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The eco enzyme application to reduce nitrite in wastewater as the sustainability alternative solution in garbage and wastewater problems

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The eco enzyme application to reduce nitrite in wastewater as the sustainability alternative solution in garbage and wastewater problems

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Abstract. The increasing population growth has an impact on increasing problems in wastewater quality and garbage volume. It is a need to develop a sustainability solution for both problems. In this research, the papaya fruit and spinach vegetable garbage were used as raw organic materials and fermented for six months. This study objective was to determine whether eco enzymes can be used to reduce nitrite concentration in wastewater. The research method was conducted on a laboratory scale with the artificial samples in a batch system. The result showed that eco enzyme made from a mixture of papaya and spinach can reduce the nitrite concentration in the water sample. The t-test showed that the effect of eco enzyme application in reducing nitrite in water samples was significant. The application of eco enzyme 0%, 2%, 4%, and 6% respectively during 10 hours exposure showed the nitrite removal were 0.3 %, 20%, 29%, and 35 %. It can be seen that the reduction of nitrite concentration in samples added by eco enzyme was caused by eco enzyme activity. The application at a higher concentration of 10%, 15%, and 20% showed that nitrite removal efficiencies were 35.7%, 36.7%, 46.7%, 49.4% respectively during 7 hours exposure. It can be concluded that a longer time exposure, and also the more eco enzyme concentration, showed the more result in the effect of nitrite reduction concentration in water. 4. By this study it is proven that application of eco enzyme made from organic garbage can be an alternative solution for garbage and wastewater quality problem.

1. Introduction

It was estimated that one-third of the world's food is wasted every year in the form of food waste. the value of the discarded food waste is USD 936 million or greater than the gross domestic product of the Netherlands as a whole [1]. Most of the food waste is dumped in the waste landfill which will eventually produce greenhouse gases from the degradation process. The Intergovernmental Panel on Climate Change (IPCC, 2010)) estimated that about 3 percent of the world's greenhouse gas emissions were the result of transporting and treating solid waste. the largest source of these emissions was generated from landfill activities.

It is estimated that 190 thousand waste is generated in Indonesia every day, and most of it is in the form of organic waste. In Indonesia, the major urban centers generate about 10 million ton of waste every year. The waste amount is estimated to increase by 2 to 4% annually (Ministry of Environment, 2008). With the continuous increase in waste along with population growth with the limited area for final landfill, it becomes a big challenge to the government. However, the waste problem is not only the duty of the government but also the responsibility of society as a whole. For this reason, the role of the community is needed to overcome the problem of waste, especially organic waste that comes from food scraps. The main sources of municipal solid waste in Indonesia generates from households andtraditional markets. The community role that makes it possible to process organic waste is the

composting system, but the survey results showed that the community does not accept that their place of residence is close to the composting facility [2].

With this condition, the use of organic waste in other useful materials can be a solution, especially if it can be used as a material agent in wastewater treatment. Dr.Rosukon Poompanvong introduced eco Enzyme intending to process enzymes from organic waste. Eco enzyme is the result of fermentation of organic waste consisting of vegetable and fruit waste, brown sugar, and water. Eco enzyme Has a characteristic dark brown to yellowish colour and has a strong sweet and sour aroma due to the fermentation process [3]

Eco Enzyme can be a potential solution to the issue of global warming due to the high production of methane gas produced by the decomposition process of organic vegetables and fruits. There are many benefits obtained from the manufacture of eco-enzymes, one of which is as a disinfectant due to the presence of organic acids and alcohol. The use of eco-enzyme as a biochemical catalyst for reducing pollutants in rivers has also begun to be studied because its ability to decompose waste is proven from previous studies [4]. From several previous studies, it was found that eco-enzyme can be used as an alternative material to reduce various pollutants in wastewater. For example, reducing COD concentrations, lowering the pH of the solution, and reducing ammonia concentrations. Organic waste that was previously only waste can be processed into eco-enzymes that have high economic value [3].

In this wastewater quality study, the parameter studied was the nitrite concentration in the water. The selection of nitrite parameter is based on the importance of this parameter in water quality standards, both drinking water, sanitation water, and wastewater. The presence of nitrite in water can occur due to contamination of underground water, wastewater from livestock, excessive use of fertilizers from agricultural land, it can also be from domestic activities [5].

