International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

Exploring The Efficacy Of Bioenzymes A Natural Alternative For Chemicals

[1]Ruchita Shrivastava, [2]Dhiraj Kumar Yadav, [3]Preetilagna Panigrahi, [4]Swarnalata Tripathy, [5*]Dhaarani Vijayakumar
[1]Guest Lecturer, Department of Botany, Govt. Homescience PG Lead College, Narmadapuram (MP)
[2]Senior Assistant Professor, Department Of Farm Forestry, Utd Sant Gahira Guru University Sarguja, Ambikapur, Chhattisgarh, India, 497001
[3]Department of Agriculture and Allied Sciences , C. V. Raman Global University
[4]Assistant Professor botany , Department of Agriculture and Allied Sciences , C .V. Raman Global University
[5*]Assistant Professor Botany, Department of Agriculture and Allied Sciences , C .V .Raman Global University , Bhubaneswar Odisha

Abstract: *Bioenzyme is a type of fermented liquid made up of kitchen wastes. The bioenzyme making required water, jaggery, and garbage in a specific amount which is 1:3:10:15, which are jaggery: kitchen waste: water: plastic container respectively. These are the requirements which are easily available in every house hold to prepare these golden liquid. It known as golden liquid because it has many benefit in every field like in medicine, farming, cleaning, self-care etc. bioenzyme can be prepared from anything like fruit waste, leaves, vegetable waste, flower etc.. Inside this desertation the given information about Orange bioenzyme, Soap nut bioenzyme, Tamarind bioenzyme, Neem bioenzyme, Hibiscus bioenzyme and Ginger bioenzyme . The enzymes having many antimicrobial property which are safe to use ,These enzymes are use in floor cleaning, dish wash cleaning, agricultural field and in every field it given positive results .This bioenzyme is environment friendly, cheap, as well as effective for every field and use . For future perspective it considered as a good substitute for chemical used harmful products in every field .*
Keywords*: Plant growth, natural cleaner, environment friendly*

INTRODUCTION

Eco-enzymes are one kind of organic compound. This complex solution is made by fermenting fresh kitchen waste, including fruit and vegetable peels. To make this type of homemade vinegar, kitchen wastes are fermented with sugar as a substrate, lowering the alcohol concentration (Xia Li and Hang Wang 2013). Bio enzymes may generally be made from organic garbage like the peels of fruits. Tangy fruit peels are used because of their special characteristics, which include their high acidity value, potent flavour and aroma, high vitamin C content, and potential medical benefits. Eco-enzyme possesses insecticidal, antifungal, and antibacterial properties. It can also be utilized as a cleaning agent.

Poompanvong was the first to produce bio-enzyme. Bio-enzymes are alternatively referred to as eco-enzymes, garbage enzymes, terrazymes, fruit enzymes, flower enzymes, and similar names. Eco-enzymes are a mixture of enzymes that catalyse many processes rapidly. In terms of chemistry, eco-enzymes are a blend of polypeptides, polysaccharides, bioactive compounds, and other materials that are formed spontaneously by the of some microorganisms and used to produce eco-enzymes. Water, Fermented fruit and vegetable scraps, brown sugar or carbohydrates are all added during the fermentation of bio-enzymes.(Sethi S.K. *et al 2021)*

In 2006 Poompanvong of Thailand used organic solid waste to create a complicated solution known as "Garbage Enzyme" (Novianti & Muliarta, 2021). EcoEnzyme and Bio-Enzyme are other names for garbage enzyme. It's a dark, grimy liquid that smells like vinegar. It's mostly made up of alcohol, acetic acid, a mix of vitamins and minerals, salts, and enzymes like cellulase, lipase, amylase, and protease. It also contains amino acids, hormones, and some helpful microorganisms. Bioenzyme is made of simply fermenting organic waste in the presence of water and molasses, such as whole fruits , vegetable peels, flowers petals and leaves. The ratio of the previously listed ingredients is 3:1:10, meaning that there are 3 parts organic waste, 1 part molasses, and 10 parts water. This combination is then set to ferment for minimum three months (Samriti *et al* 2019).

