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Analysis of Eco-Enzyme Potential to Reduce BOD, COD, Oil and Grease Concentrations in Raw Water for Treated Water in Karawang: A Laboratory Experiment

Gina Lova Sari*, Kania Ratnawati, Wilma Nurrul Adzillah, Devita Fitria Alam Sari, Saidah

Study Program of Environmental Engineering, Faculty of Engineering, Universitas Singaperbangsa Karawang, Karawang, Indonesia

* Correspondence: ginalovasari@gmail.com

ABSTRACT

Organic pollutants are produced by domestic household waste, so eco-enzymes are potentially used as bioremediation agents for community-based water quality and pollution control, as has been conducted in the Citarum Harum Program. However, scientific information regarding eco-enzymes characteristics and their ability to degrade BOD, COD, and oil and grease is limited, especially in surface water used for raw water for treated water. This research examines eco-enzymes potential as biocatalysts to reduce BOD, COD, and oil and grease in raw water for treated water in Karawang primary irrigation canals. Water samples were collected from Karawang primary irrigation canals according to the Indonesian National Standard No. 8995:2021. Meanwhile, the eco-enzyme is made from fruit peel and molasses for 624 days. The experiments were carried out with five variations of raw water and eco-enzymes mixtures (v/v) of 3,000.00:0.00 mL; 2,900.00:100.00 mL; 2,850.00:150.00 mL; 2,800.00:200.00 mL; and 2,750.00:250.00 mL with a batch flow system for ten days. The results showed that eco-enzymes contained protease, amylase, and lipase as much as 1,231.00 U/mL; 4.00 U/mL; and 9.69 U/mL, respectively, with a pH of 3.80. The concentrations of BOD, COD, and oil and grease in the current study tended to increase from 222.00 mg/L; 1,047.00 mg/L, and 2.20 mg/L to 505.00-593.00 mg/L; 1245.50-1941.50 mg/L; and 6.60-9.00 mg/L, respectively, which might be due to the addition of organic compounds through mixing eco-enzymes that makes the biodegradation process takes longer. These results indicate that eco-enzymes are less appropriate for surface water treatment with continuous flow systems and more suitable for biological treatment of communal wastewater in batch flow systems.

Keywords: BOD, COD, eco-enzyme, oil and grease, raw water for treated water

INTRODUCTION

Eco-enzyme, a dark brown liquid with a strong acidic or fresh aroma, is an organic liquid produced through a simple fermentation process. This process involves fruit or vegetable waste, sugar, and pure water, all acted upon by microorganisms (Benny *et al.*, 2023; Ratnawati, *et al.*, 2023; Wikaningrum & Pratamadina, 2022). Eco-enzyme's versatility is evident in its application as a bioremediation technology across various environmental components, including river-water (Benny *et al.*, 2023; Gaspersz & Fitrihidajati, 2022).

Eco-enzyme acts as a biocatalyst that can reduce pollutant concentrations in water by aiding the degradation of organic substances in wastewater or river water (E. Agustina, 2022; Benny *et al.*, 2023; Khasanah & Rosariawari, 2022). For instance, according to a study by Ramadhana (2023), co-enzymes at a concentration of 10.00% can reduce chemical oxygen demand (COD) and total suspended solids (TSS) by 59.00 mg/L and 68.00 mg/L, respectively. Similarly, Gaspersz & Fitrihidajati (2022) reported that 5.00% and 10.00% of eco-enzyme could reduce biochemical oxygen demand (BOD) by 0.87 mg/L and 0.66 mg/L, respectively. Furthermore, A. Agustina (2021) observed that eco-enzyme application in Tukad Bandung also reduced BOD as much as 0.90 mg/L.

In addition, eco-enzymes have been used in the water flow from Barapula Drain to the Yamuna River in India (Kumar *et al.*, 2019), which reportedly reduced total dissolved solids (TDS), TSS, BOD, COD, and nitrate each by 139.00 mg/L; 74.00 mg/L; 459.00 mg/L; 153.00 mg/L, and 2.15 mg/L. Given its benefits, eco-enzymes hold the potential as a bioremediation technology that is affordable, easily produced, and suitable for sustainable use (A. Agustina, 2021; Alamri *et al.*, 2023; Gaspersz & Fitrihidajati, 2022; Jelita, 2022; Ramadhana, 2023; Widiani & Novitasari, 2023).

