



APPLICATION OF ECO-ENZYME FOR HOSPITAL WASTEWATER TREATMENT AT SHORT CONTACT TIME

PENGOLAHAN LIMBAH RUMAH SAKIT DENGAN EKO-ENZIM PADA WAKTU KONTAK PENDEK

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Abstract

Organic solid waste management has become an important issue due to rapid population growth. Eco-enzyme (EE) is one of the many green technologies needed today to treat organic solid waste. The purpose of this study was to utilize EE to treat hospital wastewater with dilutions of 5, 10, and 15% with a contact time of 30 minutes, thus meeting the established wastewater quality standards (Regulation of the Kementerian Lingkungan Hidup No. 11/2025). The bio-physicochemical characteristics analyzed included BOD, COD, TSS, pH, and *Escherichia coli* count. The results showed a significant reduction in all bio-physicochemical characteristics and met the applicable quality standards. The effectiveness of pollutant reduction was very significant, reaching 93.67% for BOD, 97.85% for COD, and 89.71% for TSS, with 15% EE treatment. The reduction in *E. coli* numbers was also very significant, reaching 99.85%. Therefore, because EE products are simple, affordable, and environmentally friendly, they are recommended as biological products for hospital wastewater treatment.

Keywords: Eco-enzyme; Hospital wastewater; Wastewater quality

Abstrak

Pengelolaan limbah padat organik telah menjadi isu penting karena pertumbuhan penduduk yang pesat. Eko-enzim (EE) adalah salah satu dari banyak teknologi hijau yang dibutuhkan saat ini untuk mengolah limbah padat organik. Tujuan penelitian ini adalah memanfaatkan EE dan memperoleh formula terbaik untuk mengolah limbah cair rumah sakit dengan pengenceran 5; 10; dan 15% dengan waktu kontak 30 menit, sehingga memenuhi standar mutu kualitas air limbah yang ditetapkan (Peraturan Menteri Lingkungan Hidup Republik Indonesia No. 11/2025). Karakteristik bio-fisikokimia yang dianalisis meliputi BOD, COD, TSS, pH, dan jumlah *Escherichia coli*. Hasil penelitian menunjukkan penurunan yang signifikan pada semua karakteristik biofisikokimia dan telah memenuhi standar baku mutu yang berlaku. Efektivitas penurunan pencemar sangat signifikan pada perlakuan EE 15% dibandingkan dengan pengenceran 5% dan 10%, yang mencapai 93,67% untuk BOD, 97,85% untuk COD, dan 89,71% untuk TSS. Penurunan jumlah *E. coli* juga sangat signifikan, hingga 99,85%. Oleh karena produk EE adalah produk yang sederhana, terjangkau, dan ramah lingkungan, maka direkomendasikan sebagai produk hayati untuk pengolahan air limbah rumah sakit.

Kata Kunci: Air limbah rumah sakit; Eko-enzim; Kualitas air limbah

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INTRODUCTION

Indonesia produced 16.9 million tons of trash annually in 2025, of which 39.67% was organic (Tim Asri Indonesia, 2025). The handling of organic waste is an urgent problem. Although eco-enzyme technology is now advancing quickly in Indonesia, its application as a bioremediation agent is still restricted. Eco-enzymes are still being produced and developed in Indonesia, and their use is still limited to wastewater treatment.

The use of environmentally friendly enzymes for wastewater has great potential as a natural and cost-effective solution for liquid waste treatment, functioning to reduce parameters such as COD, BOD, TSS, ammonia, and kill bacteria effectively at certain doses as an additive in wastewater treatment plants (Salvi et al., 2024; Kerkar & Salvi, 2020). Moreover, it can reduce dependence on chemicals and save the operational costs of wastewater treatment plants significantly. Therefore, before being discharged into recipient bodies of water, the wastewater must be properly treated.