In a similar vein, Kanchana *et al* (2023) has created a multipurpose Bio-Enzyme solution using common home trash, such as fruit and vegetable peels and flowers. Recent research indicates that agro-industrial

wastes such fruit peels and molasses may be effectively utilized as substrates to produce bioenzymes, leading to economical and sustainable enzyme production. According to Patolia *et al* (2021), the synthesis of cellulase and pectinase enzymes was significantly increased when orange peels were used as the primary substrate. By emphasizing the great performance of the enzymes generated in applications such as wastewater treatment and textile processing, the study illustrated the promise of bioenzyme synthesis from waste as a sustainable industrial practice.

The Food Waste Index Report 2021 from the United Nations Environment Programme states that each Indian family wastes more than 50 kg of food each year. Over 7.5 tons of food are wasted daily and dumped in the trash and sewage, adding to the amount of garbage generated in India. There is not enough focus on how to solve the problem of properly utilized the food waste produced in India al well as in other country's also.

The quantity of garbage produced in India has increased along with its population, which has a detrimental Influence on both the ecosystem and the country's Financial system (Yadav & Singh, 2021). According to Singh (2020), India generate 147,613 metric tons of Daily solid waste creation. (Akusu *et al* 2019) organic wastes account for a sizable amount of municipal solid garbage, which is mostly made up of wet kitchen waste which are organic food west, and landscaping and pruning wastes. Because microorganisms perform anaerobic breakdown, which produces landfill gas, these wastes become an annoyance when they are dumped directly in landfills. These gases cause changes in the climate and are a contributing factor to global warming (Rasit and Kuan ,2018),

Up until now, waste has been drawing public attention. Any product or material that is discarded, unwanted, or useless is considered waste. Despite being an unwanted material, it may nevertheless be recycled and utilized to create eco-enzyme . However, 41.1% of Indonesia's 24 million tons of trash buildup in 2021 came from domestic rubbish, according to Waste Change. Waste management strategies are encouraged by the rise of rubbish in order to mitigate the negative impacts on society.(Serafy *et al* 2021).

The extensive use of chemical pesticides and fertilizers in agricultural production degrades the soil's quality and reduces its binding capacity. Chemical fertilizers are effective, but over time, they harm the environment by increasing soil acidity and soil surface sealing, which Influences plant development and alters Acid-base status of the soil, which promotes insect proliferation and even contributes to greenhouse gas emissions. One of the main ingredients in many of the disinfectants that are sold today is benzoalkonium chloride. The detrimental the impact of blood alcohol concentration (BAC) on the human body can include effects like skin irritation and various allergic reactions , have been shown in several research.(Oiso *et al.* 2005)

Human waste and other harsh chemicals, including BACs, find their way into the sewage system and into other aquatic bodies. This results in contamination and deteriorating aquatic quality, which upsets the Aquatic biosphere. Because bioenzymes are completely natural and minimize waste, they can help with these problems because their primary ingredients are form fruits and vegetables, which are regarded as waste and tossed in the trash. They are completely natural liquids that are non-toxic, non-corrosive, non-hazardous, and safe for the environment. In short, bio-enzymes are produced by anaerobically fermenting Fruit and vegetable waste with fermentation ingredients. The microbes have generated a combination of juvenile hormones and enzyme.(Krogsrud *et al.,* 1997).

Since industry and urbanization have increased the amount of Municipal Solid Waste (MSW) generated per person, solid waste management (SWM) has become a serious problem for several metropolitan municipal governments in India (Tiimub *et al.* 2013). Composting, incineration, and landfilling are popular methods of garbage disposal. However, because of their high energy consumption, delayed breakdown into innocuous chemicals, production of harmful methane gas, and unpleasant stink, they are not suitable for processing organic waste. Since open dumps generate methane from the anaerobic breakdown of biodegradable waste and serve as a habitat for worms and mosquitoes, which can spread infectious illnesses, the odor may also be a major problem, particularly in the summer months when daily temperatures in India can reach 45°C. (Monney *et al.*, 2013).