High concentrations of nitrite can cause a decrease in the immunity of the organism and this makes the organism susceptible to disease. Nitrite also has the potential to react with haemoglobin and trigger the formation of methaemoglobin and can be fatal because the blood cannot bind and transport oxygen to all body tissues [6].

2. Literature review

2.1 Organic waste

Household waste contributes to polluting the environment and causes aesthetic problems if there is no serious commitment to handling it. Organic waste management is still not implemented efficiently and is even dominated by irresponsible waste disposal. Meanwhile, organic waste is very useful if it can be processed into products that have economic value such as compost [7]. Improper disposal of waste can cause environmental pollution and is a serious threat to living things, including humans. Environmental problems arise as a result of ineffective waste management [8].

The increase in population and human activities in an area is in line with the increase in the amount of waste produced, therefore efficient and effective waste management is needed so that it does not cause environmental pollution. This problem cannot be handled by conventional waste management methods, namely disposal at the final waste disposal site. The weakness of this method in addition to requiring a high cost also does not reduce the volume of waste disposed of, even though the landfill hasa service life and maximum capacity [9].

2.2 Eco enzyme

Eco-enzyme solutions have many benefits, including as a pollutant-reducing agent in river water, providing nutrients for the soil and as an antiseptic [10]

Manufacturing eco enzyme can be done by mixing organic waste, brown sugar, and water in a ratio of 3:1:10 then fermented for up to three months. After fermentation is complete, the solution is filtered and stored at room temperature. The application of the eco-enzyme can also be applied to treat sludge and wastewater [11].

Fermentation is a process of chemical change in organic substrates that can survive due to the action of biochemical catalysts, namely enzymes produced by certain living microbes, such as organic acids, single-cell proteins, antibiotics, and biopolymers [10].

Enzymes are the most abundant types of proteins in living cells and have an important role as biocatalysts in biochemical reactions. One of the enzymes that play an important role is the protease enzyme. Protease enzymes function to hydrolyze peptide bonds in proteins into amino acids. Proteases are complex enzymes that have varied chemical, physical and catalytic properties. This enzyme is produced by microorganisms extracellularly and has an important function in cell metabolism [12]. Types of protease enzymes produced from plants include papain and bromelain enzymes. One of the compounds contained in the eco enzyme is acetic acid which has bactericidal properties. The types of enzymes contained in this eco enzyme are trypsin and amylase which can prevent pathogenic bacteria. [12]. The presence of NO_3 (nitrate) and CO3 (carbon trioxide) content is useful for the soil as nutrients [13].

2.3 Nitrite in water

Nitrate and nitrite ions are naturally as the are part of the nitrogen cycle. The nitrate ion (NO_3^-) is the stable form of combined nitrogen for oxygenated systems. Although chemically unreactive, it can be reduced by microbial action. The nitrite ion ($NO_2^{-)}$ contains nitrogen in a relatively unstable oxidation state [5] The organic nitrogen wastes are first decomposed to give ammonia, which is followed by an oxidation process to nitrite and nitrate (WHO).

The presence of nitrite indicates the ongoing process of biological degradation of organic matter. The degradation process of nitrogen from ammonium ions to nitrate ions is called nitrification. This process is an enzymatic oxidation process by microorganisms that occur in two continuous stages [14] The first stage involves obligate autotrophic nitrite bacteria known as Nitrosomonas and Nitrococcus with the final product nitrite ion as follows [15] :

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$$

The second stage is the conversion of nitrite ions to soluble nitrate ions involving obligate autotrophic nitrate bacteria known as Nitrobacter and Nitrocystis as follows [15]:

$$2NO_2^- + O_2 \rightarrow 2NO_3$$

The guideline for nitrite of 3 mg/l as nitrite is based on human data showing that doses of nitrite that cause methemoglobinemia in infants range from 0.4 to more than 200 mg/kg of body weight [5]. In the United States of America (USA), naturally occurring levels do not exceed 4–9 mg/l for nitrate and 0.3 mg/l for nitrite (USEPA, 1987). Nitrite levels in drinking water are usually below 0.1 mg/l [5]. In Indonesia, based on the Minister of Health Regulation No. 32 of 2017 concerning clean water for environmental health sanitation, the maximum level of nitrite is 1 mg/L and nitrate is 3 mg/L. Meanwhile, according to the wastewater quality standard No. 5 of 2014 issued by the Minister of the Environment, the maximum level of nitrite is 1 mg/L. According to the Indonesian National Standard (SNI) of 3554:2006 regarding bottled drinking water quality standards, the maximum concentration of nitrite is 0.005 mg/L and nitrate is 45 mg/L.

