

Potential of Eco-enzyme *Averrhoa bilimbi* L. Fruit as an Innovation in Tempeh Wastewater Treatment in Plaju District, South Sumatra

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Abstract

Tempeh home industries have been established in residential areas, one of which is in the assisted village of PT Pertamina Internasional RU III Plaju Factory, Palembang, South Sumatra. Eco-enzyme is one of the methods for processing and reducing pollutant organic matter in liquid waste. This study aims to determine the eco-enzyme characteristics of *Averrhoa bilimbi* L. fruit and exploit its potential to reduce chemical oxygen demand (COD), total suspended solid (TSS), and total dissolved solid (TDS) in tempeh wastewater. Eco-enzymes are characterized by measuring pH, COD, TSS, TDS, and most probable number (MPN). The reduction in COD, TSS and TDS of tempeh wastewater was observed using a simple bioreactor which was treated with the addition of 10% eco-enzyme (three replicates) and without the addition of eco-enzyme (control). The characterization results showed that the eco-enzyme of *A. bilimbi* L. fruit had a pH of 1.84, COD of 85.33 mg/L, TSS of 440 mg/L, TDS of 15,800 mg/L, protein concentration of 0.459 U/mg, and MPN value of 0.03 CFU/100 mL. The COD value of tempeh liquid waste can be reduced from the initial COD of 256 to 154.67 mg/L. The TSS value of tempeh wastewater decreased by 60% from the initial value of 4,000 mg/L. It takes 18 days to reduce the TSS content below the quality standard. The TDS value of tempeh liquid waste can be reduced by 70% from the initial TDS of 7,333.33 to 1,666.67 mg/L.

Keywords: *Averrhoa bilimbi* L., COD, Eco-Enzyme, TDS, TSS, wastewater

1. INTRODUCTION

The industrial sector is the most important contributor to economic development in Indonesia. The food industry is an industry that is growing rapidly to meet consumer food needs. One of the many established food industries is the tempeh industry. Tempeh is a fermented food made from soybeans. This food is a source of protein that is very popular with all levels of society [1]. Many of these home industries have been established in residential areas, one of which is in the assisted village of PT Kilang Pertamina Internasional RU III Plaju, Palembang, South Sumatra.

The process of making tempeh must go through several stages, boiling, soaking, washing, and fermenting the soybeans. This process requires large amounts of water. This process has a positive

correlation with the wastewater produced. Untreated sewage can pollute the water bodies, disturbing aquatic habitats and generating an unpleasant odour. Wastewater generated from the tempeh industry in the assisted village of PT Kilang Pertamina Internasional RU III Plaju, Palembang, South Sumatra, is managed before being returned to the environment. This management process still encounters several problems, such as the wastewater still smelling and the content of chemical oxygen demand (COD), total suspended solid (TSS), and total dissolved solid (TDS) being slightly high. According to Ukpong and Udechukwu, tempeh wastewater contains high levels of polluting organic matter such as COD and TSS [2]. It is necessary to treat tempeh wastewater with an appropriate and environmentally friendly method. Therefore, it is required to treat tempeh wastewater using sustainable methods using eco-enzymes.

The utilization of eco-enzymes is one of the methods for processing and reducing organic matter contaminating tempeh wastewater. Eco-enzyme is a complex liquid produced from the fermentation of fresh organic waste (fruits and vegetables), sugar, and water [3]. The mixture is fermented for three months to produce enzymes. Researchers reported that using 0.5% eco-enzyme in sewage can reduce COD values from 537 to 384 mg/L, TSS from 121

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Table 1. Characteristics of eco-enzyme of *Averrhoa bilimbi* L. fruit.

Parameters	Unit	Value
pH	-	1.840
Chemical Oxygen Demand (COD)	mg/L	85.330
Total Suspended Solid (TSS)	mg/L	440
Total Dissolved Solid (TDS)	Mg/L	15.800
Most Probable Number (MPN)	CFU/100 mL	< 0.03
Protein concentration	U/mg	0.459

to 47 mg/L and TDS from 884 to 745 mg/L [4][5]. Based on the previous literature, using eco-enzymes from *Averrhoa bilimbi* L. fruit has not been implemented yet. This study aims to determine the eco-enzyme characteristics of *A. bilimbi* L. fruit and use its potential to reduce COD, TSS and TDS in tempeh wastewater.

