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Eco Enzymes Application in Nitrite and pH Reduction Study in The River Water Samples

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Abstract
One of the pollutants that must be controlled in rivers is the level of nitrite. Excessive amounts of nitrite are detrimental because they affect the quality and health of aquatic ecosystems. This study aimed to determine the effectiveness of eco enzymes in reducing nitrite levels in polluted river water as an alternative to conventional water treatment technologies. Three types of eco-enzyme made from vegetable, tomato and orange waste were used in this study. The experiment also aimed to evaluate the potential of reducing nitrite levels and its effect on pH performance. The results showed that adding 5% Vegetables Eco Enzyme (VEE), Tomatoes Eco Enzyme (TEE) or Oranges Eco Enzyme (OEE) to river water samples with an initial nitrite level of 4.813 mg/L produced the best results, with VEE showing the greatest effectiveness. This is because the nitrite concentration was reduced by 100% in the 4^(th) hour for VEE, the 6^(th) hour for TEE and the 8^(th) hour for OEE. For the pH parameter, there was a significant decrease, but it remained within the quality standard of pH 6–9. Statistical analysis using t-tests and ANOVA showed significant differences in both nitrite levels and pH results, as well as in the type of treatment.
Keywords: *eco enzyme, nitrite, pH, water pollution, river water*

Abstrak
Salah satu pencemar yang harus dikendalikan di sungai adalah kadar nitrit. Nitrit dalam jumlah yang berlebihan akan merugikan karena mempengaruhi kualitas dan kesehatan ekosistem perairan. Tujuan penelitian adalah untuk mengetahui aplikasi eco enzyme dalam menurunkan kadar nitrit pada air sungai yang tercemar, sebagai alternatif pengolahan dalam teknologi pengolahan air. Dalam penelitian ini digunakan 3 jenis eco enzyme yang terbuat dari sampah sayur, tomat, dan jeruk. Percobaan dilakukan selain untuk mengetahui potensi penurunan kadar nitrit, juga dievaluasi pengaruhnya terhadap kinerja pH. Hasil penelitian menunjukkan bahwa penurunan kadar nitrit pada sampel air sungai yang ditambahkan masing-masing 5% *Vegetables Eco Enzyme* (VEE), *Tomatoes Eco Enzyme* (TEE), *Oranges Eco Enzyme* (OEE) dengan kadar nitrite awal 4,813 mg/L menunjukkan kinerja terbaik pada penggunaan VEE. Hal ini karena konsentrasi nitrit mencapai 100% reduksi pada jam ke-4 untuk VEE, jam ke-6 untuk TEE dan jam ke-8 untuk OEE. Untuk parameter pH terjadi penurunan yang signifikan, namun masih sesuai dengan baku mutu yaitu antara pH 6 – 9. Hasil analisis statistik pada uji t dan ANOVA menunjukkan bahwa penambahan eco enzyme menunjukkan perbedaan yang signifikan baik pada kadar nitrit dan pH yang dihasilkan maupun pada jenis perlakuan.
Kata Kunci: *air sungai, Eco enzyme, nitrite, pencemaran air, pH*

1. Introduction

The Water quality contains several parameters, namely physical parameters (turbidity, temperature, dissolved solids, etc.), chemical parameters (pH, nitrite, BOD, dissolved oxygen, metal, etc), and biological parameters (presence of bacteria, plankton, etc). In this study, the pollution caused by nitrite parameters will be discussed. This is because nitrite is an unstable compound. In the research of [1], it was found that the percentage of nitrite reduction was the smallest when compared to ammonia and nitrate. Nitrite is an intermediate (transition) step in the biological decomposition of nitrogen-containing organic compounds. Organic matter in the form of protein contained in river waste is decomposed into ammonia with the help of decomposing microorganisms. Under aerobic conditions, ammonia is oxidized to nitrite, then nitrite is oxidized again to nitrate, so that the most common chemical compound found is nitrate.

