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QUALITY OF ECO ENZYME PRODUCED THROUGH A FERMENTATION PROCESS IN VARIOUS "TEMPE" YEAST CONCENTRATIONS

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ABSTRACT

Organic waste that accumulates and is not adequately managed causes disease and environmental problems. Therefore, one method to solve the problems is to convert it into eco enzyme, a liquid created from the fermentation of organic waste, specifically vegetable and fruit peels, sugar, and water. Anaerobic fermentation is utilized to produce eco enzymes, with yeast added to shorten the fermentation time. The goal of this research is to determine the effect of "tempe" yeast concentration on the quality of eco enzymes. Eco enzyme was made from brown sugar, fruit peels (oranges and melons), and water in a 1:3:10 ratio. "Tempe" yeast concentrations of 1, 2, 3, 4, and 5 %w/v were used in the fermentation process for producing eco enzyme. The eco enzyme products were examined by analyzing pH value, acetic acid content, Total Dissolved Solids (TDS) value, organoleptic properties (aroma and color), and protease enzyme activity. The pH and TDS values of eco enzyme products increased as the concentration of "tempe" yeast increased. The results showed that eco enzyme products have pH values in the range of 3.5-4, acetic acid content of 0.81-1.08 %v/v, TDS value of 1000-2000 ppm, with fermented and orange aroma and a bright yellow color. According to the results, the addition of 3 %w/v "tempe" yeast in the production of eco enzyme was selected as a sample tested for protease enzyme activity and a clear zone was identified around the colony, indicating that there was protease enzyme activity.

Keywords: acetic acid, melon peel, orange peel, organoleptic properties, TDS

1. INTRODUCTION

The amount of garbage generated in Indonesia by commercial, industrial, livestock, plantation, and home operations is expanding in line with the country's growing human population. It could be classified into three types: organic waste, inorganic waste, and hazardous and toxic waste. Organic waste that has accumulated and is not properly handled will decompose into smaller and more stinky materials, which could have a negative impact on health, namely the emergence of viruses and bacteria that cause various diseases. Furthermore, the large amount of waste will degrade the aesthetics of the environment. As a result, this waste must be adequately controlled, one of which is by turning it into eco enzyme.

Eco enzyme is a liquid produced from the fermentation of organic waste, especially vegetable and or fruit peel, sugar, and water [1]. This liquid was first introduced by Rosukon

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Poompanvong, founder of Thai Organic Agriculture Association, who has been conducting research since the 1980s, could be used as a disinfectant, floor cleaner, vegetable cleaner, and insecticide [2]. Eco enzyme contain organic acids such as acetic acid and several enzymes (amylase, lipase, and protease enzymes). Pathogenic bacteria could be killed by acetic acid in organic waste, while enzymes, particularly protease enzymes, could eliminate contaminants with protein content [3].

There have been several researches on the production of eco enzymes. According to Rochyani et al (2021), making eco enzyme from pineapple during three months of fermentation results in a pH value of 3.15 and a TDS value of 1188 ppm, while eco enzyme from papaya produced a pH value of 3.29 and a TDS value of 1132 ppm [4]. In the other research from Islami (2022), producing eco enzyme with various orange fruit peel varieties over three months fermentation produces a pH value in the range of 3.5-3.65 with the lowest acetic acid content of 3.32% [5].

Previous researches from Rochyani et al (2021) and Islami (2022) have shown that producing eco enzymes takes three months. Thus, the production of eco enzyme requires a process that could shorten the time for fermentation process. According to Rahayu's research (2021), making eco enzyme using baker yeast (*Saccharomyces cerevisiae*) could produce eco enzyme for eight to ten days with 60-70% alcohol content and a pH value of 3.5 [6]. This process is similar to the production of bioethanol, which involves fermenting carbohydrates (cellulose) with yeast microorganisms [6][7].

