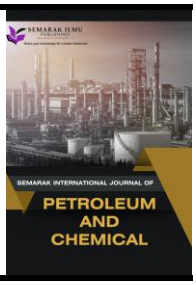
SEMARAK ILMU PUBLISHING

Semarak International Journal of Petroleum and Chemical

Journal homepage:
https://semarakilmu.my/index.php/sijpce/index
ISSN: 3083-9106



Optimization of Eco Enzyme Production Using Bacterial Starters and Fermentative Fungi

Susi Endrini[1], Siti Juariah[2,*], Waffiq Anisa Rahmi[2], Miftahul Rizqo Auliyah[2], Dewi Sekar Kinasti[2], Dewi Shinta Agustien Ayu[2], Hazli Alif Alfitra[2]

[1] Faculty of Medicine, Abdurrab University Jl. Riau Ujung No. 73, Tampan, Air Hitam, Payung Sekaki, Air Hitam, Payung Sekaki, Pekanbaru, Riau 28291, Indonesia
[2] Faculty of Pharmacy and Health Science. Abdurrab University Jl. Riau Ujung No. 73, Tampan, Air Hitam, Payung Sekaki, Air Hitam, Payung Sekaki, Pekanbaru, Riau 28291, Indonesia

ARTICLE INFO

Article history:
Received 5 November 2024
Received in revised form 17 November 2024
Accepted 2 December 2024
Available online 20 December 2024

Keywords:
Eco Enzyme; fermentation; fermentative bacteria; optimization; waste management

ABSTRACT

Eco Enzyme is a natural fermentation product made from organic waste such as leftover fruits and vegetables, which has various benefits, including serving as an environmental cleaner and pollution reducer. This research aims to optimize the production of Eco Enzyme by using specific fermentative bacterial starters to enhance fermentation efficiency and the quality of the final product. In this study, the types of fermentative bacteria used are *Lactobacillus bulgaricus, Lactobacillus acidophilus*, and the fungi *Saccharomyces cerevisiae* and *Aspergillus niger*. Parameters such as pH, temperature, and fermentation duration are strictly regulated and monitored to identify optimal conditions for Eco Enzyme production. The research results indicate that the use of certain fermentative bacterial starters can accelerate fermentation time by up to 30% and increase enzyme activity by 20% compared to natural fermentation processes without starters. The characteristics also show the same results, namely acidic pH, characteristic fresh aroma, fresh eco enzyme value and normal TDS (ranged 1,030 ppm - 1,700 ppm). This optimization not only produces a more efficient and high-quality Eco Enzyme product, but it can also serve as an innovative step towards more sustainable and environmentally friendly organic waste management.

1. Introduction

Organic waste such as fruits and vegetables that are not managed properly will cause environmental problems that affect environmental quality. Organic waste can react anaerobically so that it can cause unpleasant odors, breeding grounds for vectors and rodents and methane gas released into the atmosphere can cause global warming and damage the ozone layer [1]. Soil contaminated by organic waste also has an impact on water quality, contaminated water has a very high concentration, resulting in a decrease in dissolved oxygen levels [2]. Organic waste management needs to be carried out widely, so alternative solutions are needed that can turn organic waste into

* *Corresponding author.*
E-mail address: sitijuariah@univrab.ac.id

https://doi.org/10.37934/sijpce.1.1.2637a

a resource that can be utilized [3]. The high amount of organic waste can be overcome by processing waste into environmentally friendly products that can reduce greenhouse gases by creating Eco-enzymes that can be applied at the household level [4].

Eco-enzyme is a fermented liquid from vegetable and fruit waste with added sugar. Eco-enzyme was created by a Thai doctor named Dr. Rosukon who is also known as an environmental enzyme because it is made from food waste that is easily obtained by consumers such as fruit and vegetable peels. The characteristic of eco-enzyme liquid is dark brown with a strong vinegar or fruit aroma. The process of making Eco-enzyme only uses sugar, water, and organic waste from fruit and vegetables [5]. Eco-enzyme can help reduce the amount of organic waste produced by households whose composition is still high [6]. Eco-enzyme has many benefits, in the manufacturing process Eco-enzyme produces O3 gas (ozone) which is a gas that is useful for reducing the greenhouse effect [7]. The Acetic Acid (CH3COOH) content in eco-enzyme can be used to kill germs, viruses, and bacteria, so it can be used to repel plant pests and neutralize various pollutants that pollute the environment. Eco-enzyme converts ammonia (NH3) into NO3 nitrate which can be used to fertilize plants, in addition eco-enzyme can also be used for cleaning products in daily activities, one of which is dishwashing soap [8].

Manual eco-enzyme production takes a relatively long time, around three months. Therefore, it is necessary to develop new innovations to shorten the eco-enzyme production process time by using bacterial and fungal starters. In this study, the bacteria used were lactic acid bacteria *Lactobacillus bulgaricus, Lactobacilus acidophilus* and fungi used in saprophytic fungi, namely yeast *Saccharomyces cerevisiae*, *Aspergilus niger* is a fungus that plays a role in the fermentation process, especially fermentation for bioethanol production [9].

2. Methodology

This research method is an experimental method. In this study there are several things that need to be controlled: the placement of eco-enzymes which are located in a place away from direct sunlight, with good air circulation, and away from burning waste and chemicals, then storage containers that have wide-mouthed lids and non-glass materials such as plastic cups. The data collected were obtained from Triangulation Techniques. Organoleptic is a test based on the sensory process, namely by observing changes in the product being tested, the senses that work are the sense of sight of the eye, the sense of smell of the nose, and the sense of the skin of the fingers.