Citrus peel-based eco-enzymes can also promote plant growth (Vama & Cherekar, 2020).These can also be used to remediate effluents that include metals. It can be applied to manage substantial quantities of organic waste, heavy metals, and various pollutants that have increased as a result of industrialization

(Hemalatha & Visantini, 2020).Wastewater, which contaminates rivers where trash is dumped, may also be cleaned up using eco-enzymes derived from various citrus peels. Using Bio-enzyme can reduce pollutants and the bad smell of the water (Janarthanan & Mani, 2020).

Fermentation is a biochemical reaction that alters Carbon-based compound chemically through the action of enzymes. In biochemistry, fermentation is sometimes referred to as an anaerobic process, which is the process of turning carbohydrates into energy without oxygen. One type of carbohydrate that helps activate fruit enzymes is sugar (Parul *et al.,* 2022). Enzyme bio-cleaner, which is created during fermentation, creates microorganisms with increased yields, faster growth, reduced oxygen needs, and the capacity to operate in more concentrated medium.

Therefore, a high sugar content is ideal as it supplies the microbes with more energy for growth and enhances the production of enzymes that improve its cleaning effectiveness . There are several uses for this cleaner, including in the household, in agriculture, in animal husbandry, and in many other fields (Prerna *et al.,* 2022). It is a really good home product, like organic fertilizer and laundry detergent. Because enzyme bio-cleaner solution doesn't include any additional chemicals, it also aids in the management of environmental contamination.

These enzymes convert different components of the soil into nutrients that plants can absorb and utilize for proper growth. The variety of enzymes that may be produced allows us to produce different types of bioenzymes for different applications (Ashraf *et al., 2014).* Different fruit and vegetable wastes can be mixed to create different enzymes since different organic waste sources release different enzymes. The bulk of current research studies focus on creating bio-enzymes from citrus wastes since citrus fruits frequently produce a enough amount of powerful enzymes such amylases, pectinases, proteases, lipases, and cellulases.(Dhavale *et al., 2020).*

Metabolic conversion in any metabolic process in which sugar and starch are broken down by enzymes in microorganisms such as bacteria and yeast to produce acid and alcohol. Here, naturally occurring bacteria and yeast on the surfaces of fruits and vegetables break down sugar molecules like glucose, sucrose, and fructose without oxygen to create adenosine triphosphate (ATP), which is utilized as cellular energy (Ding *et al.,* 2022) . This procedure produces ethanol and carbon dioxide as by-products. Some yeast species, including facultative anaerobic, gram-negative bacteria like Saccharomyces cerevisiae and *Zymomonas mobilis*, may produce ethanol even when oxygen is present. (Maqtari *et al.,* 2019). Acetic acid bacteria, which are abundant in the air, water, sugary foods, fermented products with alcohol, and fruit and vegetable waste, help further oxidize the alcohol into acetic acid.

Numerous literary works have attempted to provide suggestions for minimizing food waste in the household. It may be processed into animal feed, composted and made into a soil fertilizer rich in nutritious organic matter, or utilized as biofuel to replace fossil fuels. However, current research has begun to shift toward a simpler, eco-friendly, and zero-waste approach of converting rubbish into eco-enzyme because such processes still demand energy and resources (Tian *et al.,* 2021). Eco-enzyme is a simple, multifunctional cleanser and disinfection.