It is known that fruit and vegetable waste can be fermented anaerobically for three months while sugar and water are added in a 3:1:10 ratio to produce eco-enzymes (Deepak et al., 2019). Eco-enzymes are frequently used due to their affordability and ease of manufacture (Tong & Liu, 2020). Large amounts of organic waste, such as fruits and vegetables, which are the fundamental components of eco-enzymes, are readily available and must be used to create eco-friendly products.

In the fermentation process, sugar is frequently utilized as a substrate; polyhydroxybutyrate, ethanol, pullulan, xanthan gum, and molasses are also frequently utilized as substrates in the lactic acid generation process (Zhang et al., 2021). Eco-enzymes are complex organic compounds that break down, alter, and catalyze processes. These compounds include protein chains, mineral salts, and juvenile hormones (Dhavale, 2020). Additionally, it is asserted that the eco-enzyme performs differently at various concentrations (Salvi et al., 2024).

Plant fertilizers (Tong & Liu, 2020), disinfectants (Larasati et al., 2020; Ginting et al., 2021), and fruit preservatives (Prasetyo & Maharani, 2024; Sari et al., 2020) are just a few examples of the many applications for eco-enzymes. Additionally, eco-enzymes are utilized as multipurpose solutions such as personal shampoos and detergents, household cleaners, and insect repellents for gardens (Purba et al., 2024). The use of EE for domestic wastewater processing has been widely carried out (Patel et al., 2021; Kerkar & Salvi, 2020; Salvi et al., 2024; Joseph et al., 2021) and also on laboratory scale (Wikaningrum & Dabo, 2022), however, it is still limited to hospital wastewater treatment. Thus, this study was conducted with the aim of treating hospital wastewater with EE and finding the ideal ratio to improve water quality according to the established standards, using various eco-enzyme dilutions. A very short contact period of 30 minutes was used to determine its effectiveness of hospital wastes in reducing the contaminants, as a comparison with previous studies that used a longer time, up to 21 days (Kerkar & Salvi, 2020; Salvi et al., 2024; Shivalik & Goyal, 2022; Tilana & Widyastuti, 2024). This research aimed to process hospital liquid waste with the addition of ecoenzymes. These results suggest that it could be used as an additive to hospital wastewater treatment plants to improve the efficiency of decomposing bacteria, break down organic matter, and reduce pollutants.

MATERIALS AND METHODS

Research was carried out experimentally and carried out on a laboratory scale. There are 4 research stages, namely 1) making eco-enzymes, 2) measuring bio-physicochemical characteristics of wastewater samples before administering eco-enzymes, 3) applying eco-enzymes to wastewater samples, and the last is 4) measuring again the bio-physicochemical characteristics of wastewater samples after administration of eco-enzymes.

Eco-enzyme was made from fruit and vegetable peel waste, water, and sugar/molasses in a 1:3:10 ratio (100 g of molasses, 300 g of vegetables/fruit, 1 L of water) (Deepak et al., 2019). Six grams of *Saccharomyces cerevisiae* yeast was added to speed up the fermentation time to just two weeks (Rahayu et al., 2023) compared to the three months required for the typical EE production process. Eco-enzyme was ready to use when the solution pH was 4 or lower.

Wastewater samples were collected from the Wastewater Treatment Plant (WWTP) of the hospital without chemical pre-treatment (L1) and the hospital with chemical pre-treatment (L2). Measurements of bio-physicochemical characteristics of wastewater samples were carried out before and after administration of eco-enzymes with contact time 30 minutes with 5; 10; and 15% EE. The parameters measured were pH, Biological Oxygen Demand (BOD), and Total Suspended Solids (TSS), using a Water Quality Checker (WQC), and a spectrophotometer for analysing Chemical Oxygen Demand (COD). For analysing the *E. coli* number, the membrane filter technique was used.

