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Study of Eco-Enzyme Application in Reducing Nitrite Concentration and Ph Value in Water Sample

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Abstract. *The number of high fruit loss must be resolved immediately. East Java produces the highest number of banana and orange. To prevent the fruit loss, as mitigation, the fruit loss can be used to make eco-enzymes as biodegradable agents which was made from nature to treat contaminated water from nitrite contamination and high pH value. Contaminations such as nitrite in water pose significant risks, particularly to organisms. Nitrite contamination can adversely affect humans by reacting with hemoglobin, leading to the formation of methemoglobin, which hampers the blood's ability to bind with oxygen and transport it to body tissues. Eco-enzymes serve as biocatalysts to accelerate the nitrification process, converting ammonia into nitrate with nitrite as an intermediate product. This study was conducted on a laboratory scale, using water samples comprised of distilled water and nitrite standard solution. Two types of eco-enzymes, derived from banana and orange waste, were applied at a 5% concentration to reduce nitrite levels and pH values in the water samples. The results indicated that the orange eco-enzyme reduced nitrite concentrations by 73.2% and pH values by 38.42%, while the banana eco-enzyme achieved a reduction of 93.89% in nitrite concentrations and 23.15% in pH values. As an application of this study, the 5% eco-enzyme solution holds promise for use in biological treatment within wastewater treatment plants, particularly in aeration tanks, to enhance the nitrification process, thereby aiding in the reduction of nitrite concentrations and pH values in wastewater.*

Keywords: *Eco-enzyme, Nitrite, pH, Removal.*

INTRODUCTION

In 2022, Indonesia produced 28.3 million tons of fruit (BPS, 2022). The most abundant fruit type is bananas, followed by pineapple, mango, and oranges (BPS, 2022). According to data from the Central Bureau of Statistics, East Java is the leading producer of bananas and oranges (nusantara62.com, 2022); (Safitri, 2023). Fruit loss refers to the wastage of food items like vegetables, fruits, and raw materials that are no longer usable and are discarded along the supply chain (Gustavsson et al, 2011). Data from the Ministry of National Development Planning/National Development Planning Agency (PPN/Bappenas) states that from 2000 to 2019, the majority of food loss and waste came from vegetables at 62.8%, followed by fruits at 45.5%, and meat at 30.7% (Lestari, 2022). Therefore, to prevent food loss, it is essential to utilize waste effectively.

Efforts from society are crucial to prevent and address the rising issue of food loss, as improper waste management can lead to more serious problems such as greenhouse gas emissions, land, water, and air pollution (Valizadeh & Hanie, 2018). Persistent food loss accumulation can result in unpleasant odors and the production of methane gas through decomposition, contributing to global warming (Lestari, 2022). Sustainable Development Goal (SDG) target 12 promotes the 3R (reduce, reuse, recycle) approach to mitigate food loss during production, including post-harvest losses, and manage food waste. Implementing recycling methods for food loss can help minimize the risks associated with improper waste management, emphasizing the importance of conducting studies to prevent environmental damage and adverse impacts.

As a preventive and corrective measure, one viable implementation involves utilizing food waste, such as fruit and vegetable peels, to create eco-enzymes. Eco-enzymes function similarly to enzymes, serving as catalysts. They are organic solutions produced by fermenting fresh fruits and vegetables, brown sugar, and water (Murugaiah & Visantini, 2020). Research has demonstrated a significant reduction of up to 70% in Biological Oxygen Demand (BOD) levels from metal-based effluent with eco-enzyme treatment. Moreover, eco-enzymes have proven effective in treating wastewater, showing reductions of 32.5% in Total Solids (TS), 39.5% in Total Dissolved Solids (TDS), and 33.0% in Total Suspended Solids (TSS) (Murugaiah & Visantini, 2020).

