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Role of Eco-Enzymes in Sustainable Development

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ABSTRACT

Globally organic wastes are generated from fruits, vegetables, and their peels. It is mostly decomposed in landfills or by composting methods. Food processing industries, vegetable markets, and restaurants produce a huge amount of organic waste daily, generally disposed of in the environment or composted. Producing an eco-enzyme from organic kitchen waste was an innovative solution for domestic waste pollution. It is an enzyme solution obtained from an organic waste substance that contains organic acids, enzymes, and mineral salts. It is produced by performing a simple batch fermentation that involves a mixture of brown sugar, fruit or vegetable waste, and water in the ratio of 1:3:10. Two types of the eco-enzyme were produced by a fermentation process using vegetable and fruit peels for about 90 days involving *Saccharomyces cerevisiae*. The ultimate liquid or enzyme obtained was brown. Eco-enzyme 1 from (Cucurbita maxima) contained hydrolytic enzymes like amylase and lipase. The microbial diversity was observed, and bacteria like *Yersinia sp., Bacillus sp.*, and fungi like *Trichoderma sp.* and *Penicillium sp.* No enzymes and microorganisms were observed in Eco-enzyme 2 (Citron). Eco-enzyme 1 with 50% dilution effectively reduced various parameters like BOD, COD, TDS, Nitrate, Nitrite, and Ammonium in the effluent. Also, it promoted plant growth within 10 days compared to the control. Therefore, the present study outlines how the eco-enzyme could be used to treat industrial effluent cost-effectively and environmentally friendly.

INTRODUCTION

Fruits and vegetables, as well as their peels, are organic wastes that are generated worldwide. The majority of it decomposes in landfills or through composting methods. Food processing industries, vegetable markets, and restaurants generate massive amounts of organic waste daily, typically disposed of in the environment or composted.

Pollution is caused by waste fruit and vegetable peels being disposed into the environment. To avoid such issues, they must be properly disposed of. Even though organic waste is decomposed, greenhouse gas emissions are possible during decomposition (Geetha et al. 2017). This method of producing an enzyme from organic kitchen waste was more novel than the usual method of involving them in composting (Sarabhai et al. 2019).

The result was an enzyme, after fermentation of waste fruits and vegetable peels which was named "Garbage enzyme" or "Eco-enzyme." This innovative development of producing eco-enzyme was the best alternative method for productively processing household organic waste; additionally, it leads to a zero-waste framework by reducing, reusing, and recycling organic household waste materials.

Industrial Effluent

Different industries' industrial effluents contain chemicals that should not be released into the environment. Many methods exist for treating industrial effluent. Many industries struggle with reducing organic content in wastewater and only succeed after many processing stages. The use of eco-enzymes to treat industrial effluent was a cost-effective alternative.

The leather industry is a centuries-old manufacturing sector that produces a wide range of goods, such as leather footwear, bags, and garments. The leather industry's raw material is from food waste, specifically meat processing. Humans use leather products every day. Any leather processing industry's primary raw material comes from slaughterhouses and meat industry waste. This raw material is processed and converted into usable leather in tanneries. As a result, the tanning industry is regarded as one of the industry's most important leather processing units. Fig. 1 shows various chemicals employed in the processes of tanning. The leather industrial effluent contains many chemicals and acids, as the tanning process involves many chemicals. Chemicals in leather industry effluent include ammonium salts, calcium salts, phenol, chromium, nitrogen, sulfides, solvents, surfactants, acids, and metallo-organic dyes; natural or synthetic tanning agents; sulfonated oils, and salts. Pre-tanning and tanning operations account for roughly 90% of total leather industry pollution (Sivaram & Barik 2019). The pre-tanning operation causes pH differences, which raises the chemical oxygen demand (COD), total dissolved solids (TDS), chlorides, and sulfates in tannery wastewater. Heavy chromium is widely used in the leather, electroplating, and metallurgical industries (Nur-E-Alam et al. 2020). Leather industries struggle to treat their effluent, which contains many harmful chemicals. They use many technologies to reduce the net harmful contents as much as possible. Leather industries also recycle some of their waste into value-added products for waste management. However, solid waste from leather industries isn't recycled for any purpose (CPCB 2019).