2. MATERIALS AND METHODS

2.1. Materials

Eco-enzyme *A. bilimbi* L. fruit was obtained from the assisted village of PT Pertamina Internasional RU III Plaju Factory, Palembang, South Sumatra. Eco-enzymes are characterized by measuring pH, COD, TSS, TDS, and most probable number (MPN). The decrease in COD, TSS and TDS in tempeh wastewater was observed using a simple bioreactor. The bioreactor is filled with tempeh wastewater which is treated with 10% eco-enzyme (three times replication). Observations were made for 12 days of incubation. Stirring was carried out every 24 h. COD, TSS and TDS values were measured periodically on days 0, 4, 8 and 12.

2.2. Methods

2.2.1. MPN Analysis

MPN analysis was carried out through two steps, namely the presumptive test (introduction) and the confirmative test (affirmation) using the 9-tube series (3-3-3) of the Thomas formula. The presumptive test was carried out using lactose broth (LB) to determine the presence or absence of coliform bacteria. The presumptive test was started by preparing 9 test tubes containing 10 mL of sterile LB. The first 3 test tubes were inoculated

with 10 mL eco-enzyme, the second 3 were inoculated with 1 mL eco-enzyme, and the last 3 were inoculated with 0.1 mL eco-enzyme. Inoculation was carried out aseptically. Then homogenize the LB which has been inoculated with eco-enzyme and then incubated at 37 °C for 48 h. A positive test is indicated by gas bubbles forming in the Durham tube. Positive results in the presumptive test were further confirmed with a confirmative test using brilliant green LB (BGLB). A positive test is indicated by gas bubbles in the BGLB.

2.2.2. COD Measurement

COD measurement was carried out using the titrimetric method. Measurements were carried out with three repetitions and controlled environmental factors. A total of 5 mL of sample was put into a 250 mL Erlenmeyer and added by 1 g of Hg₂SO₄, 1 mL of 0.26 N K₂Cr₂O₇, and 3 mL of reagent (a mixture of Ag₂SO₄ and H₂SO₄). Erlenmeyer was closed, homogenized, boiled, and cooled to reach room temperature. Two drops of ferroin indicator were added to the reaction mixture and the mixture was titrated with 0.1 N ferro ammonium sulphate (FAS) solution. The titration was stopped when the reaction mixture turned bluish-green to reddish brown. The used FAS volume was recorded to calculate the COD value. The same procedure was done for the blank solution by replacing 5 mL of the sample with 5 mL of distilled water. The COD value is calculated using the Equation 1.

$$COD = \frac{(A - B) \times N \text{ FAS} \times BE \text{ O}_2}{V} \quad (1)$$

where A is initial weight (mg), B is sample titrant (mL), BE O₂ is 8000, N FAS is normality of

FAS (0.1), and V is sample volume (mL) [6].

2.2.3. TSS Measurements

TSS measurements were carried out using the gravimetric method. Measurements were carried out with three repetitions and controlled environmental factors. Filter paper and a porcelain cup of known weight (W0) were prepared, placed on the filtration apparatus, and moistened with distilled water. The 5 mL of the tempeh liquid waste sample treated with 10% eco-enzyme was poured. We did the same for the control treatment (tempeh wastewater sample without adding eco-enzyme). The filtrate was accommodated in the Erlenmeyer. TSS measurement was carried out by drying the filter paper at 105 °C for 2 h, cooling, and weighing to obtain a constant weight (W1), Eq. 2 [7].

$$\text{Total Suspended Solid (TSS) (mg/L)} = \frac{(W1 - W0) \times 1000}{\text{Volume of Sample (mL)}} \quad (2)$$