Studies have shown that guava eco-enzymes, with a concentration of 10% and an 8-hour observation period, can reduce ammonia (NH3) levels in water by up to 31%. Additionally, a 2% concentration of eco-enzymes decreased phosphorus (PO4) levels by 20% over six hours of observation (Bahari & Wikaningrum, 2022); (Anindita & Wikaningrum, 2023). Furthermore, research indicates that eco-enzymes derived from various fruits can lower pH levels in textile wastewater. For instance, pH values decreased from 10.98 to 8.59 within 120 hours of applying eco-enzymes (Anindita & Wikaningrum, 2023). Another study found that nitrite elimination rates ranged from 0.3% to 35% after 10 hours of exposure to eco-enzymes at concentrations of 0% to 6% (Wikaningrum & Anggraina, 2022). These findings underscore the effectiveness of eco-enzymes in reducing pollutant levels in wastewater, highlighting their potential for environmental remediation.

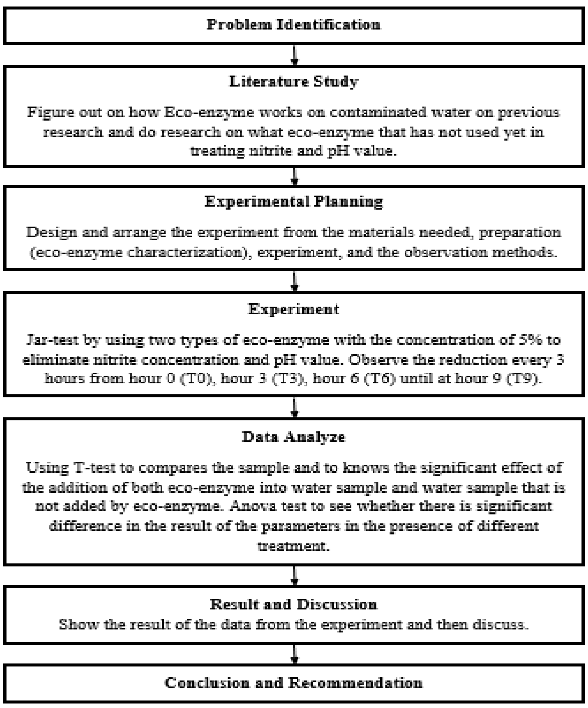
Some studies have already done the research by using eco-enzyme that made of some specific fruits and vegetables as biocatalyst in treating water and waste water. Nowadays, the study of eco-enzyme that made of citrus fruit waste, and banana waste offer potential solution to treat waste water in environmentally friendly way since in East Java it is produced in abundance and also because it is biodegradable agents which is made from nature, to implement the 3R principles, and also to make the best use of fruit loss in an area. It is needed to do research of the effect of eco-enzyme in reducing some parameters. This research is conducted especially by using eco-enzyme that made of banana and made of orange to know the reducing ability of eco-enzyme itself to some parameters such as nitrite and pH value in artificial samples that was made not meet the standard which is sanitation water quality standard of Ministry of Health Regulation No. 2 of 2023.

Several studies have explored the use of eco-enzymes derived from specific fruits and vegetables as biocatalysts for water and wastewater treatment. Presently, research on eco-enzymes derived from citrus fruit waste and banana waste presents a promising solution for environmentally friendly wastewater treatment, particularly in regions like East Java where these fruits are abundantly produced. These eco-enzymes serve as biodegradable agents derived from natural sources, aligning with the principles of reduce, reuse, and recycle (3R), and maximizing the utilization of fruit waste in the area. There is a need to investigate the efficacy of eco-enzymes in reducing various parameters. Specifically, research is required to assess the effectiveness of eco-enzymes derived from banana and orange in reducing parameters such as nitrite levels and pH values in artificial samples that do not meet the sanitation water quality standard outlined in Ministry of Health Regulation No. 2 of 2023.