The next step was to apply eco-enzymes with 5; 10; and 15% dilution to two sources of wastewater samples, for thirty minutes of contact time. The bio-physicochemical characteristics of wastewater samples were measured before and after treatment with EE, then the reduction effectiveness was calculated using the following formula (Adiputra et al., 2021). In addition, descriptive analysis was conducted based on the Regulation of the Kementerian Lingkungan Hidup (MER) No. 11/2025 (Kementerian Lingkungan Hidup, 2025) concerning domestic wastewater quality standards (Table 1). Effectiveness of reduction (%) = $((C_0 - C_i) / C_0) \times 100\%$, where C_0 = initial concentration; C_i = final concentration.

Table 1. Hospital wastewater quality standards

Parameter	Unit	Threshold limit*
pH	-	6–9
BOD	mg/L	30
COD	mg/L	100
TSS	mg/L	30
Fecal <i>Coliform</i> (including <i>Escherichia coli</i>)	CFU/100 mL	3000

Note: * = based on MER No. 11/2025 (Kementerian Lingkungan Hidup, 2025)

RESULTS

The chemical and physical characteristics of water samples are shown in Table 2. After being treated with EE, all sample characteristics were measured referring to water quality parameters pH, BOD, COD, TSS, and *E. coli* numbers, and compared with quality standards of hospital wastewater, referring to Regulation of the Kementerian Lingkungan Hidup Republik Indonesia No. 11/2025 (Kementerian Lingkungan Hidup, 2025) concerning domestic waste quality standards.

Table 2. Physicochemical characteristics of wastewater samples

Parameter	Unit	L1	L2	Standard*
pH	-	10.7	8.3	6–9
BOD	mg/L	82.65	53	30
COD	mg/L	431	110.5	100
TSS	mg/L	138	36	30
<i>Escherichia coli</i>	(CFU/100 mL)	3,103.17	325	3,000

Note: * = based on MER No. 11/2025 (Kementerian Lingkungan Hidup, 2025)

A comparison of bio-physicochemical data is presented in Table 3 after treatment with ecological enzymes (5; 10; 15% v/v) at all sampling points, namely L1 and L2, for 30 minutes of contact. Sampling point L1 is a hospital Wastewater Treatment Plant (WWTP) without chemical pretreatment (chlorine), while L2 is a hospital WWTP with chemical pretreatment (chlorine). The WWTP at L2 is only used to collect wastewater before being sent to a third party. Meanwhile, the effectiveness of EE treatment is exhibited in Figures 1 and 2. The flow of WWTP is exhibited in Figure 3.

Table 3. Bio-physicochemical characteristics of wastewater samples after EE treatment

Parameter	Units	Standard	5%		10%		15%	
			L1	L2	L1	L2	L1	L2
pH	-	6–9	7.2	6.2	6	5.4	2.4	4.8
BOD	mg/L	30	32	32.7	18.4	30.5	5.23	26.7
COD	mg/L	100	80	102.8	26.17	88	9.25	62
TSS	mg/L	30	94.2	26	22.7	20	14.2	12
<i>Escherichia coli</i>	(CFU/100 mL)	3,000	705	187	29	46	4.5	9