In the human body, nitrogen compounds cause toxic effects if the compound is nitrite. The toxic effect caused by nitrite is methaemoglobin, which inhibits the transport of oxygen in the bloodstream. If the amount of methaemoglobin is more than 10% of the total hemoglobin, a condition called cyanosis will occur. Cyanosis is a condition in which all human tissues are deprived of oxygen [2]. The acute toxic effect of nitrite is methemoglobinemia, in which more than 10% of hemoglobin is converted to methaemoglobin. If this conversion exceeds 70% it will be very fatal. The effect of nitrite in large quantities on the human body is that it can cause bloody diarrhea followed by convulsions, coma, if not treated will lead to death. Chronic poisoning can cause generalized depression and headaches. Nitrite will react with hemoglobin and will form methemoglobinemia (MetHb) **[**[3]

The content of nitrite in water that is consumed or used in daily life can be harmful to health if in high concentrations. According to the Minister of Health Regulation No. 32 of 2017 concerning Environmental Health Quality Standards and Water Health Requirements for Sanitary Hygiene Needs, the quality standard for nitrite levels is 1 mg/L. The research of [4] stated that of the 82 respondents who used river water for bathing, there were 18 respondents (22%) complained of skin disorders. Nitrite concentrations above the threshold are very risky to health and often result in death. Even in children it often causes blue baby syndrome or called methemoglobinemia.

Some previous research about reducing nitrite has been studied with the activated carbon method by coconut shell [1], reducing nitrite by activated charcoal powder adsorption method made from moringa seed [5],Other related research was removing nitrate in groundwater by activated sludge as the bacteria source [6].

Eco enzyme is an environmentally friendly liquid that can be used as a multipurpose liquid. The materials or organic waste used are still fresh (not rotten) [7]. Eco enzymes were first introduced by Dr. Rosukon Poompanvong from Thailand. Besides being able to help recycle organic waste, the Eco enzyme can also be used as an alternative to improve textile wastewater treatment processes by removing color [8] and reduce *E.Coli* level in the leachate water sample generated from Garbage temporary storage [9]

Previous research about eco enzyme application was: 10% eco enzyme made from tomatoes and oranges in the aquaculture pond sludge was able to reduce the total value of ammonia nitrogen by 91% [10]. The use of a 10% eco-enzyme solution for synthetic wastewater can reduce ammonia nitrogen by 100%[11] and application of eco enzyme made from fruit and vegetables wastes on the aquaculture sludge reported that the eco-enzyme solution (10%) can reduce total ammonia nitrogen by 94% in aquaculture solid waste [12].Research on eco enzyme application for reducing nitrite in the water sample reported a 35% reduction in 7 hours of exposure with 10% eco enzyme made from a combination of papaya and spinach application [13]. Application of 5%-eco enzyme made from banana reported reduced of 5 mg/L nitrite artificial sample in 9 hours exposure by 94%, while eco enzyme made from orange reduced by 73% [14].

2. Material and Methods

River Water Sampling method

The river water sampling method used in this research is Simple Random Sampling without paying attention to the population level. This was done because each population unit has an equal opportunity to be selected as a sample. The samples were taken at Cilemahabang River Kabupaten Bekasi Water (**Figure 1**)

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Fig 1. Cilemahabang River by Google Earth (Source: Google Earth 2025)

River Water Population

The population in this research is river water that was obtained from the Cilemahabang River Water area which is located at Jababeka, Cikarang, Bekasi. The type of the sample is homogeneity, that is the elements of the population taken have relatively uniform properties with each other.

Water sample preparation

The water sample was prepared by adding nitrite solution standard into the Cilemahabang river water sample to get about 5 mg/L concentration of nitrite. This procedure was done because the original nitrite concentration in the river water sample was < 1 mg which was too low for research purposes.

Enzyme

Eco enzyme production refers to the previous research [15], there are the procedures for making eco enzymes. Eco enzymes that were applied in the research were VEE (eco enzyme prepared from mixed vegetables trash), OEE (eco enzyme prepared from oranges peels), and TEE (eco enzyme prepared from Tomatoes trash).

Nitrite Measurement

Measurement of nitrite levels in river water using the equipment and materials referring to SNI 06-6989.9-2004. The analysis of nitrite was done by two replications.

pH Measurement

The pH parameter was measured with a pH meter Eutech Instrument PC 450 type and refers to SNI 06-6989.11-2019. The analysis of pH was done by two replications.