The industrial sludge also contains a large number of hazardous chemicals that pose a risk, out of which Chromium in the effluent of the leather industry poses a serious threat to soil and plant, so it cannot be reused for any purpose without proper treatment (Liknaw et al. 2017). Industries use various techniques to treat effluent, including biological, chemical, and oxidation. Waste management in tannery effluent is even more difficult due to numerous chemicals such as chromium, aluminum salts, and chloride (CBCP 2019). Bioremediation is one of the most recent technological advances for treating heavy metals-containing industrial wastes. (CPCB 2019). It primarily employs microorganisms such as bacteria, algae, and fungi to biodegrade toxic components (Biswas et al. 2015). Bioremediation process using microbes like bacteria (*Bacillus* spp., *Staphylococcus* spp.), yeast (*Candida* spp., *Saccharomyces* spp.,) fungi (*Paecilomyces* spp., *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp.,) and algae were carried out. (Nur-E-Alam et al. 2020, Santhosh et al. 2020). Waste treatment with bacteria entails stabilizing waste by decomposing it into harmless inorganic solids via an aerobic or anaerobic process. The aerobic process decomposes faster than the anaerobic process and produces no unpleasant odors, whereas the anaerobic process requires a longer retention period and produces unpleasant odors. In effluent treatment, microbes were used to facilitate the process to avoid these laborious methods. The eco-enzyme was an innovative method in the industrial effluent treatment process. Eco-

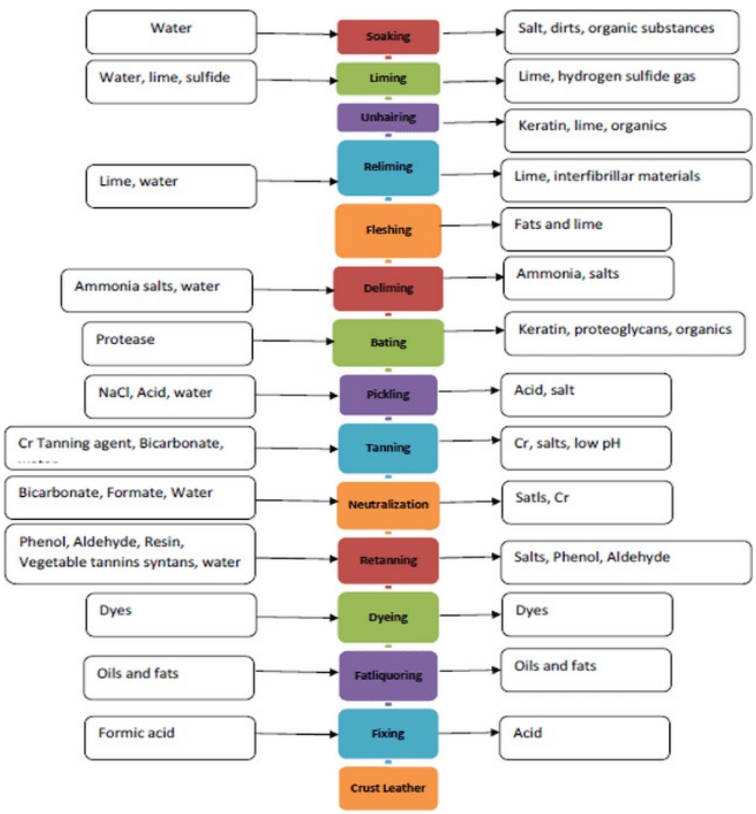


Fig. 1: Processes in the leather industry.

enzyme use in industrial effluent treatment could be a useful, nonhazardous, and time-saving method.

Eco-enzyme

An Eco-enzyme is an enzyme solution obtained from organic waste that contains organic acids, enzymes, and mineral salts. It is a simple Batch Fermentation that uses a 1:3:10 ratio of sugar, fruit or vegetable waste, and water (Neupane & Khadka 2019). The fermentation process takes place up to 60-90 days. As a result of fermentation, the final liquid or enzyme obtained was brown and contained various Organic acids, Carbon dioxide, and Ethanol. Fermentation is a chemical process in which microorganisms (non-pathogenic bacteria and yeast) break down large complex molecules into smaller ones, resulting in carbon dioxide, ethanol, and various acidic end products. The bacteria found naturally in vegetables, fruits, and their peels help convert carbohydrates into simple ones that yeast can use (Chakraborty 2020). *Saccharomyces cerevisiae* is the most commonly used yeast, producing ethanol and other acidic end products. Depending on our needs and alcoholic content, the ethanol produced in this manner can be used as a biofuel and a disinfectant. The eco-enzyme has many applications like treating domestic wastewater, contaminated ponds, lakes, and many more (Teo et al. 2021, Application of Eco-Enzyme for Domestic Waste Water Treatment 2020, Effect of Bio-Enzyme in the Treatment of Fresh Water Bodies 2019).