2.2.4. TDS Measurements

TDS was measured by transferring the filtrate to a porcelain cup of known weight (W0) and heating at 180 °C for 2 h. The porcelain cup was cooled and weighed to a constant weight (W1), Eq. 3 [7].

$$\text{Total Dissolved Solid (TDS) (mg/L)} = \frac{(W1 - W0) \times 1000}{\text{Volume of Sample (mL)}} \quad (3)$$

2.2.5. Data Analysis

The measurement data were analyzed using Microsoft Excel. Analysis of the time needed to reduce organic pollutant substances in tempeh wastewater was carried out using linear regression in SPSS Statistics 22.

3. RESULTS AND DISCUSSIONS

3.1. Characteristics Eco-enzyme of *A. bilimbi* L. Fruit

The characteristics eco-enzyme of *A. bilimbi* L. fruit were confirmed based on several parameters, namely pH, COD, TSS, TDS, MPN, protein concentration, and biocatalytic activity (amylase, lipase, and protease activities). Table 1 shows the eco-enzyme characteristics of *A. bilimbi* L. fruit produced by the Foster Village of PT Pertamina Internasional RU III Plaju Factory. Based on Table 1, the eco-enzyme of *A. bilimbi* L. fruit has an acidic pH of 1.84. The low pH is caused by the presence of organic acids in the eco-enzyme. Organic acids are produced from the fermentation of *A. bilimbi* L. fruit. It was stated that the process of making eco-enzymes produces volatile and non-volatile organic acids, such as lactic acid, acetic acid, and butyric acid [8][9].

The eco-enzyme produced from *A. bilimbi* L. fruit has a high COD value of 85.33 mg/L. The high COD value indicates the amount of organic matter from the ingredients for making eco-enzymes (*A. bilimbi* L fruit and sugar). The COD value of the fruit eco-enzyme *A. bilimbi* L. still meets the quality standard for fruit or vegetable processing. According to the Regulation of the Minister of Environment of the Republic of Indonesia Number 5 of 2014 concerning Quality Standards of Wastewater, the COD limit for processing fruits and or vegetables is 150 mg/L [10].

TSS and TDS values of the eco-enzyme of *A. bilimbi* L. fruit are 440 and 15,800 mg/L. TSS and

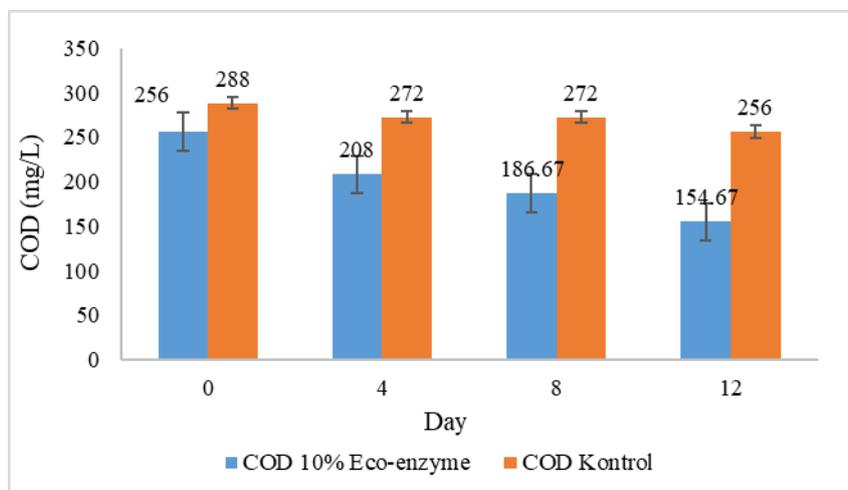


Figure 1. Reduction of COD of tempeh wastewater with 10% eco-enzyme treatment.