3. Research method

3.1Population and Sample

The samples in this study were artificial samples that were prepared by adding a standard solution of nitrite into distilled water so that a solution with a concentration of about 20 mg/L. The water sample population is homogeneous because the sample is artificial. The sampling method used in this study was a random sampling method.

3.2 Data Collection Method

The data used as a source of data in this study was through primary data generated from observations in the laboratory. The data collection technique was through an experimental method to determine the

effect of the eco enzyme on the reduction of nitrite. This research was conducted from September 2020 – April 2021

3.3 Materials and tools

The main equipment that used in the research were laboratory glassware, analytical balance, and UV-Vis double beam spectrophotometer UNICO 4082.

The materials used were eco enzyme made from mixing of papaya and spinach waste, and chemicals according to analysis procedure for nitrite measurement in water sample of SNI 06-6989.9-2004. The main chemicals are N-(1-naphthyl)-ethylenediamine dihydrochloride, sulphanilamide ($H_2NC_6H_4SO_2NH_2$).

3.4 Research framework

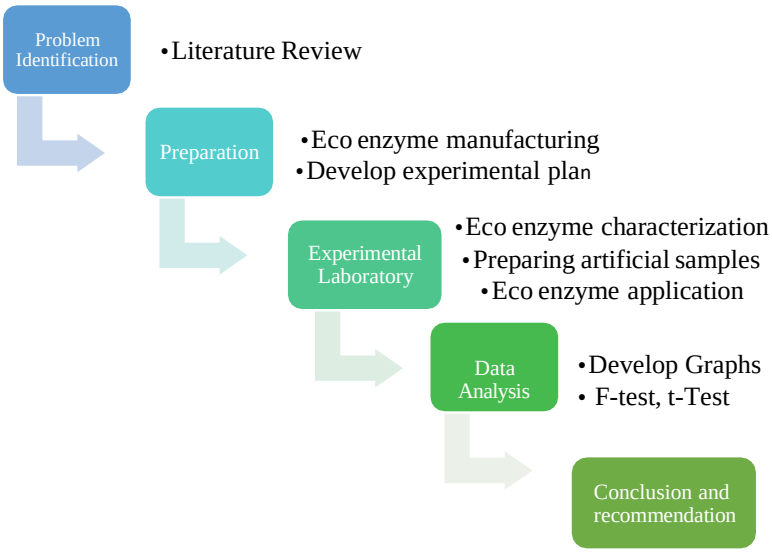

Figure 1. Research framework

3.5 Experimental methods

The steps in the research experiments were as follow:

- Prepare an artificial sample of NO_2 with a concentration range of 20 ppm which is prepared from $NaNO_2$ standard solution in 8 beaker glasses.
- Add eco enzyme with concentrations of 0 %, 2%, 4%, 6% into each artificial sample and mix well.
- Duplicate the experiments.
- The samples and control were then allowed to stand exposure for observation time.
- The nitrite concentration of the samples was measured every hour until there was a significant decrease.

3.6 Data analysis method

The hypothesis in this study was that the application of eco enzyme can reduce nitrite concentrations in the water. Hypothesis Ho, if $\mu A = \mu B$ it means the nitrite concentrations in the water sample added by the eco enzyme are the same or not lower than the control sample. Ha or Ho is rejected, if $\mu A \neq \mu B$, it means the nitrite concentration of the sample added by the eco enzyme is lower than the control sample. Statistical analysis for this study was F-test to know the variance whether it is equal or an-equal and

followed by t-Test for testing its significant concentration of nitrite reduction with a value of P =0.05, which was performed with Microsoft Excel software.

