process and has a role in antibacterial activity, not many studies have investigated the levels of hydrogen peroxide in eco-enzyme and its relationship with the activity of inhibiting bacterial growth. In addition, a study suggests that eco-enzyme storage duration may correlate to eco-enzyme's antibacterial activity. This is shown by the inhibition activity of *E. faecalis* bacteria from 6-month-old eco-enzyme which is higher than 3-month-old eco-enzyme [16].

Based on this explanation, this research will investigate the levels of hydrogen peroxide from eco-enzymes stored for a certain duration. In addition, this study will investigate the effect of eco-enzyme storage duration on antibacterial activity. It is expected that the results of this study can contribute to the scientific reference about eco-enzymes.

MATERIALS AND METHODS

Materials

The ingredients used to make eco-enzyme extract were molasses and a random mixture of orange, melon, watermelon, guava, apple and dragon fruit peels/waste. Sub-culture of *Escherichia coli* and *Staphylococcus aureus* were obtained from Faculty of Medicine, Brawijaya University. Other materials used were sulfuric acid (Merck), potassium permanganate (Merck), sodium hypochlorite (Onemed), nutrient agar (Oxoid), and nutrient broth (Himedia).

Preparation of Eco-Enzyme Extract

Prepare a plastic container with 1000 mL of water and mix 100 g of molasses in each container. About 300 g of organic material in the form of fruit peels mixture were put in each container, closed, and then harvested after 3 months fermentation at room temperature. The sample was filtered off and the filtrate was stored in glass bottles for further analysis. The samples used in this study came from eco-enzyme filtrates that had been stored for 0, 7, 10, 14, 15, 63, and 114 months.

Determination of Hydrogen Peroxide Level

Hydrogen peroxide level was determined by placing 10 mL of the sample filtrate into the Erlenmeyer and adding 2 mL of 4M sulfuric acid. The solution was heated in a water bath to a temperature of about ± 70°C, and then the solution was titrated with 0.025 M potassium permanganate solution, which was standardized by oxalic acid. The solution was titrated until the solution turned purple for approximately 15 seconds. The level of hydrogen peroxide is determined by the following formula [17], in which for each storage duration, three samples were tested:

$$H_2O_2(\text{ppm}) = \left(\frac{V\ KMnO_4 \times N\ KMnO_4 \times ME}{V\ H_2SO_4 \times V\ \text{sample}}\right) \times 10000$$

Note: V $KMnO_4$ = volume of $KMnO_4$ (mL), N $KMnO_4$ = normality of $KMnO_4$ (N), ME = equivalent factor (1,701/mg), and V H_2SO_4 = volume of sulfuric acid (mL).

Determination of Antibacterial Activity

Determination procedure of antibacterial activity was done following reference [18] with modification. A total of 20 mL of sterile nutrient agar media was poured into a petri dish and waited for the media to solidify. Then, the test culture bacteria (*Escherichia coli* and *Staphylococcus aureus*) that had been rejuvenated were spread with sterile cotton from liquid media to sterile petri dishes. Paper discs were soaked for 20 minutes consecutively with distilled water as a negative control, sodium hypochlorite solution as a positive control, and sample solutions with various concentrations of Eco-Enzyme filtrate: 100, 20, 10, 1, 0.2, and 0.1%. The soaked paper discs were aseptically transferred with sterile tweezers to a petri dish, then incubated for 1 x 24 hours at 37°C. Antibacterial activity in the form of inhibitory zone was obtained from the difference in the diameter of the clear zone and the diameter of the disc paper as measured by the caliper. Inhibitory zones of the test were grouped into 3, namely strong (diameter > 6 mm), good (diameter between 3 - 6 mm), and weak (diameter between 0 - 3 mm).

Data Analysis

Three samples were used for each storage duration and the data were expressed as mean with standard deviation (SD). Data were analyzed comparatively using one-way ANOVA followed by

the Tukey-Honestly Significant Differences (Tukey's HSD) test and Pearson's correlation test. All tests were set at a significant level of 0.05.