This study focuses on the application of eco-enzymes in water samples intended for potential wastewater treatment, specifically targeting parameters such as nitrite levels and pH. Both parameters are included in the sanitation water quality standard specified by Ministry of Health Regulation No. 2 of 2023. The negative impact of nitrite on the environment and human health underscores the importance of addressing this issue. In health aspect of human and aquatic life especially fish, the presence of high concentration nitrite possesses the ability to bind with hemoglobin and trigger the formation of methemoglobin and can be a serious problem because the failure of binding with oxygen and to transport oxygen to all body tissues (Kiswanto & Rahayu, 2020); (Florida Department of Agriculture & Consumer Services, n.d). The contamination from underground water, livestock waste water, overuse of fertilizers from agricultural land, and domestic activities can all result in the presence of nitrite (California Department of Health Services, 2000). Meanwhile, if the pH of the aquatic environment falls below 4.8 or rises above 9.2, it can adversely affect aquatic life.

METODE

The samples used in this study consist of artificial water samples created using distilled water and a standard nitrite solution with a concentration of 5 mg/L. The research was conducted at the Environmental Laboratory of President University, utilizing a random sampling method. Nitrite levels were determined colorimetrically following the American Public Health Association (APHA) method 4500-NO2, with measurements taken at a wavelength of 543 nm using a spectrophotometer. pH values were measured according to SNI 6989.11-2019 using a pH meter. The initial step involved determining the characteristics of both eco-enzymes, employing two methods for two parameters. The objective was to ascertain the concentration of each eco-enzyme. The subsequent step involved conducting experiments by combining water samples with eco-enzymes to evaluate their performance. This aimed to determine whether the eco-enzymes effectively reduced contaminants. Observations were made at various time intervals, including at 0 hours (T0), 3 hours (T3), 6 hours (T6), and 9 hours (T9), with each observation replicated twice. The samples were contained in glass bottles sealed with aluminum foil during the observation period. The equipment utilized in this study included laboratory glassware, an analytical balance, a spectrophotometer, and a pH meter. As for the materials used, they comprised eco-enzymes derived from orange and banana, distilled water, nitrite standard solution, and various chemicals. The key chemicals employed included NED-dihydrocholide, sulfanilamide, and nitrite standard solution.



Source: processed data

Figure 1
Research Framework

Experimental Method

In this study, the experimental procedure comprised the following steps:

- Preparation of artificial samples containing 5 mg/L nitrite by mixing distilled water and nitrite standard solution in six bottle samples.
- Addition of eco-enzyme with a concentration of 5% into each bottle of artificial water sample.
- Duplication of the experiments.
- Measurement of the concentration every 3 hours starting from 0 hours until 9 hours. The observation times were at 0 hours (T0 or initial), after 3 hours (T3), after 6 hours (T6), and after 9 hours (T9).

Data Analysis Method

The data analysis involves t-tests and ANOVA. In the t-test, the hypothesis aims to determine the significant effect of adding both types of eco-enzymes to water samples compared to water samples without any eco-enzyme added. This analysis entails comparing each water sample treated with eco-enzyme to those without eco-enzyme. Meanwhile, ANOVA is employed to ascertain whether there are significant differences in the parameter outcomes resulting from various treatments.

RESULT

Eco-Enzyme Characterization Result

During the initial stage of the experiment, the eco-enzyme made from banana and orange undergoes characterization. Initially, both eco-enzymes are diluted to 10x. The characterization involves testing parameters such as nitrite and pH.

Table 1
Characterization of Eco-Enzyme Results

Parameter	Eco-enzyme average result	
	Orange	**Banana**
NO_2-N	0.210 mg/L	0.172mg/L
pH	4.064	4.891

Source: processed data

The table above presents the experimental results of eco-enzyme in the NO2-N and pH value parameters. The results displayed in the table have been multiplied by 10, which represents the dilution factor. The primary objective of this experimental step is to determine the concentration of both types of eco-enzyme. Once the concentrations are determined, the next step involves collecting data through the second phase of the experiment.

