

## Effectiveness of Eco-Enzyme in Reducing pH, BOD, And NH<sub>3</sub> Levels in Wastewater from Jombang Regional General Hospital

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### Abstract

**Background:** Wastewater treatment at Jombang Regional General Hospital has not consistently met effluent quality standards for parameters such as pH, Biochemical Oxygen Demand (BOD), and ammonia (NH<sub>3</sub>). This study aimed to analyze the effectiveness of the eco-enzyme in reducing pH, BOD, and NH<sub>3</sub> levels in hospital wastewater.

**Method:** A laboratory experimental design was conducted using a post-test-only control group. Phytochemical analysis was conducted to identify the bioactive compounds present in the eco-enzyme solution. Wastewater samples were collected from the Wastewater Treatment Plant (WWTP) of Jombang Regional General Hospital and treated with eco-enzyme concentrations of 5%, 10%, and 15% for an incubation period of 5 days. The measured parameters included pH, BOD, and ammonia (NH<sub>3</sub>). Statistical analysis was performed using ANOVA, followed by Tukey's Honestly Significant Difference (HSD) post hoc test.

**Results:** Phytochemical analysis identified five main bioactive compounds in the eco-enzyme solution, namely flavonoids (14.246 mg/mL), polyphenols (3.648 mg/mL), citric acid (0.6954 g/L), lactic acid (0.05 mg/mL), and bromelain (0.714 U/mL). Eco-enzyme treatment significantly reduced all measured wastewater parameters. The mean pH decreased from 8.18 to 6.00 (26.59%), BOD decreased from 73.63 mg/L to 38.57 mg/L (47.62%), and NH<sub>3</sub> decreased from 9.45 mg/L to 2.78 mg/L (70.52%) at the 15% eco-enzyme concentration. It indicated statistically significant differences among eco-enzyme treatment groups for pH, BOD, and NH<sub>3</sub> parameters ( $p < 0.05$ ), with the greatest reductions observed at the 15% eco-enzyme concentration.

**Conclusion:** Eco-enzymes demonstrated potential effectiveness in reducing pH, BOD, and NH<sub>3</sub> levels in hospital wastewater, particularly at a concentration of 15%.

**Keywords:** BOD, Eco-enzyme, NH<sub>3</sub>, pH, Wastewater

### INTRODUCTION

Hospital wastewater is a complex mixture that contains pathogenic microorganisms, pharmaceutical residues, organic matter, heavy metals, and nutrients such as nitrogen and phosphorus. If not treated properly, this wastewater can pose a major risk to public health and environmental pollution.<sup>1</sup> In developing countries, including Indonesia, hospital wastewater management remains a serious challenge. Many hospital wastewater treatment plants (WWTPs) are not yet operating optimally, resulting in wastewater discharges that often exceed regulatory thresholds.<sup>2</sup> Untreated or inadequately treated wastewater can contaminate surface water and groundwater, degrade ecosystems, and contribute to the emergence and spread of antimicrobial resistance in the environment.<sup>3</sup>

In East Java Province, East Java Governor Regulation No. 72 of 2013 stipulates quality standards for hospital wastewater, particularly for pH (6.0–9.0), BOD ( $\leq 30$  mg/L), and NH<sub>3</sub> (NH<sub>3</sub>-N  $\leq 0.1$  mg/L). According to periodic evaluations at Jombang Regional General Hospital (RSUD Jombang), some parameters, particularly BOD and NH<sub>3</sub>, frequently exceed these thresholds despite the hospital's wastewater treatment plant using both aerobic and anaerobic biofilter systems. High BOD levels indicate a large amount of biologically degradable organic matter, which ultimately reduces dissolved oxygen levels in water bodies. Meanwhile, high NH<sub>3</sub> concentrations are toxic to aquatic life and can cause a strong odor.<sup>4</sup> This condition highlights the urgency of improving wastewater treatment performance at Jombang Regional General Hospital, a representative public hospital in East Java that continues to face substantial operational constraints in complying with effluent quality standards. Various conventional

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methods, such as coagulation, chlorination, and advanced oxidation process (AOP), have proven effective in reducing certain wastewater parameters; however, these approaches generally require high operational costs and may generate secondary pollutants.<sup>5</sup> As an alternative, eco-enzyme technology has emerged as a low-cost, nature-based biological innovation that is environmentally friendly and suitable for decentralized wastewater management. Eco-enzymes are liquid fermentation products derived from organic kitchen waste, such as fruit peels, combined with molasses and water. These products contain a mixture of enzymes, organic acids, and other bioactive compounds with potential antimicrobial and catalytic properties that may facilitate the degradation of organic pollutants in wastewater.<sup>6</sup>