4. Result and discussion

4.1 Eco enzyme characterization

Eco enzyme was prepared by anaerobic fermentation for six months made from papaya and spinach mixing garbage waste. The composition of raw materials was water: organic garbage: brown sugar was 10:3:1. The eco enzyme result was filtered before applying for removing solids materials. The laboratory analysis showed that the eco enzyme has a pH = 3.48. The pH value of eco enzyme manufactured from organic mix garbage was 3.6 [4] The pH of eco enzyme made from pineapple was reported at 3.4-3.7; made from oranges was reported at 3.2-3.3; made from tomatoes was reported at 3.1-3.4 [4], and made from *Carica papaya* L was reported at 3.29 [16]In nature, the pH of garbage enzyme solution is acidic because of its acetic acid contents [17].

In this study, there was no nitrite concentration detected in the eco enzyme by laboratory analysis procedure refer to SNI 06-6989.9-2004. The test results of the pure garbage enzyme, it was reported that it was acidic and did not contain ammonia nitrogen, and phosphorus [18].

4.2 The effect of eco enzyme application on the water samples

The effect of applying eco enzyme with concentrations of 0% (as a control), 2%, 4%, and 6% on nitrite concentration in water samples for 10 hours can be seen in Table 1. These observations were made by analyzing the concentration of nitrite concentration every hour.

Table 1. The effect of eco enzyme and time exposure on nitrite concentration

Exposure Time (hrs)	Control (0 % eco enzyme)	2% -Eco enzyme	4% -Eco enzyme	6% -Eco enzyme	Control (0 % eco enzyme)	2% - Eco enzyme	4% -Eco enzyme	6% -Eco enzyme
	NO_2 concentration (mg/L)					**NO_2 reduction (%)**		
0	21.53	21.53	21.53	21.53		0.0%	0.0%	0.0%
1	21.51	19.15	17.81	18.37	0.1%	11.0%	17.2%	14.6%
2	21.22	20.30	20.07	19.26	1.4%	4.3%	5.4%	9.2%
3	21.53	20.20	19.90	18.70	0.0%	6.2%	7.6%	13.2%
4	21.56	18.50	17.06	17.28	-0.1%	14.2%	20.9%	19.9%
5	21.42	18.67	17.39	15.83	0.5%	12.8%	18.8%	26.1%
6	21.53	17.31	15.02	16.61	0.0%	19.6%	30.2%	22.9%
7	21.48	17.16	15.16	13.98	0.3%	**20.1%**	**29.4%**	**34.9%**
8	21.48	17.31	15.25	14.82	0.3%	19.4%	29.0%	31.0%
9	21.48	17.67	16.17	14.32	0.3%	17.7%	24.7%	33.3%
10	21.28	17.27	15.94	14.93	1.2%	18.8%	25.1%	29.8%

Table 1 showed that there was a decrease in nitrite concentration in the samples that were added by eco enzyme compared to the control. The nitrite concentration of control was relatively stable within 10 hours of exposure. The stability of nitrite concentration in the control samples is proven statistically by its result regression analysis which showed equation: nitrite (mg/L) = -0.0007 time (hr) + 21.49, and value of significance F (P) = 0.528 > 0.05. A very low linear coefficient and P > 0.05 showed that there was no significant time exposure effect on the control samples during 10 hrs. It can be seen that the reduction of nitrite concentration in samples added by eco enzyme was caused by eco enzyme activity.

The key enzymes in nitrification are located in the complex internal membrane system in many species of nitrifying bacteria, are ammonia monooxygenase (which oxidizes ammonia to hydroxylamine), hydroxylamine oxidoreductase (which oxidizes hydroxylamine to nitric oxide - which

is oxidized to nitrite by a currently unidentified enzyme), and nitrite oxidoreductase (which oxidizes nitrite to nitrate) [15]. The enzyme of oxidoreductase is used by the microorganism to oxidize nitrite to nitrate. The nitrite reductases are present in Nitrobacter spp., Nitrococcus Marinus, Nitrospira spp. The microorganism of Nitrospina could reduce nitrite to nitric oxide. The oxidation of nitric oxide has been observed in Nitrobacter, but it was still unclear whether this reaction is biotic or abiotic, what kind of enzyme that responsible for this mechanism remains unknown [15]. The nitrogen sources such as NO2 is degraded by microorganism with secretes their enzyme [19]. The observations confirm that the activity of the enzymes, which are amylase, protease, and lipase, is present in the eco enzyme [3].