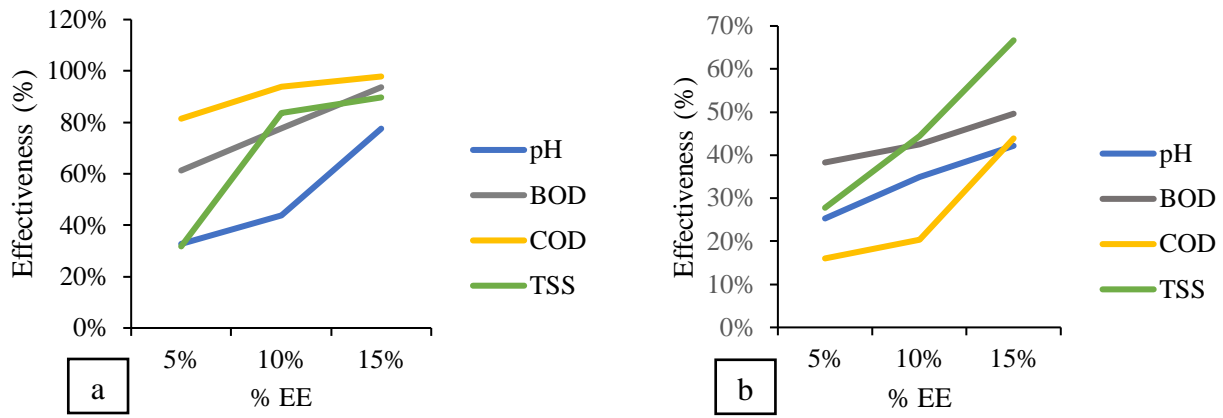


Figure 1. Effectiveness of EE treatment in improving hospital wastewater quality for L1 (a) and L2 (b)

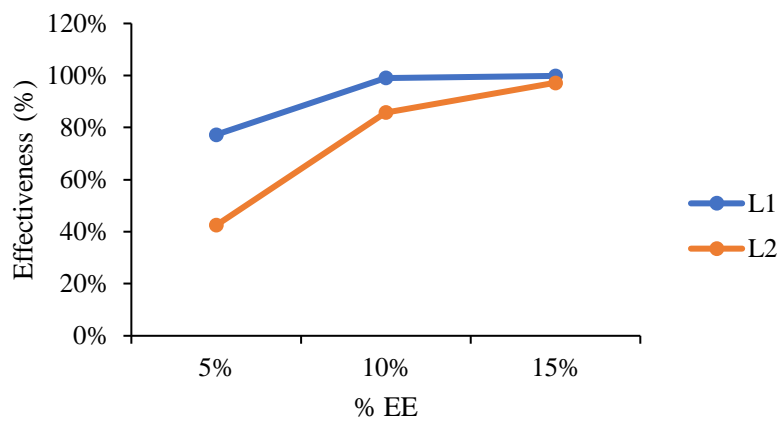


Figure 2. The effectiveness of *Escherichia coli* numbers

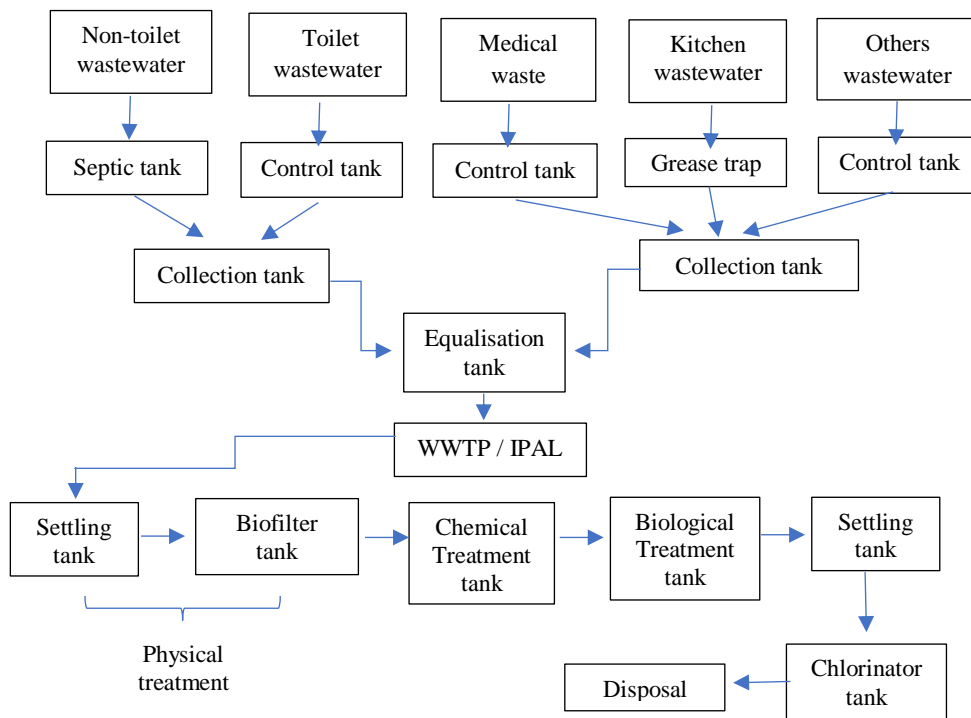


Figure 3. The flow of the wastewater treatment plant (adopted from Rahayu, 2023)