The eco-enzyme was mostly made using citrus and papaya peels or fruit as it showed greater antimicrobial activity (Saleem & Saeed 2020). Various fruits and vegetables produce the garbage enzyme depending on availability, flavor, etc. (Arora Amar et al. n.d., Narender et al. 2018). Composting organic household waste was the most efficient and cost-effective method of disposing of and biologically managing household waste materials (Nengah Muliarta & Darmawan 2021). The traditional Eco-enzyme manufacturing process varies depending on the alcoholic content and pH. The eco-enzyme has a variety of applications, and their use varies depending on their content. This research focuses on producing eco-enzymes using various fruits and vegetable peels as a substrate and their application in treating industrial effluent.

MATERIALS AND METHODS

Sample Collection

The research was carried out between 2021 and 2022. In the study, tannery effluent was collected simultaneously in two stages in the tannery industry. The following effluents were collected: i) post-tanning process and ii) final effluent.

Preparation of Samples

Because there were no larger particles in the effluent, the collected samples were used directly for analyzing physicochemical parameters.

Tanning Effluent Characteristics

BOD, COD, TDS, nitrate, nitrite, and ammonium levels were measured before and after treatment.

Biochemical Oxygen Consumption

The biological oxygen demand is the amount of oxygen organisms require over a given period to break down organic materials. The standard BOD level in industrial effluent is 30 $mg.L^{-1}$ (CETP 1986)

The biological oxygen demand was calculated by preparing all of the necessary reagents. The initial dissolved oxygen content of the effluent sample was determined first. The sample is filled to half the neck of the BOD bottle. After adding the reagents, the bottle was checked for any air bubbles. The bottles are then sealed and incubated at 20°C for 5 days.

The dissolved oxygen content of a bottle incubated at 20°C for 5 days was measured. The formula was used to calculate the BOD.

Chemical Oxygen Demand

Chemical oxygen demand measures the oxygen equivalent of organic matter content in a sample oxidizable by a strong chemical oxidant.

Potassium dichromate can almost completely oxidize a wide range of organic substances to Water and Carbon dioxide. To oxidize organic matter completely. The solution must be strongly acidic and at an elevated temperature. Standard potassium dichromate, sulphuric acid, ferrous ammonium, and mercuric sulfate were prepared as reagents.

Total Dissolved Solid Content (TDS)

The mass of residue left after a measured volume of filtered water is evaporated is used to calculate the total dissolved solid content. TDS levels were also measured before and after treatment.

Nitrate Content

Titration and UV-spectrometry methods can estimate the nitrate content present in the sample. This study employs the spectrometry method for determining nitrate in the sample. The nitrite and ammonia content were also analyzed.

Eco-enzyme Production

Two types of eco-enzyme were created using vegetable and fruit peels. Cucurbita maxima and Citron (Citrus medica) peels were collected to make eco-enzymes. It was important to avoid spoiled peel waste. The samples are air-dried before being processed to create Eco-enzyme. An airtight container was filled with 200 g of brown sugar, 600 g of peels, and 2 L of water. Optionally, yeast (Saccharomyces cerevisiae) was added to speed up the fermentation process. The container's lid was tightly closed to prevent any air exchange. Fermentation was allowed to take place for a minimum of three months. The container's lid was opened intermittently during the first and second weeks to release the gases that formed inside, preventing the contents from explosion. After three months of fermentation, the fruits and vegetable peels settle to the bottom, leaving a clear liquid on top that appeared to be a brown liquid containing organic acids, ethanol, and carbon dioxide. The eco-enzyme was then collected after being filtered to remove residues. Fig. 2 shows the fermentation process of eco-enzyme.