The reduction of nitrite is a part of the nitrification process in reducing ammonia. The reduction of ammonia by hydrolytic reaction of eco enzyme in converting ammonia, nitrogen, and ammonium into more soluble were reported [20]. Some evidence also supported the suggestion that nitrite may use by DNPAO (denitrifying polyp-P-accumulating organisms) in P uptake [21]. The application of garbage enzyme was showed remove ammonia in waste wastewater effectively [22]. Many studies have reported that the application of the eco enzyme to treat organic pollutant in domestic wastewater and greywater can remove ammonia nitrogen [17].

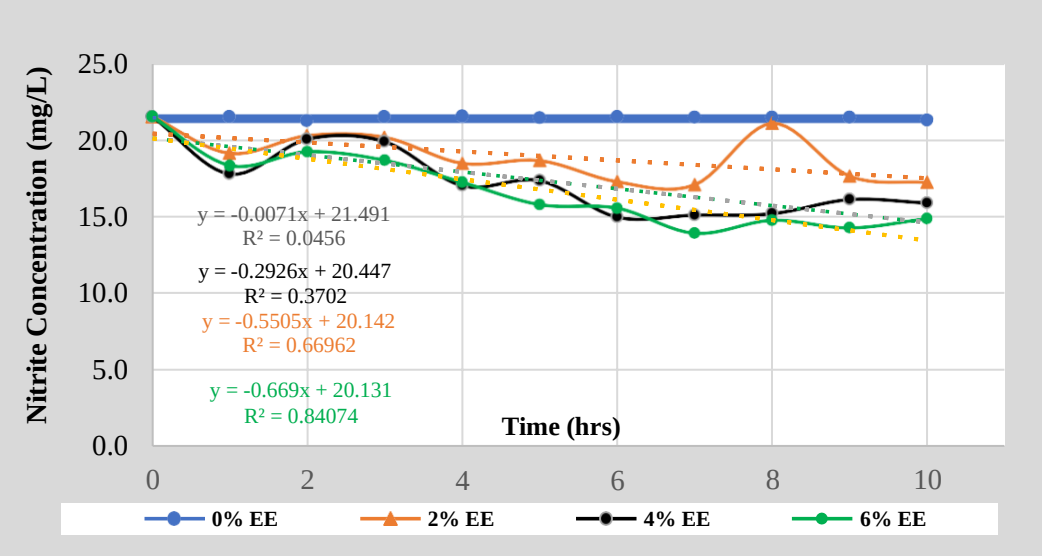

Figure 2. The effect of eco enzyme concentration and time exposure on nitrite concentration

The linear regression between nitrite concentration and exposure time on various concentrations of eco enzyme application is shown in Figure 2. The linear model was getting better with increasing the eco enzyme that was shown on a higher value of R^2 (the coefficient of multiple determination) [23]. The value of R^2 is the fit indicator of the regression equation; that is to give the proportion or percentage of the total variation in the dependent variable (time) that is explained by the independent variable (nitrite concentration).

The coefficient of the linear equation in Figure 2 showed that the more concentration of eco enzyme application has a more negative value, they are -0.29 for 2%-eco enzyme, -0.55 for the 4%-eco enzyme, and -0.67 for 6%-eco enzyme. This analysis showed that the addition of eco enzyme has an effect on reduction of nitrite concentration in the water samples, and the effect getting more on the higher eco enzyme concentration application. It showed that adding eco enzyme with a concentration of 2%, 4%, and 6% can reduce the nitrite concentration successively by 20.1%, 29.4%, dan 34.9% respectively.

It has been reported that the application of the higher concentration of eco enzyme and the longer the time exposure, given the greater results in the reduction of ammonia concentration in wastewater [17] The nitrates reduction level from 5.54 to 3.39 mg/L was reported in the 0.5%-eco enzyme testing application in the drain water [24].

