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The Study of Eco Enzymes Application For Decoloring Textile Industry Wastewater Following by pH Value Analysis

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Abstract. There is a good potential between the increase in the growth of the textile industry, especially on a small scale which also causes a lot of environmental pollution of colored water in the water bodies of the area around the textile industry With the great potential of using Eco Enzyme which can be used as a biocatalyst. Eco Enzyme contains several types of enzymes, which can be used as tools to speed up the textile wastewater decoloring process which is included in the Biological method in textile wastewater treatment. **Objective.** To find out what are the results of color removal efficiency after adding by Eco Enzymes, and its effect on the pH values. **Methods and Results**. In this research, by using 10% concentration from Orange and Dragon Fruit Peels. The sample that was added by Orange Eco Enzymes (OEE) and Dragon Fruit Eco Enzymes (DEE) has a color removal respectively in 80% and 82% in 48h then continuously slowly degrades slowly to 86% and 86% in 120h. Before adding the Eco Enzyme, the sample has a high pH of 10.98. After added by OEE and DEE, in 48h respectively the pH values decrease to 9.58 and 8.98, then continuously slowly degrades to 8.70 and 8.56 in 120h. **Conclusion**. The results show that selected Eco Enzymes are able to decolor textile wastewater also following by reducing pH and already stable starts from 48h.

1 Introduction

Based on a press release from the Ministry of Industry of the Republic of Indonesia, explaining that in the first quarter of 2019, the textile and apparel industry recorded the highest growth rate of 18.98 percent, which means an increase of 8.73 percent from the same stage in 2018 which is only 7.46 percent [1]. In the same line, every year the Batik Industry in Indonesia is growing. The latest data released by the Ministry of Industry sourced from BPS or Central Bureau of Statistics of Indonesia and processed by the Directorate General of IKMA-Kemenperin states that in 2016, there were 2,951 batik business units that had been established in Indonesia, with details of micro businesses amounting to 1,794 units, small businesses amounting to 815 units, and medium enterprises of 342 units [2]. However, the textile industry's business activities have an impact on environmental damage. This is because there are still many textile industry producers who throw their wastewater into the river due to the well-known waste management process being expensive, while the batik industry is dominated by small-scale industries.

Between 2019 until 2021, several rivers in Indonesia, especially whose surroundings are dominated by small-scale industries has changed its color into a dark colored river due to chemical pollution until the residents complained because the river was polluted, emitted an unpleasant pungent odor, and physically changed its color to cloudy, red, green, to jet black which resulted in losses for fish, and feel itching on their skin after looking for these fish [3, 4, 5]. From these cases, it shows that a solution is needed to prevent and overcome the environmental damage that comes from the coloured wastewater from the textile industry. However, the solutions must be affordable, so it can also be used by small and medium-sized industries. Taking into account the value of profit that they still have to get, which can also go hand in hand with environmental pollution actions.

Currently, research is underway on the use of Eco Enzyme, which works similarly to enzymes that act as catalysts. Eco-enzyme, also known as garbage enzyme, is an organic solution made by fermenting fresh fruit and vegetable waste, brown sugar,

and water [6]. Successful studies showed a high removal percentage of TSS, VSS, TP, TAN and COD in the optimum concentration of 10% of eco enzymes [7][22]. By utilizing the eco enzyme that is made from household or market waste generation such as fruit and vegetables, it can minimize waste generation in landfills and save money by converting organic waste into a liquid that can be used for treating the wastewater. Also already proved, that eco enzymes with the optimum of 10% concentration in several studies are enough to show a high removal efficiency in several parameters.