As part of the "Citarum Harum" program, eco-enzymes were directly applied to the Citarum Watershed. As much as 2000 L and 1000 L of eco-enzymes were added in the upstream and downstream zones of the Citarum Watershed, respectively. However, the precise effectiveness of eco-enzyme in reducing pollutant in the Citarum Watershed remains unclear. This lack of scientific data is a pressing issue, as it hinders the widespread use of eco-enzymes beyond preliminary implementations.

Moreover, considering its geographical location, the downstream zone of the Citarum Watershed in Karawang is a critical area. A significant portion of the water flows through primary irrigation canals, which are used as a raw water source for treated water in the area. Within a 50-kilometre radius, three government-owned intake points capture raw water for treated water.

The current state of the Citarum Watershed water quality is a matter of urgent concern. The water, which includes BOD, COD, oil and grease, may be at risk of increased pollutant concentrations due to the dense residential areas along the primary irrigation canals. Previous research has shown that BOD, COD, and oil and grease levels in the raw water of the canals have already reached 23.30 mg/L, 77.20 mg/L, and 162.80 mg/L, respectively (Sadidan *et al.*, 2024; Sari *et al.*, 2024). his situation underscores the urgent need for our proposed research.

Therefore, the primary goal of this study is to investigate the potential of eco-enzyme as a biocatalyst to reduce BOD, COD, oil and grease levels in the raw water for treated water in the primary irrigation canals of Karawang. The outcomes of this research could have a profound impact on sustainable water quality management and community-based pollution control efforts, thereby contributing to the achievement of the objective of the Citarum Harum Program.

METHODS

Water Sample Collection

The raw water for treated water samples were collected at a single point representing domestic activity along the primary irrigation canals in Karawang (see **Figure 1**). Sampling was performed at three different points and at three distinct depths using a water sampler, after which the samples were composited in accordance with Indonesian National Standard No. 8995:2021 (Badan Standarisasi Nasional, 2021). The depths selected for sampling were at the surface; 1.00 meter; and 2.00 meters below the water surface.

Figure 1. Water Sampling Location in the Primary Irrigation Canals of Karawang

Eco-Enzyme

The eco-enzyme used in this study was made from the waste peels of sweet oranges, citrus, apples, papayas, pineapples, pears, dragon fruit, guava, bananas, watermelon, jicama, yam peels, mustard greens, kedondong (ambarella), and molasses, aged for 624 days. This eco-enzyme was obtained from the Huma Eco-Enzyme Community, a local producer in Karawang Regency that frequently supplies eco-enzymes for various clean water management activities.

Analysis of enzyme characteristics included amylase, lipase, and protease activities. These enzyme activities were tested using specific methods: Fuwa (spectrophotometry) for amylase activity, titration for lipase activity, and Kunitz (spectrophotometry) for protease activity, as detailed in Table 1.

Table 1

Types of Enzymes in Eco-Enzyme and Testing Methods

Type	Testing Method
Amylase Activity	Fuwa (Spectrophotometry)
Lipase Activity	Titration
Protease Activity	Kunitz (Spectrophotometry)

Experiment

This study is an experimental laboratory-scale investigation conducted over 10 days, with a key feature being a batch flow system. The bioremediation process involved mixing raw water samples with eco-enzymes, which were then placed into 3,500 mL glass reactors in duplicate.

The mixtures of raw water and eco-enzyme were systematically varied across several concentration levels, ranging from raw water without eco-enzyme (control) to mixtures with higher concentrations of eco-enzyme. This meticulous approach determined the effective ratio for reducing BOD, COD, and oil and grease concentrations. The detailed composition of raw water and eco-enzyme mixtures used in the experiment is shown in **Table 2**.

