

## Ecoenzyme Production, Characteristics, and Applications: A Review

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### Abstract

The reuse and management of organic waste can reduce significantly the impact on the environment and human health, as well as improve economic value through developing value-added products of certain applications. One of the solutions to cut down is solid organic waste such as fruit and vegetable waste into ecoenzymes via fermentation. This current review discusses the production, characterization, and application of ecoenzymes. The data was gathered from international and national indexed journals and proceedings. Ecoenzyme is produced via fermentation from fruit/vegetable waste, sugar, and water with a ratio of 1:3:10. In general, the modification made is changing the type of sugar and the type of fruit/vegetable waste used as raw material. The characterization mainly focuses on evaluating the content and activity of the enzymes. Wide range of applications of ecoenzyme found as liquid fertilizers, antimicrobial agents, wastewater treatment processes, and sludge treatment processes.

**Keywords:** *ecoenzyme, fermentation, fruit waste, organic waste, vegetable waste*

### 1 Introduction

The increase in the global population and high demand for food products has caused enormous global food waste. World Health Organization (1998) has reported that approximately one-third of total food production is lost. Vegetable and fruit markets, restaurants, and households generate a significant number of biodegradable wastes, including vegetable and fruit peels and waste. Fruit processing accounts for at least 25-30% of waste [1]. Lack of infrastructure and poor storage facilities has triggered the accumulation of waste from fruits and vegetables. The decomposition of this waste is a major problem because it contributes directly to the production of greenhouse gases such as methane and nitrous oxide [2]. Most of the waste is dumped onto the ground and left to decompose in the open. Furthermore, the stench and sewage from the rotting garbage cause a nuisance. Improper disposal of vegetable waste leads to a negative impact on the environment.

One way to reduce the amount of food waste production is converting the organic waste derived from fruit and vegetable as an ecoenzyme through fermentation.

The term ecoenzyme was first introduced by Dr. Rosukon from Thailand, and refers to the process which utilized vegetable and fruit waste [3]. Another term used for similar commodities is garbage enzyme [4]. Ecoenzyme is a complex solution generated as a product from fermenting fresh kitchen waste, such as vegetable and fruit peels. For instance, orange peels were chosen as the material for producing ecoenzyme due to their distinctive natures, such as their strong aroma and taste, rich in vitamin C and medicinal properties, and high acidity value [5].

Another reason for using fruit and vegetable waste is they contain many various enzymes, which can be identified when ecoenzyme is made. For example, pineapple contains bromelain which is classified as a protease enzyme with a specific activity of 0.30 U/mg [6]. In oranges, there is a lipase enzyme, not only in the fruit flesh but also from the by-products of juice making, such as peels. Peel waste contains a lipase enzyme of 57.55 U/g [7]. Meanwhile, the amylase enzyme can be obtained from mango seeds with a specific activity of 0.858 U/mg [8].

Ecoenzymes are often claimed to be “miracle” liquids because they have several

applications such as antimicrobial, antifungal, insecticide, cleaning agent, and water purification [5,9,10]. In addition, coenzyme contains several enzymes, such as amylase, lipase, and protease which exhibited quite good activity when evaluated [11], suggesting the potential for coenzymes to replace commercial enzymes under certain conditions and interests.

## 2 Method

This study describes how the production, characterization, and application of coenzyme. The data were obtained based on literature studies from articles published in international and national journals. Here, the search of articles was expanded to Google Scholar-indexed journal and proceedings articles indexed by Scopus, since there is fewer publication found relevant when limited to the international journal indexed by Scopus and national journal accredited by Sinta as well.

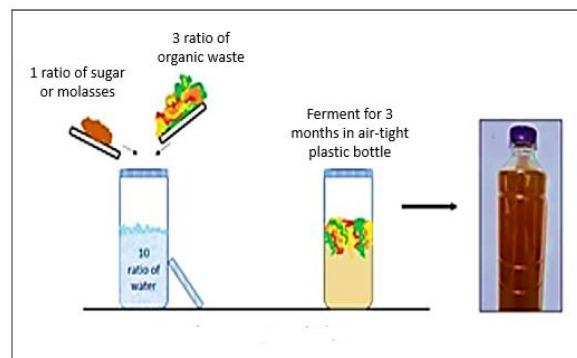
## 3 Results and Discussion

### 3.1 Production of Coenzyme

Basically, the production of coenzyme is relatively simple and does not require special chemicals. Coenzyme is made from three main components, *i.e.* water, sugar, and fruit/vegetable waste. In all articles analyzed, coenzyme was made by mixing fruit/vegetable waste, sugar, and water with a ratio of 1:3:10, respectively.