Several enzymatic research methods as a Biological textile Wastewater Treatment methods for decolorization of textile wastewater are already done, But most of the enzymes used to de-coloring textile wastewater come from plants and fungi. One of the studies came from Mahmood. et al stated that Laccase that extracted from local green algae Cladophora sp was able to decolor four types of textile dyes, especially the black dye with percentage of 91.6% [8]. So, this research is conducted to find whether eco-enzyme can be used as a formula that can decolorize textile wastewater, because there are still very few who do research on this eco enzyme even though it has a great possibility for its bio-catalyst properties and its affordable value. Another parameter is also included in the research points this time, namely to examine the effect of the addition of eco-enzyme on its high alkaline pH values of textile wastewater.

2 Method

2.1 Population and Sample

The population in this study is the Textile Wastewater, with the samples are $\pm$ 6 liters of textile wastewater for the sampling needed in each observation step, taken from Jababeka Wastewater Treatment Plant I. Before the samples are used for the experiment, it goes to the homogenization process using Agitator Engine with maximum speed in 4800 r/min during 5 (five minutes).

2.2 Sampling Method

The textile wastewater was collected from Intake Point, specifically in the Collecting Box of Textile Wastewater Pump Station of The Jababeka Wastewater Treatment Plant I which is located at Cikarang, coordinate of Lat 6°16'25.37"S, Long 107° 7'39.95"E which will be shown by the Google Earth Projection oh the Fig 1 below. The samples were taken twice at 4pm on 27th September and 8th October 2021 based on the SNI 6989.59 : 2008 Water and Wastewater – Section 59 : Regarding wastewater sampling by spot random sampling method, which is directly taken by using a container and a rope.

2.3 Experimental Method

2.3.1 Eco Enzyme Preparation

The materials that are needed are Eco Enzymes, Textile Wastewaters, Distilled Water, and Buffer Solutions for pH analysis. The eco-enzymes used in this study are made from Orange and Dragon Fruit Peels. The making process of eco enzymes in this study was carried out based on research in 2017 [9], [10], [11]. In general, it needs closed containers, a knife, and a stirrer. Meanwhile, the materials needed include Orange and Dragon Fruit Peels as the main ingredients, water, and palm sugar; cut them first, so that the fermentation process is easier to do with smaller sizes; then combine the chopped oranges, water, and brown sugar in a 1:3:10 ratio (sugar; citrus fruits; water) in a tightly closed container.

Do the same for Dragon Fruit Peels using different containers. Let stand the container that already contains a mixture of fruits and vegetables, water, and sugar for 3 months to carry out the natural fermentation process. In the first month, check once a week to make sure and remove trapped gas to be released by opening the lid of the container. If you have left it for 3 months, eco enzyme is ready to use anytime.

2.3.2 Experimental Method

This research was conducted by experimenting with the addition of 10% eco-enzyme into textile wastewater. First, textile wastewater samples were homogenized using an agitator with 4800 r/min, and then 450 mL of sample will be transferred into a bottle one by one. Based on the research needs, each sample needs 2 (two) bottles for each sample to be added by an eco-enzymes plus 2 (two) bottles more as a control/blanks; After that 50 mL of eco enzyme that already filtered by a vacuum pump and using a Whatman paper sized 42, was added to each bottles, and the other 2 (two) bottles as control/blank were added 50 mL of distilled water.

The observation process for decoloring and pH parameters was carried out in every 24 (twenty four) hours in 6 (six) days; For the decoloring analysis using Spectrophotometer, the samples are filtered manually by using Erlenmeyers, Funnels, and Whatman paper sized 42, before they are analyzed in Spectrofotometer; For the analyze process, the filtered sample are taken about 3mL to cuvette and then placed inside the Spectrofotometer; Another cuvette is also placed inside that filled by a distilled water; The set for Spectrofotometer are using a wavelength of 607 nm; Meanwhile for the pH parameter, the analysis is directly done inside of the bottle by just putting the censored to the bottle.

2.4 Data Collection Method

The entire research schedule starts from the process of making eco enzymes which must go through a fermentation process for 3 months from June to September 2021. While the observation time starts from 8 to 13 October 2021.