The F-test for variance analysis of the nitrite concentration of samples compared to the control samples showed the results are unequal for all the samples. The unequal variances can be seen by P-value are < 0.05, which are 3 x 10^{-10} for 2%-eco enzyme, 1.1 x 10^{-11} for the 4%-eco enzyme, and 4.7 x 10^{-12} for 6%-eco enzyme application. Based on the results of these F-tests, then a t-test is carried out to know the significance of different concentrations of nitrite concentration of samples and control samples. The t-test result showed that the P-value for all the samples is P<0.05, which showed there is a significant difference in each sample's nitrite concentration compared to their controls. The result of t-Test: Two-sample assuming unequal variances, P(T<=t) two-tail are 0.00045 for 2%-eco enzyme, 0.00013 for the 4%-eco enzyme, dan 7.9 x 10^{-5} for 6%-eco enzyme. These t-tests indicate that eco enzyme application on water samples can reduce the nitrite concentration significantly. ANOVA test for comparing treatment without eco enzyme (control) with eco enzyme also in line with t-Test that theresults were P = 5.16 x 10^{-5} for 2%-eco enzyme, P=6.7 x 10^{-6} for 4 % eco enzyme, and P = 3.09 x 10^{-6} for 6%-eco enzyme.

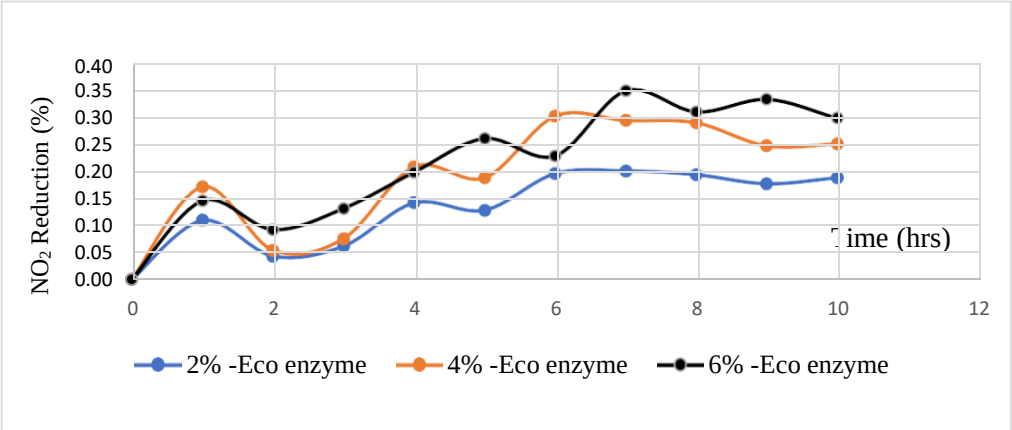

Figure 3. The effect of eco enzyme concentration and time exposure on nitrite reduction

From the data in Table 1, it can be developed a graph in Figure 3 that showed the nitrite reduction of samples at the 7th hour the highest results were achieved, while at the 8th to 10th hours there was tend to decrease.

4.3 The effect of eco enzyme concentration on the nitrite reduction

Based on the result that showed there was a significant reduction in nitrite concentration in water samples by eco enzyme addition, the next experiments were application higher concentration of eco enzyme to find more nitrite reduction. The experiments were done with eco enzyme concentration addition of 6%, 10%, 15%, and 20% application in 7 hours exposure time. The selection of 7 hours based on the previous results that nitrite reduction showed the best value at the 7th exposure time.

Table 2. The effect of eco enzyme concentration on nitrite reduction

Exposure Time (hrs)	Control (0 % eco enzyme)	6 % -Eco enzyme	10% -Eco enzyme	15% -Eco enzyme	20% -Eco enzyme
	NO_2 (mg/L)				
0	21.74	21.74	21.74	21.74	21.74
7	21.69	13.98	13.77	11.58	11.01

The experiments results can be seen in Table 2 that indicated the nitrite concentration in control samples were stable, while the samples added eco enzyme has a nitrite lower concentration after 7 hours. Figure 4 shows the effect of eco enzyme applied concentration (%) on nitrite concentration (mg/L). The graph shows that there was a negative linear relationship between the eco enzymes applied concentration and nitrite concentration with a linear coefficient of -23.79 and coefficient determination (R^2) was 91.72%.