The process for making coenzyme was started by mixing the above three components in its ratio, then continue by keeping it in a closed container, and placed in a cool place and protected from sunlight. Conventionally, the process takes three months, but sometimes it can be taken only

one month or might extend up to six months. The gas will be released during the first month of the fermentation process, therefore, it should be removed every day to prevent the container from breaking due to the pressure that builds up inside the jar [11]. After the storage period is reached, the coenzyme is harvested by filtering it to obtain the solution (Figure 1).

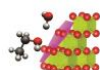


**Figure 1.** Production of coenzyme [12]

There are some modifications found in processing coenzymes, such as in the utilization of type of sugar and fruit/vegetable waste. It was found that the most common types of sugar used are brown sugar, molasses, and jaggery. However, variation in the type of fruit and vegetable waste used is still chosen as the best preference to be discovered. This variation was mostly considered due to the concern to solve the waste problem caused by the accumulation of fruit and vegetable waste. Converting those waste into coenzyme is in line with the zero-waste concept. Table 1 shows the various types of sugar and fruit/vegetable waste used in the production of coenzymes.

**Table 1.** Types of Sugar and Fruit/Vegetable Waste in Coenzyme Production

| No. | Sugar type        | Types of fruit/vegetable waste  | Reference |
|-----|-------------------|---|-----------|
| 1   | Molasses          | Tomato, cauliflower and peels of pineapple, orange and mango                    | [2]       |
| 2   | Molasses          | Pineapple and orange peels  | [4]       |
| 3   | Molasses          | Orange peel, pineapple, papaya peel   | [9]       |
| 4   | Molasses          | Papaya, dragon fruit and orange peel  | [12]      |
| 5   | Molasses          | Orange peel   | [13]      |
| 6   | Molasses          | Cabbage, potato and pumpkin   | [14]      |
| 7   | Molasses          | Fruit waste   | [15]      |
| 8   | Molasses          | Banana weevil, pineapple, ketapang, orange                                      | [16]      |
| 9   | Molasses          | Orange peel, pineapple peel   | [17]      |
| 10  | Molasses/ jaggery | Papaya, banana, sapodilla, and pomegranate; potato, gourd, eggplant, and turnip | [18]      |
| 11  | Brown sugar       | Peels of orange, lime, and lemon  | [5]       |
| 12  | Brown sugar       | Pineapple, orange, tomato and mango   | [11]      |
| 13  | Brown sugar       | Carrot, eggplant, cucumber, okra, beet; Onion, eggplant, cabbage, potato        | [19]      |



| No. | Sugar type                               | Types of fruit/vegetable waste   | Reference |
|-----|--|--|-----------|
| 14  | Brown sugar                              | Pineapple peel   | [20]      |
| 15  | Brown sugar                              | Guava  | [21]      |
| 16  | Brown sugar                              | Nutmeg, cloves, eucalyptus   | [22]      |
| 17  | Brown sugar                              | Tomato, orange   | [23]      |
| 18  | Brown sugar                              | Vegetable/fruit waste  | [24]      |
| 19  | Cane brown sugar                         | Peels of pineapple peel, mango, banana, lime, watermelon, dragon fruit and lemon | [25]      |
| 20  | Jaggery                                  | Fruit peel   | [10]      |
| 21  | Jaggery                                  | Orange seeds, marigold flowers, neem leaves                                      | [26]      |
| 22  | Jaggery                                  | Orange peel  | [27]      |
| 23  | White sugar/<br>molasses/ brown<br>sugar | Peels of orange, watermelon, melon, apple, dragon fruit, guava                   | [28]      |

Based on the data in Table 1, molasses was found as the type of sugar most widely used, followed by brown sugar and jaggery. In contrast, white sugar is the type of sugar that is rarely used in the process of making coenzymes since it has gone through the bleaching process. Brown sugar has more natural content and is also relatively more economical because it is cheaper than that white sugar.

Molasses is a byproduct of the sugar processing industry which still contains high sugar. The molasses contains high carbohydrates, especially sucrose, ranging from 48–55%. Molasses is available in dry and liquid forms in the market. The price of liquid molasses is cheaper than brown sugar for the same quantity and quality. Due to its economic value, molasses was mostly chosen as a type of sugar used in making coenzymes.

Basically, molasses, brown sugar, or other types of sugar are nutrients for microorganisms in the process of making coenzymes. During the fermentation process, carbohydrates derived from molasses are converted into volatile acids, while organic acids are extracted from fruit peels to become enzyme solutions [29].

The use of molasses as a carbon source is quite effective in making coenzymes compared to other types of sugar. This can be seen from the results of a study [28], which showed that over time of fermentation, coenzyme with molasses showed higher lipase and total acid activity compared to brown sugar and white sugar. Lipase activity was found to decrease with the increase of fermentation time in the use of white sugar and brown sugar. Yet, in the use of molasses, the lipase activity increase as a manner of fermentation time with optimum enzyme activity occurring in the third month. In addition, coenzyme molasses also

had the highest percentage of total acid compared after the third month of fermentation compared to the other two types of sugar.