Several studies have demonstrated the potential of eco-enzymes in reducing wastewater pollutant parameters. Eco-enzymes derived from orange peels have been reported to reduce  $\text{NH}_3$  and COD levels in synthetic greywater within 5–10 days, while also achieving up to a 70% reduction in BOD levels in domestic wastewater.<sup>7,8</sup> Similarly, eco-enzymes produced from pineapple peels have demonstrated effectiveness in significantly reducing  $\text{NH}_3$  concentrations in synthetic wastewater.<sup>9</sup> However, the application of eco-enzymes in hospital wastewater remains very limited, even though hospital effluents are more complex and challenging due to the presence of pharmaceutical residues and disinfectants. This complexity may influence the degradation efficiency of enzyme-based treatments.<sup>10</sup> Moreover, the enzymatic composition of eco-enzymes may vary depending on the raw materials and fermentation conditions used, resulting in inconsistent performance across studies.<sup>11</sup>

Therefore, a significant research gap regarding the reproducibility, consistency, and practical applicability of eco-enzymes in the treatment of actual hospital wastewater. Most previous studies have been conducted on a small laboratory scale using short incubation periods or low treatment concentrations, thereby limiting their relevance to high-strength wastewater, such as hospital effluent. Moreover, in tropical settings such as Jombang, no comprehensive study has evaluated the effect of varying eco-enzyme concentrations on the simultaneous reduction of pH, BOD, and  $\text{NH}_3$  parameters under real hospital wastewater conditions. Such research is crucial to support the development of sustainable and community-based wastewater treatment strategies within the healthcare sector.

In addition, many previous studies have focused primarily on the percentage reduction of pollutants without assessing whether the treated effluent complied with established regulatory standards. Consequently, evaluating the absolute effectiveness of eco-enzymes in achieving effluent quality compliance remains necessary.<sup>12</sup> Therefore, this study aimed to analyze the effectiveness of eco-enzymes at varying concentrations (5%, 10%, and 15%) in reducing pH, BOD, and  $\text{NH}_3$  levels in hospital wastewater from Jombang Regional General Hospital, to explore a sustainable and cost-effective approach to improving hospital wastewater treatment systems.

## METHOD

### Participants and Study Design

This study employed a laboratory experimental design conducted at the Wastewater Treatment Plant (WWTP) of Jombang Regional General Hospital in April 2025. The research site was selected because the hospital's wastewater exceeded permissible quality standards, particularly for pH, biological oxygen demand (BOD), and ammonia ( $\text{NH}_3$ ). The wastewater samples used in this study were collected from various hospital service units that discharge into the WWTP system. The samples in this study were collected from the aerobic biofilter tanks of the WWTP, as this unit represents the biological treatment stage where microbial activity is crucial for reducing organic pollutants. Sampling from this section allowed for evaluating the interaction between the eco-enzyme and the biological treatment process. The samples were divided into four groups: one control group (without eco-enzyme) and three treatment groups with eco-enzyme concentrations of 5%, 10%, and 15%. This study used simple random sampling and was repeated three times for each treatment.

### Measurements and Procedure

The parameters measured included pH (using a digital pH meter according to SNI 06-6989.11-2004 / APHA 4500- $\text{H}^+$  B, 2017), BOD (using the 5-day Winkler method, SNI 6989.57:2008 / APHA 5210 B, 2017), and  $\text{NH}_3$  (using a spectrophotometer based on SNI 06-6989.30-2005 / APHA 4500- $\text{NH}_3$

F, 2017). The eco-enzyme used in this study was produced from a mixture of pineapple, orange, and carrot peel waste as the raw materials. The fermentation process followed the standard eco-enzyme formulation, consisting of 3 parts fruit and vegetable waste, 1 part brown sugar (molasses), and 10 parts clean water (v/v).

All ingredients were placed in a tightly sealed plastic container, leaving approximately 10–15% headspace to allow for gas expansion during the fermentation process. The mixture was subsequently fermented under anaerobic conditions at room temperature (25–30°C) for 90 days. To prevent excessive pressure buildup, the container was briefly opened every two weeks to release accumulated gas.