The results showed that eco-enzyme is widely used in the home (instead of soap and mouthwash), in health , and in agriculture (as liquid organic fertilizer and insecticides) (Jabhade *et al., 2022)* . The discovery of bio-enzyme as a possible sanitizers was not included in the earlier publications, despite the fact that it may eliminate bacteria, viruses, and germs, including COVID-19. During the pandemic, eco-enzyme was able to function as a natural disinfectant by combining acetic acid with enzymes such as lipase, amylase, and protease. (Kumar *et al.,* 2017)

Natural alternatives are replacing petroleum-based toxic cleaning solutions. Fermented fruit solutions offer a natural alternative for cleaning. While many natural cleaning products can be expensive and less eco-friendly, enzyme-based detergents provide an effective option, delivering cleaning performance comparable to that of synthetic detergents (Kurniawan *et al.,* 2024). A fruit enzyme solution might be useful for eliminating phosphate, nitrogen, and ammonia from wastewater treatment. Natural cleaning products can also help slow down global warming by lowering pollution levels and developing a greener method of removing stains, oil, and grime. Consequently, a natural, eco-friendly, and biodegradable cleaning solution was created by our study.

Eco-friendly enzymatic cleaner has proven to be as successful as commercially available artificial cleaning agents without adding to pollution (Ways to Save Energy, 2008). This cleanser, which is created from

fruit waste like lime, pineapple, and lemon, has a lot of potential as a multipurpose cleaning agent. Research indicates that lime fruit has a high citric acid content, which indicates that its acidity is probably in the range of 1 to 3. Lemons are acidic because their pH ranges from 2 to 3. The pineapple's pH is between 3 to 5.2, indicating that lemons are more acidic than pineapples. It is possible for the weight percentage of the bio enzyme used for cleaning to be greater than or equal to 5%. (Lakra *et al.,* 2022).

Even if eco-enzyme is effective, its performance in an Pressurized spray should be considered. Studies have shown that eco-enzyme is suitable for sanitizing and cleaning surfaces. We need disinfectant to prevent diseases caused by bacteria that stick to surfaces like hands, phones, and other objects. Bacterial infections, such as COVID-19, can cause mild to severe flu by producing diarrhea or even attacking our digestive systems (Medina-Ramón *et al.,* 2005). Ecoenzymes can thus be utilized as disinfectants to maintain the health of our bodies. Although it may have an unpleasant odor, eco-enzyme serves as an excellent bio-waste solution for chemical-free sanitization. Through a systematic review, we explored and addressed the current knowledge gap regarding its potential as an eco-friendly air disinfectant spray (Ominski *et al.*, 2021). People are becoming increasingly concerned about the waste that surrounds us. By turning it into an eco-enzyme, this might get rid of a lot of organic waste.

Natural floor and pest cleansers, cleaning agents, personal care items, natural antiseptics, fertilizers, and substitutes for traditional detergents may all be made with these bio-enzymes. Furthermore, these enzymes help to remove noxious air pollutants and smells from vehicle exhaust. It keeps drainage pipes clean and aids in the purification of water bodies. It also helps lower greenhouse gas emissions by minimizing the organic waste sent to landfills (Thirumurugan *et al.,* 2016)

Though many studies have been carried out upon bioenzyme the present study is an attempt to document the making and uses with future perspective showing following objectives:-

1. To prepare bioenzyme from kitchen waste.
2. To compare the efficiency of different bioenzymes.
3. To prepare a natural cleansing substitute

MATERIALS AND METHODS

3.1 Materials used for making bioenzyme

In this experiment 6 types of bioenzyme was prepared which are 1) Orange 2) Soap nut 3) Ginger 4) Hibiscus 5) Tamarind 6) Neem . The materials which are helped in the preparation of above bioenzymes are

- Jagery
- Water
- Plastic container
- Peels, fruit and leaves (Mention above)

Fig.1 Fruit peels

Fig.2 water

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

Fig.3 plastic container

Fig.4 jaggery

3.2 Preparation of bioenzyme

Bio-enzymes are produced through a simple fermentation process using biodegradable kitchen waste—such as fruit and vegetable peels, flowers, or leaves combined with water. The standard mixture follows a 3:1:10 ratio, consisting of 3 parts organic waste, 1 part molasses, and 10 parts water. Three months are then allowed for this combination to ferment (Samriti *et al.*, 2019).