The materials used in making 5-liter eco-enzyme are sugar: fruit peel: water. 500 grams of brown sugar, 1.5 kg of fruit peel (600 grams of orange peel, 300 grams of watermelon peel, 200 grams of dragon fruit peel, 100 grams of mango peel, 100 grams of avocado peel, 100 grams of papaya peel, 50 grams of pear peel and 50 grams of melon peel.), and 5 L of water. The materials used in making bacterial and fungal suspensions are dissolved with NaCl at a certain concentration. The suspension added to each treatment is 9 ml.

2.1 Environmentally Friendly Enzyme Production

This study will observe 2 variables, namely the aroma and color of ecoenzyme products before and after harvest (0 days, 10 and 35 days), with the tools and materials used coming from the tools and materials of the "ladder" that we generally use everyday, including: a 10 L plastic jar, a teaspoon of rice, a plastic container, and a weight.

Eco enzyme production uses the method by Istanti *et al.,* [10] with slight modifications. Remove the fruit skin into small slices. The composition of the treatment of the materials used is fruit skin waste (S1); fruit skin + bacteria/fungi without dilution (S2); fruit skin + 10 times dilution of bacteria/fungi (S3); vegetable waste + fruit skin + bacteria/fungi dilution 100 times and (Control); fruit skin. The weight ratio of granulated sugar, fresh ingredients, and water with a maximum water volume of 60% is 1:3:10, then the ingredients are put in according to the order of water, sugar, fruit pieces and then stirred evenly. The container is tightly closed and marked with the date of manufacture and harvest date.

2.2 Preparation of Microorganisms

Lactobacillus bulgaricus, Lactobacilus acidophilus, Saccharomyces cerevisiae, Aspergiluus niger used in this study from the Microbiology and Parasitology Laboratory of Abdurrab University. To preserve the stock culture, bacterial strains were subcultured at 37°C for 24 hours in Man Rogosa Sharpe Agar (MRSA) and fungal strains were subcultured at 25°C in Potato Dextrose Agar (PDA) for 72 hours and then cooled to 4°C. At 37°C and 25°C for 16-18 hours in an incubator, full oxygen from the growing bacterial and fungal cultures was injected into three-quarters of the tubes. contains 10 ml of nutrient container. The inoculum size of each bacterial strain is standardized by adjusting the optical density of the nutrient container to a thickness of 0.5 and a 10-fold and 100-fold dilution [11].

2.3 Antibacterial Activity Test

Antibacterial activity tests were carried out using a modified disc diffusion method [12]. The first step is to prepare the concentration of bacteria and fungi, then adjust it to the Farland Standard Mc 0.5. Prepare 50 ml of MHA (Muller Hilton Agar) media and 50 ml of PDA on each plate. Then, tests were carried out on four discs containing the concentration of each test (without dilution, 10-fold dilution, and 100-fold dilution), and control (without adding bacterial/fungal suspension) and allowed to dry. Then place the disc on the MHA and PDA media at regular intervals. Then incubated at 37°C and 25°C for 24 hours. Observations were made by measuring the lymphatic zones formed. All treatments were repeated three times to minimize errors.

2.4 pH Measurement

The pH measurement procedure of Eco Enzyme was carried out using a previously calibrated pH meter. The first step in this procedure is to calibrate the pH meter using standard buffers with pH values of 4.01, 7.00, and 10.01 to ensure measurement accuracy. After calibration, 80 ml of Eco Enzyme sample was taken and placed in a clean beaker. The pH meter electrode was then dipped into the sample, and the pH value was recorded after it stabilized. It is important to ensure that the electrode does not touch the bottom of the beaker to avoid inaccurate results. After the measurement is complete, the electrode should be cleaned with distilled water and dried using a lint-free tissue to prevent contamination in subsequent measurements [13].

2.5 TDS Test

A Total Dissolved Solids (TDS) testing on Eco Enzyme is carried out to measure the number of dissolved solids including minerals, salts, and organic compounds in solution. This procedure begins with the calibration of the TDS meter using a standard solution to ensure accuracy. A total of 80 ml

of Eco Enzyme sample is taken and placed in a clean beaker. The TDS meter probe is then dipped into the sample, and the TDS value is recorded after it is stable. After measurement, the probe is cleaned with distilled water and dried using a lint-free tissue to avoid contamination in subsequent measurements. This TDS measurement is important for assessing the quality of Eco Enzyme fermentation, where similar procedures are often described in the literature that focuses on the analysis of water quality and fermentation fluids, as described by Sawyer *et al.,* [14] in Chemistry for Environmental Engineering and Science and guidelines from the American Public Health Association [15].

2.6 Data analysis

The determination was done in triplicate and the data analysed by one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS version 16.0, IBM Corp., Armonk, NY, USA)

3. Results

Processing waste into an eco-enzyme product provides added value and is able to reduce greenhouse gas production. Basically, eco enzyme is a fermentation product from vegetable and fruit waste with the addition of molasses. Fermentation in the field of biochemistry means a catabolic process that produces energy, while fermentation in the field of microbiology means producing new products by utilizing the activity of microorganisms which includes the production of cells, enzymes, metabolites, recombinant products, and transformation products [16]. In eco enzyme during the fermentation process happen metabolism microorganisms from material skin waste used. With existence addition molasses in a way No direct fermentation process This give quality biochemistry and microbiology product fermentation by controlling rotting microorganisms [17]. Microorganisms found in fruit peel waste will emit a sour aroma because acetic acid is formed which then becomes a medium for the growth of bacteria, mold, and yeast [18]. Figure 1 shows the washed fruit skin and eco enzyme before fermentation.

Fig. 1. Washed fruit skin (left), eco enzyme before fermentation (right)

Testing for eco enzyme solution was done by taking a sample of eco enzyme solution as much as 100 ml and then checked and analyzed after the time was reached. This study was conducted to determine the characteristics of eco-enzymes from organic materials derived from several types of fruit skins by measuring pH, observing color, aroma, testing antibacterial and antifungal activity and TDS testing on eco enzymes.