Table 2
Variations in Raw Water and Eco-Enzyme Composition in the Experiment

Composition Variation (mL)		Retention Time (days)	Reactor Code
Raw Water	Eco-Enzyme		
3,000.00	0.00	10.00	Control
2,900.00	100.00	10.00	A
2,850.00	150.00	10.00	B
2,800.00	200.00	10.00	C
2,750.00	150.00	10.00	D

Furthermore, measurements were performed during the experiment for several primary and environmental parameters. Information on the parameters, measurement periods, and testing methods is provided in **Table 3**. Data from the experiment were processed through simple data tabulation. The tabulated results were analyzed using qualitative descriptive methods, comparing the experimental outcomes with those from similar prior studies to derive fundamental insights related to the study's objectives.

Table 3
Parameters and Testing Methods

Parameter	Measurement Period	Testing Method/Instrument
Temperature	Daily	Thermometer
pH	Daily	pH meter
BOD	Initial raw water characteristics and Day 10	Winker (Badan Standarisasi Nasional, 2009)
COD	Initial raw water characteristics and Day 10	Titrimetric (Badan Standarisasi Nasional, 2019)
Oil and Grease	Initial raw water characteristics and Day 10	Gravimetry (Badan Standarisasi Nasional, 2011)

RESULTS AND DISCUSSION

Characteristics of Raw Water for Treated Water in the Primary Irrigation Canal of Karawang

The results revealed a concerning trend-the concentrations of BOD, COD, oil and grease in the raw water for treated water in Karawang significantly exceeded the water quality standards for the second water class in Indonesia. This is a matter of immediate concern. **Table 4** provides detailed information on these characteristics. The high BOD, COD, oil and grease levels in the water samples are likely due to domestic waste discharge around the sampling location (Sadidan *et al.*, 2024; Sari *et al.*, 2024).

Table 4
Characteristics of Raw Water in the Primary Irrigation Canals of Karawang

Parameter	Value	Class II Water Quality Standard*
pH	7,10	6,00-9,00
BOD	222,00 mg/L	3,00 mg/L
COD	1.047,00 mg/L	25,00 mg/L
Oil and Grease	2,20 mg/L	1,00 mg/L

* Source: Government Regulation of the Republic of Indonesia No. 22 of 2021 on Environmental Protection and Management

Characteristics of the Eco-Enzyme

The eco-enzyme used in this study had a pH of 3.80 (see Table 5). According to Benny *et al.* (2023), one factor affecting the pH of eco-enzymes is the fermentation period; the longer the process, the lower the pH. This finding aligns with the current study, as the fermentation of organic materials from various fruit peels continued up to 624 days, resulting in a pH level of around 3.80.

Additionally, the pH level observed in this eco-enzyme is consistent with previous research findings, which report eco-enzyme pH values between 3.50 and 3.70 (Suprayogi *et al.*, 2022). These studies, conducted by Putra & Suyasa (2022); Widiani & Novitasari (2023), have contributed significantly to our understanding of eco-enzymes. Their further explain that an optimal eco-enzyme should have a pH below 4.00, as a low pH indicates high levels of acetic and lactic acid produced through the anaerobic decomposition process facilitated by microorganisms during fermentation (Gumilar *et al.*, 2023; Larasati *et al.*, 2020; Rasit *et al.*, 2019; Samriti *et al.*, 2019; Suprayogi *et al.*, 2022)

Table 5
Characteristics of the Eco-Enzyme

Parameter	Value
pH	3,80
Amylase Activity	4,00 U/mL
Lipase Activity	9,60 U/mL
Protease Activity	1.231,00 U/mL

Table 5 also indicated the presence of several biocatalytic enzyme activities in the eco-enzyme—specifically, amylase, lipase, and protease—with varying activity levels. The presence of these enzymes is consistent with prior studies that produced eco-enzymes from tomato and citrus waste, kitchen organic waste, fruit peels, and other organic materials (Permatananda *et al.*, 2023; Rasit *et al.*, 2019; Samriti *et al.*, 2019; Selvakumar & Sivashanmugam, 2017). However, the activity level of each enzyme depends on the type of waste used as the eco-enzyme base material (Samriti *et al.*, 2019).

The three enzyme activities in the eco-enzyme of protease, lipase, and amylase are of paramount importance, as they play essential roles in breaking down complex organic materials like proteins, fats, carbohydrates, and others into simpler compounds (Widyastuti, Sutrisno, *et al.*, 2023). The highest enzyme activity identified is protease at 1,231.00 U/mL, which aids in breaking down protein into smaller molecules (Benny *et al.*, 2023).