According to the preceding discussions, eco enzyme could be produced in this research through a fermentation process with the addition of "tempe" yeast, which contains the *Rhizopus oligosporus* microorganism [8]. The concentration of "tempe" yeast added was 1 to 5 %w/v and the fermentation process was carried out for ten days. The goal of this research is to determine the effect of "tempe" yeast concentration to the quality of eco enzymes.

2. RESEARCH METHOD

The production of eco enzyme with "tempe" yeast was studied experimentally in several stages, including equipment and material preparation, raw material preparation, fermenter preparation, fermentation process, and product analysis. The fermentation process was conducted for ten days with various "tempe" yeast concentrations (1, 2, 3, 4, and 5 %w/v) at the same mass ratio of brown sugar, fruit peels (orange and melon), and water in a 1:3:10 [9]. The eco enzyme product was analyzed for pH value, acetic acid content, TDS value, organoleptic properties (aroma and color), and protease enzyme activity, which was carried out in Research and Bioprocess Laboratory at Department of Chemical Engineering, Politeknik Negeri Malang.

2.1. Equipment and Material Preparation

This research involved the use of both experimental and analytical equipment. The experiment needs 10 mineral water bottles (1.5 L of volume), a ¼-inch diameter of waterpass hose, a knife, a cutting board, a measuring cup of 1 L, and an analytical balance. The equipment for product analysis consisted of a filter, plastic container, pH meter, TDS meter, dropper pipette, ball pipette, 10 mL measuring pipette, burette, clamp and stative, 250 mL erlenmeyer, glass funnel, 10 mL volumetric flask, 500 mL beaker

glass, 100 mL beaker glass, measuring cup, watch glass, petri dishes, test tube, burner, micro pipette tips, micro pipette, vortex mixer, hotplate, incubator oven, and autoclave.

Materials used in this research consisted of 150 g of orange peel and 150 g of melon peel, 100 g of coconut brown sugar, 1000 mL of water, “tempe” yeast from the Raprima brand (10, 20, 30, 40, and 50 g), and hot glue. Materials used for product analysis consisted of 0.1 N of NaOH solution, phenolphthalein indicator, distilled water, skim milk agar (250 mL of distilled water, 7 g of skim milk powder, 0.625 g of yeast extract, 0.25 g of dextrose from the Himedia brand, 1.25 g of tryptone from the Himedia brand, and 3.75 g of plain agar from the Swallow brand), 70% alcohol, matches, gauze, cotton, and thread.

2.2. Raw Material Preparation

The container was filled with 40 liters of water and set aside for 24 hours to precipitate the impurities. Orange and melon peels were weighed at 150 g each, while brown sugar was weighed at 100 g. Each fruit peel and brown sugar were finely chopped. For the next step, "tempe" yeast was weighed at 10, 20, 30, 40, and 50 g.

2.3 Fermenter Preparation

Ten 1.5-liter bottles of mineral water were washed with warm water to remove impurities and bacteria. The bottle caps were perforated to fit the diameter of the hose. A 50 cm waterpass hose was connected to a perforated bottle cap to allow air bubbles to escape. The waterpass hose was hot glued to the bottle top so that there were no air spaces. Five bottles were used as fermenters, while the remaining five served as air bubble bottles. As a space for the air bubbles to escape, the air bubble bottle was filled with water to the same height as the fermenter, which already contained eco enzyme.

2.4 Fermentation Process

In the fermenter, 100 g of brown sugar was dissolved in 1000 mL of water and mixed until homogeneous. The sugar solution was thoroughly mixed with 300 g of fruit peel mixture (orange and melon) then added 1 %w/v of “tempe” yeast into it (made by diluting 10 g of yeast in 1000 mL of water). Furthermore, the fermenter was labeled with the date of production, materials used, type of yeast, yeast concentration, and harvest time for the eco enzyme. The same procedure was carried out with yeast concentrations of 2, 3, 4, and 5 %w/v. After ten days of anaerobic fermentation process, eco enzyme could be filtered for analyzing the products.