3.1. Eco-Enzyme pH Analysis

The characteristics of a good *eco-enzyme* are those that have a pH equal to 4 or below pH 4. The pH of *the* eco-enzyme obtained from the results of research with the addition of two types of microorganisms, namely *Lactobacillusbulgaricus, Lactobacillus acidophilus, Saccaromyces cerevisiae* and *Aspergillus niger* as seen in Figure 2.

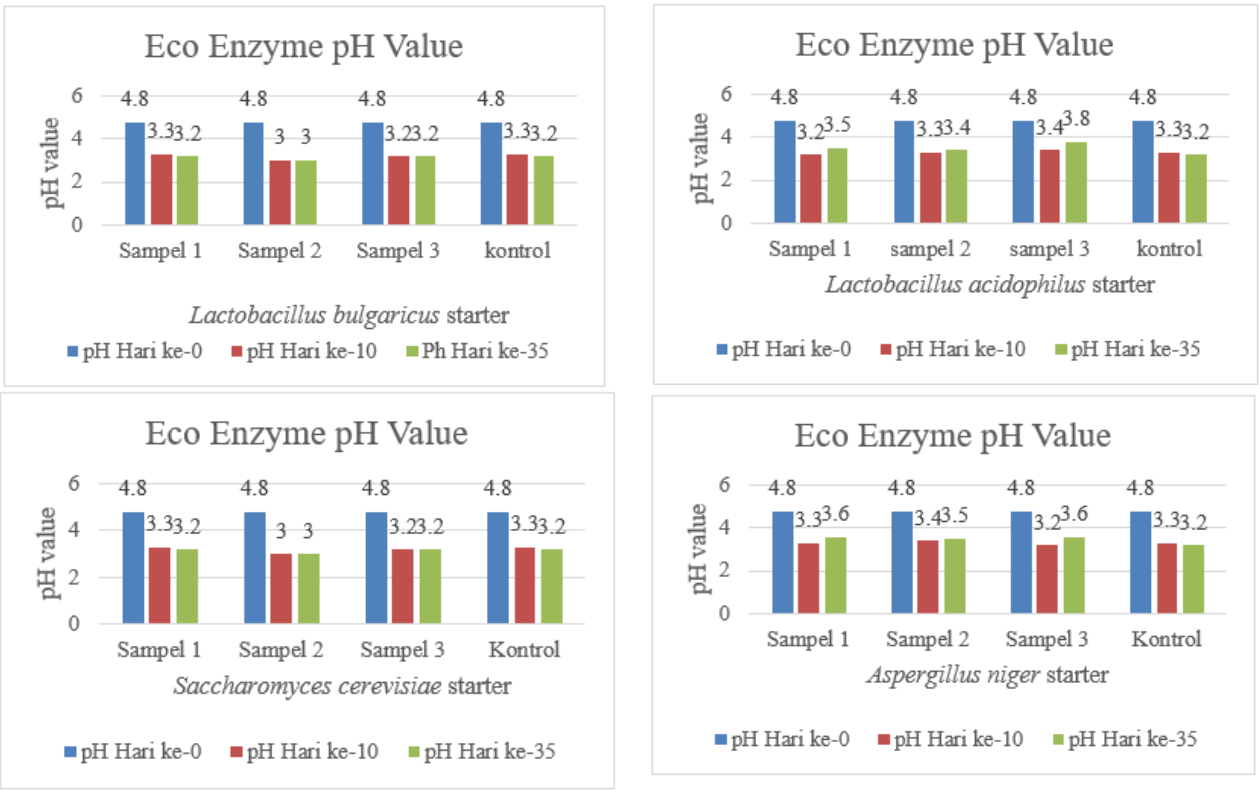


Fig. 2. Results of measuring the pH of Eco Enzyme with the addition of suspension

Information:
Sample 1 = Addition of bacteria/fungi without dilution
Sample 2 = addition of bacteria/fungi dilution 1
Sample 3 = addition of bacteria/fungi dilution 2
Control = No addition of bacteria/fungi

Based on Figure 2, it can be seen that the pH measurement results obtained have met good standards in making eco enzyme, namely pH below 4. The results of this study are in line with previous studies where eco enzyme is chemically acidic between pH3-pH4 [19] . The low eco enzyme product is caused by the high organic acid content. The higher the organic acid content, the lower the pH of the eco enzyme product [20] .

pH is a measure of the acidity or alkalinity of a solution, usually measured on a scale of 0–14, with 7 being neutral. This pH range can vary depending on the enzyme and the environment. When the pH is outside the optimal range of an enzyme, it can cause the enzyme to denature and stop functioning. The pH of eco-enzymes made from fruit waste is usually slightly acidic. The pH of eco-enzymes can range from 4.5 to 6.5 depending on the type of fruit waste used and the composition of the mixture. The pH of eco-enzymes will also depend on the amount of water added and the fermentation time. Generally, the longer the fermentation time, the lower the pH [5].

3.2 Organoleptic Analysis

In this study, fermentation was carried out for 10 days and 35 days, based on Table 1, the organoleptic results of color and aroma obtained in eco enzyme using *Lactobacillus bulgaricus bacteria* and *Saccharomyces cerevisiae* fungi were brown and had a fresh aroma. Good Eco enzyme fermentation produces a brownish solution and a fresh sour aroma typical of fermentation [21]. The brownish color of eco enzyme is influenced by the original color of the brown sugar used, namely brown, which is then mixed with pulp from the fruit skin [22].