Lipase activity, measured at 9.60 U/mL, is significantly lower than protease, suggesting a relatively low citric acid content (Kavitha *et al.*, 2014; Rasit *et al.*, 2019). This result aligns with the findings of Selvakumar & Sivashanmugam (2017), who reported that the highest lipase activity in eco-enzymes derived from organic waste was 39.00 U/mL. They explained that optimal lipase activity forms at a pH range of 8.00–9.00. Lipase activity assists microorganisms in breaking down oils, fats, and peptides, thereby facilitating the decomposition process (Benny *et al.*, 2023; Permatananda *et al.*, 2023; Selvakumar & Sivashanmugam, 2017).

Amylase activity, like protease, also plays a crucial role, generally serving as a biocatalyst for microorganisms to decompose compounds in detergents, agricultural waste, and carbohydrates into simpler molecules (Benny *et al.*, 2023; Rasit *et al.*, 2019). In the current study, the amylase activity in the eco-enzyme was measured at only 4.00 U/mL, a level influenced by pH value of eco-enzyme. Optimal amylase activity typically requires a pH range of 6.00–7.00 (Rasit *et al.*, 2019).

Bioremediation of Raw Water for Treated Water Using Eco-Enzyme

As shown in **Figure 2**, During the bioremediation process, pH fluctuations were observed across all experimental variations during the bioremediation process. In the control reactor, pH remained neutral over the 10-day period of bioremediation, ranging between 6.60 and 7.20. In contrast, the addition of eco-enzyme led to a decrease in pH for other variations in reactors A, B, C, and D, which reached values of 4.10, 4.00, 3.90, and 3.90, respectively. The decrease can be attributed to the acidic pH of the eco-enzyme (3.80), with more generous eco-enzyme concentrations causing lower pH values in the mixtures.

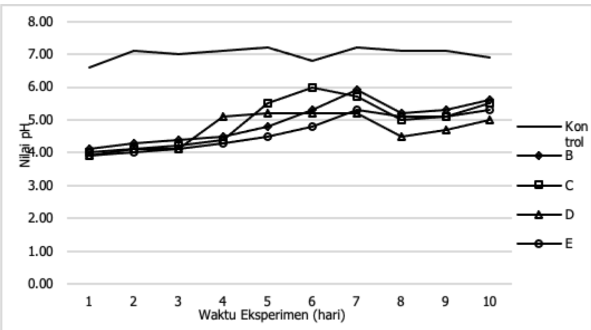

Figure 2. pH Changes During the Experiment

Furthermore, **Figures 3 and 4** illustrate the analysis of BOD and COD levels in the raw water for treated water before and after eco-enzyme addition. These figures reveal a significant finding: only the control reactor, with no eco-enzyme added, exhibited a reduction in BOD and COD levels. This underscores the crucial role of indigenous microorganisms in the raw water for treated water in the primary irrigation canal of Karawang to conduct natural bioremediation under aerobic conditions.

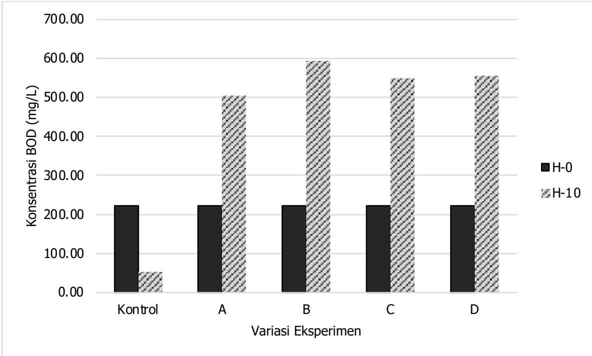

Figure 3. BOD Concentration Changes During the Experiment

However, different results were noted in reactors A, B, C, and D, with BOD concentrations increased to 505.00 mg/L, 593.00 mg/L, 549.50 mg/L, and 555.00 mg/L, respectively. These findings are in line with previous studies by Tilana & Widyastuti (2024) and Muliarta *et al.* (2023), which also concluded that eco-enzymes were ineffective in reducing BOD and COD levels in landfill leachate.