DISCUSSION

The hospital's liquid waste used in this study was supplied by the hospital's wastewater treatment facility and comprises household wastewater from restrooms, kitchens, and laundry rooms, derived from patients, visitors, and hospital staff. Hospitals generate liquid waste from both medical and non-medical operations, which may include radioactive elements, dangerous chemicals, and infectious bacteria. This category of waste comprises laboratory wastes (chemical, biological, and physical pollutants, including hazardous waste), clinical wastes (wound cleaning fluids, blood, and IV fluids), and household wastes (from the toilet, kitchen, and laundry). Some hospital liquid waste can be categorized as domestic waste (such as from toilets, bathrooms, kitchens, and laundry), but hospitals also produce medical/infectious and chemical waste that is much more dangerous, so all liquid waste must be processed in an integrated WWTP, not just disposed of like ordinary domestic waste, because it contains pathogenic microorganisms, chemicals, and drug residues. Thus, in this study, all the samples were derived from the WWTP of the hospitals.

Based on Table 2, all physicochemical parameters of hospital wastewater samples before EE treatment were higher than the quality standard. This was in line with observations made by Yuwati (2021), Dwitaningtyas (2025), Joseph et al. (2021), and Salvi et al. (2024), that the quality of hospital wastewater, including domestic wastewater, had high BOD, COD, and TSS. The BOD and COD values were related to the type and source of hospital wastes, which were from toilet and non-toilet, kitchen, medical wastes, and also from laboratory wastes. The higher organic pollutants, the higher BOD and COD values, which correlated with oxygen use for biochemical processes. The BOD and COD values in this study were above the standards, more than 30 mg/L (BOD) and 100 mg/L (COD). Thus, it needs to be treated to meet the standard.

The parameter of TSS is a measure of haze in water, due to the presence of suspended particles such as silt, algae, microorganisms, and organic matter, thus blocking light from passing through and making it opaque (Kerker & Salvi, 2020). In hospital wastewater, high TSS values are caused by contamination from organic and inorganic matters from the decomposition of incompletely treated hospital waste. The TSS value in this study was not too high, namely 138 mg/L (L1) and 36 mg/L (L2) before EE treatment. This result was in line with Rahayu's study (2023) and Dirgahayu et al. (2024), which found 71.43–100 mg/L of TSS value. The lower TSS values, such as at L2, are due to the application of conventional pre-treatment processes. However, most of the TSS value in hospital wastewater was still above the standard (MER No. 11/2025) (Kementerian Lingkungan Hidup, 2025).

Monitoring the physicochemical quality of hospital wastewater effluents showed that their characteristics change globally according to their origin, which is linked to the activities in the various establishments' premises. Thus, several physicochemical parameters showed a relatively significant variability at the same sampling point and during the study period. This variability may be associated with the release of high-complexity effluents due to the different uses of water (Grimah et al., 2024). Thus, the pH of the wastewater remains relatively neutral to alkaline for all sampling points. This alkaline trend could be related to their organic nitrogenous contents (i.e., ammonia <0.031 mg/L) with generally alkaline characteristics (Svehla et al., 2015) and to their low content of nitrate (<0.016–10.58 mg/L). This effluent's alkalinity would also be caused by the discharge of chemical solutions, especially alkaline ones, which originate from laboratories and medical waste, such as at L1. However, the pH at L1 was already normal before the pre-treatment process. This study found the neutral to alkaline pH in wastewater samples, which was the same as that of Yuwati (2021), Dwitaningtyas (2025), and Dirgahayu et al. (2024).