RESULTS AND DISCUSSION

Hydrogen Peroxide Level

Analysis of hydrogen peroxide levels was carried out by a redox titration of potassium permanganate and the results of the analysis were expressed as ppm. From the results of this study, as shown in Table 1, eco-enzyme stored for 0 months has a hydrogen peroxide level of 1323.95 ppm. Hydrogen peroxide levels increased to 2283.60 ppm after 7 months of storage and reached the highest level of 2664.90 ppm after 10 months of storage. After 10 months of storage, the hydrogen peroxide content started to decrease and reached its lowest level (691.70 ppm) at 114 months of storage.

Table 1. Hydrogen Peroxide Levels in Various Storage Duration

No	Storage Duration (month)	H_2O_2 (ppm)
1	0	1323.95 ± 2.46^{c}
2	7	2283.60 ± 0.00^{f}
3	10	2664.90 ± 2.46^{g}
4	14	2038.40 ± 2.46^{e}
5	15	1620.20 ± 0.00^{d}
6	63	891.60 ± 2.46^{b}
7	114	691.70 ± 2.46^{a}

*Data are means of 3 replications and followed by the different letter shown significantly different in Tukey's Honestly Significant Differences (HSD) test with ($p<0.05$)

Eco-enzyme storage duration has a strong relationship with hydrogen peroxide levels, with a correlation coefficient of -0.604. This can be seen from the increased levels of hydrogen peroxide up to 10 months of storage, and levels decreased the longer the eco-enzyme was stored, in this study up to 114 months. Hydrogen peroxide levels are related to the condition of the bacterial cells contained in the eco enzyme. This is because hydrogen peroxide is a by-product produced by lactic acid bacteria against unfavorable environmental conditions [19]. An unfavorable environment is generally related to the presence of oxygen, because lactic acid bacteria themselves are aerotolerant anaerobic bacteria, which means that these bacteria grow optimally in an anaerobic environment but can tolerate an aerobic environment to a certain degree [14]. As a result of the excess oxygen, lactic acid bacteria will produce hydrogen peroxide as a form of self-defense against this unfavorable environment. The metabolism of lactic acid bacteria can be divided into two, depending on the type of lactic acid bacteria itself. Homofermentative lactic acid bacteria have an EMP fermentation pathway, in which the main product of this pathway is lactic acid. Whereas heterofermentative lactic acid bacteria have the HMS fermentation pathway, in which the products of this pathway are lactic acid, acetic acid, and/or ethanol. The difference between these two pathways is that the HMS pathway occurs because heterofermentative lactic acid bacteria are not able to produce aldolase enzymes, but the glucose phosphate dehydrogenase and xylulose phosphoketolase enzymes. This causes the HMS pathway to have two phases during the fermentation process, namely the oxidative phase and the non-oxidative phase [20]. During the oxidative phase, there is a process of re-oxidation of NADH from NAD^+ assisted by the enzyme NADH:H_2O oxidase, as shown in equation 3.1 below. Related to unfavorable environmental conditions, there is an imbalance in [NADH]/[NAD^+] levels so that the NADH re-oxidation process is assisted by the enzyme NADH:H_2O_2 oxidase, which is shown in equation 3.2. This causes the product of oxygen reduction to become H_2O_2 [19].

$NADH + 2H^+ + O_2 \rightleftharpoons NAD^+ + H_2O$, catalysted by NADH:$H_2O$ oxidase (3.1)

$NADH + H^+ + O_2 \rightleftharpoons NAD^+ + H_2O_2$, catalysted by NADH:$H_2O_2$ oxidase (3.2)

In this study, the eco-enzyme that was stored for a certain time in the form of filtrate and the organic waste used in the eco-enzyme production process were separated, so the amount of H_2O_2 formed is related to the number of bacteria present in the eco-enzyme. Based on data from Table 1, the amount of H_2O_2 increased from 0 months to 10 months of storage, indicating an increase in the number of lactic acid bacteria during 10 months of storage. The decrease in H_2O_2 levels starting from storage for 14 months to 114 months indicates that the number of lactic acid bacteria has decreased drastically. It is possible that after storage for more than 10 months, the lactic acid bacteria in eco-enzyme have entered the death phase, a phase where there is degradation of the number of bacterial cells due to the absence of nutrients for bacterial cell growth [14]. As a note, in this study no definite calculation of the number of lactic acid bacteria cells was carried out, so the explanation regarding the number of lactic acid bacteria refers to the

amount of H_2O_2 and the growth phase of the bacteria theoretically.