Characteristics of Eco-enzymes

After 3 months of fermentation, the characteristics of the purified eco-enzymes were examined. The pH, enzyme activity, and physicochemical properties of the enzyme extract, such as color, and odor, were measured.

pH

The pH meter was used to determine the PH of the eco-enzyme. The meter was first calibrated using a buffer solution to pH 4, 7, and 9. The electrode was then gently dipped in the eco-enzyme sample. The pH of the eco-enzyme was displayed and recorded in the digital reader.

Enzyme Assay

The enzyme assay was carried out using qualitative analysis - A method of Agar diffusion

Activity of amylase: The activity of amylase in the medium was tested using starch as the substrate. The starch agar was prepared and sterilized by autoclaving it for 15 minutes at 121ºC. After cooling to 55ºC, the agar was poured onto sterile petri plates. After that, the plates were left alone to solidify.

10 µL of the eco-enzyme sample was inoculated in the plates. The plates were then incubated for 24 h at 37°C. The plates were flooded with iodine, and a clearance zone was determined. The presence of amylase was indicated by the clearance zone, which was compared to control plates.

Lipase activity: The eco-enzyme's lipase activity was discovered using tributyrin as a substrate in the agar medium. After incubation, the zone of clearance is observed.

Protease activity: The presence of protease activity in the agar medium is determined by using casein 1 percent as a

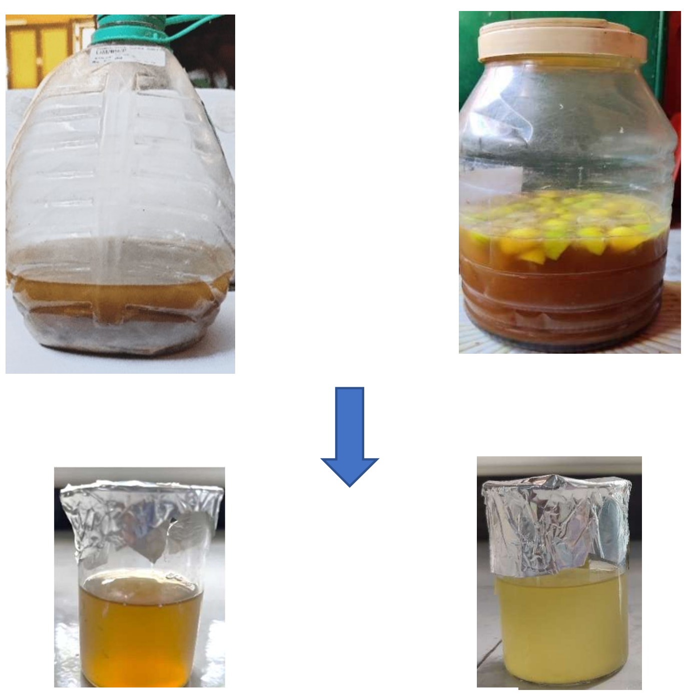


Fig. 2: Fermentation process of Eco-enzyme.

substrate (1.5 percent). After inoculating the plates with the sample, they were incubated at 37°C for 24 h. The clearance zone is observed and recorded

Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry was used to detect enzymes both qualitatively and quantitatively. Gas chromatography-mass spectrometry (GC-MS) is a technique for identifying various substances in a test sample that combines the advantages of gas chromatography and mass spectrometry.

Microbial Diversity

The spread plate method was used to examine the microbial diversity present in the eco-enzyme. Gram staining and LPCB staining were used to identify bacteria and fungi. 0.1mL of the eco-enzyme sample was spread on the Nutrient agar and SDA mediums using a glass L- rod. The plates had been incubated. SDA plates were incubated at room temperature for 48-72 h, while nutrient agar plates were incubated at 37°C for 24 h (Sarabhai et al. 2019).

Treatment of Effluent Using Eco-enzyme

The treatment was carried out for approximately 30 days in two dilutions of the eco-enzyme (25 percent and 50%). The parameters were analyzed before the treatment process to compare the results after treatment with the eco-enzyme.

Dilution by 25%: The industrial effluent was placed in a conical flask (375 mL), to which the eco-enzyme sample (125 mL) was added. The flask was then sealed with aluminum foil. The same setup was used for both eco-enzyme samples. For a month, the flask was left alone. After 30 days of treatment, the color change and parameter reduction were tested.