Based on Table 1, it was revealed that the types of fruit and vegetable waste for coenzyme production are varied. In general, fruit waste is used more than vegetables. The fruit waste used is mainly peels because usually, this part is the largest component of fruit waste originating from markets and households.

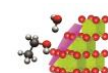
Among the fruits, oranges are the most widely used type of fruit used for making coenzyme. Distinctive aroma and taste, as well as vitamin C content and high acidity values are some of the reasons why oranges are widely used [5]. In addition, the content of the lipase enzyme found in oranges is an added value when used as raw material for coenzyme.

Although the amount is not as much as fruit waste, the use of vegetable waste has also been reported by several researchers. Cabbage, potato, and eggplant are examples of several types of vegetables that are commonly used as raw materials for coenzymes [14,18,19].

### 3.2 Characterization of Coenzyme

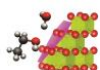
Coenzyme characterization is an important step to determine the characteristics of the coenzyme being made. This is especially useful when we use coenzymes for certain applications. However, not all the articles analyzed provided information related to the characterization of coenzymes.

Based on the literature review, it appears that there is no standard regarding what parameters must be determined in the coenzyme characterization process. Thus, there are quite a variety of differences from the specified parameters, as shown in Table 2.

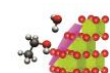


**Table 2.** Characterization and Application of Ecoenzyme

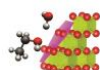
| No. | Parameters  | Methods  | Value  | Application  | Reference   |
|-----|---|--|--|--|---|
| 1   | Organic acid content<br>• Acetic acid<br>• Lactic acid<br>• Oxalic acid<br>• Malic acid<br>• Citric acid  | RP-HPLC (Reversed Phase-High Performance Liquid Chromatography)  | After 15 days of incubation:<br>• 11.12 g/L<br>• 26.02 g/L<br>• 44.81 g/L<br>• 11.05 g/L<br>• 39.05 g/l  | Liquid fertilizer, antimicrobial agents, treatment of domestic wastewater, municipal and industrial sludge treatment | [2]   |
| 2   | pH<br>TS (mg/L)<br>TDS (mg/L)<br>BOD (mg/L)<br>COD (mg/L)<br>citric acid (mg/L)<br>Activity of lipase (µ/ml)<br>Activity of protease (µ/ml)<br>Activity of amylase (µ/ml) | pH meter<br><u>Apha Standard Method 2540 B</u><br>Apha Standard Method 2540 C<br>Apha Standard Method 5210 B<br>Apha Standard Method 5220 C<br>High Performance Liquid Chromatography (HPLC) Method<br>Casein Digestion Unit (CDU) Analytical Method<br>3,5-dinitrosalicylic Acid (DNS) Method<br>Titrimetric Method | 2.79 (TE); 2.86 (OE)<br>14,000 (TE, OE)<br>17,000 (TE); 19,000 (OE)<br>40 (TE), 33 (OE)<br>80,000 (TE); 96,000 (OE)<br>14,130 (TE); 35,281 (OE)<br>At pH 8: 330 (TE); 690 (OE)<br>At pH 7.5: 45 (TE); 55 (OE)<br>At pH 6.5: 2.62 (TE); 2.37 (OE)   | Industrial Wastewater treatment agents   | [3]<br>TE: Tomato Eco Enzyme<br>OE: Orange Eco Enzyme |
| 3   | Activity of lipase (U/ml)<br>Activity of protease (U/ml)<br>Activity of amylase (U/ml)  | Spectrophotometer (using p-nitrophenyl palmitate (p-NPP))<br>Spectrophotometer<br>Spectrophotometer  | At pH 6: 74.990<br>At pH 6: 44.309<br>At pH 6: 56.409  | Industrial waste and activated-sludge  | [4]   |
| 4   | Activity of lipase<br>Activity of protease<br>Activity of amylase<br>Secondary metabolites content<br>Antimicrobial activity  | Clear zone formation<br>Clear zone formation<br>Clear zone formation<br>Qualitative test of flavonoids, alkaloids, quinones, cardenolides, and saponins.<br>Diffusion method   | Lipase (+)<br>Protease (+)<br>Amylase (+)<br>Flavonoids (+), alkaloids (+), quinones (+), cardenolides (+), and saponins (+)<br>Zone of inhibition:<br><i>Bacillus</i> spp. (18 mm) ;<br><i>Pseudomas</i> spp (11 mm), and<br><i>E.coli</i> (5 mm) | Bionutrient for plant growth hormone, Household cleaning agent.  | [5]   |
| 5   | pH  | pH meter   | • YP<br>7.8 (2014)<br>7.4 (2015)   | Water cleaning effect in drain water, reducing   | [10]<br>YP: Yamuna river at Palla                     |