Antibacterial Activity of Eco-Enzyme

In this study, the antibacterial activity was seen from the size of the inhibition zone produced by the eco-enzyme filtrate. From the results shown in Table 2, it can be seen that there are significant differences in the inhibition zones for both *E. coli* and *S. aureus*. Eco-enzyme with pure concentration stored for 0 months showed strong antibacterial activity against *E. coli* and *S. aureus* with inhibition zones of 6.37 and 6.59 mm, respectively. The inhibition zone of eco-enzyme which had been stored for 7 months showed good results, with a diameter of 5.89 mm for *E. coli* and 4.72 mm for *S. aureus*. Eco-enzymes that had been stored after 10 and 14 months showed weak inhibition zones of 2.89 mm and 0.51 mm respectively against *E. coli* and 3.00 mm and 0.57 mm against S. aureus. Eco-enzymes stored for more than 14 months did not show antibacterial activity. The inhibition zone against *E. coli* and *S. aureus* shows that the antibacterial activity of eco-enzyme against *E. coli* and *S. aureus* is not much different.

Table 2. Inhibitory zone of Eco-Enzyme against *Escherichia coli* and *Staphylococcus aureus*

No	Storage Duration (month)	Inhibitory Zone (mm)	
		E. coli	*S. aureus*
1	0	6.37 ± 0.06^{d}	6.59 ± 0.04^{d}
2	7	5.89 ± 0.04^{c}	4.72 ± 0.04^{c}
3	10	2.89 ± 0.08^{b}	3.00 ± 0.08^{b}
4	14	0.51 ± 0.03^{a}	0.57 ± 0.04^{a}

*Data are means of 3 replications and followed by the different letter shown significantly different in Tukey's Honestly Significant Differences (HSD) test with ($p<0.05$)

Research conducted by Rahman *et al*. [21], reported that the diameter of the zone formed as an inhibitory effect on *E. coli* and *S. aureus* respectively is 7.5 and 5 mm. Another study conducted by Saramanda and Kaparapu [22], reports that the inhibition zones formed by *E. coli* and *S. aureus* bacteria were 11 and 10 mm, respectively. Even though using the same bacteria in the antibacterial test, the differences in the inhibition zones of eco-enzyme against *E. coli* and *S. aureus* bacteria were influenced by the composition of the organic waste used. This is because organic waste has a specific microbiota that participates in fermentation [23], which not only produces organic acids as the main product, but the microbiota also produces enzymes and other compounds that enrich the active compounds in eco-enzymes and indirectly affect antibacterial activity.

Eco-enzyme storage time has a very strong relationship with antibacterial activity against E coli (-0.896) and S aureus (-0.908). This indicates that the longer eco-enzyme storage causes a decrease in its antibacterial activity. The antibacterial activity of eco-enzymes depends on the metabolites produced by lactic acid bacteria, such as organic acids and ethanol [24]. The amount of metabolites depends on the number of lactic acid bacteria after storage for a certain period. Because in this study the number of lactic acid bacteria was not determined, the number of lactic acid bacteria refers to the H_2O_2 level and the growth phase of the bacteria theoretically. Right after the fermentation was carried out (0-month storage), lactic acid bacteria were still in the growth phase so that the rate of formation of the primary metabolite which is responsible for the antibacterial activity reached its highest point. After 7 months of storage, the bacterial cells have entered the stationary phase which can be seen from the activity of forming an inhibition zone which is quite good. This stationary phase ended after 10 months of storage, seeing H_2O_2 levels reaching their highest levels and inhibition zone activity starting to decrease. After storage for more than 14 months, the lactic acid bacteria cells have entered the death phase and the antibacterial activity is almost no longer visible, along with a decrease in H_2O_2 levels based on data in Table 1.