Figure 1. Eco enzyme products by fermentation process for ten days with “tempe” yeast at a concentration of (a) 1 %w/v, (b) 2 %w/v, (c) 3 %w/v, (d) 4 %w/v, and (e) 5 %w/v

2.5 The Analysis of Eco Enzyme Product

1. pH Test
   pH test is used to determine the degree of acidity of the eco enzyme product [6]. The test was carried out by filling 40 mL of eco enzyme sample in a beaker glass, then the sensor of pH meter was immersed into the sample, and the pH value of the product could be determined.
2. Acetic Acid Content Test
   This test is to determine the acetic acid content of eco enzymes. It was conducted by pouring 2 mL of the eco enzyme sample into a 10 mL volumetric flask and diluted with distilled water up to the mark, so that a dilution factor of 5 was obtained. Then, added 3-4 drops of phenolphthalein indicator (PP) into it. The sample was titrated with 0.1 N NaOH as titrant until it turned pink. The amount of NaOH required for the titration was recorded. The acetic acid content was then calculated using Equation (1) [5].

$$\text{Acetic Acid } (\%) = \frac{V_{NaOH} \times N_{NaOH} \times Mr_{\text{acetic acid}} \times DF}{(M_{sample} \times 1000)} \text{x } 100\% \quad (1)$$

   Molarity of sample (M_{sample}) obtained through the Equation (2).

$$M_{sample} = \frac{m_{substrate} + m_{brown\ sugar}}{Mr_{glucose}} \times \frac{1000}{V_{water}} \quad (2)$$

   Note:
   V_{NaOH} = Volume of NaOH (mL)
   V_{water} = Volume of water (mL)
   N_{NaOH} = Normality of NaOH (N)
   $Mr_{acetic\ acid}$ = Relative molecular mass of acetic acid (g/gmol)
   $Mr_{glucose}$ = Relative molecular mass of glucose (g/gmol)
   $m_{substrate}$ = Mass of substrate (orange and melon peels) (g)
   $m_{brown\ sugar}$ = Mass of brown sugar (g)
   DF = Dilution factor
3. TDS Test
   The TDS test is used to determine the amount of dissolved solid in eco enzyme [10]. This test was carried out by filling 40 mL of eco enzyme sample in beaker glass, then the TDS meter was immersed into the sample, and the pH value of the product could be determined.
4. Organoleptic Properties Test
   Organoleptic properties test is used to investigate the quality of eco enzyme products through the human senses. This test consisted of aroma and color tests, which were conducted by filling 40 ml of eco enzyme in a plastic cup and testing it on 25 participants [11], ranging in age from 20 to 22 years.
5. Protease Enzyme Activity Test
   Protease enzyme activity was tested on eco enzyme with a “tempe” yeast concentration of 3 %w/v for the product, which accords with the best range of pH value, TDS value, organoleptic properties, and acetic acid content. The eco

enzyme samples were diluted 10^{-1} to 10^{-4}. A 10^{-1} dilution was made by homogenizing 1 ml of the eco enzyme sample with 9 ml of sterile distilled water. Following that, 1 ml of sample was taken from the first test tube and placed in the next test tube containing 9 ml of sterile distilled water for a 10^{-2} dilution. This dilution method was used until a 10^{-4} dilution was obtained. The diluted sample was aseptically placed in a sterile petri dish. In a sterile petri dish, the sample was added to skim milk agar medium (250 mL distilled water, 7 g skim milk powder, 0.625 g yeast extract, 0.25 g dextrose, 1.25 g tryptone, and 3.75 g of plain agar) [12]. The samples in the dishes were incubated at 37°C for three and six days.