Table 1
Organoleptic test of aroma and color of eco enzyme

Treatment	Day	Sample 1	Sample 2	Sample 3	Control
Starter *L. bulgaricus*	10 days	Strong sweet fresh aroma and brown color	Strong sweet fresh aroma and brown color	Fresh sweet aroma and brown color	Fresh sweet aroma and brown color
	35 days	Strong sweet fresh aroma and brown color	Strong sweet fresh aroma and brown color	Fresh sweet aroma and brown color	Fresh sweet aroma and brown color
L. acidophilus starter	10 days	Strong sweet fresh aroma and brown color	Strong sweet fresh aroma and brown color	Fresh sweet aroma and brown color	Fresh sweet aroma and brown color
	35 days	Strong sweet fresh aroma and brown color	Strong sweet fresh aroma and brown color	Fresh sweet aroma and brown color	Fresh sweet aroma and brown color
S. cerevisiae starter	10 days	Strong sweet fresh aroma and brown color	Strong sweet fresh aroma and brown color	Fresh sweet aroma and brown color	Fresh sweet aroma and brown color
	35 days	Strong sweet fresh aroma and brown color	Strong sweet fresh aroma and brown color	Fresh sweet aroma and brown color	Fresh sweet aroma and brown color
Starter *A. niger*	10 days	Strong sweet fresh aroma and brown color	Strong sweet fresh aroma and brown color	Fresh sweet aroma and brown color	Fresh sweet aroma and brown color
	35 days	Strong sweet fresh aroma and brown color	Strong sweet fresh aroma and brown color	Fresh sweet aroma and brown color	Fresh sweet aroma and brown color

All types of samples and controls have a certain sour fermentation aroma. The acid produced is a product of the fermentation process. This compound contributes to the sour aroma of eco enzyme products. The level of fresh aroma differs between samples and controls. In the sample, the higher the concentration of bacterial or fungal suspension, the stronger the fresh aroma and the control has a fresh aroma but not too strong. Under anaerobic conditions (without oxygen), the fermentation process is an attempt by microbes to obtain energy from carbohydrates. This process produces alcohol and organic acids as by-products. The level of fresh aroma differs between samples and controls [23].

Pitera fungus is a good fungus resulting from the fermentation process of making eco-enzyme solutions, pitera cannot be used as a benchmark for the success of eco-enzyme, but eco-enzyme that produces pitera fungus during the fermentation process will be more expensive, because the organic acid contained is greater so that the pH value is also lower. The growth of fungi or microbes in each container during the fermentation process is shown in Figure 3 as follows;

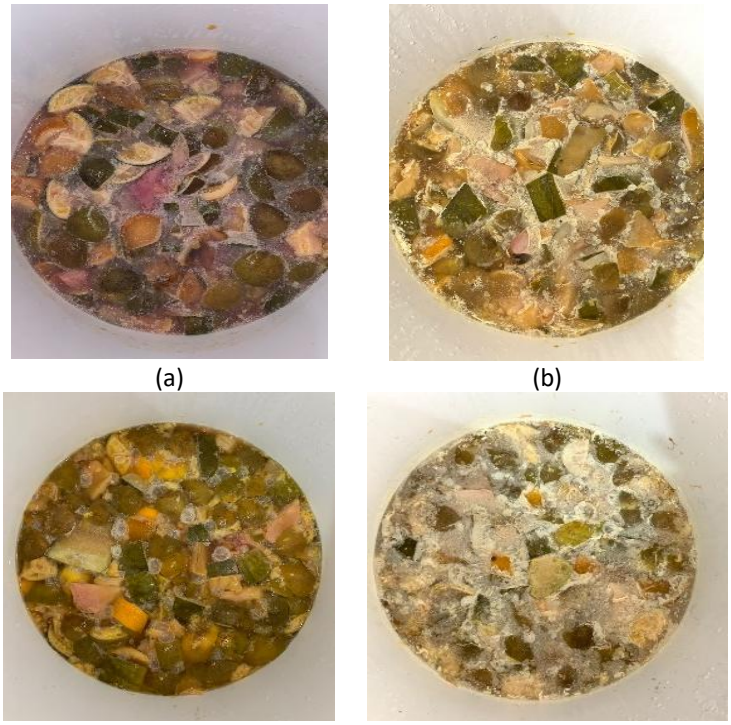


Fig. 3. Appearance of fungi or microbes

From Figure 3, we can see the difference in appearance of the treatment that was added with the bacterial starter, namely there are bubbles between the pitera fungi.

3.3 Antibacterial and Antifungal Analysis

Eco enzyme has uses as an antifungal, antibacterial, and insecticide. From Table 2 it can be seen that eco-enzyme can inhibit the growth of bacteria and fungi as seen from the inhibition zone formed.

Table 2
Inhibition Zone Diameter in Various Fungi Using *Saccharomyces cerevisiae* and *Aspergillus niger* Starters

Types of bacteria	Treatment	Day	Starter *L. bulgaricus* (mm)	Starter *L. acidophilus* (mm)	Types of bacteria	treatment	Day	Starter *L. bulgaricus* (mm)	Starter *L. acidophilus* (mm)
S. aureus	S1	0	6 ± 0.00	6 ± 0.00	*E. coli*	S1	0	6 ± 0.00	6 ± 0.00
		10	8 ± 1.41	17 ± 0.00			10	8.5 ± 0.71	8 ± 0.00
		35	10.5 ± 0.71	1.5 ± 0.71			35	9.5 ± 0.71	8 ± 2.83
	S2	0	6 ± 0.00	6 ± 0.00		S2	0	6 ± 0.00	6 ± 0.00
		10	9.5 ± 0.71	12.5 ± 4.95			10	7.5 ± 0.71	7.5 ± 0.71
		35	9.5 ± 0.71	14 ± 1.41			35	8.5 ± 0.71	8.5 ± 0.71
	S3	0	6 ± 0.00	6 ± 0.00		S3	0	6 ± 0.00	6 ± 0.00
		10	6.5 ± 0.71	11 ± 5.66			10	7 ± 0.00	6 ± 0.00
		35	8 ± 0.00	11.5 ± 0.71			35	7.5 ± 0.71	7 ± 0.00
	control	0	6 ± 0.00	6 ± 0.00		control	0	6 ± 0.00	6 ± 0.00
		10	6 ± 0.00	6 ± 0.00			10	6.5 ± 0.71	8.5 ± 0.71
		35	6.5 ± 0.71	6 ± 0.00			35	6.5 ± 0.71	8 ± 1.41