Result of 2 types of eco-enzyme addition in water sample

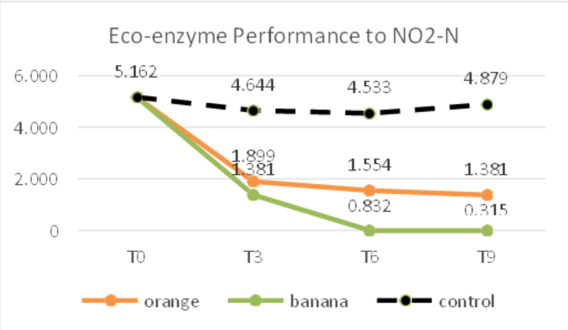
During this phase of the experiment, observations were conducted over a period of 9 hours at intervals of T0, T3, T6, and T9. The water samples initially contained a nitrite concentration of 5 mg/L. Subsequently, each water sample was supplemented with 5% eco-enzyme derived from oranges and bananas.

Table 2
Experiment Result

Time (hour)	Ecoenzyme	Initial		Average Result of water sample		% Removal	
		NO_2-N (mg/L)	pH	NO_2-N (mg/L)	pH	NO_2-N	pH
T3	Orange			1.899	3.76	62.3%	36.91%
	Banana			1.381	4.66	73.24%	21.81%
T6	Orange	5.162	5.96	1.554	3.85	70.08%	35.40%
	Banana			0.832	4.61	92.66%	22.65%
T9	Orange			1.381	3.67	73.24%	38.42%
	Banana			0.315	4.58	93.89%	23.15%

Source: processed data

a. $\boldsymbol{NO_2}$*-N*



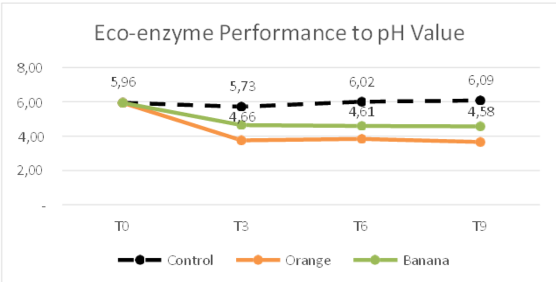
Source: processed data

Figure 2
Eco-enzyme performance to NO2-N

The results depicted in Figure 2 illustrate the performance of eco-enzymes on the NO2-N parameter. Initially, at T0, the control sample registered a value of 5.162 mg/L, while water samples treated with eco-enzymes derived from oranges and bananas recorded concentrations of 1.899 mg/L and 1.381 mg/L, respectively, at T3. Subsequently, after 6 hours (T6), these concentrations further decreased to 1.544 mg/L for the orange eco-enzyme-treated sample and 0.832 mg/L for the banana eco-enzyme-treated sample. Over time, both treated water samples exhibited a continuous reduction in concentration, reaching 1.381 mg/L for the orange eco-enzyme-treated sample and 0.315 mg/L for the banana eco-enzyme-treated sample at T9. The orange eco-enzyme achieved a reduction of 73.24% in NO2-N, while the banana eco-enzyme resulted in a 93.89% reduction from T0 to T9.

b. pH Value

The performance of eco-enzymes on the pH value indicates a gradual decrease in NO2-N water samples treated with eco-enzymes derived from oranges and bananas, although the reduction is not substantial. Specifically, the pH of water samples treated with orange eco-enzyme decreased from 5.96 at T0 to 3.67 at T9, resulting in a reduction percentage of 38.42%. Similarly, the pH of water samples treated with banana eco-enzyme decreased from 5.96 at T0 to 4.58 at T9, representing a reduction percentage of 23.15%.



Source: processed data

Figure 3
Eco-enzyme Performance to pH Value of NO2-N Water Sample

Data Analysis

The table below presents the results of the T-test and ANOVA conducted in this study. The data analysis was performed using Microsoft Excel software with an alpha value of 0.05, and data from each observation hour (T0, T3, T6, T9) were utilized. In the T-test, the P-value indicates whether there is a significant difference before and after the addition of eco-enzymes derived from oranges and bananas. On the other hand, in ANOVA, the P-value determines whether both eco-enzymes have an effect on the analyzed parameters. The table demonstrates the P-values for NO2-N and pH value parameters, with a threshold of < 0.05 to indicate significance. The P-values from the T-test assess the difference before and after, while those from ANOVA evaluate the impact of both eco-enzymes on NO2-N and pH value parameters.