After the fermentation period, the liquid was filtered through a fine cloth to obtain a clear eco-enzyme solution, which was then stored in dark glass bottles at room temperature until further use. Phytochemical analysis was conducted to identify and quantify the bioactive compounds present in the eco-enzyme, including flavonoids, polyphenols, organic acids, and bromelain. Experimental data were analyzed descriptively by calculating mean values and percentage reductions for each parameter, and the results were presented in tables and graphs.

### Statistical Analysis

Normality testing was performed using the Kolmogorov-Smirnov test. Differences among treatment groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey’s Honestly Significant Difference (HSD) post hoc test to identify pairs of groups with statistically significant differences. Homogeneity of variance was assessed using Levene’s test, with a p-value > 0.05 indicating homogeneous variances. All statistical analyses were performed using statistical software at a significance level of  $\alpha = 0.05$ .

## RESULTS

Table 1 shows that the results of phytochemical characterization quantitatively indicate that eco-enzyme contains five main groups of bioactive compounds, namely flavonoids (14,246 mg/mL), polyphenols (3,648 mg/mL), citric acid (0.6954 g/L), lactic acid (0.05 mg/mL), and bromelain (0.714 U/mL).

**Table 1. Results of Concentration of Bioactive Compounds in Ecoenzyme**

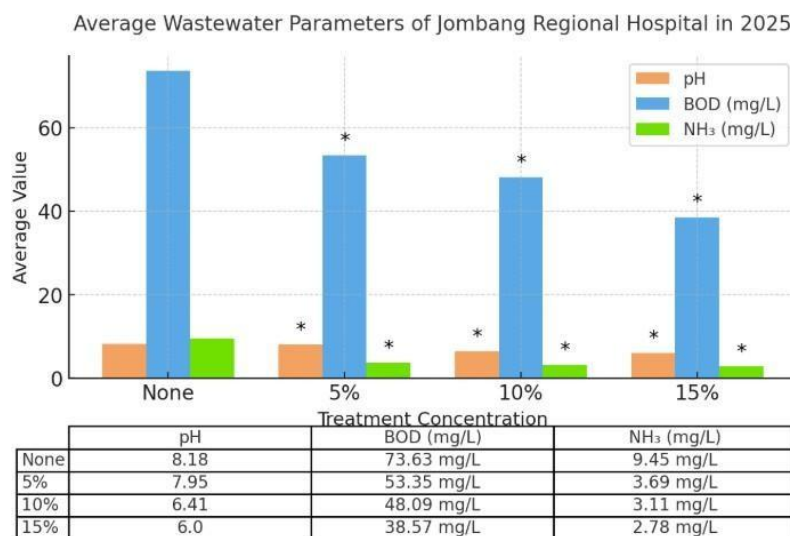
| Parameter                 | Results |
|---------------------------|---------|
| Flavonoid (mg/mL)         | 14.246  |
| Phenol (mg/mL)            | 3.648   |
| Acetic acid citrate (g/L) | 0.6954  |
| Lactic acid (mg/mL)       | 0.05    |
| Bromelain enzyme (U/mL)   | 0.174   |

This study measured changes in wastewater parameters, including pH, BOD, and NH<sub>3</sub>, at the Jombang Regional General Hospital Wastewater Treatment Plant after treatment with eco-enzymes at concentrations of 5%, 10%, and 15%. Incubation was carried out for five days at room temperature ( $\pm 27$ –30°C), and each treatment was replicated nine times.

The table in Figure 1 above shows the average wastewater parameters of Jombang Regional General Hospital for each treatment group. The graph shows a downward trend in pH as the enzyme concentration increases. The highest concentration has the greatest impact on the decrease in pH levels. A 5% eco-enzyme concentration resulted in only a slight decrease in pH to 7.96. In contrast, concentrations of 10% and 15% reduced the pH to 6.49 and 6.00, respectively. Nevertheless, all treatment groups remained within the effluent quality standards established by East Java Governor Regulation No. 72 of 2013, which specifies an acceptable pH range of 6.0–9.0.

The BOD parameter demonstrated a decreasing trend with increasing eco-enzyme concentration. The mean BOD level in the control group was 73.63 mg/L, exceeding the maximum permissible limit for hospital wastewater established under East Java Governor Regulation No. 72 of 2013 (30 mg/L). Higher eco-enzyme concentrations produced greater reductions in BOD levels. A 5% concentration resulted in a modest decrease to 53.35 mg/L, whereas concentrations of 10% and 15% reduced BOD levels to 48.09 mg/L and 38.57 mg/L, respectively.