The data was collected every 24 (twenty four) hours for 6 (six) days. But specifically for the decoloring parameter, observations were made after 2 (two) days of treatment on the sample, because in the 24h there still no significant differences in the color removal. Observation was conducted using several analyzing tools. First, for decoloring parameters using Spectrophotometer 4802 UV/VIS Double Beam method based on modified SNI 6989.80:2011 [12] standard with a focus on measuring the absorbance value of the color sample using the original sample

wavelength of 607 nm as it standards due to the unstable conditions of textile wastewater color. But, before that, the sample had gone through a manual filtering process using Whatman Filter Paper measuring number 42 nm so that the analyzed results only come from true colors.

For the pH analysis using pH Meter PC 450 based on the SNI 6989.11:2019 [13] Water and wastewater – Part 11 : How to test the degree of acidity (pH) using a pH meter, with a principle that The pH measurement method is based on potentiometric/electrometric measurements of hydrogen ion activity using a pH meter.

3 Results and Discussion

3.1 Eco Enzyme Characterization

The characteristics of Orange Eco Enzyme (OEE), and Dragon Fruits Eco enzyme (DEE) was observed for the pH parameter only. Those eco enzymes were acidic with the low pH values which were 3.96 for OEE and 3.29 for DEE. The acidity condition inside those eco enzymes means a lot for balancing the alkaline high value of textile wastewater. The other thing that is found here is the low pH value of OEE and DEE. This may be caused by the pH condition of those Eco Enzymes main ingredients, which are fruits that have an acidic content for both commodities. This may be proved also by fruits that contain natural acids, such as citric acid, malic acid, tartaric acid, ascorbic acid, until Vitamin C contributes to lowering the fruit's pH [14, 15].

Several studies also show an acidic or low pH condition in their Eco Enzymes that are made from fruits and vegetables. [7] Stated, this is caused by Eco Enzyme containing Acetate Acid from their fermentation process and their got pH 2.79 with acetate acid 14,130 mg/L and 2.86 with acetate acid 35,281 mg/L respectively for eco enzymes made from tomato and orange. The others studies also found that the pH of Eco Enzyme made from a mixture of fruits and vegetables which are tomato, cauliflower, pineapple, orange and mango dregs is 3.6 [9]; a mixture of pineapple, orange, tomato, and mango dregs is 3.07 [18]; apple and apple mixed Chinese

honeylocust are 4.4 and 5.1 [19]. Several studies also stated that Eco Enzymes made from fruits and vegetables contain the Hydrolysis Enzymes such Amylase, Lipase, and Protease that are possible as a biocatalyst enzymes [10, 11, 18, 20].

3.3 Textile Wastewater Characterization

Table 1 shows the characterization of textile wastewater that was used in this experiment. The sample was tested not far from the sampling time, just one hour after it, to avoid any changes that may occur during storage. The first step is analyzing the wavelength for dyes absorbance standard. The analysis was done by using Spectrophotometer at wavelength rate between 400-1100 nm, and then the graph shows a peak at 607 nm that became a wavelength standard for all analysis during the observation. After analyzing the sample, the result shows that the value of dyes absorbance in the sample is 0.570. Accompanying the additional parameters, which is pH of 10.98.

Table 1. Textile Wastewater characteristics

Dyes Absorbance (λ=607 nm)	pH
0.570	10.98

The others related studies about Textile Wastewater Treatment, found that the wavelength peak point or maximum wavelength of their sample that also used for their wavelength standard analysis is 661 nm, and shows the initial dyes absorbance value of 1.556 [21] and another one shows dyes absorbance value of 4.317 in maximum wavelength of 351 nm [22]. Another study also found that there was a high alkaline pH content of the textile industry wastewater in Pekalongan of 14 [23], then another study also found a pH of 10.5 in textile wastewater from individual scale textile industries in Pekalongan [24].