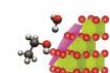
| No. | Parameters     | Methods  | Value  | Application   | Reference                                |
|-----|----------------|--|--|---|--|
|     |                |  | 7.8 (2016)   | solids, suspended solids<br>hardness, and chlorides | YN:<br>Yamuna<br>river at<br>Nizammudin  |
|     |                |  | • YN<br>7.5 (2014)<br>7.8 (2015)<br>7.1 (2016)     |   |  |
|     |                |  | • YAC<br>7.7 (2014)<br>7.8 (2015)<br>7.1 (2016)    |   | YAC:<br>Yamuna<br>river at Agra<br>canal |
|     | DO (mg/L)      | Indian standard lab<br>authority for<br>monitoring pollution | • YP<br>11.0 (2014)<br>5.5 (2015)<br>9.2 (2016)    |   |  |
|     |                |  | • YN<br>1.2 (2014)<br>N.d. (2015)<br>0.9 (2016)    |   |  |
|     |                |  | • YAC<br>0.9 (2014)<br>N.d. (2015)<br>0.6 (2016)   |   |  |
|     | COD (mg/L)     | Indian standard lab<br>authority for<br>monitoring pollution | • YP<br>8 (2014)<br>35 (2015)<br>9 (2016)          |   |  |
|     |                |  | • YN<br>42 (2014)<br>83 (2015)<br>92 (2016)        |   |  |
|     |                |  | • YAC<br>117 (2014)<br>75 (2015)<br>82 (2016)      |   |  |
|     | BOD (mg/L)     | Indian standard lab<br>authority for<br>monitoring pollution | • YP<br>2 (2014)<br>3 (2015)<br>2 (2016)           |   |  |
|     |                |  | • YN<br>13 (2014)<br>29 (2015)<br>45 (2016)        |   |  |
|     |                |  | • YAC<br>29 (2014)<br>27 (2015)<br>21 (2016)       |   |  |
|     | Ammonia (mg/L) | Indian standard lab<br>authority for<br>monitoring pollution | • YP<br>- (2014)<br>- (2015)<br>- (2016)           |   |  |
|     |                |  | • YN<br>6.8 (2014)<br>12.3 (2015)<br>16.4 (2016)   |   |  |
|     |                |  | • YAC<br>12.5 (2014)<br>14.1 (2015)<br>11.8 (2016) |   |  |



| No. | Parameters  | Methods  | Value  | Application                                 | Reference  |
|-----|---|--|--|---|--|
|     | Total Coliform (MPN/100 mL)   | Indian standard lab authority for monitoring pollution   | <ul style="list-style-type: none"> <li>• YP<br/>450 (2014)<br/>17000 (2015)<br/>1400 (2016)</li> <li>• YN<br/>35000000 (2014)<br/>3500000 (2015)<br/>3500000 (2016)</li> <li>• YAC<br/>4600000 (2014)<br/>5400000 (2015)<br/>9200000 (2016)</li> </ul> |   |  |
| 6   | Antimicrobial activity  | MIC and MBC method   | 50% concentration of P-EE and M-EE had antibacterial activity against <i>Enterococcus faecalis</i>   | Antimicrobial                               | [9]<br>P-EE: Papaya eco-enzyme<br><br>M-EE: Natural pineapple-orange eco-enzyme [11] |
| 7   | BOD (mg/L)<br>COD (mg/L)<br><br>pH<br>TDS (mg/L)<br><br>TS (mg/L)<br><br>Activity of lipase (U/mL)<br><br>Activity of protease (U/mL)<br><br>Activity of amylase (U/mL)         | DO meter<br>Closed reflux method<br>pH meter<br>Alpha Standard Methods 2540 C<br>Alpha Standard Methods 2540 B<br>Spectrophotometry & Titrimetric Method<br>Titrimetric method Casein Digestion unit, (CDU method)<br>DNS method | 87.53<br>133760<br><br>3.07<br>15,900<br><br>17,744.44<br><br>At pH 8: 450<br><br>At pH 6.5: 68<br><br>At pH 6: 110  | Sludge treatment                            |  |
| 8   | pH<br>Density (g/mL)<br>Methanol (%)<br>Ethanol (%)<br>TSS (mg/L)<br><br>TDS (mg/L)<br>BOD (mg/L)<br>COD (mg/L)<br>Antibacterial activity (%)<br><br>Activity of amylase (U/mL) | pH meter<br>-<br>-<br>-<br>SNI 06-6989 3/2004 method<br>-<br>-<br>-<br>ASTM 2315:2008 method.<br>DNS method  | 3.17<br>1.02<br>0.04<br>2.22<br>5<br><br>2200<br>634<br>880<br><i>E. Coli</i> : 99.95<br><i>P. Aeruginose</i> : 99.90<br>2.15  | Reducing organic waste, antibacterial agent | [12]   |