Data Analysis of The Addition of 2 Types of Eco-enzyme

Table 3
T-Test Result on NO_2-N Parameter

Eco-enzyme	P-Value one-tailed T-Test		
	T3	T6	T9
Orange	0,000017	0,000090	0,000494
Banana	0,000011	0,000061	0,00005

Source: processed data

Table 4
Anova Result on NO_2-N Parameter

Eco-enzyme	P-Value ANOVA		
	T3	T6	T9
Orange Banana	0,00001	0,00001	0,00004

Source: processed data

For the NO2-N parameter, the T-test results depicted in Table 3 indicate that the P-values for all observation hours (T) are < 0.05. This suggests that the null hypothesis (H0) is rejected, and the alternative hypothesis (H1) is accepted. In other words, both types of eco-enzymes exhibit a significant difference in the water sample. Similarly, the P-values from the ANOVA analysis in Table 4 are all < 0.05, indicating the rejection of H0 and acceptance of H1. This implies that the water samples treated with both types of eco-enzymes have an effect on the NO2-N parameter.

Table 5
T-Test Result of pH in Water Sample

Eco-enzyme	P-Value one-tailed T-Test		
	T3	T6	T9
Orange	0,000016	0,000021	0,000034
Banana	0,000741	0,000081	0,000197

Source: processed data

Table 6
Anova Result of pH in Water Sample

Eco-enzyme	ANOVA		
	T3	T6	T9
Orange Banana	0,00003	0,000002	0,00001

Source: processed data

Table 5 presents the T-test results for the pH parameter in the NO2-N water sample. The table indicates that all P-values are < 0.05, leading to the rejection of H0 and acceptance of H1. This suggests that the addition of both types of eco-enzyme results in a significant difference in the water sample's pH. Similarly, Table 6 displays the ANOVA results, with all P-values < 0.05. This implies the rejection of H0 and acceptance of Ha, indicating that the addition of eco-enzyme made of orange and eco-enzyme made of banana affects the pH value in the water sample.

Eco-enzyme characteristic

The eco-enzymes examined in this study exhibit an acidic nature, as evidenced by their pH values below 7. Specifically, the orange eco-enzyme has a pH value of 4.064, while the banana eco-enzyme has a pH value of 4.891. Additionally, the concentration of NO2-N for the orange eco-enzyme is measured at 0.210 mg/L. Previous research has demonstrated that eco-enzymes typically possess low pH values and acidic characteristics due to the presence of citric acid and enzymes such as amylase, lipase, and protease, which act as biocatalysts (Galintin & Hamzah, 2021),

Table 7
Characteristics result compare to literature

Parameter	Average result in this study		Literature		Reference
	Orange	Banana	Result	Type of EE	
NO2-N	0.210 mg/L	0.172 mg/L	3.48	Papaya + spinach	Wikaningrum & Anggraina (2022)
pH	4.064	4.891	3.96	Orange	Anindita et al (2023)
			3.47	Orange	Wikaningrum et al (2022)
			2.86	Orange	Galintin & Hamzah (2021)
			3.30	Orange	Permatananda & Pandit (2023)
			3.88	Banana	Nurlatifah et al (2022)
			5.60	Banana	Yuliana et al (2023)

Source: processed data

The literature presented in Table 7 indicates that the pH of the orange eco-enzyme falls below 4, whereas in our study, the eco-enzyme utilized exhibited a pH of 4.064. Conversely, the banana eco-enzyme in another study possessed a pH of 3.88 (Nurlatifah et al, 2022), which is more acidic than the banana eco-enzyme employed in our research. In yet another study, the pH of banana eco-enzyme was reported to be 5.6 (Yuliana et al, 2023), which is less acidic compared to our findings. In our investigation, the orange eco-enzyme exhibited greater acidity than the banana eco-enzyme. This disparity in pH levels could potentially be attributed to the inherent acidity present in the fruits themselves, characterized by natural acids such as citric acid, acetic acid, malic acid, tartaric acid, and ascorbic acid, including vitamin C (Lagha-Benamrouche & Khodir, 2013); (Franke et al, 2005),