3.4 Decolorization of Textile Wastewater Added by Eco Enzymes

The samples are placed in a closed bottle to measure the color absorbance using a Spectrophotometer. About 10 mL of them are taken in every day to go to the

filtration process to get the true color before being moved again to the cuvette about 3 mL to be observed in Spectrophotometer. The observation was done in 4 (four) days after 2 (two) days treated by the eco enzyme due to the color removal starting to change significantly from 48h. After waiting for 2 (two) days, the sample was observed and the value on the 4th (fourth) day is already shown near to zero results.

Fig 1. The experimental Eco Enzymes Application to Textile Wastewater using Anaerobic process (OEE)

Fig 2. The experimental Eco Enzymes Application to Textile Wastewater using Anaerobic process (Control)

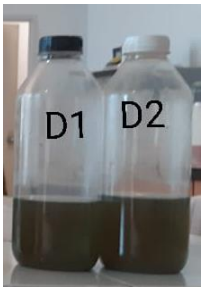

Fig 3. The experimental Eco Enzymes Application to Textile Wastewater using Anaerobic process (DEE)

It shows that the process of decoloring was done with good results. Even textile wastewater can naturally do the docoloring process because day by day the sample that was not added by Eco Enzyme also shows a slow decoloring result, but by using the eco enzyme it will show better results, especially starting from 48h as shown in the Table 3. Meanwhile, the Color Removal Efficiency is calculated based on a formula shown below, where the A0 is the initial value of dyes absorption and A is the after-treat value of dyes absorbance in each observation day **[25; 26]**.

$$\frac{A0 - A}{A0} x\ 100$$

Table 2 presents the results of the average values from duplicate sampling results of color absorbance measurements and % color removal calculation. The treatment uses 2 (two) kinds of eco enzymes which are made from Orange, and Dragon Fruit Peels.

Table 2. The experimental result of dyes absorbance and its decolorization absorption

Sample	Color Absorbance					% Color Removal					Description
(10%EE)	0h	48h	72h	96h	120h	0h	48h	72h	96h	120h	The Results are shows
Control	0.57	0.40	0.29	0.19	0.11	0%	29%	49%	66%	82%	as the **average values**
OEE	0.57	0.12	0.11	0.10	0.08	0%	80%	80%	82%	86%	from **duplicate**
DEE	0.57	0.10	0.09	0.08	0.08	0%	82%	84%	85%	86%	**sampling** method.

The table above shows the average values from the duplicate sampling method, both values of Color Absorbance and % Color Removal in 0h, 48h, 72h, 96h, and 120. The results show a high color removal rate and already shows a stable phase starting from 48h and resulting in the sample that was added by OEE and DEE decreased the color absorbance respectively 0.12 and 0.10 when the Control sample still has a high value, 0.40. Then, slowly degrade to 0.08 and 0.08 in 120h. Means those values have a color removal respectively in 80% and 82%, when the Control sample still has a low value of 29% color removal. Then continuously slowly degrades until 86% and 86% in 120h. This study also shows that the Control sample in 48h and 120h, respectively, showing about 29% and 82% color removal results.

Sample data from experimental results using eco enzymes showed significant results when compared to those not added samples. However, when viewed from the sample group that was added with Eco Enzyme only, the results did not show a significant difference, only a few digits behind the comma.