3. RESULTS AND DISCUSSIONS

In this research, eco enzyme was produced by fermentation of organic ingredients such as orange and melon peels, brown sugar, water, and “tempe” yeast. The yeast contains *Rhizopus oligosporus*, a microorganism that serves as a catalyst in "tempe" yeast. The fermentation was formed as a result of the reaction of cellulose on the fruit peels with water (H_2O) using “tempe” yeast as a catalyst, which is shown in Equation (1) [13].

$$\underset{\text{Cellulose}}{C_6H_{10}O_5} + \underset{\text{Water}}{H_2O} \xrightarrow{\text{"tempe" yeast}} \underset{\text{Acetic Acid}}{3CH_3COOH} \quad (1)$$

The fermentation process was conducted for ten days at 25°C, 1 atm to produce eco enzyme. After that, the eco enzyme products were determined by analyzing the pH value, acetic acid content, TDS value, and organoleptic properties for each addition of “tempe” yeast concentrations. Selected sample was tested for protease enzyme activity. The results could be shown in Table 1.

Table 1. The result of eco enzyme product analysis by fermentation process for ten days with various “tempe” yeast concentrations

“Tempe” yeast concentration (%w/v)	pH	TDS (ppm)	Aroma	Color	Acetic Acid Content (%v/v)
1	3.8	1272	Fermented and orange fruit aroma	Bright yellow	0.95
2	3.8	1405	Fermented and orange fruit aroma	Bright yellow	1.08
3	3.8	1475	Fermented and orange fruit aroma	Bright yellow	0.81
4	3.9	1475	Fermented and orange fruit aroma	Bright yellow	0.81
5	4.0	1405	Fermented and orange fruit aroma	Bright yellow	0.81

3.1 The effect of “tempe” yeast concentration on pH value of eco enzyme

A pH measurement was conducted to determine the degree of acidity of the eco enzyme product. The pH value ranges from 1 to 14, with neutral solutions has a pH value of 7, acid has a pH value less than 7, and alkaline has a pH value higher than 7 [14]. Figure

2 shows the pH value of eco enzyme products at yeast concentrations of 1, 2, 3, 4, and 5 %w/v.

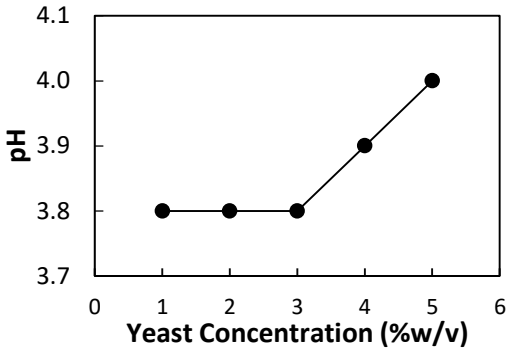


Figure 2. The pH value of eco enzyme by fermentation process for ten days with various "tempe" yeast concentrations

According to Figure 2, the pH value of eco enzyme tends to increase with increasing yeast concentrations. The increased pH value could be due to the increased amylase enzyme content associated with "tempe" yeast concentrations [15]. The eco enzymes with the addition of "tempe" yeast at a concentration of 1-5 %w/v have pH values of around 4 (acidic solution). These results are in line with the literature, which states that eco enzyme has a pH value range of 3.5-4 [6]. The acid content in eco enzyme indicates the presence of organic acids such as acetic acid, lactic acid, malic acid, oxalic acid, and citric acid [16].

3.2 The effect of "tempe" yeast concentration on acetic acid content

The acetic acid content test is used to detect the presence of acetic acid in eco enzyme product. Acetic acid is an organic acid that could hinder the growth of microorganisms. The acetic acid in eco enzyme could be used to clean the equipment, floors, and to kill mosquitos [12]. Figure 3 shows the acetic acid content test result for eco enzyme products.