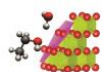


| No.                          | Parameters                     | Methods  | Value   | Application                                   | Reference |
|------------------------------|--------------------------------|--|---|---|-----------|
| 9                            | Activity of cellulase (U/mL)   | DNS method   | 1.69  | Substrat in microbial fuel cell               | [13]      |
|                              | pH                             | pH meter   | <ul style="list-style-type: none"> <li>One pair: 3.45</li> <li>Three pairs: 3.45</li> </ul>   |   |           |
|                              | Brix level                     | -  | <ul style="list-style-type: none"> <li>One pair<br/>0 rpm: 4%<br/>200 rpm: 3%</li> <li>Three pairs<br/>0 rpm: 4%<br/>200 rpm: 3%</li> </ul> |   |           |
| 10                           | pH<br>Nutrient content         | pH meter<br>-  | 4.16<br>C-organic: 0.9%<br>N: 0.09%<br>P: 0.01%<br>K: 0.12%   | Liquid organic fertilizer (LOF), disinfectant | [15]      |
| 11                           | pH                             | pH meter   | <ul style="list-style-type: none"> <li>0%: 7.20</li> <li>5%: 6.11</li> <li>10%: 5.3</li> </ul>  | LAS remover from water                        | [17]      |
|                              | Temperature (° C)              | Thermometer  | <ul style="list-style-type: none"> <li>0%: 26.73</li> <li>5%: 26.76</li> <li>10%: 26.8</li> </ul>   |   |           |
|                              | TDS (ppm)                      | TDS meter  | <ul style="list-style-type: none"> <li>0%: 0.65</li> <li>5%: 242.16</li> <li>10%: 409.66</li> </ul>   |   |           |
|                              | LAS content (ppm)              | MBAS method  | <ul style="list-style-type: none"> <li>0%: -8.30</li> <li>5%: -7</li> <li>10%: -5.90</li> </ul>   |   |           |
|                              | DO (ppm)                       | DO meter   | <ul style="list-style-type: none"> <li>0%: 4.02</li> <li>5%: 1.43</li> <li>10%: 1</li> </ul>  |   |           |
|                              | BOD (ppm)                      | DO meter   | <ul style="list-style-type: none"> <li>0%: 1.19</li> <li>5%: 0.54</li> <li>10%: 0.77</li> </ul>   |   |           |
|                              | 12                             | pH   | pH meter  |   |           |
| Turbidity                    | -                              | <ul style="list-style-type: none"> <li>GE-1: less viscoud</li> <li>GE-2: more viscous</li> </ul> | GE-1:<br>Garbage enzyme papaya, banana, sapodilla pomegranate<br><br>GE-2:<br>Garbage enzyme potato, gourd, eggplant turnip                 |   |           |
| Acetic acid content (g/mL)   | Titrimetric                    | <ul style="list-style-type: none"> <li>GE-1: 4.2±0.42</li> <li>GE-2: 5.4±0.28</li> </ul>         |   |   |           |
| Protein content (g/mL)       | Biuret method                  | <ul style="list-style-type: none"> <li>GE-1: 4.225±0.261</li> <li>GE-2: 4.47±0.480</li> </ul>    |   |   |           |
| Carbohydrate content (mg/mL) | Anthrone method                | <ul style="list-style-type: none"> <li>GE-1: 14.295±0.219</li> <li>GE-2: 4.47±0.480</li> </ul>   |   |   |           |
| Alcohol content (mL/mL)      | Acidified potassium dichromate | <ul style="list-style-type: none"> <li>GE-1: 0.18</li> <li>GE-2: 0.13</li> </ul>                 |   |   |           |
| Activity of protease (cm)    | Agar plate diffusion           | <ul style="list-style-type: none"> <li>GE-1: 1.6±0.01</li> </ul>                                 |   |   |           |