Meanwhile, high levels of *E. coli* were found in wastewater samples, reaching 3,103.17 and 325 CFU/100 mL in L1 and L2, respectively. This indicates that the quality is very poor or above the safe standard based on MER No. 11/2025 and must be treated in an environmentally friendly manner, one of which is with eco-enzymes. Reports from various studies indicate that most hospital wastewater has been contaminated by high levels of *E. coli*, 1,800–35,000 CFU/100 mL (Nugroho & Purwaningrum, 2025; Ulfa et al., 2020; Wahyuningsih et al., 2020). This study shows that the level of *E. coli* contamination in large hospitals (L1) is higher than in small hospitals (L2). This also occurred in large hospitals in Bangkalan, East Java, studied by Wahyuningsih et al. (2020), produced

very high levels of waste, up to 35,000 CFU/100 mL *E. coli*, and even up to 550,000 CFU/100 mL *E. coli* in a central hospital in Wisconsin, USA (Bojar et al., 2025).

Comparing the two wastewater sources, the wastewater from L1, which originated from no chemical pre-treatment of WWTP, showed poorer wastewater quality compared to the wastewater from L2, which had already undergone chemical pre-treatment. Nevertheless, the wastewater quality indicators for both still did not meet the established quality standards. The implementation of chemical pre-treatment was still unable to improve the quality and meet the applicable standards. Therefore, further treatment is needed to achieve the standards, one of which is the addition of EE as an additive in the wastewater treatment plant.

The results of bio-physicochemical data are presented in Table 3 after treatment with eco-enzymes (5; 10; 15%) at all sampling points, namely L1 and L2, for 30 minutes of contact. Sampling point L1 is a hospital WWTP without chlorine pretreatment, while L2 is a hospital WWTP with chlorine pretreatment. The WWTP at L2 is only used to collect wastewater before being sent to a third party, so no processing is carried out. However, most hospital wastewaters have been treated using physicochemical methods, including collection, separation, filtration, settlement, chemical treatment using $Al_2(SO_4)_3$ or alum, and an aeration process to meet the standard, then finally adding chlorine to reduce *E. coli* number (Wahyuningsih et al., 2020; Yuwati, 2021; Rahayu, 2023).

The pH values in all EE treatments moved to alkaline conditions in 30 minutes contact time. This study showed that the higher EE concentrations were the more acidic, for both sampling points. This was caused by the acidic nature of the eco-enzyme, which has a pH of 4 or less (Patel et al., 2021; Kerkar & Salvi, 2020; Salvi et al., 2024, Joseph et al., 2021), whereas in this study, the pH of EE was 3,8. The 5% and 10% EE were within normal pH levels for aquatic environments. However, compared to BOD, COD, TSS, and *E. coli* values, an EE of 15% represented a significant reduction for wastewater remediation. To address acidic pH, wastewater is diluted before being discharged into water bodies, until the pH returns to normal and is environmentally safe. The studies by Salvi et al. (2024), Patel et al. (2021), Kerkar and Salvi (2020), and Joseph et al. (2021) showed the same thing. The higher the EE concentration, the more acidic the solution. Then, it will decrease again when a higher dosage of EE is added with a contact time of 5 days.

Meanwhile, BOD values showed a decrease in all eco-enzyme treatments (Table 3). This indicates that eco-enzyme treatment of wastewater can support microbial activity in optimally decomposing organic matter. This is related to the biological process in which eco-enzymes convert organic matter into simpler particles (Urry et al., 2021) using dissolved oxygen to decompose organic matter into carbon dioxide and water (Manahan, 2017). Furthermore, the action of eco-enzymes during fermentation also plays a role in increasing dissolved oxygen in water samples. This study showed that higher EE concentrations resulted in a decrease in BOD values. However, according to Putri and Wati (2023), BOD values increased again in the 20% and 30% EE treatments. This is because the solution was too acidic, preventing biochemical processes from running smoothly. The same thing happened with COD (Table 3). The higher the EE treatment percentage, the lower the COD value. This means that at 15% EE, the quality of hospital wastewater has improved and is already below the recognized standard threshold. This study showed more effective BOD and COD reduction at L1, reaching 93.67% and 97.85%, respectively. Although the effectiveness of BOD and COD reduction at L2 was not as high as at L1, reaching only 49.62% and 43.89% at L2, it was still below the applicable standards.