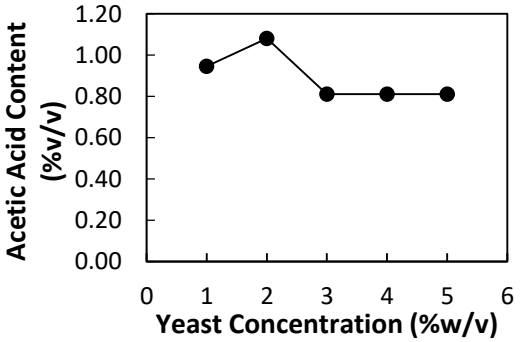


Figure 3. The acetic acid content of eco enzyme by fermentation process for ten days with various "tempe" yeast concentrations

Based on Figure 3, the lowest acetic acid content is found at "tempe" yeast concentrations of 3-5 %w/v (0.81 %v/v) and the greatest quantity is obtained at "tempe" yeast concentration of 2 %w/v (1.08 %v/v). The result is lower than Islami's research (2022), which showed that eco enzyme contained 3.32% acetic acid [5]. The difference

between the results of this study and the literature could be due to the type of organic material used, which affects the amount of acetic acid in the eco enzyme.

3.3 The effect of "tempe" yeast concentration on TDS value of eco enzyme

TDS is the amount of all dissolved solids in eco enzyme, which are organic ions, compounds, or colloids [10]. In this research, TDS measurements were performed using a TDS meter, with values expressed in parts per million (ppm), which could be shown in Figure 4.

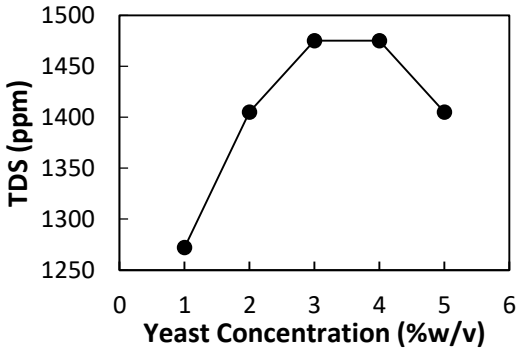
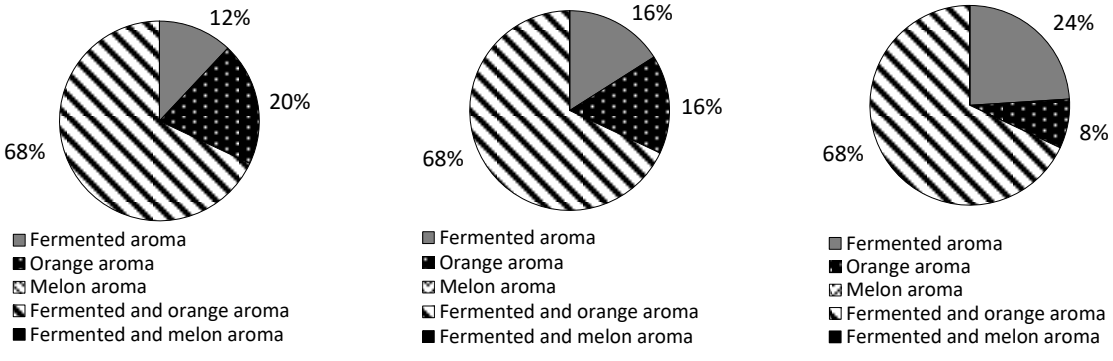


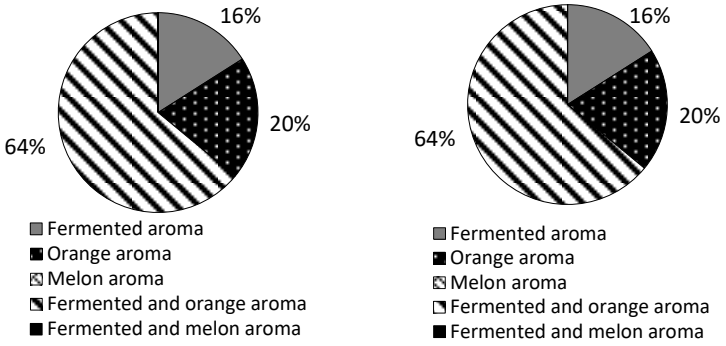
Figure 4. The TDS value of eco enzyme by fermentation process for ten days with various "tempe" yeast concentrations

Figure 4 shows that the TDS values for various yeast concentrations are in the 1000-2000 ppm range. This value corresponds to the literature, which suggests that the range of eco enzyme values is 1000 to 2500 ppm [5, 15]. The presence of organic substances such as fruit peels, brown sugar, and the addition of yeast could cause a high TDS value, with increasing yeast concentration, the TDS value rises [17]. It implies that the higher the organic substances in eco enzyme, the more organic solids are dissolved, which leads to a high TDS value. The TDS and pH values increased as yeast concentration increased whereas had no effect on acetic acid content.

