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A Systematic Review on Enzyme Extraction from Organic Wastes and its Application

Indrani Barman, Shrijona Hazarika, Jyotchna Gogoi, Nayan Talukdar*

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Abstract

Eco-enzymes or Garbage enzymes have emerged as a solution to managing organic wastes. These organic wastes containing carbon compounds are discharged from houses that ultimately end up in landfills. Eco-enzymes or Garbage enzymes are mainly concerned with the enzymes produced from the anaerobic fermentation of fresh vegetables or fruit wastes. The longer the fermentation period, the better its efficiency. This review is carried out with the intention of bringing forth all the enzymes isolated so far from household organic wastes and their various applications, including the microorganisms involved in the process. Production of Eco-enzymes or Garbage enzymes is very straightforward. It requires fresh vegetable and/or fruit wastes, a sugar source in the form of jaggery, molasses or brown sugar, water, and airtight plastic containers. These are mixed in a 3:1:10 ratio respectively and kept for three months, whereby through anaerobic fermentation, the wastes get converted to enzymes. After three months, this organic liquid loaded with hydrolytic enzymes can be used in a wide variety of ways. Applying Eco-enzymes or Garbage enzymes includes treating wastewater sludge as a potent disinfectant and fertilizer, antibacterial agent in endodontic treatments, and hand sanitizer. These bio-enzymes are a one-stop solution to managing many issues. From being a disinfectant and biofertilizer to having great potential in cleaning waste water while reducing the need for landfills that releases methane gases which play a significant role in global warming, it is a multipurpose liquid (Kirilmaz, 2022).

Keywords: Eco-Enzymes, Garbage enzymes, Bio-Enzymes, Anaerobic fermentation, Organic wastes

Introduction

With the ever-increasing population and the surge of medical conditions and food wastage in society, people are opting for eco-friendly measures to solve such problems (Shahmars & Valiev, 2022).

Indrani Barman, Jyotchna Gogoi
Program of Biochemistry, Faculty of Science, Assam down-town University, Guwahati, India.

Shrijona Hazarika, Nayan Talukdar*
Program of Biotechnology, Faculty of Science, Assam down town University, Guwahati, India.

***E-mail:** nayan.new16@gmail.com

Using biofertilizers, biopesticides, and biofuels instead of the harmful chemicals that have a detrimental effect on the environment are being practiced (Çora & Çora, 2022) Among such measures, eco-enzymes have been found to play a positive role in bringing a balance in nature (Kuthiala *et al*., 2022; Nurlatifah *et al*., 2022).

When fresh vegetables or/and fruit wastes are left to get fermented in a mixture of sugar and water, it produces a complex; concentrated organic liquid termed an eco-enzyme or garbage enzyme (Syakdani *et al*., 2021). During the process of weathering these organic wastes, hydrolytic enzymes are produced, potentially replacing commercial enzymes. This liquid can be used in almost many ways, ranging from household activities to treating contaminated soil and water, as well as in agriculture. These are so-named because of their environment-friendly characteristics and multipurpose potential (Rasit & Mohammad, 2018; Rahayu & Situmeang, 2021).

According to the discoverer Dr. Rosukon Poompanvong, Eco-enzymes or Garbage enzymes are prepared by mixing fresh vegetable and fruit wastes, sugar (jaggery or molasses or brown sugar), and water in a 3:1:10 ratio respectively. These are stored in an airtight container in a cool place at room temperature for approximately three months (Patel *et al*., 2021). It is a complex liquid that produces alcohol in the first month, vinegar in the second, and enzymes in the third month (Janarthanan *et al*., 2020; Novianti *et al*., 2021; Rusdianasari *et al*., 2021). Anaerobic fermentation of the kitchen squander produces eco-enzymes that consist of natural proteins (enzymes), mineral salts, and organic acids (Neupane *et al*., 2019). The general parameters of eco-enzymes are listed in **Table 1** (Janarthanan *et al*., 2020; Muliarta & Darmawan, 2021; Novianti & Muliarta, 2021).