This research shows that *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* have an inhibitory effect on the growth of Staphylococcus aureus and Escherichia coli after incubation for 10 and 35

days. In *S. aureus*, S1 treatment with *L. bulgaricus* showed a significant increase in the diameter of the inhibition zone from 6 mm on day 0 to 10.5 mm on day 35. Treatment *with L. acidophilus* on S1 also resulted in a significant zone of inhibition, reaching 17 mm on day 10, although it decreased to 1.5 mm on day 35. For *E. coli*, inhibition by both probiotic starters was lower compared to *S. aureus*, but still showed an increase from day 0 to day 35, especially in treatments S1 and S2.

The results of this study are consistent with previous studies which reported that *Lactobacillus sp.* effective in inhibiting the growth of pathogenic bacteria. Kim *et al.,* [24] stated that *Lactibacillus* species have high antibacterial potential through the production of organic acids, hydrogen peroxide and bacteriocins which inhibit the growth of bacteria such as *Staphylococcus aureus*. The greater inhibition of *S. aureus* compared to *E. coli* may be due to the cell wall structure of gram-positive bacteria which is more susceptible to lactic acid and antibacterial compounds produced by Lactobacillus compared to the more complex cell walls of gram-negative bacteria.

This study also supports the findings of Meng *et al.,* [25] which shows that *Lactobacillus acidophilus* is effective in inhibiting the growth of gram-positive and gram-negative bacteria, although gram-positive bacteria tend to be more sensitive. Zhang *et al.,* [26] also reported that probiotics such as *L. bulgaricus* and *L. acidophilus* can play a role in improving the immune system and inhibiting pathogens through competition for nutrients and the production of antimicrobial compounds. Thus, this study strengthens the evidence that the use of *Lactobacillus sp.* as a probiotic has significant potential in reducing pathogenic bacterial infections through direct and indirect inhibitory mechanisms.

The Table 3 below shows the activity of *Saccharomyces cerevisiae* and *Aspergillus niger* starters on the growth of *Candida sp.* and *Aspergillus sp.* on days 0, 10, and 35. A significant effect was seen on day 10, where the *S. cerevisiae* starter provided a larger zone of inhibition on both types of fungi than the *A. niger* starter. In *Candida sp*., the highest zone of inhibition with *S. cerevisiae* starter was recorded on day 10 for treatment S2 at 14.5 mm, while *A. niger* starter showed overall lower inhibition. On *Aspergillus sp.,* the *S. cerevisiae* starter also provided a significant inhibitory effect, with the highest inhibition zone reaching 19.5 mm on day 35 for S2 treatment, while the *A. niger* starter showed minimal inhibitory activity, consistently at around 6 mm throughout the observations.

Table 3
Diameter of inhibition zones for various fungi using *Saccharomyces cerevisiae* and *Aspergillus niger* Starters

Types of mushrooms	treatment	day	*S. cerevisiae* starter (mm)	Starter *A. niger* (mm)	Types of mushrooms	treatment	day	*S. cerevisiae* starter (mm)	Starter *A. niger* (mm)
Candida sp.	S1	0	6 ± 0.00	6 ± 0.00	*Aspergillus sp.*	S1	0	6 ± 0.00	6 ± 0.00
		10	14 ± 1.41	6.5 ± 0.71			10	15.5 ± 0.71	6 ± 0.00
		35	8 ± 0.00	9 ± 0.00			35	9 ± 1.41	6 ± 0.00
	S2	0	6 ± 0.00	6 ± 0.00		S2	0	6 ± 0.00	6 ± 0.00
		10	14.5 ± 0.71	7.5 ± 0.71			10	16 ± 1.41	6 ± 0.00
		35	9 ± 1.41	8.5 ± 0.71			35	19.5 ± 3.54	6 ± 0.00
	S3	0	6 ± 0.00	6 ± 0.00		S3	0	6 ± 0.00	6 ± 0.00
		10	12 ± 0.00	7 ± 0.00			10	13.5 ± 0.71	6 ± 0.00
		35	7.5 ± 0.71	8.5 ± 0.71			35	19 ± 1.41	6 ± 0.00
	control	0	6 ± 0.00	6 ± 0.00		control	0	6 ± 0.00	6 ± 0.00
		10	6.5 ± 0.71	6.5 ± 0.71			10	8.5 ± 0.71	6 ± 0.00
		35	6 ± 0.00	6.5 ± 0.71			35	17 ± 1.41	6 ± 0.00

These results support previous research which reported that *Saccharomyces cerevisiae* has stronger antifungal activity than *Aspergillus niger* against certain fungal species, especially Candida

sp.. According to research by Pairera [27], *S. cerevisiae* produces enzymes and metabolites such as ethanol and carbon dioxide which can inhibit the growth of fungal pathogens, especially in the early logarithmic phase. This was seen on day 10 where the inhibition zone of *S. cerevisiae* showed a significant size compared to A. niger, which tended to maintain a smaller barrier size. The lower inhibition by *A. niger* may be due to its primary role as a producer of digestive enzymes rather than direct antimicrobial compounds.