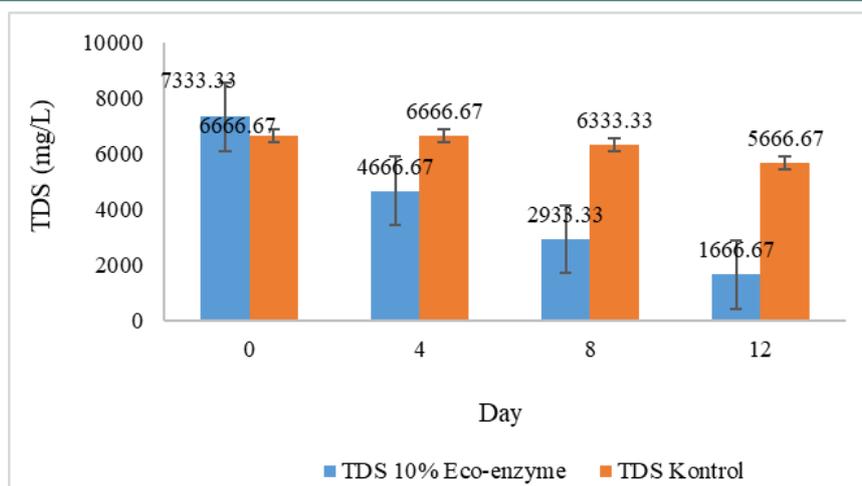


Figure 2. Reduction of the TSS in tempeh wastewater treated with 10% eco-enzyme *Averrhoa bilimbi* L fruit.

TDS show a high value and are above the standard value for the quality of fruit and or vegetable processing. It is also reported that eco-enzymes from oranges and tomatoes produce high TSS and TDS values [11]. TSS eco-enzyme from oranges is 19,000 mg/L, and from tomatoes is 17,000 mg/L while eco-enzymes from oranges and tomatoes have the same TDS value of 14,000 mg/L. According to Rasti et al., the high TSS and TDS values in eco-enzymes are caused by organic solid waste originating from the main ingredients [11].

In the eco-enzyme characterization, coliform and colifecal bacteria were detected using the MPN method [12]. This is because the water pollution caused by the faeces of warm-blooded animals can be detected through the presence of coliform and colifecal bacteria using the MPN method. The test results showed that the eco-enzyme produced by PT Kilang Pertamina Internasional RU III Plaju did not contain coliform and colifecal bacteria with an MPN value of less than 0.03 CFU/100 mL (Table 1) United States Department of Agriculture (2014) stated that the requirement for microbiological water that is clean and safe for consumption is an MPN value of less than 0.03 CFU/100 mL [13].

Table 1 shows the protein concentration in eco-enzyme is 0.459 U/mg. Detection of protein concentration was carried out to determine the specific activity of amylase, lipase, and protease. Several studies have reported that eco-enzymes consist of complex components, one of which is an enzyme. Enzymes are protein biomolecules that act as catalysts in a biochemical reaction [14], thus the

eco-enzymes contain enzymes (proteins) [15]. The proteins have optimum amylolytic, lipolytic and proteolytic activities at a given pH treatment.

3.2. Reduction of Polluting Organic Substance

The reduction of polluting organic compounds was analyzed through several parameters, namely by looking at the reduction in COD, TSS, TDS, and pH as additional parameters. These parameters are determining factors for the level of contamination of water bodies based on quality standards [16]. COD analysis carried out for 12 days of incubation showed a decrease in COD content (Figure 1).

The COD value of tempeh wastewater with the addition of 10% eco-enzyme *A. bilimbi* L. fruit showed a decrease of 39.6%. Adding 10% eco-enzyme of *A. bilimbi* L. fruit into tempeh wastewater can reduce the initial COD of 256 to 154.67 mg/L. Tempeh wastewater without adding *A. bilimbi* L. fruit eco-enzyme (control) showed an insignificant decrease of 11.0% [16]. The control treatment could only reduce the COD content of tempeh wastewater by 32 mg/L from the initial COD content of 288 to 256 mg/L.

