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Eco Enzyme Production from Fruit Peel Waste and its Application as an Anti-Bacterial and TSS Reducing Agent

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Abstract

The increasing volume of organic waste in garbage dump facilities will emit methane gas, which is one of the greenhouse gases that cause global warming. By producing eco enzymes from fruit peel and vegetable waste, we can minimize organic waste while also creating beneficial products. In this research, eco-enzyme was produced from papaya, dragon fruit, and orange peel waste mixed with water and molasses. The weight ratio between water, organic compounds, and molasses was 10:3:1. The mixture was fermented for 3 months. The DNS method was used to test enzyme activity; the ASTM 2315:2008 method was used to measure bacteria killing power; and the SNI 06-6989-3-2004 method was used to measure TSS levels in liquid waste.The Eco enzyme product generated from this research has an amylase enzyme activity of 2.15 and a cellulase activity of 1.69. Eco enzyme effectiveness in killing E. coli bacteria reaches 99.95% and P. aeruginosa bacteria at 99.90% with only 20% of eco enzyme concentration within 15 seconds of contact time. The results of liquid waste processing using this eco enzyme show that TSS reduction rate is 65-88% on an initial TSS concentration of 345 ppm.

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1 Introduction

Methane gas generated from organic compounds is one of the greenhouse gases that can destroy the ozone layer that causes global warming (Friedrich & Trois, 2016; Jegannathan & Nielsen, 2013; Panda et al., 2015). A number of methods to reduce waste emissions can be implemented, for example, by managing waste and utilizing technology to generate power (Rajaeifar et al., 2017; Nathao et al., 2013). One method for reducing organic waste is to use fruit peel and vegetable waste to make eco enzyme. An eco enzyme is a complex organic liquid that contains a number of organic enzymes and acids. Arun & Sivashanmugam (2015), developed an eco enzyme from waste cabbage, pineapple, orange, and mango peels. Meanwhile, Arun & Sivashanmugam (2017), produced eco enzymes out of tomato and orange peel wastes. Besides that, Mavani et al. (2020), also produced eco-enzyme out of orange peel, pineapple hump, and papaya peel waste. Fruit peel waste mixture can also be utilized as eco-enzyme material as practiced by Mavani et al. (2020). The result of this research also shows that the previous researchers detected that eco enzymes contain organic acids such as lactate acid, malate acid, oxalate acid, acetate acid, and citric acid. Besides that, the compound also shows amylase, lipase, cellulase, and protease enzyme activities. With the existence of organic acids and enzymes, eco enzymes can be utilized as cleaning agents and waste processing agents (Arun & Sivashanmugam, 2015; Sambaraju & Sree Lakshmi, 2020; Etienne et al., 2013), while also having the nature of anti-bacteria and anti-fungi. In this research, eco-enzyme was produced from a mixture of papaya, dragon fruit, and orange peel waste. Produced eco-enzyme liquid was utilized to lower TSS levels in order to study its potential to process wastes that contain TSS. Besides that, it also aims to see the potential of eco-enzyme as an anti-bacterial agent (Selvakumar & Sivashanmugam, 2017).

2 Materials and Methods

Eco enzyme production

300 grams of organic waste (papaya, dragon fruit, and orange peels) were mixed with 100 grams of sugar cane drops (molasses) as a carbon source and one liter of water inside an airtight container with a 2 liter capacity. Fermentation was conducted for 3 months in sun-protected conditions at room temperature. After 3 months, the compound was filtered and the filtered result was collected as eco enzyme.

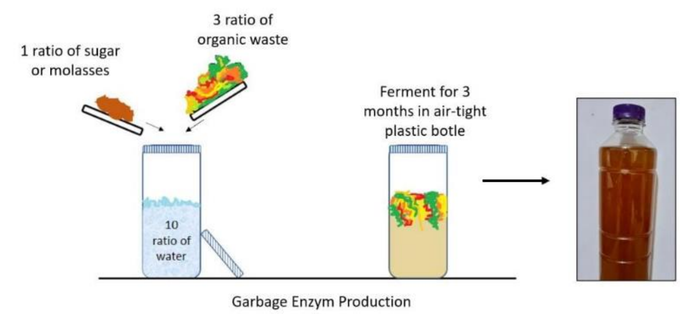

Figure 1. Eco Enzyme production from fruit peel wastes

Eco enzyme characteristics include pH, density, Total Suspended Solid (TSS), Total Dissolved Solid (TDS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), enzyme activities, and killing power against bacteria measurements.