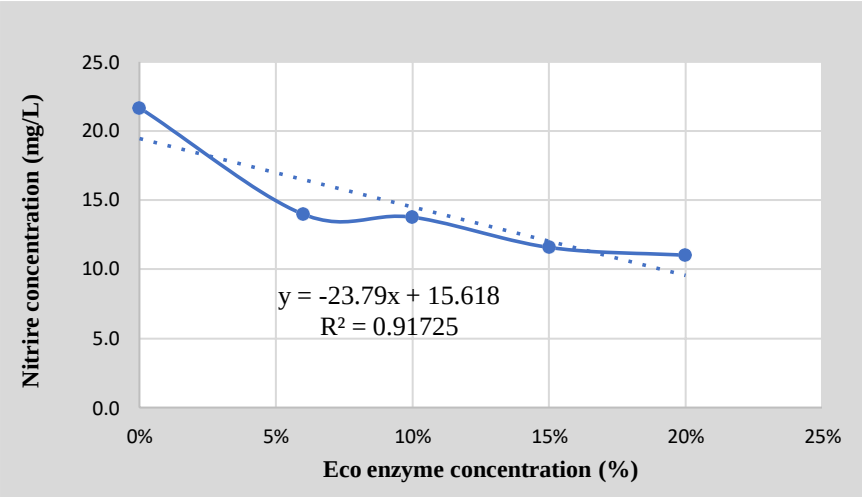

Figure 4. Nitrite reduction with various concentration eco enzyme application

Figure 5 showed more clearly that after 7 hours of exposure, the nitrite concentration in the control sample was relatively stable, while the sample with the eco enzyme 2%, 4%, and 6% have a negative coefficient or there was a decrease in nitrite concentration.

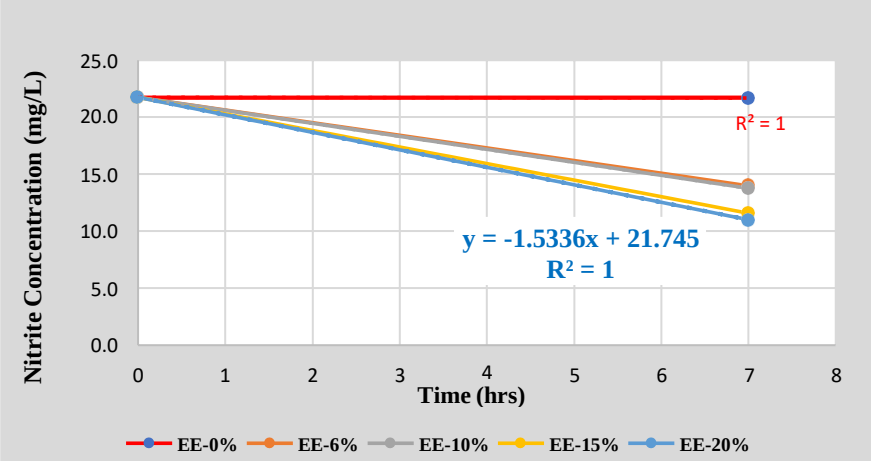

Figure 5. The Effect of eco enzyme concentration on nitrite concentration after 7 hours application

The effect of eco enzyme application concentration on nitrite can be seen in Figure 6, which showed a positive linear relationship. The data is shown in Table 3. The effect showed that the higher the concentration of added eco-enzyme, the greater the decrease in the concentration of nitrite.

The application for 5 days of 10% eco enzyme in artificial greywater samples reported can reduce the ammonia levels from 9.6 mg/L to 0 mg/L [17].

Table 3. The effect of eco enzyme concentration on nitrite reduction

Exposure Time (hrs)	Control (0 % eco enzyme)	6 % -Eco enzyme	10% -Eco enzyme	15% -Eco enzyme	20% -Eco enzyme
	NO_2 reduction (%)				
7	0.3	35.7	36.7	46.7	49.4

The positive linear correlation has a coefficient of + 2.954 which reaches a 49.4% reduction with 20% -eco enzyme addition. In this study, the initial nitrite of 21.74 mg/L can be reduced to 11.01 mg/L or a 49.4% reduction in applying 20% eco enzyme. The achievement of the nitrite concentration from the original 21.74 mg/L to 11.07 mg/L still does not meet the quality standard of 1 mg/L according to the Minister of the Environment No. 5 of 2014 concerning the wastewater quality standard. However, the result of a 49.4% decrease in nitrite concentration has significantly reduced the level of water pollution.