The COD content of tempeh wastewater with the adding 10% eco-enzyme from *A. bilimbi* L. fruit decreased significantly. The decreasing COD was due to an enzymatic process to degrade the organic matter contained in tempeh wastewater. The enzymes contained in eco-enzymes utilize these organic materials as substrates. Reducing the COD content in wastewater by treating 10% eco-enzyme meets the quality standards set by the Regulation of

the Minister of Environment of the Republic of Indonesia Number 5 of 2014 [10]. The quality standard for COD content for soybean waste-making tempeh is 300 mg/L [17].

The decrease in COD content in tempeh wastewater was followed by a reduction in TSS and TDS values. TSS and TDS values in tempeh wastewater decreased by 60% and 70%, respectively (Figures 2 and 3). Figure 2 shows tempeh wastewater without treatment only experienced a 20% decrease in TSS value. The TSS value of the treated wastewater decreased from 4,000 to 1,600 mg/L (60%). When compared to the TSS of the control, the TSS value of tempeh wastewater treated with 10% eco-enzyme *A. bilimbi* L. fruit decreased significantly. After being incubated for 12 days, the reduction in TSS values did not meet the quality standard values stipulated by the Regulation of the Minister of Environment of the Republic of Indonesia [10]. The decrease in TSS values was greatly influenced by the length of treatment time [18]. Therefore, a linear regression test was carried out to predict the treatment time needed to reduce the TSS content to below the standard quality. The analysis produces the following regression equation 4.

$$y = -183,33x + 3466,7 \tag{4}$$

Based on equation 4, it can be seen that there is a negative correlation between the time of treatment and the TSS content of -183.33. This correlation indicates that the longer the treatment time, the

lower the TSS content. The results show that reducing the TSS value to 90 mg/L takes 18 days. Figure 3 shows the control and treatment tempeh wastewater experienced a decrease in TDS values. Control experiment decrease in TDS value of 15%. The TDS value of wastewater with the addition of 10% eco-enzyme of *A. bilimbi* L. fruit decreased by 77%, from 7,333.33 to 1,666.67 mg/L. The TDS value already meets the quality standards set by the government whereas the quality standard for Total Dissolved Solid (TDS) is 2,000 mg/L [19].

The pH value of tempeh wastewater tends to be acidic and easily changed. The acidic pH of tempeh wastewater indicates a process of decomposition of the organic substance. These organic substances will be broken down with by-products in the form of organic acids. Figure 4 shows that the treatment can increase the pH value from day 0 to 12 of incubation. The final pH value of treated tempeh wastewater did not meet the quality standards set by the government. The lack of incubation time is needed to increase the pH value to reach a predetermined quality standard value. Regulation of the Minister of Environment of the Republic of Indonesia Number 5 of 2014 set the pH quality standard for tempeh industrial wastewater ranges from 6 – 9 [10].

A linear regression test was carried out to predict the time needed to increase the pH value to reach the quality standard. The resulting regression equation follows equation 5.

$$y = 0,0858x + 3,453 \tag{5}$$

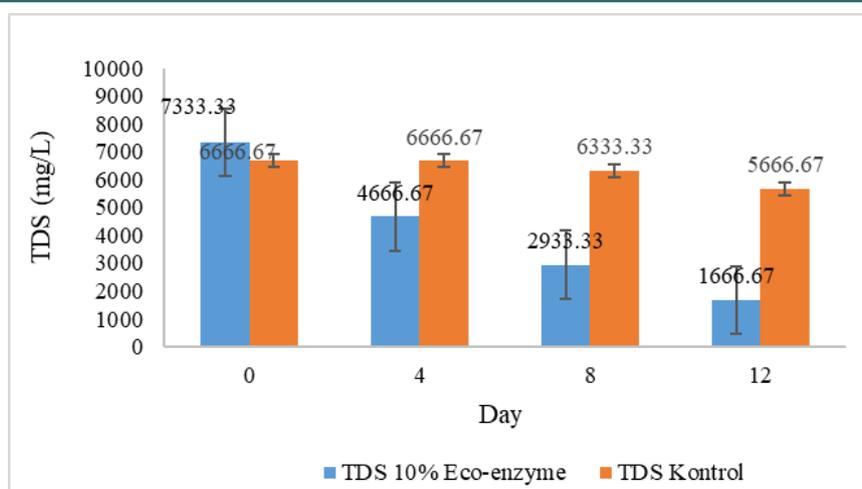


Figure 3. Reduction of the TDS in tempeh wastewater treated with 10% eco-enzyme *Averrhoa bilimbi* L. fruit.