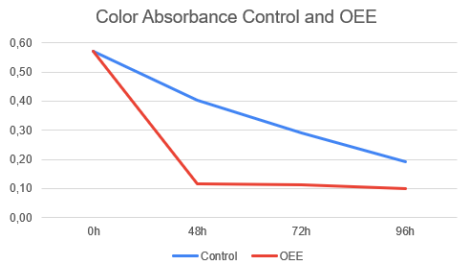

Fig. 4 Graph of Color Absorbance Differences Between Control and OEE

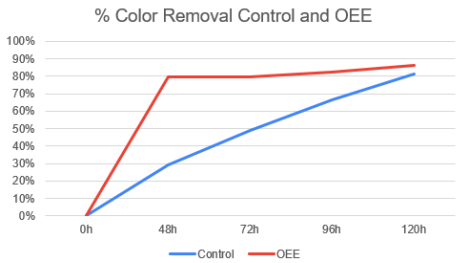

Fig. 5 Graph of % Color Removal Differences Between Control and OEE

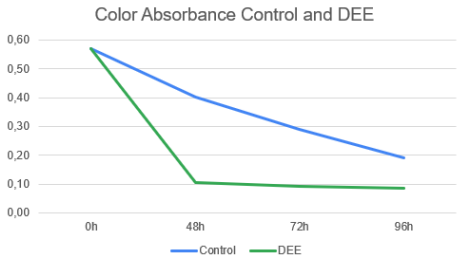

Fig. 7 Graph of Color Absorbance Differences Between Control and DEE

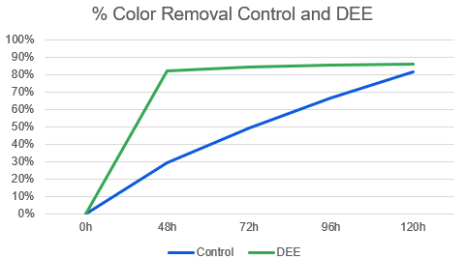

Fig. 8 Graph of % Color Removal Differences Between Control and DEE

The graph above shows that the results between Control (not added by Eco Enzymes) with OEE and DEE shows a difference, especially in 48h and then continuously slowly degrades until 120h. Another thing that happened in this study is textile wastewater can decolor slowly naturally. This may be possible, because according to a number of studies, there are decoloring bacteria isolated from colored wastewater that can help the textile wastewater that are used in this study to decolor naturally. In the dye waste there are bacteria that have been proven to be used as dye decolorizing agents, namely Pseudomonas stutzeri and Bacillus Sp., but this also requires some further actions such as optimization of parameters such as pH, temperature, to the concentration of wastewater or with the addition of enzymes to help optimize the performance of these bacteria [27,28,29].

Even though, by using Eco Enzyme the process is done faster than the original. This Eco Enzymes as fermented solution contains lipase enzymes whose concentrations can be influenced by the type and materials used [10]. The reason is because Orange and Dragon fruit peels that are used in this study may contain high Antioxidants that can also help the activity of Lipase Enzymes [16,17], which acts as a biocatalyst in the degradation process of textile wastewater. The picture below is a simple illustration of the reaction that can occur in the textile wastewater decoloring process, where what was originally an Azo complex bond is reduced to an aromatic amine bond with the help of lipase enzyme.

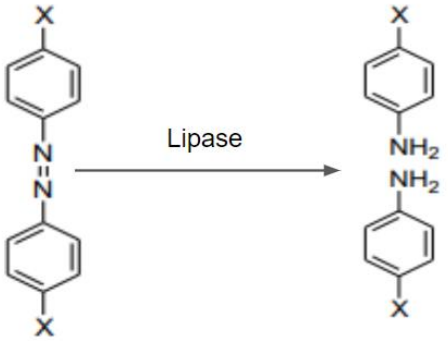

Fig 9. The reaction of decoloring process by Lipase Enzyme resulting Azo Bonds become Aromatic Amines with Direct Enzymatic Method

Waste from textile dyes containing azo takes a long time. This is due to the presence of a double bond (–N=N-) in azo compounds. This double bond (-N=N-) must be broken in order to describe the environmental pollution caused [31]. The illustration of reactions that happen inside the azo before and after adding Eco Enzyme containing the enzyme lipase are shown in Figure 12. The results will be a colorless solution which does not contain the double bonds again as the azo bond has, which is amines. Aromatic amines will arise after the process of reducing the azo bond which can be passed by several different mechanisms [32].