3.4 The effect of "tempe" yeast concentration on organoleptic properties of eco enzyme product

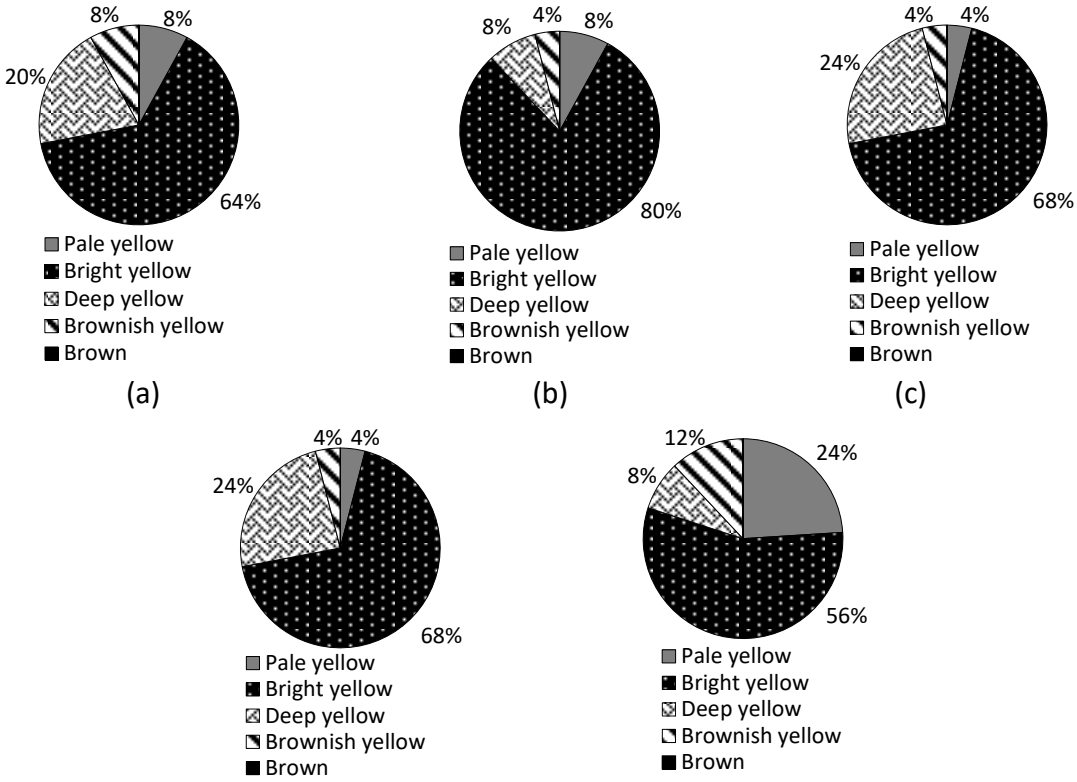
An organoleptic properties test measures the acceptability of a product using the human senses. According to the stimuli received by the senses, the ability of these sensory organs determines the impression and assessment of the product being tested [18]. Organoleptic properties tests in the form of aroma and color were performed on 25 respondents [11]. Figures 5 and 6 show the organoleptic properties test results.