The research was continued by testing the antibacterial activity of the eco-enzyme. Eco-enzymes were prepared in various concentrations as shown in table 3 and 4. Except for eco-enzymes which were stored for 0 months, eco-enzymes with various concentrations had significant differences in inhibition zones when compared to pure eco-enzyme concentrations, this happened both to *E. coli* and *S. aureus* bacteria. Eco-enzymes stored for 0 months had insignificant differences in antibacterial activity against *E. coli* bacteria at concentrations between 0.1% and 1% and between 10% and 100%. The same results also occurred in *S. aureus* bacteria, where the antibacterial activity against bacteria did not show a significant difference at concentrations of 10% and 20%. The same thing was found by Mavani et al., 2020 [16], eco-enzyme activity at 50% and 100% concentrations showed insignificant differences in the inhibition zone data.

Table 3. Inhibitory Zone of Eco-Enzyme in Various Concentrations against *E. coli*

Storage Duration (month)	Inhibitory Zone against *E Coli* (mm)					
	100%	**20%**	**10%**	**1%**	**0.2%**	**0.1%**
0	6.37 ± 0.06^{c}	$6.07 \pm 0.07^{b,c}$	$5.75 \pm 0.06^{b,c}$	$5.11 \pm 0.06^{a,b}$	$4.62 \pm 0.04^{a,b}$	4.37 ± 0.06^{a}
7	5.89 ± 0.04^{e}	5.13 ± 0.08^{d}	4.99 ± 0.08^{d}	4.69 ± 0.04^{c}	4.22 ± 0.04^{b}	3.73 ± 0.13^{a}
10	2.89 ± 0.08^{e}	2.43 ± 0.19^{d}	2.14 ± 0.06^{c}	$2.06 \pm 0.05^{b,c}$	1.86 ± 0.06^{b}	1.44 ± 0.09^{a}
14	0.51 ± 0.03^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

*Data are means of 3 replications and followed by the different letter shown significantly different in Tukey's Honestly Significant Differences (HSD) test with ($p<0.05$)

Table 4. Inhibitory Zone of Eco-Enzyme in Various Concentrations against *S. aureus*

Storage Duration (month)	Inhibitory Zone against *S Aureus* (mm)					
	100%	**20%**	**10%**	**1%**	**0.2%**	**0.1%**
0	6.59 ± 0.04^{e}	5.93 ± 0.06^{d}	5.85 ± 0.04^{d}	5.59 ± 0.09^{c}	5.30 ± 0.04^{b}	4.75 ± 0.06^{a}
7	4.72 ± 0.04^{e}	4.35 ± 0.04^{d}	3.84 ± 0.06^{c}	3.75 ± 0.06^{c}	3.41 ± 0.06^{b}	3.07 ± 0.07^{a}
10	3.00 ± 0.08^{d}	2.92 ± 0.11^{d}	1.91 ± 0.07^{c}	$1.69 \pm 0.08^{b,c}$	$1.55 \pm 0.14^{a,b}$	1.33 ± 0.11^{a}
14	0.57 ± 0.04^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

*Data are means of 3 replications and followed by the different letter shown significantly different in Tukey's Honestly Significant Differences (HSD) test with ($p<0.05$)

Relationship between Hydrogen Peroxide in Eco-Enzyme toward Antibacterial Activity

In the consortium of lactic acid bacteria on eco-enzyme, several fermentation pathways occur, namely homofermentative lactic acid fermentation and heterofermentative lactic acid fermentation. Homofermentative lactic acid fermentation produces lactic acid as the main product, while heterofermentative lactic acid fermentation produces lactic acid, acetic acid, and/or ethanol [24]. The production of acetic acid or ethanol in the heterofermentative lactic acid fermentation process depends on the ability of microorganisms to re-oxidize the NADH produced in the oxidation phase of the fermentation process [19]. As a result of the excess oxygen in unfavorable environmental conditions, lactic acid bacteria will produce hydrogen peroxide as a form of self-defense against this unfavorable environment. Although hydrogen peroxide is a by-product of lactic acid fermentation, this compound can inhibit the growth of pathogenic bacteria. Hydrogen peroxide will oxidize lipid membranes and together with other metabolites, such as organic acids and bacteriocins, damage the molecular system of nucleic acids and proteins [25].