The bio-enzyme was made in fifteen sealed plastic vials. After adding 250 grams of jaggery to the bottle and increasing the water level to dissolve it, then used three litters, or 10 times as much water, and then added the raw material you wish to manufacture the bio-enzyme to the bottle three times. Then included 500g of raw ingredients in that water.

Total number of bioenzyme created six different kinds of bio-enzymes: orange (including the entire orange with its fruit and peel), hibiscus (leaf and flower), soap nut (15–20), ginger, tamarind (with its seed), and neem (leaf alone).

After the combination is ready, seal the plastic jar air tight and keep it for 3 months. However, during the first one month, we must release the gas that has created. After one month, we must lock the lid and wait for two more months without touching it. Three months later, the bio-enzyme-prepared strain was stored for later use in an airtight container.

To determine whether the bioenzyme is ready, there are a few visual indicators to observe:

- **Whitish Layer**: If a whitish layer forms on the surface after three months, it is a good sign that the fermentation process is complete and the bioenzyme is ready for use.
- **Brown Layer**: If a brown layer appears, it indicates that the fermentation is incomplete. In this case, an equal amount of sugar or jaggery (as used initially) should be added, and the mixture should be left to ferment for an additional month.
- **Green Layer**: If any green discoloration is observed, it means the mixture has spoiled. Unfortunately, spoiled bioenzymes cannot be salvaged and should be discarded immediately.

These bio-enzyme making process have to be done in plastic bottle because if we use glass bottles it may expand and break after the fermentation process started by producing gas inside but if we use plastic container it expand as it required because of its elastic property.

3.3 Characters of bioenzymes

a) pH of bioenzyme

To determine the pH of the bioenzymes laboratory pH meter was used , the following table shows the pH of various Bioenzymes respectively.(Vama *et al* ,2020)

b) Determination of TDS

To check the TDS of bio-enzymes a market available hand held TDS meter was used.(Vama *et al* ,2020)

- **Bioenzymes biochemical analysis**

Primary tests for the quality analysis of bio enzymes were used to assess the presence of various biochemical components. This was done because the fermented organic garbage results in the production of bio-enzymes, which show the presence of organic materials. Because of the fermentation process, the samples already included alcohol and carboxylic groups.

Identification of metabolites

Tests confirmed the presence of several bioactive compounds, including flavonoids, alkaloids, quinones, cardenolides, and saponins.

c) Alkaline reagent test

Following the addition of diluted HCL, 1 to2 drops of a 20% NaOH solution added to the 2 mL sample to see if the intense yellow hue transformed into a colorless solution.(Vama *et al*,2020)

d) Wanger's reagent

Two milliliters of the sample were mixed with three to five drops of Wanger's reagent (1.27 grams of iodine and 2 grams of KI in 100 milliliters of water), and the formation of a reddish-brown precipitate was seen.(Vama *et al* ,2020)

e) Keller test

In a test tube with 5 mL of sample that had been treated with 2 mL of glacial acetic acid, a few drops of a 5% FeCl3 solution were added. This was carefully layered with 1 mL of concentrated H2SO4, and the development of the brown ring at the buttom between the chemicals caused by the deoxy sugars in the cardenolides was seen.(Vama *et al* ,2020)

f) Ferric chloride test

The development of a deep blue hue, which indicates the absence of phenols, was seen after two milliliters of the extracts were treated with 5% ferric chloride in water.(Vama *et al*,2020)

g) Foam test

After adding two mL of the bioenzyme to six mL of water and giving it a good shake, the foam production was monitored.(Vama *et al* ,2020)

h) Test for Quinones

Concentrated HCl was added to 2 mL of the sample, and the development of a yellow precipitate was monitored.(Vama *et al* ,2020)

i)Quantification of Acetic acid

The 10 mL of sample was titrated using the titrant 1M NaOH. After applying a few drops of phenolphthalein indicator, the formation of a pink tint was seen.(Vama *et al* ,2020)