In addition, this research is in line with the findings of Freimoser *et al.,* [28], who stated that *Saccharomyces cerevisiae* has potential as a biocontrol agent against pathogenic fungi through competition for nutrients and the release of toxic compounds. The decrease in the size of the inhibition zone on day 35 may be due to the adaptation of the fungus to antimicrobial compounds produced by *S. cerevisiae* and *A. niger*. However, the consistent activity of *S. cerevisiae* in inhibiting the growth of *Aspergillus sp.* day 35 indicates that this strain may have a more long-lasting antifungal effect, which is important in long-term control of fungal pathogens.

In this study, based on the data above, there is inhibition of bacterial growth and fungal growth by eco-enzyme. This has the potential to make eco-enzyme a natural disinfectant because it has cleaning and antimicrobial characteristics [5]. This is supported by the following antibacterial and antifungal anova data.

Based on the Least Significant Difference (LSD) test on the comparison of antibacterial inhibition zones measured on day 0, day 10 and day 35. From these results, it can be seen that there are significant differences in the inhibition zone between day 0 and day 10, and day 0 and day 35. This difference is significant at the 0.05 level, as indicated by a p value (Sig.) of less than 0.05. However, the comparison between day 10 and day 35 did not show a significant difference, because the p value = 0.348, greater than 0.05. This means that the zone of inhibition did not experience significant changes between day 10 and day 35.

The significant differences observed between day 0 and day 10, as well as day 0 and day 35, indicated a significant increase in the zone of inhibition after 10 and 35 days of antibacterial treatment. This shows that the antibacterial agent used in this study was effective in inhibiting bacterial growth after an incubation period of 10 and 35 days. The significant increase in the inhibition zone indicates that the process of inhibiting bacteria by antibacterial agents may be more efficient with time, because the deposition or penetration of the active ingredient into the medium or microbial network requires a certain time to reach maximum effectiveness. This is consistent with studies showing that the timing of exposure to antimicrobial agents influences the magnitude of inhibition of bacterial growth [29].

However, the absence of significant differences between day 10 and day 35 indicates that after 10 days, antibacterial effectiveness begins to decline or reaches a saturation point. In some cases, bacteria may begin to develop resistance or adapt to environmental conditions, which may explain the absence of a significant increase in the zone of inhibition. This is also supported by other research which found that bacteria can develop resistance mechanisms after long-term exposure to certain antibacterial agents [30].

Based on Least Significant Difference (LSD) test for the antifungal inhibition zone measured on day 0, day 10 and day 35. From these results, there are significant differences in the inhibition zone between day 0 and day 10, and day 0 and day 35. The difference was significant at the 0.05 level (p value < 0.05), indicating a significant increase in the zone of inhibition after 10 and 35 days of antifungal treatment. However, the comparison between day 10 and day 35 did not show a significant difference, with a p value of 0.972, which is greater than 0.05. This means there is no significant change between the inhibition zone on day 10 and day 35.

These results indicate that the antifungal agent began to have a significant effect on the zone of inhibition after 10 days and remained effective until day 35, as seen from the significant differences between day 0 and day 10 and day 0 and day 2. -35. The increase in the antifungal inhibition zone after treatment indicates that the antifungal agent works more effectively with increasing time, as also reported by other studies. The study by Zhang *et al.,* [26] stated that longer exposure times to antifungal agents can increase effectiveness due to deeper penetration and more efficient inhibition of fungal growth.

However, the absence of significant differences between day 10 and day 35 indicated that the antifungal effect reached its maximum on day 10, and there was no further increase after that time. This could be because the antifungal agent has reached maximum effectiveness, or the fungus has begun to adapt to the environment affected by the agent. This study found that some fungi can develop adaptations to antifungal agents after a certain exposure time, which may explain why there was no significant difference between day 10 and day 35 in the zone of inhibition.

3.4 Total Dissolved Solid (TDS) Analysis

From the Table 4 TDS (Total Dissolved Solids) data measured in ppm on *Lactobacillus bulgaricus, Lactobacillus acidophilus, Saccharomyces cerevisiae*, and *Aspergillus niger*, it can be seen that all samples have lower TDS values compared to the control (1,700 ppm). For *Lactobacillus bulgaricus*, the TDS value ranged from 1,030 ppm to 1,340 ppm in the samples tested, while for Lactobacillus acidophilus, the TDS value ranged from 910 ppm to 1,290 ppm. In *Saccharomyces cerevisiae*, the TDS value range is 1,060 ppm to 1,340 ppm, and for *Aspergillus niger*, the TDS value varies from 930 ppm to 1,500 ppm. In general, controls showed higher TDS values than samples that had been treated with microorganisms. Based on research conducted by Suprayogi, *et al.,* [16], the range of TDS values in eco enzymes is 1,180 ppm-3,680 ppm with orange peel and pineapple peel waste.

Table 4
TDS test results

Treatment	*Lactobacillus bulgaricus* (ppm)	*Lactobacillus acidophilus* (ppm)	*Saccharomyces cerevisiae* (ppm)	*Aspergillus niger* (ppm)
Sample 1	1,300	910	1,330	1,500
Sample 2	1,030	920	1,060	930
Sample 3	1,340	1,290	1,340	1,460
control	1,700	1,700	1,700	1,700

These results indicate that the activity of microorganisms significantly influences the TDS level in the solution. Microorganisms such as *Lactobacillus bulgaricus, Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, and *Aspergillus niger* are known to have the ability to consume and decompose dissolved organic substances in the medium, there by reducing the TDS value. This decrease can be caused by the metabolic processes of microorganisms, where they utilize organic compounds for growth and production of secondary metabolites. The study conducted by Velmurugan & Pandian [31] showed that microorganisms such as Lactobacillus and Saccharomyces are able to ferment organic components in the substrate, which causes a decrease in TDS values due to consumption of dissolved compounds.