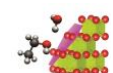


| No. | Parameters                             | Methods  | Value  | Application                                   | Reference |
|-----|--|--|--|---|-----------|
|     | Acitivity of lipase (cm)               | Agar plate diffusion   | <ul style="list-style-type: none"> <li>• GE-2: 1.4±0.05</li> <li>• GE-1: 2.35±0.1</li> <li>• GE-2: 2.45±0.05</li> </ul>  |   |           |
|     | Activity of amylase (cm)               | Agar plate diffusion   | <ul style="list-style-type: none"> <li>• GE-1: 1.13±0.1</li> <li>• GE-2: 1.43±0.5</li> </ul>   |   |           |
|     | Activity of papain (cm)                | Agar plate diffusion   | <ul style="list-style-type: none"> <li>• GE-1: 1.49±0.1</li> <li>• GE-2: 1.23±0.5</li> </ul>   |   |           |
|     | Acitivity of amylase in garbage (U/mL) | Spectrophotometry  | <ul style="list-style-type: none"> <li>• GE-1: 151.6±25.02</li> <li>• GE-2: 198.5±36.6</li> </ul>  |   |           |
|     | Activity of papain in garbage (U/mL)   | Spectrophotometry  | <ul style="list-style-type: none"> <li>• GE-1: 153.57±11.73</li> <li>• GE-2: 78.38±22.35</li> </ul>  |   |           |
| 13  | pH                                     | pH meter   | <ul style="list-style-type: none"> <li>• 1: 9.2 to 4.79</li> <li>• 2: 11.2 to 10.05</li> </ul>   | Purification of contaminated water            | [19]      |
| 14  | Antibacterial activity                 | Paper disc diffusion   | <ul style="list-style-type: none"> <li>• At 100%</li> <li>• S. aureus: 12.33±1.37</li> <li>• P. acnes: 8.67±0.52</li> </ul>  | Anti-acne bacteria                            | [20]      |
|     | Secondary metabolites content          | Qualitative test of flavonoids, alkaloids, tannin, and saponins. | Flavonoids (-), alkaloids (-), tannin (+), and saponins (+)  |   |           |
| 15  | pH                                     | pH meter   | 3.36   | Reduce waste generation                       | [21]      |
|     | COD (mg/L)                             | Close reflux method  | 116800   |   |           |
|     | NH <sub>3</sub> (ppm)                  | Spectrophotometer  | 1.4  |   |           |
|     | PO <sub>4</sub> (ppm)                  | Spectrophotometer  | 11.2   |   |           |
| 16  | Antibacterial activity                 | Agar diffusion   | <ul style="list-style-type: none"> <li>• Highest zone of inhibition: From DK (eucalyptus leaf waste)</li> <li>• E. coli: 14.42 mm</li> <li>• S.aureus: 12.58 mm</li> </ul> | Antibacterial                                 | [22]      |
| 17  | pH                                     | Potentiometric   | <ul style="list-style-type: none"> <li>• Eco Enzyme: 3.50</li> <li>• Tomato: 3.50</li> <li>• Orange: 3.47</li> </ul>   | Industrial waste activated sludge degradation | [23]      |
|     | COD (mg/L)                             | Closed Reflux Spectrophotometric                                 | <ul style="list-style-type: none"> <li>• Tomato: 17.108</li> <li>• Orange: 20,817</li> <li>• Industrial Waste Activated</li> </ul>   |   |           |

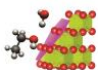




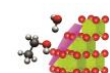
| No. | Parameters   | Methods               | Value   | Application          | Reference  |
|-----|--|-----------------------|---|----------------------|--|
|     | TSS (mg/L)   | Gravimetric           | Sludge:<br>22.400<br>Reduction<br>of TSS by:<br><ul style="list-style-type: none"> <li>• Tomato eco enzyme applications were 39%</li> <li>• Orange eco enzyme: 45%</li> </ul> |                      |  |
|     | VSS (mg/L)   | Gravimetric & Furnace | Reduction<br>of VSS by:<br><ul style="list-style-type: none"> <li>• Tomato eco enzyme applications were 41%</li> <li>• Orange eco enzyme: 46%</li> </ul>                      |                      |  |
| 18  | pH   | pH meter              | 4.1-5.24  | Wastewater treatment | [24]   |
|     | Water parameters of Ca <sup>2+</sup> (ppm)             | -                     | <ul style="list-style-type: none"> <li>• VE: 107.8</li> <li>• FE: 20.5</li> </ul>   |                      | VE:<br>Vegetable-produced<br>eco-enzyme  |
|     | Water parameters of Na <sup>+</sup> (ppm)              | -                     | <ul style="list-style-type: none"> <li>• VE: 303.2</li> <li>• FE: 256.7</li> </ul>  |                      |  |
|     | Water parameters of K <sup>+</sup> (ppm)               | -                     | <ul style="list-style-type: none"> <li>• VE: 2055.6</li> <li>• FE: 1098.3</li> </ul>  |                      |  |
|     | Water parameters of NO <sub>3</sub> <sup>-</sup> (ppm) | -                     | <ul style="list-style-type: none"> <li>• VE: 3422.2</li> <li>• FE: 135</li> </ul>   |                      | FE: Fruit-produced<br>eco-enzyme   |
| 19  | pH   | -                     | 2.4-2.8   | Cleaning agent       | [25]   |
|     | Aroma of Ecoenzyme Products                            | Qualitative approach  | All variant<br>ecoenzymes are<br>acidic   |                      |  |
|     | Ecoenzyme Product Color                                | Qualitative approach  | <ul style="list-style-type: none"> <li>• Ecoenzyme products on all variables</li> <li>• brown with different levels of color density</li> </ul>                               |                      |  |
|     | Final product volume                                   | Qualitative approach  | <ul style="list-style-type: none"> <li>• V1 and V2 there was an increase in volume to 133%</li> <li>• V3 there was a reduction in product volume to 86%.</li> </ul>           |                      |  |
| 20  | pH   | pH meter              | <ul style="list-style-type: none"> <li>• OE: 3.1</li> <li>• ME: 2.9</li> <li>• NE: 3.2</li> </ul>   | Wastewater treatment | [26]   |
|     | TDS (mg/mL)  | TDS meter             | <ul style="list-style-type: none"> <li>• OE: 1300</li> <li>• ME: 1400</li> <li>• NE: 2500</li> </ul>  |                      | OE: Orange<br>peel waste<br>Eco-enzyme<br>ME:<br>Marigold<br>flowers<br>waste Eco-<br>enzyme |