The Mechanism Involved in Enzyme Production

As mentioned above, garbage enzyme results from anaerobic fermentation and involves the utilization of sugar besides kitchen wastes. Although enzymes are produced by the indigenous microflora present in the wastes, sometimes Baker's yeast or Saccharomyces cerevisiae is also used during the fermentation of vegetable wastes (Low *et al*., 2021). An airtight plastic container is used in order to create an anaerobic environment. The absence of oxygen and the acidic nature of the mixture provides a suitable environment for the production of eco-enzymes (Gu *et al*., 2021; Patel *et al*., 2021). Adding sugar in the form of jaggery, brown sugar, or molasses aids in the growth of microorganisms by providing nutrition. When the sugar content is higher, the volume produced is higher (Low *et al*., 2021). With the progression of the process, gases are released due to the microbial activity causing bubbles to form in the container (Gu *et al*., 2021; Patel *et al*., 2021). Carbohydrates get converted into volatile acids. As the pH lowers, the organic acids dissolve into fermentation solutions. Glucose breaks down into pyruvic acid, which in turn is decomposed to acetaldehyde by pyruvate decarboxylase. Acetaldehyde is converted to ethanol and carbon dioxide by the action of alcohol dehydrogenase. Acetobacter bacteria convert alcohol to acetaldehyde and water, after which acetaldehyde ultimately gets converted to acetic acid (Rusdianasari *et al*., 2021). This results in the formation of a vinegar-like liquid commonly known as eco-enzyme. The reaction involved in the production of this multipurpose liquid is (Muliarta & Darmawan, 2021):

$$CO_2 + N_2O + O_2 \rightarrow O_3 + NO_3 + CO_3 \quad (1)$$

Advantages

Even though it is required to wait for a minimum of three months for eco-enzyme production, the result proves advantageous as it can be used for multiple purposes (Rusdianasari *et al*., 2021).

1. O_3 gas (ozone) is produced during the breakdown of the organic matter in the container. The release of ozone gas reduces the greenhouse gases and heavy metals in the atmosphere.
2. Moreover, NO_3 and CO_2 gases are produced, which provide nutrition to the plants in the soil.

Table 1. General Parameter of Eco-enzyme

Parameters	Values
pH	3.5
TDS	1107 mg/L
BOD	88.6 mg/L
COD	178 mg/L
MPN	< 3 (CFU/100ml)
Enzymes	Protease, lipase, amylase, cellulase
Colour	Yellow to dull dark brown
Odor	Strong, sweet, and sour

Importance

Following are the various ways in which eco-enzymes can be utilized (Neupane & Khadka, 2019; Janarthanan *et al*., 2020; Low *et al*., 2021; Novianti & Muliarta, 2021; Patel *et al*., 2021; Rusdianasari *et al*., 2021).

1. Eco-enzymes can be used as disinfectants, hand sanitizers, and cleaning stains, thereby replacing the use of chemicals like bleach, phenyl, etc.
2. The left-out solid wastes or dregs are used as organic fertilizers.
3. It also acts as a potent insecticide.
4. It can purify the air by absorbing pollutants released during smoking and motor vehicle fumes.
5. It degrades pollutants or hazardous materials in sewage or wastewater.
6. The acetic acid component acts as an anti-fungal and antibacterial agent that helps kill germs, viruses, and bacteria.
7. It can lower the car's temperature when mixed with the radiator.
8. Accumulation of wastes in landfills is greatly reduced.
9. It requires less space and can be carried out in used plastic bottles or containers.
10. Moreover, it can release residual deposits on water pipes, thereby preventing the clogging of water pipes.
11. Enzymes that get released underground consequently purify the river and sea.

Microorganisms Involved

During fermentation, a white mold-like formation is observed on the surface of the mixture. This growth is believed to be due to the activity of Yeast B Complex and Yeast vitamin C (Novianti & Muliarta, 2021). The microflora was found in a study on garbage enzyme composed of apple scraps and Chinese Honeylocust garbage enzyme having Chinese Honeylocust fruit powder in addition to sugar and water (Gu *et al*., 2021).