A similar trend was observed for the NH<sub>3</sub> parameter, in which NH<sub>3</sub> concentrations decreased as the eco-enzyme concentration increased. The control group showed a mean NH<sub>3</sub> level of 9.45 mg/L, substantially exceeding the quality standard for hospital wastewater, which sets a maximum limit of 0.1 mg/L. The greatest reduction was observed at the highest eco-enzyme concentration. A 5% concentration reduced NH<sub>3</sub> levels to 3.69 mg/L, whereas concentrations of 10% and 15% produced NH<sub>3</sub> levels of 3.11 mg/L and 2.78 mg/L, respectively. Although none of the treatment groups achieved BOD and NH<sub>3</sub> levels below the established quality standards, the consistent decline in concentrations with increasing eco-enzyme dosage indicates that eco-enzymes show promising potential for improving wastewater quality.



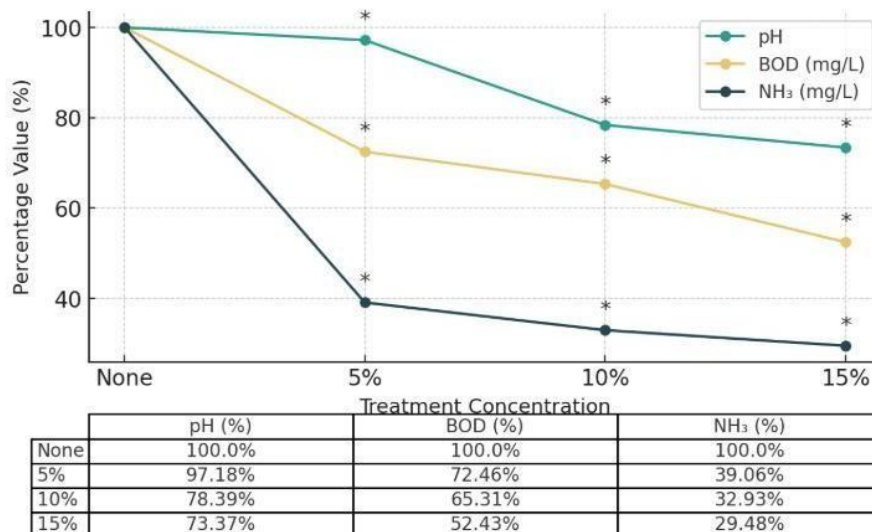
**Figure 1. Bar chart of Average Wastewater Parameters of Jombang Regional Hospital**

Based on Figure 2, the largest decrease in pH occurred at 15% (26.63%), followed by 10% (21.61%) and 5% (2.82%). This indicates that the eco-enzyme is more effective at lowering pH at higher concentrations, particularly at 15%. The percentage decrease in BOD showed that the 15% treatment achieved the best result, decreasing by 47.57%, followed by the 10% treatment, which decreased by 34.69%. The 5% treatment reduced BOD by only 27.54%. The percentage reduction in NH<sub>3</sub> showed that the most effective treatment was at 15% (70.52%), followed by 10% (67.07%) and 5% (60.94%). This indicates that the eco-enzyme is more effective at reducing wastewater parameters when applied at higher concentrations, specifically at 15%. Furthermore, the graph showing the percentage (%) decrease in wastewater parameters at each concentration can be seen in the image below:

Table 2 presents the results of the one-way ANOVA and Tukey HSD post hoc analyses evaluating the effects of eco-enzyme concentrations (5%, 10%, and 15%) on the pH, BOD, and NH<sub>3</sub> parameters of hospital wastewater at Jombang Regional Hospital in 2025. Overall, the statistical analysis demonstrated significant differences among treatment groups for all measured parameters ( $p < 0.05$ ), indicating that eco-enzyme concentration significantly influenced wastewater quality.

For the pH parameter, the one-way ANOVA yielded an F-value of 2.1114 with  $p < 0.05$ , indicating statistically significant differences among treatment groups. Tukey HSD analysis further showed that all eco-enzyme concentration groups differed significantly from one another. These findings indicate that increasing the eco-enzyme concentration led to a gradual, consistent decrease in wastewater pH, with the 15% concentration producing the greatest pH reduction.