Eco enzyme activity and killing power testing against bacteria

Enzyme activity testing was conducted by using the DNS method.
The Eco Enzyme sample was gradually dissolved from 10X, 100X, 500X, and 1000X. 250 L substrate + 250 L Enzyme were reacted for 15 minutes at 30ºC temperature. After that, 500 L of DNS was added and heated at 100

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C for 15 minutes. The compound was then cooled on ice for 10 minutes and measured with a spectrophotometer at a wavelength of 540 nm. Substrate control: 250 µL substrate + 250 µL buffer sodium citrate pH 3.0 0.05 M reacted on 30ºC temperature for 30 minutes. The next process was similar to before. The absorbance value generated was converted into sugar reducer concentration (ppm) by using a formula generated from the glucose standard curve.

$$\text{Amylase enzyme activity: } \frac{\text{Enzyme concentration (mg/ml) x dilution factor x 1000}}{\textit{reaction time}\ (15\ \textit{minute}) \times \textit{starch molecular weight}\ (180)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

250 L substrate + 250 L enzyme were reacted for 15 minutes at a temperature of 25ºC. After that, 500 L of DNS was added and heated at a 100 oC temperature for 15 minutes. The compound was then cooled on ice for 10 minutes and measured with a spectrophotometer at a wavelength of 540 nm. 250 L substrate + 250 L buffer sodium citrate pH 3.0 0.05 M was reacted for 15 minutes at 25ºC temperature. The next process was similar to before. The absorbance value generated was converted into sugar reducer concentration (ppm) by using a formula generated from the glucose standard curve.

$$\text{Cellulase enzyme activity: } \frac{\textit{Enzyme concentration}\left(\frac{mg}{liter}\right) \times \textit{dilution factor} \times 1000}{\textit{reaction time}\ (15\ \textit{minute}) \times \textit{glucose molecular weight}\ (180)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

A killing power test against E. coli and P. Aeruginosa bacteria was conducted by using the ASTM 2315:2008 method. Pure eco enzyme was dissolved in aquadest to produce 40%, 60% and 80% rates. After that, each concentration was further dissolved up to 40%, 60%, and 80%. A killing power test against bacteria was conducted on each dissolving process to obtain 9 dissolving variations.

TSS rate testing was conducted by using the SNI 06-6989 3/2004 method. 1. Filter paper was placed on a filtration vacuum before pouring in 20 mL of water. After that, the vacuum was turned on in order to ensure that the filter paper was perfectly installed. Filter paper in a crus cup was heated up to 105 oC for 15 minutes. The tested compound was placed inside a pipet tube with a volume of 220 ml, poured onto dry filter paper, and heated for 3 minutes to reach perfect condition.
A = filter weight + dried residue (mg),
B = filter weight (mg)

$$\text{Total Suspended Solid (mg/l) : } \frac{(A-B) \times 1000}{Sampel\ \ (ml)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

TSS treatment on waste water by using eco enzyme

10 ml of eco enzyme solution with a 20% concentration was mixed with waste that contained 345 ppm of TSS. After that, the solution was inserted into the incubator shaker at 100 RPM speed for 10 minutes at 35 oC temperature. After the shaker is turned off, the solution is set aside for 30 minutes before being tested for TSS using the SNI 06-6989 3-2004 method. The same procedure was conducted by using 20% of the eco enzyme concentration in 30 ml of volume.

3 Results and Discussions

Based on this research, we generated eco enzyme characterization as shown in Table 1.

Table 1
Eco Enzyme (EE) Characterization Results

Parameter	Unit	Test Results
Color		Brown
Smell		Specific Fragrant
pH		3.17
Density	g/mL	1.02

Methanol	%	0.04
Ethanol	%	2.22
Total Suspended Solid (TSS)	mg/L	5
Total Dissolved Solid (TDS)	mg/L	2200
Biological Oxygen Demand (BOD)	mg/L	634
Chemical Oxygen Demand (COD)	mg/L	880
E-Coli Bacteria Killing Power (20% content, 15 seconds)	%	99.95
P. Aeruginosa Bacteria Killing Power (20% content, 15 seconds)	%	99.90
Amylase enzyme activity (100x dissolving)	U/mL	2.15
Cellulase enzyme activity (100x dissolving)	U/mL	1.69

Table 1 shows that eco enzyme with an acidic nature at a pH level of 3.17, which possesses a low TSS level of 5 ppm, has significant bacteria killing power of up to 99.97 %, while also containing amylase and cellulase enzyme activities. In previous research, amylase, protease, and lipase enzyme activities were discovered by Arun & Sivashanmugam (2015), Rasit & Mohammad (2018), Samriti et al., (2019). However, the tests were unable to discover the cellulase enzyme. In this research, Eco Enzyme that was dissolved 100 times showed amylase enzyme activity of 2.15 U/m and cellulase enzyme activity of 1.69 U/mL.

Table 1 also shows significant Eco Enzyme killing power against bacteria. In just 15 seconds of contact time, 20% of eco enzyme concentration can kill E. coli with a killing rate of up to 99.95% and P. aeruginosa bacteria with a killing rate of up to 99.90%. This discovery was also generated by Arun & Sivashanmugam (2015), who measured eco enzyme killing power against E. coli and salmonella.