| No. | Parameters        | Methods | Value   | Application          | Reference                             |
|-----|-------------------|---------|---|----------------------|---------------------------------------|
| 21  | pH                | -       | <ul style="list-style-type: none"> <li>• 2%<br/>Day 1: 7.11<br/>Day 5: 7.8</li> <li>• 4%<br/>Day 1: 6.32<br/>Day 5: 7.2</li> <li>• 6%<br/>Day 1: 5.84<br/>Day 5: 6.8</li> </ul>   | Wastewater treatment | NE: Neem leaves waste Eco-enzyme [27] |
|     | Alkalinity (mg/L) | -       | <ul style="list-style-type: none"> <li>• 2%<br/>Day 1: 387<br/>Day 5: 307</li> <li>• 4%<br/>Day 1: 295<br/>Day 5: 190</li> <li>• 6%<br/>Day 1: 216<br/>Day 5: 114</li> </ul>      |                      |                                       |
|     | BOD (mg/L)        | -       | <ul style="list-style-type: none"> <li>• 2%<br/>Day 1: 559.5<br/>Day 5: 239.5</li> <li>• 4%<br/>Day 1: 240<br/>Day 5: 97.5</li> <li>• 6%<br/>Day 1: 92<br/>Day 5: 59.5</li> </ul> |                      |                                       |
|     | COD (mg/L)        | -       | <ul style="list-style-type: none"> <li>• 2%<br/>Day 1: 760<br/>Day 5: 459</li> <li>• 4%<br/>Day 1: 400<br/>Day 5: 239</li> <li>• 6%<br/>Day 1: 247<br/>Day 5: 210</li> </ul>      |                      |                                       |
|     | Chlorides (mg/L)  | -       | <ul style="list-style-type: none"> <li>• 2%<br/>Day 1: 220<br/>Day 5: 179</li> <li>• 4%<br/>Day 1: 239<br/>Day 5: 205</li> <li>• 6%<br/>Day 1: 254<br/>Day 5: 213</li> </ul>      |                      |                                       |
|     | TS (mg/L)         | -       | <ul style="list-style-type: none"> <li>• 2%<br/>Day 1: 1800<br/>Day 5: 1500</li> <li>• 4%<br/>Day 1: 1400<br/>Day 5: 1000</li> <li>• 6%<br/>Day 1: 800<br/>Day 5: 600</li> </ul>  |                      |                                       |
|     | TDS (mg/L)        | -       | <ul style="list-style-type: none"> <li>• 2%</li> </ul>  |                      |                                       |



| No. | Parameters                 | Methods     | Value  | Application            | Reference |
|-----|----------------------------|-------------|--|------------------------|-----------|
|     |                            |             | Day 1: 1200<br>Day 5: 1000   |                        |           |
|     |                            |             | • 4%<br>Day 1: 1000<br>Day 5: 800  |                        |           |
|     |                            |             | • 6%<br>Day 1: 600<br>Day 5: 400   |                        |           |
|     | TSS (mg/L)                 | -           | • 2%<br>Day 1: 600<br>Day 5: 500   |                        |           |
|     |                            |             | • 4%<br>Day 1: 400<br>Day 5: 200   |                        |           |
|     |                            |             | • 6%<br>Day 1: 200<br>Day 5: 200   |                        |           |
|     | Oil & Grease (mg/L)        | -           | • 2%<br>Day 1: 0.33<br>Day 5: BDL  |                        |           |
|     |                            |             | • 4%<br>Day 1: BDL<br>Day 5: BDL   |                        |           |
|     |                            |             | • 6%<br>Day 1: BDL<br>Day 5: BDL   |                        |           |
| 22  | Acitivity of Lipase (U/mL) | Titrimetric | • White Sugar<br>Month 1:<br>37.33±0.58<br>Month 2:<br>17.33±1.15<br>Month 3:<br>12.67 ± 0.58                | Reducing organic waste | [28]      |
|     |                            |             | • Brown<br>Coconut<br>Sugar<br>Month 1:<br>13.33±0.58<br>Month 2:<br>14.00 ± 1.00<br>Month 3: 6.33<br>± 0.58 |                        |           |
|     |                            |             | • Molasses<br>Month 1:<br>18.33 ± 0.58<br>Month 2:<br>18.00 ± 0.00<br>Month 3:<br>20.33 ± 0.58               |                        |           |
|     | Total titrated acid        | Titrimetric | • White Sugar<br>Month 1:<br>14.67±0.58<br>Month 2:<br>13.00 ± 0.00<br>Month 3:<br>13.67 ± 0.58              |                        |           |