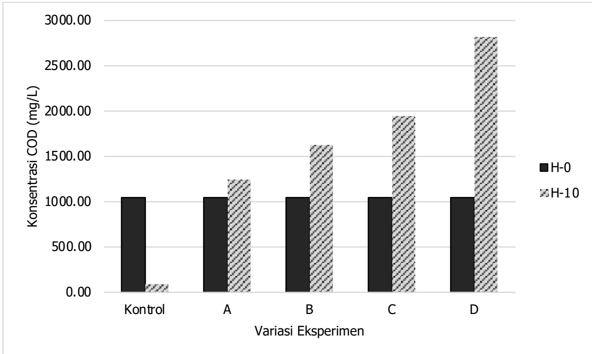

Figure 4. COD Concentration Changes During the Experiment

The suboptimal activity of protease and amylase enzymes during the experiment underscores the importance of understanding enzyme activity in the context of organic pollutant decomposition. This understanding is crucial as it may influence the inhibition of the breakdown of proteins, carbohydrates, and other organic compounds. Despite the relatively high protease activity in the eco-enzyme at 1,231.00 U/mL, the pH during days 0 to 10 was not within the optimal range for supporting its catalytic function (pH 3.90–5.90). Protease activity is most effective at a pH of 6.50–7.00 (Arun & Sivashanmugam, 2015; Gómez *et al.*, 2019).

Conversely, the amylase enzyme activity was relatively low, only 4.00 U/mL, and was also influenced by the acidic pH (3.90–5.90). It's important to note that amylase activity can be optimal in the pH range of 6.00–8.00 (Arun & Sivashanmugam, 2015; Liew *et al*., 2020). These findings not only contribute to our understanding of amylase activity but also have potential applications in various fields. Galintin *et al*. (2021) reported that amylase activity formed at a pH of 3.07 was 25.00–26.00 U/mL, further highlighting the potential of these findings. Similarly, Suliestyah *et al*. (2022) reported that eco-enzymes derived from papaya peel, dragon fruit, and oranges had an amylase activity of 2.15 U/mL formed at pH 3.17.

However, refer to the definition of BOD and COD, which are the oxygen demand for organic material decomposition. In that case, it is possible that another factor affecting the decomposition process is the lack of oxygen. The higher the BOD and COD concentrations, the greater the oxygen demand for aerobic bioremediation (Benny *et al*., 2023). Given this condition, the aerobic bioremediation process using eco-enzymes may require longer. Some studies report that BOD and COD reduction occurs on day 20 (Widyastuti, Sutrisno, *et al*., 2023), day 30 (Patel *et al*., 2021), or day 70 (Hemalatha & Visantini, 2020). This suggests that the degradation process may not be effective in a continuous flow system due to the relatively short duration.

Other studies indicate that the catalytic function of protease and amylase activity works optimally in anaerobic conditions, enabling microorganisms to solubilize and break down carbohydrates, proteins, and lignin (Liew *et al*., 2020; Roman *et al*., 2006). The addition of eco-enzymes, with its disinfectant properties, in aerobic conditions may inhibit the growth and reproduction of aerobic microorganisms, which drive suboptimal activity (Hemalatha & Visantini, 2020; Patel *et al*., 2021; Suliestyah *et al*., 2022; Tilana & Widyastuti, 2024; Vidalia *et al*., 2023).

Furthermore, the concentration of oil and grease in all experimental variations increased. The initial concentration of oil and grease in the raw water for treted water was 2.20 mg/L, which, over 10 days, increased to 5.10 mg/L, 6.60 mg/L, 7.50 mg/L, 8.40 mg/L, and 9.00 mg/L for the control reactors A, B, C, and D, respectively (see **Figure 5**).