Although hydrogen peroxide can inhibit the growth of pathogenic bacteria, the results of this study indicate that hydrogen peroxide in eco-enzymes does not significantly correlate with the inhibition of *E. coli* and *S. aureus* bacteria. Similar results were presented by Bucekova [26], who stated that although hydrogen peroxide in honey was able to interact with bacterial cell proliferation signals, there was no clear relationship between hydrogen peroxide level and the antibacterial activity of honey samples. However, polyphenol compounds and their interactions with hydrogen peroxide show high antibacterial activity in honey. Hydrogen peroxide does not enhance the antibacterial effect, but this compound is capable of acting as a short-lived free radical that disrupts the integrity of the cell wall. Antibacterial ability will be high if there are compounds that are not dissociated, where these compounds will penetrate cells and interfere with the essential metabolic functions of pathogenic bacteria [26].

CONCLUSION

It can be concluded that the storage duration of eco-enzymes affects the level of hydrogen peroxide and antibacterial activity. Hydrogen peroxide reached the highest level when the eco-enzyme was stored for 10 months, which is 2664.9 ppm. Antibacterial activity based on the inhibitory zone against *E. coli* and *S. aureus* bacteria reached its highest point at 0 months of storage, and the activity tend to decrease until 14 months of storage. There is a strong and significant correlation between storage duration and the antibacterial activity of eco-enzymes. The longer the eco-enzyme storage duration, the lower the

antibacterial activity against *E. coli* and *S. aureus*. The same relationship is also shown between storage duration and hydrogen peroxide levels. The longer the eco-enzyme storage duration the lower hydrogen peroxide levels decrease. However, there is no significant relationship between levels of hydrogen peroxide and eco-enzyme's antibacterial activity. Future research in this field can be focused on investigating other eco-enzyme fermentation products, i.e. organic acids, ethanol, and enzymes.

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REFERENCES

[1]. Kementerian Lingkungan Hidup dan Kehutanan, "Sistem Informasi Pengelolaan Sampah Nasional," 2022. [Online]. Available: http://sipsn.menlhk.go.id/sipsn/. [Accessed: 07-February-2023]

[2]. Eco-Enzyme Nusantara. 2020. "Modul Belajar Pembuatan Eco-Enzyme." 1–62.

[3]. Hasanah, Y. 2021. "Eco Enzyme and Its Benefits for Organic Rice Production and Disinfectant." *Journal of Saintech Transfer* 3(2), 119–28.

[4]. Rahayu, M.R, Muliarta, I.N. & Situmeang, Y.P. 2021. "Acceleration of Production Natural Disinfectants from the Combination of Eco-Enzyme Domestic Organic Waste and Frangipani Flowers (Plumeria Alba)." *Sustainable Environment Agricultural Science* 05(01), 15–21.

[5]. Kerkar, S. S. & Salvi, S. S. 2020. "Application of Eco-Enzyme for Domestic Waste Water Treatment." *International Journal for Research in Engineering Application & Management,* 05(11), 114–116.

[6]. Arun, C. and P. Sivashanmugam. 2015. "Solubilization of Waste Activated Sludge Using a Garbage Enzyme Produced from Different Pre-Consumer Organic Waste." *RSC Advances* 5(63), 51421–51427.

[7]. Tong, Y. & Liu, B. 2020. "Test Research of Different Material Made Garbage Enzyme's Effect to Soil Total Nitrogen and Organic Matter." *IOP Conference Series: Earth and Environmental Science* 510(4), 042015.

[8]. Wei, X., Cao, P. Wang, G. & Han, J. 2020. "Microbial Inoculant and Garbage Enzyme Reduced Cadmium (Cd) Uptake in Salvia Miltiorrhiza (Bge.) under Cd Stress." *Ecotoxicology and Environmental Safety* 192(151), 110311.

[9]. Gao, Y. & Liu, P. 2020. "Research Progress in Application of Garbage Enzyme in Soil Improvement, Agricultural Production and Environmental Management." *Advances in Environmental Protection* 10(04), 541–548.

[10]. Wang, Y., Zhao, J., Wang, J., Luo, Y., Liu, Y., Bai, D., Shao, P. & Li, Y. 2020. "Inhibitory Activity of Home-Made Garbage Enzyme against Aquatic Pathogens and Isolation and Identification of Its Fermentation Strains." *Nanfang Shuichan Kexue*, 16 (6), 97–104.