In addition, the control that has the highest TDS value shows that there is no microorganism activity that affects the amount of dissolved substances in the solution, so the TDS value remains high. Microorganism activity not only reduces TDS values, but can also produce metabolites that affect the chemical composition of the solution. A study by Tostain *et al.,* [32] confirmed that

Aspergillus niger can produce enzymes that break down complex organic compounds into simpler molecules, which can explain the quite large variations in TDS values in this fungus.

Total Dissolved Solids (TDS) is the total amount of inorganic and organic substances, including minerals, salts, or metals, dissolved in water. TDS in eco-enzymes indicates that organic solids are dissolved in the eco-enzyme solution. TDS will vary depending on the type and amount of fruit waste used in eco-enzymes. In general, the TDS levels of eco-enzymes through fruit waste will be relatively low because most of the waste components will decompose during the fermentation process [5].

4. Conclusions

After conducting research on optimizing eco enzyme production with the addition of *Saccharomyces cerevisiae, Aspergillus niger, Lactobacillus acidophilus* and *Lactobacillus Bulgaricus*, it can be concluded that *Saccharomyces cerevisiae, Aspergiluus niger, Lactobacillus and Lactobacillus bulgaricus* can accelerate the fermentation process in making eco enzymes. This is observed by referring to the similarities between the results of 100-day eco enzymes without the addition of starter and the results of 35-day eco enzymes with the addition of starter.

In future research to develop this research, it is necessary to conduct additional tests or detailed checks related to the levels and components of acetic acid, enzymes and compounds contained in the eco enzyme.

Acknowledgement

This research was funded by Abdurrab University, Grant Number 016/LPPM/KH-UNIVRAB/PTUPT-K/2023.

References

[1] Reza, Para Mesti Anna, and Syuhriatin Syuhriatin. "Pengolahan Sampah Organik Menggunakan Lalat Tentara Hitam (Hermetia illucens) Di Tempat Pengolahan Sampah Terpadu (TPST) Lingsar, Kabupaten Lombok Barat." *Lombok Journal Of Science* 2, no. 3 (2020): 27-31.

[2] Muslimah, M. S., and S. Si. "Impact of Soil Pollution and Preventive Measures." *Agrisamudra* 2, no. 1 (2017): 11–20. https://doi.org/10.33059/jpas.v2i1.224

[3] Wuni, C., A. Husaini, and P. Wulandari. "Training on Making Eco-Enzymes from Household Organic Waste as an Alternative to Natural Cleaning Liquids." *J-ABDI: Journal of Community Service* 1, no. 4 (2021): 589–94. https://doi.org/10.53625/jabdi.v1i4.253

[4] Arun, C., and P. J. Sivashanmugam. "Investigation of Biocatalytic Potential of Garbage Enzyme and Its Influence on Stabilization of Industrial Waste Activated Sludge." *Process Safety and Environmental Protection* 94 (2015): 471–78. https://doi.org/10.1016/j.psep.2014.10.008

[5] Benny, N., R. Shams, K. K. Dash, V. K. Pandey, and O. Bashir. "Recent Trends in Utilization of Citrus Fruits in Production of Eco-Enzyme." *Journal of Agriculture and Food Research* 13 (2023): 100657. https://doi.org/10.1016/j.jafr.2023.100657

[6] Mardiani, I. N., N. Nurhidayanti, and M. Huda. "Socialization of the Use of Organic Waste as Raw Material for Making Eco Enzymes for Residents of Jatireja Village, East Cikarang District, Bekasi Regency." *Pelita Bangsa Community Service Journal* 2, no. 1 (2021): 42–47.

[7] Widiani, N., and A. Novitasari. "Production and Characterization of Eco-Enzymes from Kitchen Organic Waste." *BIOEDUKASI: Journal of Biology Education* 14, no. 1 (2023): 110–17. https://doi.org/10.24127/bioedukasi.v14i1.7779

[8] Muliarta, I. N., and I. K. Darmawan. "Processing Household Organic Waste into Eco-Enzyme as an Effort to Realize Zero Waste." *Agriwar Journal* 1, no. 1 (2021): 6–11.

[9] Mehta, P., and D. K. Chelike. "Utilizing Fungal Biodegradation for Valorisation of Lignocellulosic Waste Biomass and Its Diverse Applications." *Applied Research* 3, no. 4 (2024): e202300119. https://doi.org/10.1002/appl.202300119

[10] Istanti, A., A. B. Indraloka, and S. W. Utami. "Characteristics of Eco-Enzyme Liquid Fertilizer Based on Vegetable and Fruit Waste Regarding Nutrient Content and Organic Materials." *Agriprima: Journal of Applied Agricultural Sciences* 7, no. 1 (2023): 79–85. https://doi.org/10.25047/agriprima.v7i1.503