Bacterial genera: The dominant genera in garbage enzyme included Caproiciproducens, an unidentified species, Tyzzerella, Sporomusa, and Lachnoclostridium_5 while in Chinese Honeylocust garbage enzyme, Lactobacillus was found to be dominant among other bacterial species. Apart from the dominant species, the other bacterial genera in both garbage enzyme and Chinese Honeylocust garbage enzyme are Acetobacter, Neisseria, Anaerosopra, Ruminococcaceae_NK4A214_group, Clostridium_sensu_stricto_3, Eubacterium_fissicatena_group, Methylobacterium, Ruminococcaceae_UCG-010, Anaerosporobacter, Clostridium_ sensu_stricto_1, Mobilitalea, Paenibacillus, Oscillibacter, Ruminococcaceae_ UCG-014.

Fungal genera: The fungal species in both garbage enzyme and Chinese Honeylocust garbage enzyme are Talaromyces, Inocybe, Aspergillus, Cladosporium, Hyonectria, Apiotrichum, Oidiodendron, Chrysosporium, Gibberella, Plectosphaerella, Cadophora, Chloridium. Among the fungal species, the dominant fungal species found in garbage enzymes were unidentified, Mortierella, Dactylonectria, Guehomyces, Fusarium, Penicillium, and Rhodotorula. While in the Chinese Honeylocust garbage enzyme, the dominant genera found was Candida. While in another study, the presence of bacteria that produces nitrate reductase was indicated in garbage enzymes containing waste fruit and vegetable. These were thought to be Escherichia coli, Paracoccusdenitrificans, and Achromobacterxylosoxidans (Low *et al*., 2021).

The presence of Yersinia, Pseudomonas in the bacterial community and Aspergillusniger, Trichodermaviride, Saccharomyces cerevisiae, and Rhizopusstolonifera in the fungal genera was found in the garbage enzyme prepared from fruit peels of papaya, banana, sapodilla, pomegranate and vegetable peels of potato, gourd, eggplant (brinjal) and turnip (Samriti & Arya, 2019).

Applications of Garbage Enzymes

Aquaculture Sludge

The effect of garbage enzyme on aquaculture sludge was tested using different fruit peels and was found to have the potential to treat the aquaculture sludge to a great extent (Rasit & Mohammad, 2018). Moreover, in the previous studies on the treatment of synthetic grey water, garbage enzymes were able to remove phosphates and nitrogen (Nazim & Meera, 2013). It was shown to be potent in reducing the TDS and COD on domestic wastewater and waste-activated sludge (Tang & Tong, 2011; Arun & Sivashanmugam, 2015a, 2015b). The results related to the treatment of the aquaculture sludge experiment are as follows:

Table 2. Different Parameters of Garbage enzyme obtained from Pineapple, Mango, Orange and Tomato

Parameter	Pineapple garbage enzyme	Mango garbage enzyme	Orange garbage enzyme	Tomato garbage enzyme
pH	3.3	2.95	3.25	2.80
TSS (mg/L)	897.67	567.67	600.67	455.67
VSS (mg/L)	512.67	497.67	488.67	412.67
COD (mg/L)	7160	5660	7020	7110
Citric acid (mg/L)	45.01	19.92	29.18	16.41
Enzymes	Highest protease activity at pH 7 and lowest at pH 3.5. At pH 7, amylase activity was the lowest. Lipase activity was found to be the highest at pH 7	Lowest protease and amylase activity at pH 3.5 and 7. While lipase activity was highest at pH 7.	Lowest protease and amylase activity at pH 3.5 and 7. While lipase activity was highest at pH 7.	Lowest protease activity at pH 3.5 and 7. Amylase and lipase activity was highest at pH 7.
Reduction of TSS and VSS after pretreatment of sludge.	Showed the highest reduction, which might be due to the presence of high citric acid content.	-	-	-
Reduction of COD after pretreatment of sludge.	-	-	The highest COD removal was found at pH 7 because of the presence of citric acid. The acidic nature might have broken down the insoluble to a soluble form.	-
Reduction of TAN after pretreatment of sludge.	-	-	The highest removal of TAN can be because of carbon sources that convert insoluble substances to soluble ones.	-