[11]. Samriti, S. S. & Arti, A. 2019. "Garbage Enzyme: A Study on Compositional Analysis of Kitchen Waste Ferments." *The Pharma Innovation Journal* 8(4), 1193–1197.

[12]. Gänzle, M. G. 2015. "Lactic Metabolism Revisited: Metabolism of Lactic Acid Bacteria in Food Fermentations and Food Spoilage." *Current Opinion in Food Science* 2, 106–117.

[13]. Neupane, K. & Khadka, R. 2019. "Production of Garbage Enzyme from Different Fruit and Vegetable Wastes and Evaluation of Its Enzymatic and Antimicrobial Efficacy." *Tribhuvan University Journal of Microbiology* 6(1), 113–118.

[14]. Sopandi, T. & Wardah. 2014. *Mikrobiologi Pangan - Teori Dan Praktik*. Jilid Pertama. Yogyakarta: ANDI.

[15]. Juven, B. J. & Pierson, M. D. 1996. "Antibacterial Effects of Hydrogen Peroxide and Methods for Its Detection and Quantitation." *Journal of Food Protection* 59(11), 1233–1241.

[16]. Mavani, H. A. K., Tew, I-M, Wong, L., Yew, H-Z, Mahyuddin, A., Ghazali, R.A. & Pow, E. H. N. 2020. "Antimicrobial Efficacy of Fruit Peels

Eco-Enzyme against *Enterococcus faecalis*: An in Vitro Study." *International Journal of Environmental Research and Public Health,* 17(14), 1–12.

[17]. Muawanah, Afiah, N. & Mashudi, E. 2020. "Penetapan Kadar Hidrogen Peroksida (H_2O_2) pada Tahu Dengan Metode Permanganometri." *Jurnal Medika,* 5(2):9–13.

[18]. Nurhamidin, Anastasia, P. R., Fatimawali, & Antasionasti, I. 2021. "Uji Aktivitas Antibakteri Ekstrak N-Heksan Biji Buah Langsat (*Lansium domesticum Corr*) Terhadap Bakteri *Staphylococus aureus* dan *Klebsiella pneumoniae*." *Pharmacon,* 10(1), 748.

[19]. Condon, S. 1987. "Responses of Lactic Acid Bacteria to Oxygen." *FEMS Microbiology Letters* 46(3), 269–280.

[20]. Hutkins, R. W. 2007. "*Microbiology and Technology of Fermented Foods*." Balckwell Publishing.

[21]. Ng, M. Y., Shafiei, Z., Rahman, M. A., Nagarajan, S. & Sockalingam, M. P. 2020. "Antibacterial Effects of Effective Ecoproduce on Enterococcus Faecalis: An in Vitro Study." *Journal of International Dental and Medical Research* 13(3), 861–867.

[22]. Saramanda, G. & Kaparapu, J. 2017. "Antimicrobial Activity of Fermented Citrus Fruit Peel Extract." *Journal of Engineering Research and Application* 7(11), 25–28.

[23]. Di Cagno, R., Coda, R. De Angelis, M.& Gobbetti, M. 2013. "Exploitation of Vegetables and Fruits through Lactic Acid Fermentation." *Food Microbiology* 33(1), 1–10.

[24]. Prayitno, T. A. & Hidayati, N. 2017. *Pengantar Mikrobiologi*. Vol. 1. Media Nusa Creative, Malang.

[25]. Kang, D. K., Oh, H. K., Ham, J. S., Kim, J. G., Yoon, C. H., Ahn, Y. T. & Kim, H. U. 2005. "Identification and Characterization of Hydrogen Peroxide-Generating Lactobacillus Fermentum CS12-1." *Asian-Australasian Journal of Animal Sciences* 18(1), 90–95.

[26]. Bucekova, M., Buriova, M., Pekarik, L., Majtan, V. & Majtan, J. 2018. "Phytochemicals-Mediated Production of Hydrogen Peroxide Is Crucial for High Antibacterial Activity of Honeydew Honey." *Scientific Reports* 8(1), 1–9.