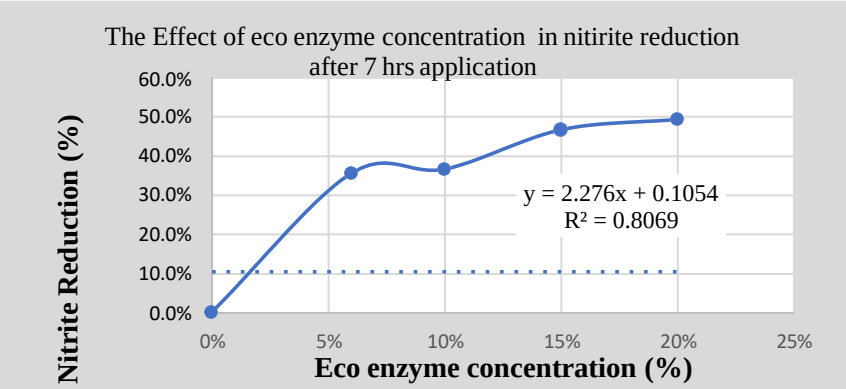

Figure 6. The Effect of eco enzyme concentration on nitrite reduction after 7 hours application

The reduction of TAN (Total ammonia Nitrogen) was sharply more effective with the exposure time. The study result showed that the reduction rates of TAN with 15 % eco enzyme were significantly higher than 10 % and 5 % [3]. It also found that eco enzyme not only very efficient in removal TAN, but also very efficient in removal Total Suspended Solid (TSS), Volatile Suspended Solids (VSS), Total Phosphorus (TP), and stabilization of the Chemical Oxygen Demand [3]. The effectiveness of eco enzyme solution application for removing the concentration of ammonia, nitrogen, and phosphorus also reported that the result is highly effective [24]. The effectivity in removing Total Phosphate (TP) and TAN that achieved up to 90 by eco enzyme was initiated by its characteristic and biocatalytic property [3] The optimum concentration of eco enzyme application was found 10% in removing TSS, VSS, TP, TAN, and COD by the reduction efficiency of 87%, 67%, 99%, 91%, and 77% respectively [3].

In this study, the decrease in nitrite concentration continued to occur up to 49% even in an acidic environment. This will be an advantage of the nitrite removal by eco enzyme because nitrification occurs naturally at the pH range of 6.45-8.95 and lower than 6.45 for complete inhibition nitrification to take place.

5. Conclusion and recommendation

5.1 Conclusion

Based on the study, conclusions and recommendations can be summarized as follows:

The study on the application of eco-enzyme made from a mixture of papaya and spinach in artificial water samples to reduce nitrite levels showed the following results:

1. Eco enzyme made from a mixture of papaya and spinach can reduce the nitrite concentration in the water sample. The t-Test showed that the effect of eco enzyme application in reducing nitrite in water samples was significant.

2. The application of eco enzyme 0%, 2%, 4%, and 6% respectively during 10 hours exposure showed the nitrite removal were 0.3 %, 20%, 29%, and 35 %. It can be seen that the reduction of nitrite concentration in samples added by eco enzyme was caused by eco enzyme activity. The application at a higher concentration of 10%, 15%, and 20% showed that nitrite removal efficiencies were 35.7%, 36.7%, 46.7%, 49.4% respectively during 7 hours exposure.
3. The result showed that longer time exposure, and also the more eco enzyme concentration, showed the more result in the effect of nitrite reduction concentration in water samples.
4. By this study it is proven that application of eco enzyme made from organic garbage can be an alternative solution for garbage and wastewater quality problem.

5.2 Recommendation

Further research should be carried out by measuring the concentration of nitrate to determine whether a decrease in nitrite indicates an increase in nitrate in the water sample. In addition, further research should use the wastewater samples to determine whether the reduction of nitrite is still effective in the presence of other materials contained in the real wastewater samples.

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