| No. | Parameters                      | Methods  | Value  | Application                  | Reference |
|-----|---------------------------------|--|--|------------------------------|-----------|
| 23  | Root length and leaves quantity | non-factorial randomized block design (RAK) method | <ul style="list-style-type: none"> <li>Brown Coconut Sugar</li> <li>Month 1: 6.00 ± 1.00</li> <li>Month 2: 5.67 ± 1.15</li> <li>Month 3: 4.67 ± 0.58</li> <li>Molasses</li> <li>Month 1: 14.33±1.15</li> <li>Month 2: 13.67 ± 0.58</li> <li>Month 3: 17.00 ± 0.00</li> </ul> At dose 1.75 mL/L | Liquid organic fertilization | [30]      |

Based on Table 2, it was shown that pH is a fairly common parameter used in the characterization of ecoenzyme. A good ecoenzyme is indicated by a low pH, because organic acids, such as acetic acid and citric acid, will be produced during the fermentation. As can be seen in Table 2, the pH of ecoenzyme is generally below 4 with the smallest pH reported at 2.4 [25] and 2.79 [3]. Organic acids are an important key in the determination of acidity. This means that the lower the pH value, the higher the organic acid content.

Other parameters such as the bioactivity of the ecoenzyme are also considered to evaluate its application in daily life, industry, wastewater treatment, agriculture, etc. Particular bioactivity measurements highlight the activity of the enzymes such as lipase, protease, and amylase. Each enzyme has its own optimum pH. Based on research [11], lipase activity in ecoenzyme solutions increased dramatically when the pH reached 7; 7.5; and 8. The maximum lipase activity occurs at pH 8, and the lowest lipase activity occurs at pH 3.07. The optimum activity for protease and amylase was observed at pH 6 and 6.5, respectively.

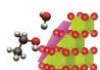
### 3.3 Ecoenzyme Applications

The production of ecoenzyme, which is an environmentally friendly enzyme, is part of efforts to recycle waste from households, markets, and restaurants. Reuse of waste provides an excellent way to reduce waste, as well as provide economic benefits because ecoenzymes can be applied in several applications, including cleaning agents,

detergent remediation agents, antibacterial/antimicrobial, water treatment, aquaculture sludge treatment, industrial waste treatment, organic fertilizer, and as microbial fuel cell (Table 2).

Based on Table 2, it was shown that ecoenzyme was applied more in wastewater treatment. According to [19], the utilization of ecoenzymes is developing as part of a viable strategy to treat contaminated water. The addition of ecoenzyme is proven can increase the BOD of wastewater. The more amount of ecoenzyme added, the higher BOD of the wastewater, suggesting that the ecoenzyme can be used as an additive in wastewater treatment, especially to eliminate ammonia nitrogen, and phosphorus.

The application of ecoenzyme in aquaculture sludge treatment shows that ecoenzyme solutions are very efficient in removing Total Suspended Solid (TSS), Volatile Suspended Solid (VSS), Total Phosphorus (TP), Total Ammonia Nitrogen (TAN) and stabilizing Chemical Oxygen Demand (COD). The results significantly show that the most efficient and economical ecoenzyme concentration is 10% dilution which has a percentage of TSS removal of 89%, a percentage of VSS removal of 78%, a percentage of COD reduction of 88%, a percentage of TAN removal of 94%. and the percentage of TP removal was 97%. This result introduced that ecoenzyme can be used as an alternative strategy to treat aquaculture sludge with a low-cost and environmentally friendly [11].

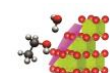


#### 4 Conclusion

Ecoenzyme is a complex solution produced from organic wastes such as vegetable and fruit peels through fermentation. Ecoenzyme production involves a simple process with simple components. It's made from three main components, *i.e.* water, sugar, and fruit/vegetable waste in a ratio of 1:3:10, respectively. The characteristics of the generated ecoenzymes were determined by measuring the pH and activity of lipase, protease, and amylase. Ecoenzyme has been reported as a 'multipurpose' solution with a wide range of applications, including as a cleaning agent, antibacterial/antimicrobial, organic fertilizer, detergent remediation agent, water treatment, industrial waste treatment, aquaculture sludge treatment, and microbial fuel cell (MFC). Ecoenzymes not only provide an effective and efficient strategy to reduce waste in the environment but also increase the value of the waste into value-added products, as well as provide alternative enzymes for replacing commercial enzymes. Ecoenzyme conversion from household waste introduced an effort to contribute to climate change mitigation.

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