Based on this characterization, eco enzyme can be utilized as a cleaning agent in soaps, a material to produce hand sanitizer, a disinfectant, and to process waste. In this research, eco enzyme was applied as a waste processing agent, in order to lower the TSS rate in liquid waste. TSS Waste processing results in a lower TSS rate. This can be seen in Table 2.

Table 2
TSS Results by Utilizing Eco Enzyme (EE)

Initial Concentration TSS (mg/l)	Initial pH	EE Concentration (ml)	TSS Treatment Results	TSS Reduction Percentage (%)	pH After Treatment
345	5.7	30 ml EE 20%	35	88.333	3.68
345	5.7	20 ml EE 20%	115	66.667	3.36
345	5.7	10 ml EE 20%	120	65.217	3.51

Table 2 shows the influence of Eco Enzyme addition against TSS concentration on liquid waste, which also pictured in Figure 2.

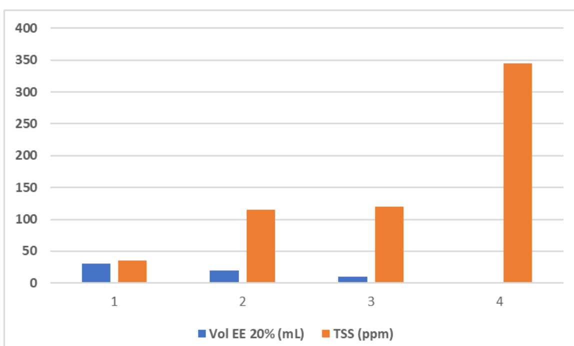

Figure 2. The Influence of Eco Enzyme addition against TSS

Figure 2 shows that higher Eco Enzyme addition on liquid waste will produce a higher TSS drop rate. The highest TSS reduction occurred on a 20% Eco Enzyme addition of 30 ml, where the TSS rate dropped from 345 ppm to 35 ppm.

This research shows that eco enzyme production from fruit peel and vegetable waste can bring two benefits. The first is to lower organic waste volume and actively contribute to global warming prevention. If every house can consistently create eco enzyme, it can lead to significant organic waste volume reduction (Jegannathan & Nielsen, 2013; Panda et al., 2016; Jaya et al., 2022)), which is a valuable contribution from every house. The second benefit is that eco enzyme products possess many advantages. Based on our research, the compound is proven to be able to significantly lower TSS in waste streams by up to 65-88%. This result outperforms a study conducted by Arun & Sivashanmugam (2015), which found a reduction in TSS of between 37 and 39%. Sambaraju & Sree Lakshmi (2020), also published a TSS reduction rate by using Eco Enzyme with a reduction rate of 26-29%. Meanwhile, Rasit & Mohammad (2018) discovered that eco enzyme reduces TSS by 55-65%. The existence of enzymes in eco enzymes as biocatalysts is useful for degrading polymer molecules in wastes such as fat, amylum, protein, and cellulose as TSS components into monomers with smaller molecule sizes. By doing so, these monomers will be dissolved and no longer suspended, which leads to a lower TSS rate in liquid waste. This research and previous research show that TSS reduction rate is affected by initial TSS concentration, eco enzyme dose, pH of solution, and contact time.

Eco enzyme had a killing power of 99.95% of E. coli bacteria and 99.90% of P. Aeruginosa bacteria at a concentration of 20% with a contact time of 15 seconds in 9 variations of dilution. This research shows that eco enzyme can also be utilized as an anti-bacterial agent. This result is similar to that of research conducted by Arun and Sivashanmugam (2015), which shows the anti-bacterial nature of eco enzyme against E. coli and Salmonella, with a lower killing rate percentage. Besides containing enzymes, eco enzymes also contain organic acids that kill bacteria, such as citric, acetate, lactate, malate, and oxalate acids. The anti-bacterial nature and effectiveness of eco enzyme is influenced by organic compounds used to generate eco enzyme as well as pH, eco enzyme concentration, and contact time (Rasit & Mohammad, 2018; Etienne et al., 2013).

4 Conclusion

Eco enzyme production can bring many benefits. Besides reducing organic waste, it can also be used as an effective compound to process waste and as an anti-bacterial agent. This research shows effective TSS reduction on liquid waste with a percentage of 65-88% and anti-bacterial agent effectiveness of 99.90–99.95%. Further research is recommended in order to discover other types of eco enzyme utilizations and to produce larger waste processing abilities, such as reducing BOD, COD, anions such as ammoniac and phosphate, along with cationic materials such as metal ions commonly found in liquid waste.

Conflict of interest statement
The authors declared that they have no competing interest.

Statement of authorship
The authors have a responsibility for the conception and design of the study. The authors have approved the final article.

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