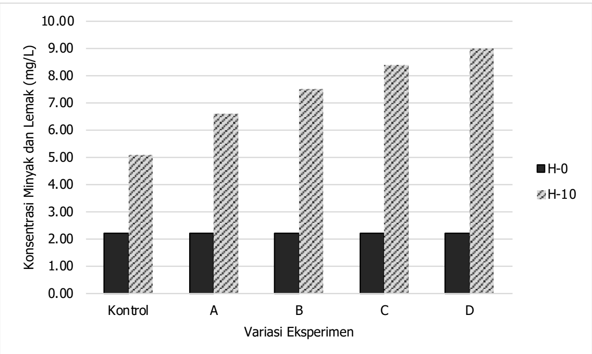

Figure 5. Changes in Oil and Grease Concentration during the Experiment

The increase in oil and grease concentrations in reactors A, B, C, and D can be attributed to the potential presence of oil and grease in the eco-enzyme, as its raw material was predominantly fruit peel waste. As explained by Kurniawan *et al*. (2008); Nianti *et al*. (2018), citrus peels contain essential oils, which could contribute to the elevated oil and grease levels in the experiment.

Additionally, the low lipase enzyme activity detected in the eco-enzyme affected the inefficiency of oil and grease breakdown through hydrolysis into monoglycerides, diglycerides, and fatty acids (Kasih & Hendrasarie, 2023; Noviana *et al*., 2024). Lipase enzyme activity can optimally performed as a biocatalyst at a pH range of 7.00–10.00 (Galintin *et al*., 2021; Gumilar *et al*., 2023; Liew *et al*., 2020; Shu *et al*., 2006), while the pH during the experiment remained between 3.90 and 5.90. Galintin *et al*. (2021), show that biocatalytic activity tends to be lower or decreased under acidic conditions. This is because acidic environments affect hydrogen and ionic bonds within the enzyme.

However, in this case, the control reactor showed a different trend than the results of BOD and COD. The concentration of oil and grease in the control reactor also increased, a finding that underscores the importance of understanding the limitations of indigenous bacteria in the raw water to break down complex molecules in the oil and grease (Liew *et al*., 2020).

Recommendations for Eco-Enzymes Utilization

Referring to the results of the current study and several previous studies, aerobic bioremediation using eco-enzymes requires a period ranging from 20 to 70 days, depending on the concentration of pollutants. This condition indicates that the flow system used in eco-enzyme treatment should be batch-type, a crucial aspect that significantly influences the effectiveness of the process. Therefore, it can be confirmed that eco-enzymes are less suitable as bioremediation agents for organic materials in water bodies with continuous flow, particularly for raw water

Eco-enzymes are more suitable for biologically treating domestic wastewater in communal wastewater treatment units before being discharged into surface water. Their application in a batch flow system, which allows for a longer residence time, is particularly beneficial. This setup optimizes the function of eco-enzymes as biocatalysts in the decomposition of organic pollutants.

Furthermore, during the treatment process, the pH of the mixture of water and eco-enzyme should be maintained within the range of 6.50–8.00 to maximize the activity of the enzymes formed. According to Benny *et al*. (2023), the optimal composition ratio of eco-enzyme to treated wastewater should be 50:50 (v/v). This ratio is crucial as it ensures the most effective use of eco-enzymes, thereby contributing significantly to the preservation of the quality of aquatic ecosystems.

CONCLUSION

The use of eco-enzyme made from waste materials such as sweet orange peel, squeezed orange, apple, papaya, pineapple, pear, dragon fruit, guava, banana, watermelon, jicama, cassava peel, mustard greens, kedondong, and molasses aged for 624 days was not able to reduce the concentrations of BOD, COD, oil and grease in raw water for treated water from the primary irrigation canals of Karawang. In other words, it was found that eco-enzymes are ineffective for the bioremediation of polluted surface water with continuous flow systems. However, under the appropriate conditions, eco-enzymes have the potential to be effective. Eco-enzymes contain organic compounds that, when used in processes with short periods, may increase the concentration of organic pollutants, thus requiring a more extended biodegradation period. Therefore, eco-enzyme is recommended as a biocatalyst in biological wastewater treatment processes using a batch system with a retention time of more than 10 days.

RECOMMENDATION

Further research is needed on similar bioremediation using eco-enzymes aged for approximately 90 days (3 months) and with a higher composition than in the current study. The comparison of the composition of treated water with eco-enzyme should have a (v/v) ratio ranging from 1:1 to 1:5. Furthermore, the experimental period should be extended beyond 10 days using a semi-continuous flow system to assess the optimal residence time and flow system for aerobic bioremediation.

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