Percentage (%) Reduction in Wastewater Parameters at Jombang Regional Hospital in 2025



**Figure 2. Graph showing the percentage (%) Reduction in Wastewater Parameters at Jombang Regional Hospital in 2025**

Similarly, the Biochemical Oxygen Demand (BOD) parameter showed significant differences among treatment groups, with a one-way ANOVA F-value of  $1.238 \times 10^3$  and  $p < 0.05$ . Post hoc Tukey HSD analysis demonstrated significant differences across all treatment combinations, with the largest difference observed between the control group and the 15% eco-enzyme concentration group. These findings suggest that the highest eco-enzyme concentration was the most effective in reducing BOD, suggesting enhanced enzymatic activity in degrading organic pollutant levels in hospital wastewater.

Regarding the ammonia (NH<sub>3</sub>) parameter, the one-way ANOVA yielded an F-value of  $3.422 \times 10^3$  with  $p < 0.05$ , indicating statistically significant differences among treatment groups. Post Hoc Tukey HSD analysis confirmed significant differences between all eco-enzyme concentrations. Among the treatments, the 15% eco-enzyme concentration achieved the greatest reduction in NH<sub>3</sub> levels, indicating enhanced pollutant removal efficiency at higher eco-enzyme concentrations.

**Table 2. Summary of One-Way ANOVA and Post Hoc Tukey HSD Results on the Effect of Eco-Enzyme Concentration on Hospital Wastewater Parameters**

| Parameter       | Statistical Test | F-value             | Significance (p-value) | Statistical Decision                 | Post Hoc Tukey HSD Result   |
|-----------------|------------------|---------------------|------------------------|--------------------------------------|---|
| pH              | One-Way ANOVA    | 2.1114              | $p < 0.05$             | Significant differences among groups | All eco-enzyme concentration pairs differed significantly; 15% showed the greatest pH reduction           |
| BOD             | One-Way ANOVA    | $1.238 \times 10^3$ | $p < 0.05$             | Significant differences among groups | All treatment combinations differed significantly; the largest difference was between the control and 15% |
| NH <sub>3</sub> | One-Way ANOVA    | $3.422 \times 10^3$ | $p < 0.05$             | Significant differences among groups | All concentrations differed significantly; 15% achieved the greatest NH <sub>3</sub> reduction            |

## DISCUSSION

### Bioactive Compounds in Eco Enzyme

Based on the results of phytochemical testing, quantitative characterization shows that eco-enzyme contains five main groups of bioactive compounds, namely flavonoids (14.246 mg/mL),

polyphenols (3.648 mg/mL), citric acid (0.6954 g/L), lactic acid (0.05 mg/mL), and bromelain (0.714 U/mL). These bioactive constituents may contribute to the reduction of wastewater parameters, including pH, BOD, and NH<sub>3</sub> in hospital wastewater at Jombang Regional General Hospital. The presence of these bioactive components, both biologically and chemically, significantly improves wastewater quality. The combination of phytochemicals indicates that eco-enzymes are not only environmentally friendly but also effective as biocatalytic agents for hospital wastewater treatment.<sup>13</sup>

The use of fruit peels and vegetable scraps in eco-enzyme production enhances biodegradability, enzymatic activity, and pollutant removal efficiency. During fermentation, microorganisms produce enzymes such as protease, lipase, and amylase that accelerate the degradation of organic and nitrogenous pollutants.<sup>14</sup> Similar findings were reported by those who observed that eco-enzymes derived from citrus waste improved biodegradation efficiency in greywater treatment. This aligns with the current results, confirming the role of diverse enzymes and organic acids in reducing pollutants.<sup>15</sup>

### **The Effectiveness of Eco Enzyme in Reducing the pH Parameter of Wastewater at Jombang Regional General Hospital**

The pH measurements of wastewater from Jombang Regional General Hospital following a 5-day incubation period showed a significant decrease with increasing eco-enzyme concentrations. The findings indicate a linear relationship between eco-enzyme concentration and the effectiveness of pH reduction in wastewater. The control group showed a mean pH value of 8.18, which remained within the effluent quality standards established by East Java Governor Regulation No. 72 of 2013, which range from 6.0 to 9.0. Progressive decreases in pH were observed with increasing eco-enzyme concentrations. The 5% treatment caused only a minor reduction (pH 7.95), whereas the 10% and 15% treatments showed more substantial decreases to 6.41 and 6.00, respectively. These results indicate that higher concentrations of eco-enzyme enhanced acidification, likely due to the presence of organic acids formed during fermentation. Overall, the pH values remained within the permissible range set by the environmental quality standards.