3.5 pH Levels on Decolorization Process

As additional data, the authors also do an analysis of the pH parameters. The results that will show in the table below are the values of the average of pH analysis results for 5 (five) days.

Table 3. The Experimental Result of pH Values (shows in average value from duplicate sampling)

Sample (10%EE)	pH					
	0h	24h	48h	72h	96h	120h
Control	10.95	10.81	10.66	9.66	9.23	9.13
OEE	10.45	10.35	9.58	9.95	8.81	8.70
DEE	10.22	10.02	8.98	8.87	8.71	8.56

The data obtained from analysis of the pH value of the sample after adding Eco Enzyme resulted in a better value than that which was not given. Respectively for

OEE and DEE in 48h resulting the pH values are all decreasing until 9.58 and 8.98, then continuously decreasing until 8.70 and 8.56 in 120h.

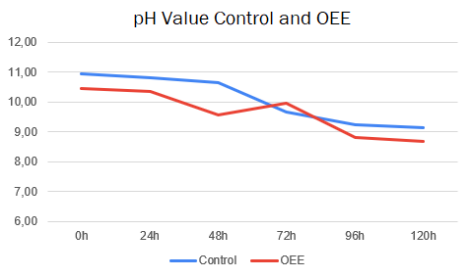

Fig 10. Graph of pH Value Differences Between Control and OEE

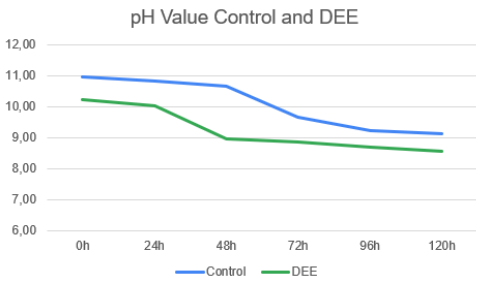

Fig 11. Graph of pH Value Differences Between Control and DEE

The graph shows that there are differences between Control (not added by Eco Enzymes) with OEE and DEE (added by Eco Enzymes). In 48h, which is the highest color removal point and already starts the stable phase. Surprisingly it also happens to the pH parameter. The values are all decreased from 10.98 to the range of 9 values for those that are added and still in the range of 10 for those that are not added. Then continuously degrade to the range of 8 for OEE and DEE, where this value is sufficient for the value of the Textile Wastewater Quality Standard **[33]**, but for the Control it is still in the range of more than 9, over the standard.

Theoretically, in the presence of a decolorization process, the biological reduction of azo bonds could result in an increase in pH due to the formation of aromatic amine metabolites, which are more basic than the original azo compounds **[34]**. While the pH of textile wastewater shows a high alkaline value, but after mixing with Eco Enzyme which has an acidic pH value, it shows that the pH value of textile wastewater that has been added with Eco Enzyme has its own resistance to decrease slowly and its condition may allow it to run slightly unstable due to the resistance between the alkaline decolorization process and the acidic Eco Enzyme.

4 Conclusions

It can be concluded that based on the experimental results that have been completed that:

1. From the color absorbance measurements on Spectrophotometer using wavelength of 607 nm and shows a high color removal rate and already shows a stable phase starts from 48h and resulting the sample that added by OEE and DEE has a color removal respectively in 80% and 82% then continuously slow degrade until 86% and 86% in 120h. In addition, if compared to the Control, it also has 29% and 82% color removal respectively in 48h and 120h, means that textile wastewater can decolor naturally, but by using Eco Enzyme it can be more faster due to the bio-catalyst ability of those Eco Enzymes.
2. For the pH value measurements using pH meter, before adding the Eco Enzyme, the sample has a high pH of 10.98. After the samples of Textile Wastewater added by OEE and DEE, respectively in 48h, the pH values are all decreasing until 9.58 and 8.98, then continuously decreasing until 8.70, and 8.56 in 120h. This is sufficient for the value of the Textile Wastewater Quality Standard.

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