Eco-enzymes performance in reducing NO_2-N and pH value

The initial phase of the experiment involved characterizing the eco-enzyme to determine its original concentration and the values of the parameters under analysis. Subsequently, in the second phase of the experiment, eco-enzymes derived from both oranges and bananas were introduced. The observation focused on NO2-N levels and the pH value within the NO2-N water sample. As depicted in (Table 4), the eco-enzymes exhibited substantial effects on the water sample compared to its initial concentration. Specifically, banana-derived eco-enzyme facilitated a remarkable reduction in NO2-N levels, amounting to 93.89% from T0 to T9. Meanwhile, eco-enzyme derived from oranges achieved a significant decrease of 73.24% in NO2-N concentration over the same time span, from T0 to T9.

Source: processed data

Figure 4

Water sample before and after the addition of orange eco-enzyme

Source: processed data

Figure 5

Water sample before and after the addition of banana eco-enzyme

Based on the T-Test results presented in Table 5, where all P-Values at every time interval (T) are < 0.05, it can be concluded that there are significant differences before and after the addition of both types of eco-enzymes to the NO2-N water samples, compared to the control sample. Similarly, the Anova results in Table 6 also indicate P-Values < 0.05 across all time intervals, signifying significant differences in parameter outcomes with different treatments. Both types of eco-enzymes play a substantial role in reducing NO2-N levels, likely facilitated by the oxygen content in the water sample (Said & Hartaja,. 2015), which aids in the nitrification process for eliminating NO2-N concentrations. Additionally, the enzymatic activity inherent in each type of eco-enzyme (as shown in Table 2) contributes to this reduction. The presence of oxygen may be attributed to the periodic sample observations conducted every 3 hours, allowing oxygen to enter the container during pipetting, despite the container being sealed after each observation.

Similar trends were observed in the pH value, where both types of eco-enzymes reduced the pH value from T0 to T9. Orange eco-enzyme exhibited a reduction of 38.42%, while banana eco-enzyme showed a reduction of 23.15% from T0 to T9. Although orange eco-enzyme demonstrated the largest reduction in pH value, there was an increase observed from T3 to T6, followed by a decrease at T9. This fluctuation can be attributed to the citric acid concentration in the eco-enzyme, along with the presence of amylase, lipase, and protease as biocatalysts

(Galintin & Hamzah, 2021).. On the other hand, banana eco-enzyme exhibited a gradual reduction from T0 to T9. The varying concentrations of eco-enzyme contributed to the changes in pH value, with higher concentrations resulting in greater reductions (Bahari & Wikaningrum, 2022). However, the pH values of both types of eco-enzymes in this study (orange eco-enzyme: pH 4.064, banana eco-enzyme: pH 4.891) were higher compared to a previous study Bahari & Wikaningrum (2022), which reported a pH of 3.36 using guava eco-enzyme. This disparity may explain why the percentage reduction in pH value in this study was not as substantial as in the previous research.

The acidity of the eco-enzyme itself could also influence the pH reduction, as both types of eco-enzyme in this study exhibited an acidic nature with pH < 7. The acidity is attributed to the conversion of carbohydrates in fruit organic waste into highly volatile acids during fermentation, along with the processing of organic acid compounds during the fermentation process (Wibowo, 2023). Statistically, the T-Test results for the pH in NO2-N water samples indicate a P-Value < 0.05 for all time points (T), signifying a significant difference when both types of eco-enzymes were added to the water samples compared to the control sample. Similarly, the ANOVA results reveal a P-Value < 0.05 for all time points, indicating a significant difference in the parameter outcomes under different treatment conditions.