The fermentation of organic materials produces various organic acids that contribute to lowering pH levels. In wastewater treatment, this decrease in pH reflects increased biological activity and the accumulation of compounds generated through microbial decomposition.<sup>16</sup> Moderate acidification is considered beneficial because it supports the activation of certain enzymes and microbial processes responsible for ammonia (NH<sub>3</sub>) conversion. However, excessively low pH levels may inhibit microbial performance; therefore, maintaining pH within the range of 6–7 is generally considered optimal for efficient wastewater treatment.

Phytochemical analysis of the eco-enzyme solution showed the presence of lactic acid (0.05 mg/mL) and citric acid (0.6954 g/L), both of which contribute to the acidifying properties of the solution. Statistical analysis using one-way ANOVA showed significant differences in pH levels among the eco-enzyme treatment groups. These findings were further supported by the Tukey HSD post hoc test, which showed significant differences across all treatment pairs, indicating that the addition of eco-enzyme significantly reduced wastewater pH. One of the main mechanisms underlying this pH reduction is the accumulation of organic acids derived from orange and pineapple peels during the fermentation process. Through microbial metabolism, these acids accumulate and produce an eco-enzyme solution with an acidic pH ranging from 3.3 to 4.8.<sup>17</sup> When applied to wastewater, these organic acids neutralize alkaline conditions, thereby lowering pH levels and creating a more optimal environment for pollutant degradation processes.<sup>18</sup>

### **The Effectiveness of Eco Enzyme in Reducing BOD Parameters in the Wastewater Treatment Plant of Jombang Regional General Hospital**

BOD measurements of wastewater from Jombang Regional General Hospital after 5 days of treatment with eco-enzyme showed a significant decrease in BOD. The average BOD value in the control group was 73.63 mg/L, exceeding the threshold limit. The addition of eco-enzyme consistently decreased BOD as the concentration increased. BOD levels showed a clear decreasing trend with increasing eco-enzyme concentrations. The 5% treatment produced a moderate reduction, while the 10% and 15% concentrations led to larger decreases, reaching the lowest BOD value of 38.57 mg/L. This pattern indicates that higher eco-enzyme concentrations enhanced the biodegradation of organic matter, likely due to the increased activity of bioactive compounds and microbial enzymes.

A previous study has reported that eco-enzyme doses of 5–15 mL are effective in reducing BOD levels in domestic and industrial wastewater.<sup>19</sup> The effectiveness of homemade eco-enzymes in reducing BOD appears to be concentration-dependent. Fluctuations in BOD values among replicates may be due to variations in the organic composition of hospital wastewater, which can vary daily depending on service activities such as laundry, laboratory, and kitchen operations.

Statistical analysis using one-way ANOVA demonstrated significant differences in BOD values among the eco-enzyme treatment groups. These findings were further supported by the Tukey HSD post hoc test, which showed significant differences between all treatment pairs, indicating that increasing eco-enzyme concentration was statistically associated with greater reduction in BOD levels. The results suggest that eco-enzymes have promising potential for reducing BOD in hospital wastewater. However, further technical optimization remains necessary. Extending the incubation period beyond 5 days, combined with bioaugmentation or an aeration system, may enhance the efficiency of organic matter degradation.<sup>1</sup>

The activity of lactic acid bacteria (*Lactobacillus sp.*) during the fermentation of orange and pineapple peels using eco-enzymes produces lactic acid, which helps accelerate the decomposition of organic matter.<sup>20</sup> Lactic acid also converts organic matter into simpler compounds, accelerating the decomposition process. This decomposition indirectly reduces the BOD level in wastewater.<sup>21</sup> Eco-enzymes containing natural enzymes and organic acid compounds can efficiently reduce BOD levels by accelerating the biodegradation process of dissolved organic matter.<sup>22</sup>

### The Effectiveness of Eco Enzyme in Reducing NH<sub>3</sub> Levels in the Wastewater Treatment Plant of Jombang Regional General Hospital