Observation Method

The experiment was conducted every 2 hours, this is because each measurement will take time to prepare, and a gap of 2 hours is enough time to prepare for the next measurement. The sample was observed until the color of the sample changed from pink to clear. Analysis of NO_2 parameter analysis was carried out using a spectrophotometer UNICO type (4802 UV/VIS Double Beam Spectrophotometer) and referring to SNI 06-6989.9-2004. The sample has been diluted 25 times, this is used because the sample has added a nitrite concentration of 5 mg/L, while the limit on the spectrophotometric reading is in the range of 0.01 mg/L to 1.00 mg/L NO_2-N.

Type and concentration of eco enzyme application

In this study, 3 types of eco enzyme which had been applied were eco enzyme which made from vegetables (VEE), eco enzyme made from tomatoes (TEE), and eco enzyme made from oranges Eco (OEE). The concentration of all eco enzymes used in this study was 5%.

3. Results and Discussion

Eco enzyme characteristic

The characteristics of eco enzymes after 3 months of fermentation are observed that eco enzymes were acidic with low pH values which were 5.06 for VEE, 3.59 for TEE, and 3.93 for OEE. pH of OEE was reported 4.064 [14], and 3.96 [8]. Eco enzyme prepared from banana was 4.891 [14], prepared from papaya and spinach combination was 3.48 [13], and from dragon fruit was 3.29 [8].

The nitrite content in all eco enzymes VEE, VEE, and OEE was 0 mg/L. Nitrite content in eco enzyme prepared from papaya and spinach combination was reported zero [13]. The research [14] mentioned that eco enzyme prepared from bananas showed a nitrite level was 0.172 mg/L and prepared from orange was 0.210 mg/L[14].

Experimental result of eco enzyme application in nitrite performance

The initial value of nitrite was 4.813 mg/L. The reduction of nitrite concentration after 5% adding VEE, TEE, and OEE is shown in **Table 1.**

Table 1. The decrease of nitrite concentration after VEE, TEE, and OEE application

Time (hour)	Control Sample (mg/L)	5% VEE application (mg/L)	5% TEE application (mg/L)	5% OEE application (mg/L)
0		4.813		
2	4.269	3.117	4.110	3.587
4	4.031	0	3.501	1.938
6	3.971	0	1.924	0
8	3.938	0	0	0
% - reduction	18%	100%	100%	100%

The initial concentration of nitrite was 4.813 mg/L. By application of VEE, at the second hour, the nitrite concentration has reduced to 3.117 mg/L (35%), and at the 4th, 6th, and 8th hours, it can already be reduced to 0 mg/L (100%). By application of TEE, at the second hour the nitrite concentration has reduced to 4.110 mg/L (15%), 4th at 3.501 mg/L (27%), 6th at 1.924 mg/L (60%), and 8th hours it can already reduce to 0 mg/L (100%). By application of OEE, at the second hour, the nitrite concentration has reduced to 3.587 mg/L (25%), and at the 4th, 6th, and 8th hours, it can already be reduced to 0 mg/L (100%). The comparison of the application of 5% eco enzyme VEE, TEE, and OEE in the nitrite performance with the same initial sample is shown in **Figure 2**. It showed that the best nitrite reduction was done by VEE only within 4 hours, nitrite reduction achieved 100%, followed by OEE in 6 hours and TEE in 8 hours.

These study results were aligned with previous research [13] with a water sample made by distillation water that was added by nitrite solution standard up to 21.53 mg/L nitrite concentration. The research [13] has reported that the application of 6% eco enzyme prepared from papaya and spinach combination can reduce nitrite level by 29.8% within 10 hours, and 36.7 % reduction with 10% eco enzyme concentration in 7 hours. Application 5% of BEE and OEE in water sample reported [14] that can reduce nitrite 5.162 mg/L 93.89% by BEE and 73.24% by OEE in 9 hours application. Application of BEE also reported [16] can reduce nitrite in aquaculture effluent water by 66.39% in 60 minutes of treatment.