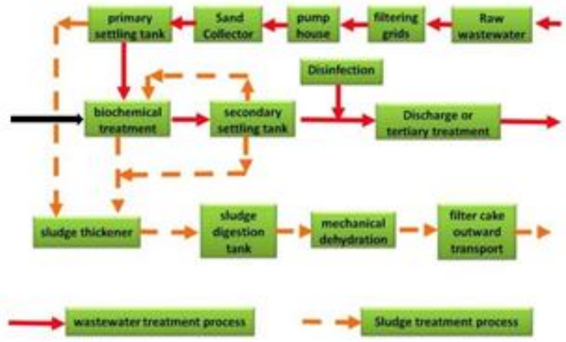
Applicability in water treatment plant

Given that nitrification is facilitated by oxygen, it is crucial to ensure that water treatment processes provide sufficient oxygen levels. Therefore, aeration can be recommended as a method to enhance nitrification by increasing the supply of oxygen into the water (Bagastyo et al, 2023).

$NH_4^+ + 1.5\ O_2 \rightarrow 2H^+ + H_2O + NO_2^-$

$NO_2^- + 1.5O_2 \rightarrow NO_3^-$

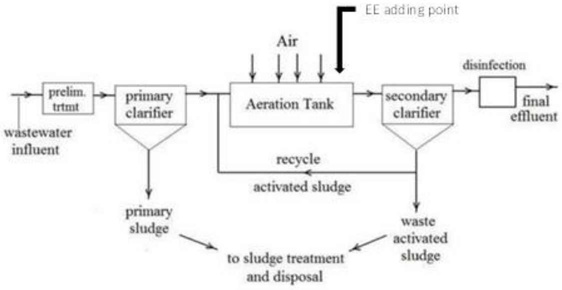
EE adding point



Source: Guo et al (2019)

Figure 6
Flow diagram process on municipal waste water treatment

An instance of biological treatment can be observed in municipal wastewater treatment, which incorporates biological treatment as a green chemistry technology in the treatment process (Guo et al, 2019). Eco-enzymes can be utilized in the biochemical treatment step, complemented by aeration. Aeration aids in removing COD (Chemical Oxygen Demand) and facilitates the nitrification process (Guo et al, 2019). Another potential application of eco-enzymes is in the treatment of wastewater from the dairy industry, with the assistance of aeration (Figure 7), which employs activated sludge to break down organic matter and nutrients (Azadi & Shahram, 2015). Enhancing the aeration of activated sludge can be achieved through oxidation ditches (Wikaningrum & Rijal, 2020). Activated sludge is a regulated aerobic biological treatment method for wastewater, where microorganisms are aerated to ensure the removal of organic matter and nutrients (Nurtaç et al, 2010). To improve nitrification, the implementation of oxidation ditches as the aeration system in wastewater treatment is considered, with a hydraulic retention time ranging from 15 to 30 hours, aiding in the nitrification process (Mackenzie, 2011). Therefore, eco-enzymes function as biocatalysts to accelerate the biological processes occurring in aeration, presenting significant potential in facilitating nitrification, the process by which ammonia is converted into nitrate.



Source: Azadi & Shahram (2015)

Figure 7

Flow process of diary wastewater treatment plant

CONCLUSION

Some points to be concluded based on the personal experiments, it can be concluded that:

1. The characterization of both type of eco-enzyme shows a present in nitrite concentration which is for eco-enzyme that made of orange is at 0.210 mg/L and for eco-enzyme that made of banana is at 0.172 mg/L. The pH value of both eco-enzyme is acidic since the pH for orange eco-enzyme is at 4.064 and banana eco-enzyme is at 4.891.
2. After treated by both types of eco-enzyme with the concetration of 5% shows the reduction in concentration of NO_2-N and pH value of water sample in 9 hours observations. The reduction of nitrite is 73.24% for orange eco-enzyme and 93.89% for banana eco-enzyme.
3. Eco-enzyme that made of orange and that made of banana with the concentrations of 5% shows a reduction in pH value during 9 hours of observations. Orange eco-enzyme has reduced 38.42% and banana eco-enzyme reduce 23.15% of pH value.
4. Eco-enzyme that made of banana and eco-enzyme that made of orange can be used as the applicability on waste water treatment plant to treat water. The addition of eco-enzyme can be on biological process and it can be put into aeration tank so that the nitrification process would be maximal and it can speed up the reaction to decompose ammonia becomes nitrate.

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