Measurements of NH<sub>3</sub> levels in wastewater from Jombang Regional General Hospital showed a significant decrease following eco-enzyme treatment. The control group showed a mean NH<sub>3</sub> concentration of 9.45 mg/L, considerably exceeding the effluent quality standard of 0.1 mg/L established by the local government. Eco-enzyme concentrations of 5%, 10%, and 15% reduced NH<sub>3</sub> levels to 3.69 mg/L (60.94%), 3.11 mg/L (67.08%), and 2.78 mg/L (70.52%), respectively. Although none of the treatments reduced NH<sub>3</sub> levels below the regulatory threshold, the findings indicate that eco-enzymes effectively reduced ammonia concentrations in hospital wastewater. Phytochemical analysis of the eco-enzyme solution identified the presence of bromelain (0.174 U/mL) and flavonoids (14.246 mg/mL), which may support the growth of nitrifying microorganisms, such as *Nitrosomonas* and *Nitrobacter*. The results of the one-way ANOVA showed highly significant differences among treatment groups in reducing NH<sub>3</sub> levels, while the Tukey HSD post hoc test confirmed significant differences across all treatment pairs. These findings suggest that higher eco-enzyme concentrations were associated with greater NH<sub>3</sub> reduction efficiency.

The decrease in NH<sub>3</sub> levels in hospital wastewater may be attributed to the activity of proteolytic enzymes, such as bromelain derived from pineapple peel, as well as flavonoids from orange peel. These compounds may enhance eco-enzyme fermentation through their antioxidant and antimicrobial properties.<sup>23</sup> They accelerate organic matter degradation and promote microbial activity, including nitrifying microorganisms involved in the oxidation of NH<sub>3</sub> into nitrite and nitrate.<sup>24</sup> Although NH<sub>3</sub> levels consistently decreased with increasing eco-enzyme concentrations, variability among replicates was still observed. This variation may be related to differences in raw material composition and the lack of standardization in the fermentation processes. Therefore, further studies are required to identify the dominant microbes in eco-enzymes to better understand the biological mechanisms. In addition, pilot-scale investigations are required to assess the long-term stability and effectiveness of eco-enzyme applications in hospital wastewater treatment systems.

The reduction of NH<sub>3</sub> is important for improving water quality, as elevated ammonia concentrations are toxic to aquatic organisms and may contribute to environmental pollution. Enzymatic activity plays an important role in the degradation of nitrogen-containing compounds, including NH<sub>3</sub>, during wastewater treatment processes. In the present study, eco-enzymes demonstrated the potential to reduce NH<sub>3</sub> levels at specific concentrations, although lower concentrations appeared less effective, possibly due to insufficient enzymatic activity.<sup>25</sup> Nevertheless, this study has several limitations. The composition of homemade eco-enzymes may vary depending on the types of raw materials used and the fermentation conditions, potentially affecting treatment consistency and reproducibility. Furthermore, treatment effectiveness was evaluated only within a single incubation period (24 hours),

and the long-term stability and operational performance of eco-enzyme treatment were not assessed. Therefore, the findings may not fully represent the effectiveness of eco-enzymes under different operational conditions or extended treatment durations.

## CONCLUSION

The study demonstrated that eco-enzymes derived from fruit and vegetable waste contain bioactive compounds, including flavonoids, polyphenols, organic acids, and bromelain, which play essential roles in improving hospital wastewater quality. Eco-enzyme treatment for 5 days effectively reduced pH, BOD, and NH<sub>3</sub> levels, with maximum reductions of 26.59%, 47.62%, and 70.52%, respectively.

These results demonstrate that increasing the eco-enzyme concentration enhances both biochemical and microbial degradation processes, resulting in significant improvements in wastewater quality parameters. The findings highlight the potential of eco-enzymes as a low-cost, sustainable, and environmentally friendly alternative for hospital wastewater treatment, offering a promising substitute for conventional chemical-based methods. Future studies should therefore focus on optimizing fermentation conditions, extending treatment duration, and evaluating large-scale applications to assess long-term stability and economic feasibility.

## ETHICS APPROVAL

This study received ethical approval from the Health Research Ethics Committee of Jombang Regional General Hospital under approval number 48/KEPK/IV/2025.

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## COMPETING INTEREST

All authors declare that there are no conflicts of interest.

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This study received no external funding.

## UNDERLYING DATA

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

## DECLARATION OF ARTIFICIAL INTELLIGENCE USE

We confirm that there is no artificial intelligence (AI) used at any stage of the study, including data collection, analysis, visualization, etc. All work in this study was conducted manually by the authors without the assistance of AI-based tools or systems

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