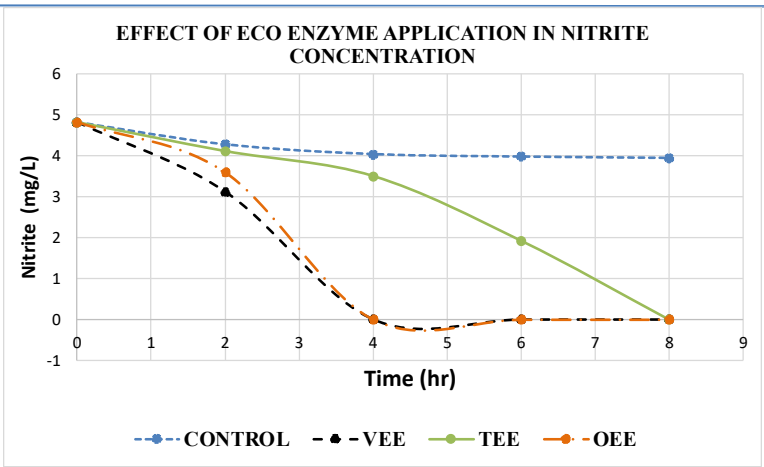

Fig 2. Effect of eco enzyme application in nitrite concentration

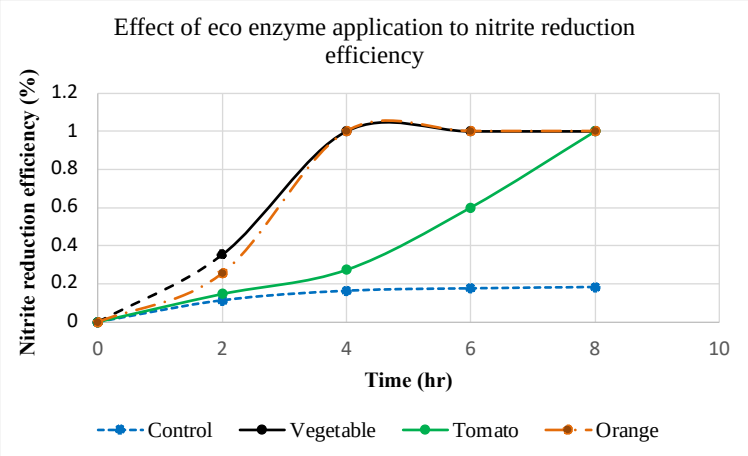

Fig 3. Effect of eco enzyme application in nitrite reduction efficiency

Experimental result of eco enzyme application in pH performance

The initial value of pH was 7.12 mg/L. The changes in pH level after adding 5% of VEE, TEE, and OEE are shown in **Table 2.** It showed that pH values were immediately dropped after 5% eco enzyme application to 6.42 for VEE, 6.05 for TEE, and 6.54 for OEE. This lowering pH also was reported [14] to 5.96 after 5% of OEE in the water sample. Application of 10% of OEE in textile wastewater with a pH value was 10.95 could be lowered to 10.45, and by 10% DEE was lowered to 10.22 [8].

Table 2. pH performance after VEE, TEE, and OEE application

Time (hour)	Control Sample	5% VEE application (mg/L)	5% TEE application (mg/L)	5% OEE application (mg/L)
0	7.12	6,42	6.05	6.54
2	7.32	6,92	6.35	6.80
4	7.32	7.01	6.49	6.70
6	7.55	7.06	6.44	6,59
8	7.64	7.04	6,59	6.40

The comparison of the application of 5% eco enzyme VEE, TEE, and OEE in pH level from the same initial sample is shown in **Figure 4**