Treatment of Contaminated Water

In order to purify contaminated water, two solutions of garbage enzyme were prepared; solution 1 contained carrot, brinjal, cucumber, ladies finger, beetroot wastes, and onion, brinjal, cabbage, and potato wastes in solution 2. Solution 1 showed better results after the treatment of contaminated water (Janarthanan *et al*., 2020). Domestic wastewater was treated with a solution of garbage enzyme having an orange peel, marigold flower, and neem leaves. The results obtained after the treatment of wastewater are:

Table 3. Garbage enzyme in domestic wastewater treatment

After treatment	Orange garbage enzyme	Marigold garbage enzyme	Neem garbage enzyme
pH	3.9	4.7	3.2
TDS (50 days)	2800 mg/L	2900 mg/L	3600 mg/L
COD (30 days)	1816 mg/L	1028 mg/L	1252 mg/L

Thus garbage enzymes have been shown to have good results in domestic wastewater treatment to an extent (Patel *et al*., 2021).

After treatment with wastewater, fresh fruit-based eco-enzyme has been shown to reduce NO3- to NH4+, which was released as deionized ammonia into the surroundings. In contrast, when vegetable waste eco-enzyme are fermented with Saccharomyces cerevisiae, they increase the NO3- content in the sewage, which is believed to be due to the absence of nitrate reductase action in the yeast that cannot further break down NO3- (Low *et al*., 2021).

Disinfectant Production

A study conducted on garbage enzymes containing orange peel, pineapple, and papaya showed that the phenolic content increased after three months of fermentation. It was thought to be because of enzymatic reactions on the substrate (Khuc *et al*., 2022; Maralov *et al*., 2022). The phenolic content of the garbage enzyme reduces the growth of bacteria. These phenol compounds cause the denaturation of proteins by interacting with the cell wall of the microorganisms, following which structural changes in the proteins occur, thereby increasing cell permeability. This hinders cell growth and thus proves to be bactericidal. Moreover, in the hard water emulsion stability test, no precipitate or floc was formed in the mixture, which shows that garbage enzyme can be used to treat hard water as well (Rusdianasari *et al*., 2021).

Endodontic Treatment

Sodium hypochlorite (NaOCl), the 'gold standard' among endodontic irrigants, is commonly used because of its antibacterial and proteolytic activity. However, NaOCl causes irreversible damage in periradicular tissues, including the neuromuscular structures and soft tissue spaces. Therefore, pineapple and papaya fruit peel as eco-enzyme were experimented on for their effects in the endodontic treatment. Unripe papaya peels have been found to minimize the chronic inflammatory process in apical periodontitis and tissue destruction (Pandey *et al*., 2016). Moreover, pineapple and orange peel have high polyphenolic compounds and flavonoids, showing better antimicrobial and antioxidant activities (Li *et al*., 2014; Ana *et al*., 2018). Furthermore, pineapple extracts have bromelain, which disrupts the peptidoglycan and polysaccharide components of the cell wall of Enterococcus faecalis, thereby killing it (Liliany *et al*., 2018). A study conducted to test the effect of pineapple orange eco-enzyme and papaya eco-enzyme on the sensitivity against Enterococcus faecalis showed that at least 50% concentration, the activity of protease and amylase in pineapple orange eco-enzyme and protease in papaya eco-enzyme destroys the structure of *Enterococcus faecalis* that ultimately leads to cell death (Mavani *et al*., 2020).

Organic Rice Production

When eco-enzyme treatment is done to grow rice, the number of tillers increases, and the plants absorb more nitrogen, for which chlorophyll content increases, resulting in better crop yields and healthy growth (Anas *et al*., 2020; Hasanah *et al*., 2020).