50 percent dilution: 250 mL of effluent was mixed with 250 mL of eco-enzyme sample. The flask was sealed with aluminum foil. Fig. 3 shows the treatment of effluent using Eco-enzyme in 2 dilutions.

Sludge Treatment

The dry leather industrial sludge was collected. The sludge was mixed in a 1:1 ratio with eco-enzyme and left for 2-3 days. Before treating with sludge, the eco-enzyme was diluted to about 1:500. After about 2-3 days, the sludge was mixed in a 1:1 ratio with the sand. It was then used to control plant growth. (Nabila et al. 2021)

Four pots were selected as controls: soil alone, raw sludge without enzyme treatment, sludge treated with eco-enzyme 1, and sludge treated with eco-enzyme 2. Each pot was labeled and filled with Indian mustard, *Brassica juncea*. The pots were watered regularly, and the plant growth was monitored. The heights of the plant shoots were measured.

RESULTS AND DISCUSSION

Production of Eco-enzyme

The batch fermentation process produced two eco-enzymes. After fermentation, the enzyme extract was obtained by filtration method. Centrifugation was done at 5000 rpm for 5 mins to obtain the same enzyme extract. Fig. 4

Fig. 3: Treatment of effluent using Eco-enzyme in 2 dilutions.

(a) (b)

Fig. 4: Eco-enzyme 1. Eco-enzyme 2.

shows the enzyme obtained after the batch fermentation process.

Examination of Microbial Diversity in Eco-enzyme

Microscopic examination revealed Gram-negative bacilli and Gram-positive bacilli observed, and other biochemical tests were performed. It was found that there was the presence of *Bacillus* sp., *Yersinia* sp. Figs. 5 and 6 show the preliminary tests (Gram staining and biochemical tests performed). Fig. 7 shows the LPCB staining and growth on the SDA plate.

The eco-enzyme was inoculated in SDA plates and incubated for 48-72 h. Upon further staining using LPCB, fungi like Trichoderma viride, Saccharomyces cerevisiae, and Penicillium sp. were present in both the

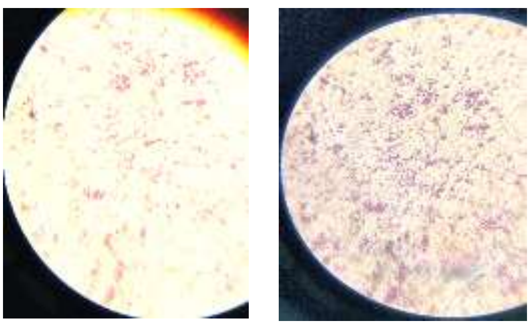
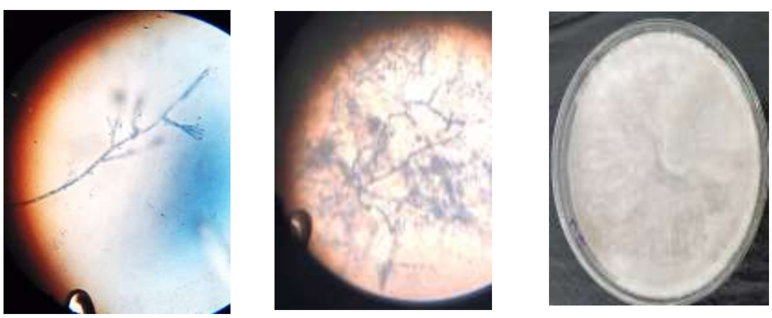


Fig. 5: Gram staining (a) Gram negative bacilli (b) Gram positive bacilli.

Fig. 6: Biochemical test.



(a) (b) (c)

Fig. 7: LPCB staining (a)- *Penicillium* sp. (b)- *Trichoderma* sp., (c) – SDA plate with growth.

Table 1: Characteristics of the Eco-enzyme.

Physical characteristics	Eco-enzyme -1	Eco-enzyme -2
Color	Brown colored liquid	Pale yellow colored liquid
pH	4.10	3.83
Turbidity	Clear liquid, non-viscous	Clear liquid, non-viscous
Odor	Pungent	Sweet sour

Table 2: Biochemical test done for Eco-enzyme 1.