(d) (e)

Figure 5. The aroma test diagram of eco enzyme products by fermentation process for ten days with "tempe" yeast at concentrations of (a) 1 %w/v, (b) 2 %w/v, (c) 3 %w/v, (d) 4 %w/v, and (e) 5 %w/v to 25 respondents



(a) (b) (c)

(d) (e)

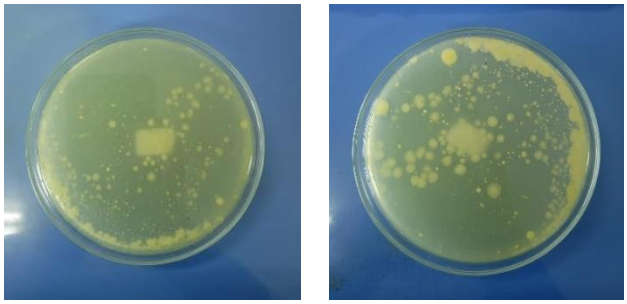
Figure 6. The color test diagram of eco enzyme products by fermentation process for ten days with "tempe" yeast at concentrations of (a) 1 %w/v, (b) 2 %w/v, (c) 3 %w/v, (d) 4 %w/v, and (e) 5 %w/v to 25 respondents

According to the aroma test results shown in Figure 5, 64-68% of respondents agreed that eco enzyme had a fermented and citrus aroma at various yeast concentrations. These results are consistent with the results of Titiaryanti et al (2022), who showed that eco enzyme has a fresh, sour aroma characteristic of fermentation [19]. Based on the color test results shown in Figure 6, 56-80% of respondents agreed that eco enzyme had a bright yellow color at various yeast concentration variations. According to Larasati (2020),

the fermentation process is successful if it has a brownish color [2]. The amount of "tempe" yeast used had no effect on the aroma or color changes of the eco enzyme produced.

3.5 The effect of "tempe" yeast concentration on protease enzyme activity

The protease enzyme is one of numerous enzymes found in eco enzyme. According to Arun and Sivashanmugam (2015), the presence of protease enzymes tends to decrease the presence of harmful microorganisms. This ability of protease enzymes is related to the usage of eco enzyme as an antibacterial cleanser [3]. The protease enzyme activity test was performed on eco enzyme at 3 %w/v "tempe" yeast concentration based on the test results of pH value, TDS value, organoleptic properties, and acetic acid content. The test was carried out after three and six days of incubation. The test results could be seen in Figure 7.



(a) (b)

Figure 1. Protease enzyme activity of eco enzyme products by fermentation process for ten days with "tempe" yeast at concentration of 3 %w/v (a) three days incubation (b) six days incubation

Figure 7 indicates that several colonies formed on the third day of incubation, whereas a clear zone appeared on the sixth day, indicating the activity of the protease enzyme. This analysis corresponds with the literature, which states that the presence of a clear zone around the colony shows the presence of protease enzyme activity [12]. The presence of protease enzymes in *Rhizopus oligosporus* stimulates protease in eco enzyme production [8].

4. CONCLUSIONS AND SUGGESTIONS

Based on the results and discussions, increasing the concentration of "tempe" yeast (1, 2, 3, 4, and 5 %w/v) affects the quality of eco enzymes such as pH value, acetic acid content, TDS value, organoleptic properties (aroma and color), and protease enzyme activity. The pH and TDS values of eco enzyme products tend to rise when the concentration of "tempe" yeast increases. The pH values obtained were in the 3.5-4 range, with TDS values in the 1000-2000 ppm range. The produced eco enzyme has a fermented and orange fruit aroma and a bright yellow color. The three eco enzyme quality parameters are consistent with previous research. The acetic acid content produced ranges between 0.81 and 1.08 %v/v. The addition of 3 %w/v "tempe" yeast produced the best eco enzyme with a pH value 3.8, acetic acid content of 0.81 %v/v, TDS value 1475 ppm, and fermented and orange fruit aroma with a bright yellow color, which was then tested for protease enzyme activity and a

clear zone was discovered around the colony indicating that there was protease enzyme activity. According to the results, a rise in yeast concentration leads to an increase in pH and TDS values but not in acetic acid content because other components other than acetic acid are present.

For further research of eco enzyme, protease activity could be tested in skim milk agar with other types of materials and these isolates could then be tested in liquid culture with casein as a substrate.

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