Treatment of Metal-Based Effluents

Effluents of electronic-based industry were treated with garbage enzyme that showed a reduction in TS, TDS, COD, BOD, and no bacterial growth was observed. Moreover, when chili and aloe vera were grown in the sludge treated with 25% eco-enzyme, it produced better results than the soil with eco-enzyme and control in ten weeks (Hemalatha & Visantini, 2020; Chattopadhyay *et al*., 2022).

Natural Disinfectants

Based on the results obtained from the combination of domestic organic waste having rambutan fruit skin, corn cobs, chayote skin, Saccharomyces cerevisiae 10% rangipani sandalwood extract, it has proved to have antimicrobial activity against Staphylococcus aureus with an excellent inhibition power ranging from 31.85-34.41 mm; showing eco-enzymes to be a good alternative for chemical-based disinfectants (Kerkar & Salvi, 2020; Rahayu & Situmeang, 2021).

Hand Sanitizer

With a three months fermentation time, pH 4.5, and a dilution ratio of 5:40, eco-enzyme having an orange peel, pineapple peel, and papaya skin has been shown to inhibit the growth of microbes and thus act as a potent hand sanitizer spray (Rusdianasari *et al*., 2021).

Natural Detergents

A comparative study on apple scraps and Chinese Honeylocust fruit powder-based eco-enzyme found that the Chinese Honeylocust garbage enzyme has higher enzyme activity, including amylase, cellulase, and lipase than the standard garbage enzyme. However, protease activity was found to be the same in both cases. This is an important factor as these enzymes are quite crucial in the washing process. In detergents, amylase is responsible for degrading the residue of starchy foods, cellulose revives the colors in the clothes, and lipase forms a stable fabric-lipase compound by getting adsorbed on it, after which it hydrolyses the oil stains on the fabric. Moreover, the viscosity of both these garbage enzymes was high (Çakar *et al*., 2022; Do *et al*., 2022; Hoang *et al*., 2022; Üzüm *et al*., 2022). The Chinese Honeylocust garbage enzyme did better removing soil stains than the commercial detergent WhiteCat and apple-based garbage enzyme. Chinese Honeylocust garbage enzyme had high detergency of oil, more whitening power, and a high pesticide removal rate than the apple-based garbage enzyme. Both these eco-enzymes showed excellent results in removing dichlorvos and chlorpyrifos residues than the commercial detergent. Furthermore, the Chinese Honeylocust garbage enzyme is better suited as a detoxification agent and natural detergent because of reduced indigenous microbial species, which are non-pathogenic and have high enzymatic activity (Gu *et al*., 2021).

Conclusion

The treatment of solid and liquid wastes has emerged as an important research topic. From various industries to households, lots of waste is discarded into water bodies or landfills, producing methane gas. This poses a threat not just to mankind but to biodiversity as a whole. Therefore, several eco-friendly measures have been adopted to check these issues. This study confirmed that eco-enzyme or garbage enzyme has great potential in dealing with a lot of the issues created by waste.

This eco-enzyme primarily contains protease, amylase, lipase, and cellulase enzymes and has a pH of around 3-4.5 in a three months fermentation time, along with acetic acid and other secondary metabolites. Although the production of eco-enzymes does not require the addition of other microorganisms since the peels and scraps of the fruits and vegetables already have their own microbial community, sometimes Baker's yeast is used to aid in the fermentation.

The number of ways that this organic liquid can be used opens up employment and will probably reduce the emission of harmful gases as well as reduce the piling up of wastes. As eco-enzyme is produced from the utilization of organic wastes, it is economically sound.

Even though many studies have confirmed the excellent potential of eco-enzymes, further investigation on different fruits and vegetables is required so that the common people can start preparing and using this multipurpose liquid, thereby reusing and recycling the wastes.

Future Scope

Conversion of waste material to a useful product is the key demand of current times. This paper mainly focused on the significant application of waste material into valuable products, enhancing the general awareness among people to use waste as a valuable product. This article will help the researchers design research on organic waste, which can be converted into valuable products.

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