Salvi et al. (2024) found lower reduction effectiveness than this study, reducing only 90.54% for BOD and 83.33% for COD with a 20% EE treatment and a longer contact time of 5 days. The use of eco-enzyme often requires a long incubation period of 10–20 days for a significant BOD/COD reduction of 79.75% (Widyastuti et al., 2023). However, in this study, the incubation time could be shortened to just 30 minutes, resulting in improved wastewater quality. This is very effective and efficient when used on a large scale or in industrial settings, as it saves costs and time.

The TSS value also showed conditions above the quality standard at all sampling points and decreased after being given EE treatment, which was significantly purified in 15% EE. High TSS levels in domestic wastewater, including hospital wastewater, were caused by the large number of

water-soluble organic and inorganic compounds, minerals, and salts (Patel et al, 2021; Salvi et al, 2024; Joseph et al, 2021), which come from various wastes from anthropogenic activities. This is related to the biological process that occurs where the enzymes contained in the eco-enzyme solutions change it into simpler suspended solid particles. Organic nitrogen and ammonium, contained in wastewater, are then converted into nitrate by bacteria such as *Nitrosomonas* and *Nitrobacter* (Urry et al., 2021).

In accordance with Kementerian Lingkungan Hidup, Regulation No. 11/2025 (Kementerian Lingkungan Hidup, 2025), the biological properties of water samples, including *E. coli*, which is included in the fecal *Coliform* group, were also measured. Using the Total Plate Count (TPC) method, *E. coli* data in 100 mL samples are shown in Tables 2 and 3, before and after EE treatment. The number of *E. coli* decreased significantly in all samples treated with EE (Table 3). This reduction reached 99.85% (L1) and 97.23% (L2), respectively, at 15% EE treatment. This finding was higher than that reported by Yuwati (2021), who found an 83.27% reduction in *E. coli* after chlorination. Before EE treatment, the *E. coli* number was 325–3,103 CFU/100 mL, higher than the quality standard (MER No. 11/2025). This was in line with research reports by Putri and Wati (2023), Sebayang et al. (2025), and Imamah (2025), which found that most domestic wastewater in Indonesia is contaminated with *Coliform* and *E. coli*. This can even reach 9,300 CFU/100 mL in a large hospital in Bojonegoro city (Nugroho & Purwaningrum, 2025). Therefore, it is found that the higher EE concentration indicates a reduction in *E. coli* in wastewater. The 15% EE treatment can inhibit the growth of *E. coli*, indicating that a significant number of bacteria are killed due to the enzymes or the acidic nature of the eco-enzyme.

CONCLUSION

Based on the results obtained, the use of environmentally friendly enzymes can be implemented in wastewater treatment plants (WWTPs) in hospitals and other industries, with 15% EE treatment showing the best results in improving the quality of wastewater samples. The use of EE is highly recommended for restoring the quality of domestic wastewater, as it is an environmentally friendly, easy, and inexpensive bioremediation agent for wastewater. All bio-physicochemical profiles of hospital WWTPs showed significant improvements in water quality, with 15% EE treatment being the most optimal for a short contact time of 30 minutes. However, it is important to note that increasing the EE dosage does not always improve water quality; other factors, such as temperature, pH, contact time, and waste load, also play a role. Another advantage of using EE as a wastewater bioremediation agent is its fresh aroma, which minimizes unpleasant odors during the process and makes it safe for discharge into water bodies. A significant obstacle in hospital wastewater treatment is the formation of relatively large amounts of sediment and acidic pH. This can be overcome by filtering the wastewater multiple times before EE treatment and using smaller doses of EE and shorter contact times.

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