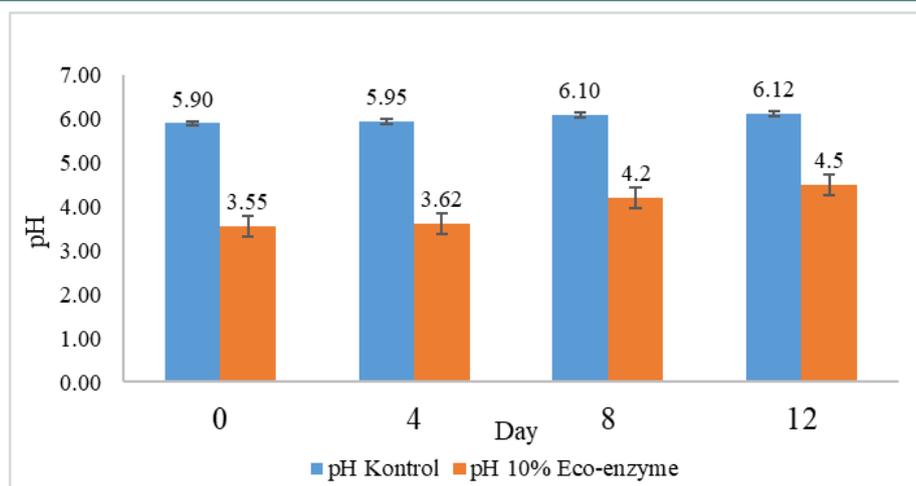


Figure 4. pH of tempeh wastewater with 10% eco-enzyme treatment of *Averrhoa bilimbi* L. fruit.

The results showed that there was a positive correlation between the treatment time and the increase in the pH value. The positive value of the x coefficient illustrates that the longer the treatment time, the higher the resulting pH value. To raise the pH value to 6, it takes 29 days.

Based on the parameters, 10% eco-enzyme treatment can reduce the organic substance contained in tempeh wastewater. The decrease in COD, TSS and TDS was followed by an increase in the pH of the tempeh wastewater. The pH enhancement is caused by the use of oxygen to decompose complex organic substances into simpler ones so that CO₂ levels decrease. An increase in pH and a decrease in COD content had a negative correlation value [20]. It can be interpreted that the pH enhancement and decrease in COD content are inversely proportional. The reduction in COD content affects the increase in the pH value due to less organic substance being degraded. Consequently, the resulting pH will be more alkaline [21].

4. CONCLUSIONS

Eco-enzyme from *Averrhoa bilimbi* L. fruit has a pH of 1.84, COD 85.33 mg/L, TSS 440 mg/L, TDS 15,800 mg/L, protein concentration of 0.459 U/mg, and MPN value of 0.03 CFU/100 mL. Pollutant organic matter (COD, TSS, and TDS) in tempeh wastewater can be reduced by adding 10% (v/v) *A. bilimbi* L. fruit eco-enzyme with an incubation time of 12 days. The COD value of tempeh liquid waste can be reduced by 101.33 mg/L from the initial

COD of 256 mg/L. The TSS value of tempeh wastewater decreased to 1,600 mg/L from the initial value of 4,000 mg/L, which takes 18 days to reduce the TSS content to below the quality standard. The TDS value of tempeh liquid waste can be reduced by 5,666.67 mg/L from the initial TDS of 7,333.33 mg/L.

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Author Contributions

S. S. contributed in planning, conducting experiments, developing theory, doing calculations, interpreting results, and writing the manuscript. D. E. M. assisted in verifying analytical methods and monitoring findings in experiments. A. S. helped

carry out experiments and edit the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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