Identification of Carbohydrates

The bioenzyme samples should include carbohydrates since jaggery was added to the solution during the fermentation process. Market-available sugar was used as the reference in two tests to detect the presence of carbohydrates.

j) Molisch's test

One milliliter of the sample solution was mixed thoroughly with α-naphthol. Concentrated sulfuric acid (H_2SO_4) was then carefully added along the sides of the test tube, resulting in the formation of purple rings at the interface between the two layers.(Lakra *et al* ,2022)

k) Benedicts test

This test aimed to detect the presence of reducing sugars in the samples. Two milliliters of Benedict's reagent a commercially available solution containing sodium citrate, sodium carbonate, and copper(II) sulfate pentahydrate were added to the samples. The mixture was then heated in a water bath for three to five minutes. A color change in the precipitate, from clear blue to greenish-blue and eventually to yellow-orange, indicated the presence of reducing sugars. (Lakra *et al* ,2022)

Identification of Proteins

Bio-enzyme solutions are made from yeast, which produces the enzymes needed for growth metabolic activities before dying from malnutrition and leaving the enzymes in the solution. The bio-enzyme solution contains proteins as well since enzymes are complex proteinsTwo tests were conducted to confirm the presence of proteins in the reference sample, which was commercially available Amul Full Fat Milk.

l) Ninhydrin test

One milliliter of Ninhydrin solution was mixed with one milliliter of sample, agitated, and placed in a water bath for five to ten minutes until it boiled. After then, the color changed from dark purple to light purple. (Lakra *et al* ,2022)

m) Xanthoproteic test with HNO3

The samples were heated after a few drops of strong nitric acid were added. A yellow precipitate was seen to form. (Lakra *et al* ,2022)

n) Biuret test

Two milliliters of diluted sodium hydroxide were mixed with a few drops of copper sulphate solution, and then the sample's diluted solution was added. The color of each sample ranged from blue to greenish blue. (Lakra *et al* ,2022

RESULT AND DISCUSSION

In the present study six types of bio enzyme were prepared, with most preparations aged between five to six months. During the initial stage of fermentation, the mixture primarily consists of water and does not emit any significant odour. Over time, particularly after three months of fermentation, the colour of the liquid changes from brown to a golden yellow. This golden liquid is what we refer to as the **bio enzyme**. For more precise to know if our enzyme prepared or not we have to observe it have that white layered or not. If it is present then bio enzyme is completely prepared.

Fig.5 (Preparatory phase of citrus bio-enzyme)

Fig.6 (Preparatory phase of ginger bio-enzymes

Fig.7 Orange bioenzyme after fermentation

Fig.8 Ginger bio enzyme after fermentation

Fig.9 Tamarind bio enzyme after fermentation

Fig.10 Soap nut bioenzyme after fermentation

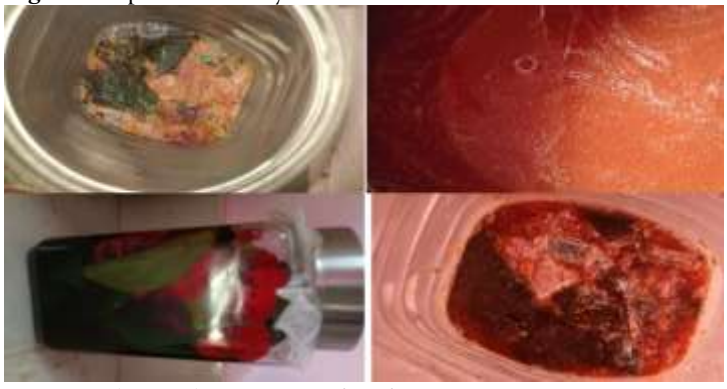

Fig.11 Hibiscus bioenzyme after fermentation

Fig.12 Neem bioenzyme after fermentation

Different bio enzymes exhibit varying pH levels, all of which are acidic in nature. Orange bio enzyme with acidic nature having pH 3.05 . In the study Synthesis, Physiochemical Analysis and Applications of Bio-Enzymes Based on Fruit and Vegetable Peels Lakra *et al* 2022 concluded the pH of the orange bio enzyme is 5.31 which is also showing the acidic nature of that bioenzyme(table.1).