[11] Pandey, D. "Antibacterial Efficacy of *Curcuma caesia* from Bastar District of Chhattisgarh, India." 5, no. 6 (2014): 2294–2301.
[12] Juariah, S., F. I. Abu Bakar, M. F. Abu Bakar, and S. E. Susi Endrini. "Antibacterial Potential of *Curcuma caesia Roxb* Ethanol Extract Against Nosocomial Infections." *Bali Medical Journal (Bali MedJ)* 12, no. 2 (2023): 1959–63. https://doi.org/10.15562/bmj.v12i2.4320
[13] Harris, Daniel C. *Quantitative Chemical Analysis.* New York: Macmillan, 2010.
[14] Sawyer, C. N., P. L. McCarty, and G. F. Parkin. *Chemistry for Environmental Engineering and Science.* New York: McGraw Hill, 2003.
[15] American Public Health Association. *Standard Methods for the Examination of Water and Wastewater.* Vol. 6. New York: McGraw Hill, 1926.
[16] Suprayogi, D., R. Asra, and R. Mahdalia. "Analysis of Eco-Enzyme Products from Pineapple (*Ananas comosus* L.) and Berastagi Orange (*Citrus X sinensis* L.) Peels." *Redox Journal* 7, no. 1 (2022): 19–27. https://doi.org/10.31851/redoks.v7i1.8414
[17] Meiyasa, F. *Microbiology of Fishery Products.* Banda Aceh: Syiah Kuala University Press, 2021.
[18] Mardiyah, S. "The Effect of Heating Time on Alcohol Content in Palm Sap (*Borassus flabellifer*)." *The Journal of Muhammadiyah Medical Laboratory Technologist* 1, no. 1 (2017): 9–15. https://doi.org/10.30651/jmlt.v1i1.977
[19] Rochyani, N., R. L. Utpalasari, and I. Dahliana. "Analysis of Eco-Enzyme Conversion Results Using Pineapple (*Ananas comosus*) and Papaya (*Carica papaya* L.)." *Redox Journal* 5, no. 2 (2020): 135. https://doi.org/10.31851/redoks.v5i2.5060
[20] Nururrahmani, Azmah, M. Rio Hibatulloh, Revani Ayu Nabila, Kusnadi, and Peristiwati Djuarsa. "Ecoenzymes from Various Types of Orange Peel." *HYGIENE: Journal of Environmental Health* 9, no. 1 (2023): 30–35.
[21] Safrida, Suryani, and Zuhra Amalia. "The Effect of Addition of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on Eco-Enzyme Characteristics and Its Application in Making Antiseptic Solid Soap." *Journal of Technology* 23, no. 1 (2023): 20. https://doi.org/10.21548/39-2-3172
[22] Dewi, S. P., S. Devi, and S. Ambarwati. "Preparation and Organoleptic Testing of Eco-Enzyme from Orange Peel." In *Proceedings of the National Seminar on Law, Business, Science and Technology*, Vol. 2, no. 1 (2022): 649–49.
[23] Chidi, B. S., F. F. Bauer, and D. Rossouw. "Organic Acid Metabolism and the Impact of Fermentation Practices on Wine Acidity: A Review." *South African Journal of Enology and Viticulture* 39, no. 2 (2018): 1–15. https://doi.org/10.21548/39-2-3172
[24] Kim, H. J., M. S. Kwon, H. Hwang, H.-S. Choi, W. Lee, S.-P. Choi, ... and S. W. Hong. "A Review of the Health Benefits of Kimchi Functional Compounds and Metabolites." *Microbiology and Biotechnology Letters.* Korean Society for Microbiology and Biotechnology, 2023. https://doi.org/10.48022/mbl.2310.10011
[25] Meng, F., X. Zhu, H. Zhao, T. Nie, F. Lu, Z. Lu, and Y. Lu. "A Class Ⅲ Bacteriocin with Broad-Spectrum Antibacterial Activity from *Lactobacillus acidophilus* NX2-6 and Its Preservation in Milk and Cheese." *Food Control* 121 (2021): 107597. https://doi.org/10.1016/j.foodcont.2020.107597
[26] Zhang, Z., J. Lv, L. Pan, and Y. Zhang. "Roles and Applications of Probiotic *Lactobacillus* Strains." *Applied Microbiology and Biotechnology* 102 (2018): 8135–43. https://doi.org/10.1007/s00253-018-9217-9
[27] Pereira, B. D. F. "Activity of Essential Oils and Hydroalcoholic Extracts from 12 Plants Against Food Spoilage Yeasts: Evaluation of Their Potential as New Biopesticides." Master's thesis, Universidade do Minho, 2021.
[28] Freimoser, F. M., M. P. Rueda-Mejia, B. Tilocca, and Q. Migheli. "Biocontrol Yeasts: Mechanisms and Applications." *World Journal of Microbiology and Biotechnology* 35, no. 10 (2019): 154. https://doi.org/10.1007/s11274-019-2728-4
[29] Li, Z. L., R. Cheng, F. Chen, X. Q. Lin, X. J. Yao, B. Liang, ... and A. J. Wang. "Selective Stress of Antibiotics on Microbial Denitrification: Inhibitory Effects, Dynamics of Microbial Community Structure and Function." *Journal of Hazardous Materials* 405 (2021): 124366. https://doi.org/10.1016/j.jhazmat.2020.124366
[30] Li, T., Z. Wang, J. Guo, C. de la Fuente-Nunez, J. Wang, B. Han, ... and X. Wang. "Bacterial Resistance to Antibacterial Agents: Mechanisms, Control Strategies, and Implications for Global Health." *Science of The Total Environment* 860 (2023): 160461. https://doi.org/10.1016/j.scitotenv.2022.160461
[31] Velmurugan, L., and K. D. Pandian. "Enhancing Physico-Chemical Water Quality in Recycled Dairy Effluent Through Microbial Consortium Treatment." *Heliyon* 10, no. 21 (2024). https://doi.org/10.1016/j.heliyon.2024.e39501
[32] Tostain, G. C., J. Hoarau, M. Watson, L. Adelard, A. Shum Cheong Sing, Y. Caro, ... and T. Petit. "Production of *Aspergillus niger* Biomass on Sugarcane Distillery Wastewater: Physiological Aspects and Potential for Biodiesel Production." *Fungal Biology and Biotechnology* 5 (2018): 1–12. https://doi.org/10.1186/s40694-018-0045-6