PRELIMINARY TEST	RESULT
Gram staining	Gram positive bacilli and Gram-negative bacilli
BIOCHEMICAL TEST	**RESULT**
Indole	Negative
Methyl red	Negative
Voges Proskauer	Positive
Citrate	Negative
Triple sugar ion	Yellow slant and yellow butt, no gas production
Urease	Negative
Nitrate	Positive

eco-enzyme samples. Table 1 shows the characteristics of eco-enzyme.

Table 2 shows the biochemical tests performed for Eco-enzyme 1 as there was growth in the Nutrient agar and SDA plates. Eco-enzyme 1 was found to contain Gram positive and negative bacilli.ss There was no growth observed in the eco-enzyme 2 solution.

Enzyme Assay

There was a zone of clearance after adding iodine in starch ag, indicating the presence of amylase activity in both the eco-enzymes produced. Figs. 8, 9 and 10 show the amylase, protease, and lipase activities respectively. There was no protease activity seen in the eco-enzymes. There was a small zone of clearance in case of lipase activity in both the eco-enzymes.

Gas Chromatography-Mass Spectrophotometry

Gas Chromatography-Mass Spectrophotometry was performed for both the eco-enzyme samples after batch fermentation. It was found that Eco-enzyme 1 consists of active ingredients like alcohol, and acids. Fig. 11 a and b show the graphical peaks of various compounds present in the eco-enzyme sample.

Fig. 8: Amylase activity of eco enzyme 1 and eco enzyme 2.

Fig. 9: Protease activity -Nil in both eco enzyme 1 and eco enzyme 2.

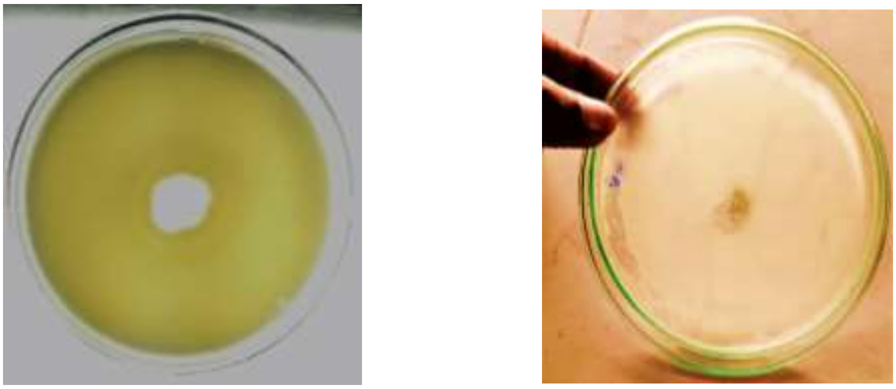


Fig. 10: Lipase activity - positive for Eco enzyme 1 and nil for Eco enzyme 2.

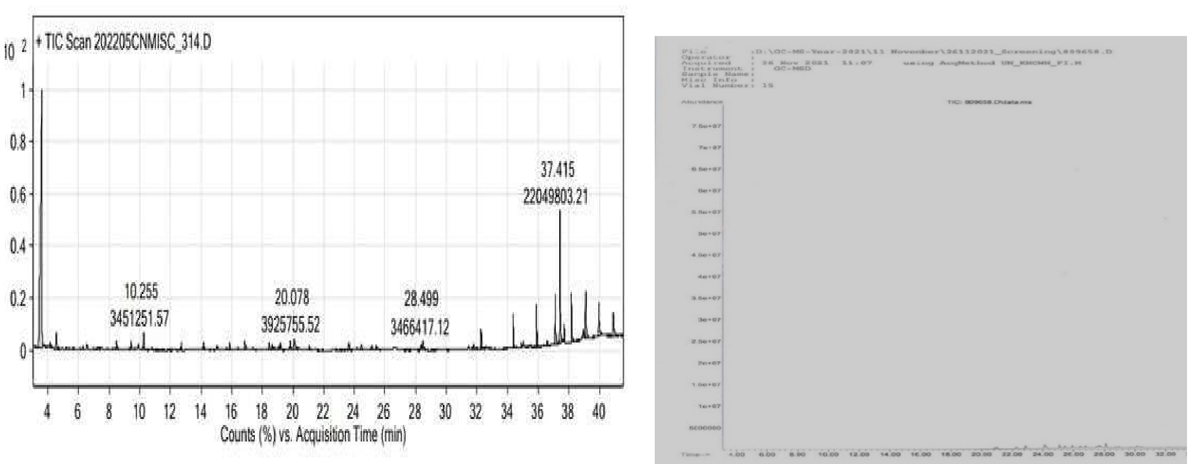


Fig. 11: (a) GC- MS of Eco-enzyme 1 (b) GC- MS of Eco-enzyme 2.