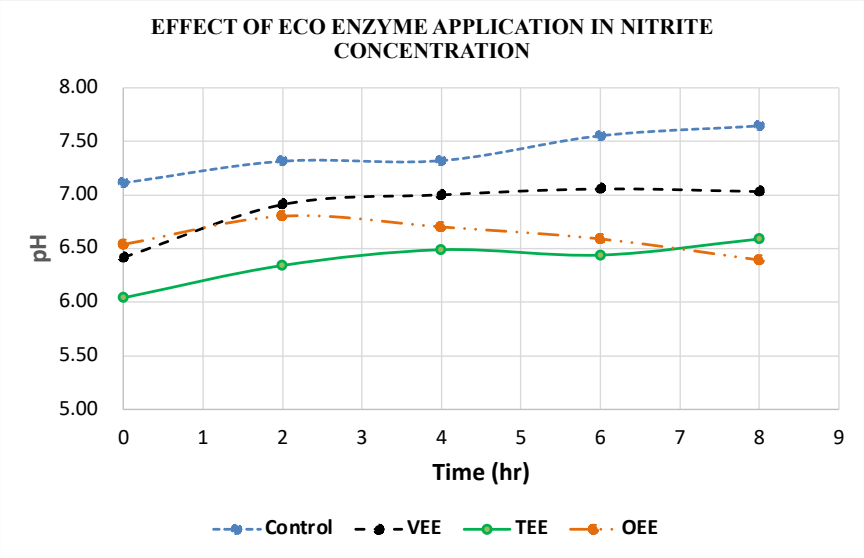

Fig 4. Effect of eco enzyme application on pH performance

Table 3 and **Figure 3** showed that by the exposure time, their pH consistently increased to 7.64 for the control, 7.04 for the sample added by VEE, and 6.59 sample added by OEE. For sample added by TEE had a different performance which increased in the second hour, but after that got lowered and reached 6.40 in the 8th hour. The application of 5% BEE reduced the pH water sample from 5.96 to 4.66 and by OEE to 3.76 in 3 hours [14]. In textile wastewater application, 10% OEE can be reduced from 10.45 to 8.81 and 10% DEE from 10.45 to 8.71 in 96 hours [8]

Statistical analysis

A t-test statistical analysis was done to know if was there any significant difference in performance between the control and each sample that was added by each eco enzyme. An ANOVA analysis was done to know if there was any significant difference in treatment between the control and each sample that was added by each eco enzyme. The statistical analysis for nitrite concentration was done for data from the 2nd until the 8th hours as shown **in Table 3.**

Table 3. Summary of t-test statistical analysis of nitrite performance

Time	Summary of t-test result, alfa 5%, P (T<=t) two tail		
(hrs)	Control vs VEE	Control vs TEE	Control vs OEE
2	0.103	0.108	0.010
4	0.001	0.007	0.000
6	0.000	0.002	0.000
8	0.000	0.000	0.000

Data in **Table 3** showed that an application of 5% VEE and TEE in the water river sample had a significantly different nitrite concentration compared to the control sample (no eco enzyme application) at 4, 6, and 8 hours of exposure because alfa values were less than 0.05. The application of OEE has a significantly different nitrite concentration compared to the control sample from 2 hours up to 8 hours exposure. ANOVA single analysis factor for comparing treatments without eco enzyme, VEE, TEE, and OEE application has a result P value was 0.038, which meant there was a significant difference among those treatments.

The statistical analysis for pH values was done for data from the 2nd until the 8th as shown in **Table 4.**

Table 4 Summary of t-test statistical analysis of pH performance

Time	Summary of t-test result, alfa 5%, P (T<=t) two tail		
	Control vs VEE	Control vs TEE	Control vs OEE
0	0.003	0.002	0.005
2	0.015	0.001	0.000
4	0.050	0.006	0.011
6	0.006	0.001	0.001
8	0.005	0.000	0.002

Data in **Table 4** showed that an application of 5% VEE, TEE, and OEE in the water sample had significantly different pH levels compared to the control sample (no eco enzyme application) at 2, 4, 6, and 8 hours of exposure because alfa value was less than 0.05. ANOVA single analysis factor for comparing treatment without eco enzyme, VEE, TEE, and OEE application has a result of P value was 9.3 x 10^{-6}, which means there was a significant difference among those treatments. ANOVA single analysis factor for comparing treatment without eco enzyme, VEE, TEE, and OEE application has a result of P value was 0.038, which means there was a significant difference among those treatments.

4. Conclusion

The application of eco enzymes prepared from vegetables, tomatoes, and oranges can reduce nitrite levels in river water samples significantly. The best reduction was performed by vegetables (Anon., n.d.) eco enzyme which can reduce 100% of nitrite 4.813 mg/L in 4 hours, while oranges eco enzyme and tomatoes eco enzyme required 6 and 8 hours respectively. The effect on the pH parameter showed a significant decrease, but it was still following the quality standard between pH 6 - 9.

5. Acknowledgment

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6. Abbreviations

ANOVA	Analysis of variance
hr	Hour
BEE	Banana eco enzyme
DEE	Dragon fruit eco enzyme
OEE	Oranges eco enzyme
TEE	Tomatoes eco enzyme
VEE	Vegetables eco enzyme
%	Percentage

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