Characterization of Industrial Effluent

The characteristics of industrial effluent were studied before treatment. Both the effluent was found to be acidic and black in color. Table 3 shows the characteristics of industrial effluent used in this study.

Effluent Treatment

Table 4 shows the results of parameters tested before and after treatment of the effluent. It was found that Eco-enzyme diluted to 50% which was found to be effective in the treatment of leather industrial effluent.

Table 3: Characteristics of industrial effluent.

Parameters	Raw effluent	Tannery effluent
P^H	3.42	2.76
Color	Black	Black
Odor	Unpleasant foul odor	Repulsive, offensive chemical smell

Fig. 12 shows the process of effluent treatment after 30 days. There was a considerable amount of reduction in parameters and color as well. Eco-enzyme 1 was found to be effective when used in the dilution of 50%.

Sludge Treatment

The sludge without eco-enzyme treatment showed no plant growth, indicating that it cannot be used to grow plants directly. Plant growth was aided when the sludge was treated with the eco-enzyme. The conversion of ammonium in the sludge into nitrogen, which the plant readily utilizes for growth, could cause plant growth. The seeds were planted in each pot, and the pots were watered daily. Plant growth was monitored, and shoot heights were measured. When compared to the control, sludge treated with eco-enzyme

Table 4: Treatment of Industrial effluent using Eco-enzyme.

Parameters	Before Treatment	After Treatment			
		Raw Effluent		Tannery Effluent	
		25%	50%	25%	50%
BOD	1080	344 mg.L^{-1}	297 mg.L^{-1}	372 mg.L^{-1}	324 mg.L^{-1}
COD	4250	678 mg.L^{-1}	606 mg.L^{-1}	730 mg.L^{-1}	576 mg.L^{-1}
TDS	5096	1560 mg.L^{-1}	1420 mg.L^{-1}	2510 mg.L^{-1}	1680 mg.L^{-1}
Nitrate	0.90	0.30 mg.L^{-1}	0.17 mg.L^{-1}	0.32 mg.L^{-1}	0.11 mg.L^{-1}
Nitrite	0.73	0.21 mg.L^{-1}	0.18 mg.L^{-1}	0.26 mg.L^{-1}	0.12 mg.L^{-1}
Ammonia	0.36	0.11 mg.L^{-1}	0.09 mg.L^{-1}	0.13 mg.L^{-1}	0.06 mg.L^{-1}

Fig. 12: Effluent treatment process.

showed greater plant growth. Fig. 13 a and b shows the sludge taken from the industry and (c) shows the growth of plants when sludge was treated with eco-enzyme.

Table 5 shows the plant shoot height measured after 10 days. These results show that Eco-enzyme 1 was found to be effective in the treatment of industrial sludge when compared with the control (sludge alone). Further, the active component present in eco-enzyme which makes it a better solution for treating the effluent and sludge should be studied in detail.

Fig. 13: Sludge treatment (a) (b) Sludge mixed with eco-enzyme and sand (c) Plant growth after 10 days.

Table 5: Plant shoot height measurement after 10 days.

Treatment	Plant Height (in cm)
Control – Soil +Sludge	1.0
Control- Soil	3.5
Soil + Sludge treated with the Eco-enzyme 1	5.7
Soil + Sludge treated with the Eco-enzyme 2	4.2

DISCUSSION

The production of the eco-enzyme from organic waste is a novel method that replaces the traditional method of composting waste. Numerous studies have been conducted to test various applications of the eco-enzyme. These eco-enzymes effectively treated water bodies such as ponds and lakes. Similar to the study conducted, the antimicrobial activity of the eco-enzyme was found to be effective against a wide range of microorganisms (Arun & Sivashanmugam 2015). According to (Made Rai Rahayu et al. 2021), the eco-enzyme contains hydrolytic enzymes such as amylase and lipase.

Leather industrial effluent contains several chemicals introduced at various stages of the leather manufacturing process. Both humans and the environment are endangered by the chemicals used. Proper treatment and waste management methods must be implemented in industries to ensure the safety of humans and the environment. Waste management in industries has traditionally been a time-consuming process. Many industries use enzyme technologies and microorganisms to treat effluent (Choudhary et al. 2004, Pandey et al. 2017). Microbial isolates like *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* from watermelon were used to treat raw tannery effluent. They were found to degrade the physicochemical parameters and could be used to treat tannery effluent biologically (Okoduwa et al. 2017). The concept of using eco-enzymes to treat industrial effluent is novel. The hydrolytic enzymes were discovered in eco-enzyme 1, made from vegetable peel waste.

These enzymes are involved in the breakdown of compounds found in effluent. In about 50% dilution of the eco-enzyme, there was a significant reduction in major parameters such as BOD, COD, and TDS. Similarly, Rasit and Chee Kuan (2018) used eco-enzymes to treat Palm oil mill effluent. There was a decrease in oil and grease content observed. The eco-enzyme was also used to treat metal-based effluents, which were observed by Hemalatha and Visantini (2020)

Similarly, Teo et al. (2021) discovered decreased nitrate content in wastewater samples. In contrast to the previous study, there was a significant reduction in nitrate content in the effluent sample in the current study.

Similarly, a study found that the yeast Saccharomyces cerevisiae reduced various parameters in tanneries. Galintin et al. (2021) conducted a study similar to the current one. To treat aquaculture sludge, they used eco-enzymes. It was discovered that 10% dilution was effective in lowering various parameters. The study used tannery sludge that had been collected in dry form. Compared to the control, sludge treated with eco-enzymes 1 and 2 promoted plant growth. This was similar to the research by Hemalatha and Visantini (2020). The beneficial effect of the eco-enzyme on plants was investigated by Nabila et al. (2021)

The plant growth of Turi (*Sesbania grandiflora*) increased in the study. In a study conducted by discharging leather industrial effluent into the soil, there was an increase in soil microbiota and enzyme activity (Reddi & Narasimha 2012). Plant growth could be attributed to the organic acids found in the eco-enzyme. Production and usage of the eco-enzyme effectively treated leather industry effluent and paved the way to sustainable development. The current study used eco-enzyme production as an innovative approach. Using eco-enzymes to treat effluent would be a promising method and the best alternative to the chemicals commonly used in industry. It protects both humans and the environment. It is also less expensive and saves time. Enzyme stabilization would be a better way to increase their efficacy.

CONCLUSION

Organic and agricultural waste is being used in a variety of fields. They are becoming more popular as value-added products, and many industrial firms use them as raw materials. Fruit and vegetable peels contain a variety of bioactive components. When organic waste is disposed of in trash or landfills, its components and nutrients are lost. Eco-enzyme production is a new and innovative method of reducing and reusing waste as a value-added product.

Eco-enzymes are being developed, and it has been discovered that they can be used in various fields. It also replaces the traditional composting method of disposing of organic waste. The Eco-enzyme is a collection of enzymes that reduce organic compounds in industrial effluent. Agar diffusion and GC-MS methods revealed that Eco-enzyme 1 contained enzymes such as amylase and lipase. The eco-enzyme 2, made from citrus peels, contained no hydrolytic enzymes.

Hydrolytic enzymes were discovered to cause a decrease in parameters such as BOD, COD, TDS, nitrate, nitrite, and ammonium. Eco-enzyme diluted to 50% was effective in treating leather industrial effluent. The eco-enzyme contained a diverse group of microbes. Bacteria such as *Yersinia sp*. and *Bacillus sp*. were present, as well as fungi such as *Trichoderma* sp. and *Penicillium* sp., More research is needed to test the microorganism species found in the eco-enzyme. Dry sludge collected from the industry was also used in the sludge treatment. Within 10 days, plant growth was observed. The increased shoot heights suggest that the eco-enzyme aids plant growth. The challenging approach in producing Eco-enzyme is that there is no standard protocol for production. Because it takes longer to produce an Eco-enzyme, it is still not used in industries. There has been no research on the mechanism of action of eco-enzymes in component reduction, which should be looked into further.

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