The bio enzyme of neem with a pH3.13 .Upon the study of physicochemical property of bioenzymes produced from organic household waste and their application in daily life Kurniawan *et al* 2024 concluded that pH of Neem bio enzyme having 5 to 6 pH.

Hibiscus having a pH 3.20 is also acidic in nature. From the study *Hibiscus rosasinensis* A newer analytical detection bio-enzyme method Kapadi *et al* 2022 concluded that pH of Hibiscus bio enzyme having 3 to 5 pH i.e. naturally acidic.

Tamarind bio enzyme an acidic bio enzyme is having pH value 2.73. In a study *Phytochemical Studies of Seeds of Tamarindus indica* Kumar *et al* 2014 concluded Tamarind bio enzyme as acidic bio enzyme with pH value between 2 to 3 because it is the most acidic in nature.

The bio enzyme from Soap nut with a pH value 3.09 is acidic bio enzyme. It is concluded by Ariharan *et al* 2015 in the study physico chemical studies on soap nut (*sapindus trifoliatus*) oil for source as biodiesel that Soap nut bio enzyme is acidic with a pH between 3 to 6.

Ginger is also acidic bio enzyme having pH 3.35 . in the study Nafi *et al* 2013 documented the pH from 3 to 5 value in Properties of proteolytic enzyme from ginger (*Zingiber officinale Roscoe*) which shows its acidic nature .

TDS stands for total dissolved solute. The existence of solute particles in the enzyme is indicated by performing TDS experiment(table.2). Soap nut bioenzyme having TDS of 236pm.In the study by Ariharan *et al* 2015 in physico chemical studies on soap nut (*sapindus trifoliatus*) oil for source as biodiesel that the TDS between 150 to 500 ppm for surface and laundry cleaning .

The Orange bioenzyme having TDS of 136ppm , in paper, production, extraction and uses of eco-enzyme using citrus fruit waste: wealth from waste study by Vama *et al* 2020 that the TDS ranges between 100 to 300ppm for gardening and dishwashing.

Tamarind made bioenzyme have TDS of 153ppm, in the study by Kumar *et.al* 2014 in paper *Phytochemical Studies of Seeds of Tamarindus indica* coated that the range for TDS is 150 to 700ppm used for dish washing ,gardening, laundry and personal uses.

In Ginger bioenzyme the TDS meter shows 139ppm, in proteolytic enzyme from ginger (*Zingiber officinale Roscoe*) documented by Nafi *et al* 2013 that 100 to 300ppm can used for gardening, lundry, and personal care.

Hibiscus infused bioenzyme showing TDS of 148ppm, documented by Kapadi *et al* 2022 in *Hibiscus rosasinensis*: A newer analytical detection bio-enzyme method that 100 to 500ppm used for gardening and dish washing.

Neem bioenzyme having TDS of 149ppm shown in TDS meter ,in physicochemical property of bioenzymes produced from organic household waste and their application in daily life documented by

Kurniawan *et al* 2024 concluded that TDS having 100 to 200ppm could use for gardening and personal care .
In Alkaline reagent test it shows some result which shows dark before in colour but after experiment it fadede a bit and more transparent in colour(fig.14) , which indicate the presence of protein in bio enzyme . Lakra *et al* 2022 also concluded the presence of protein in bio enzymes in the study Physio-Chemical Analysis and Applications of Bio-Enzymes Based on Fruit and Vegetable Peels .
In Killer test a few sample shows the brown ring which form after the test completed, the bioenzyme which shows brown rings(fig.15) are soap nut and tamarind in the bioenzymes. This test shows the availability of deoxy sugar documented by Vama *et al* 2020 in extraction and uses of eco-enzyme using citrus fruit waste: wealth from waste.
Ferric chloride test does not shows any blue colours in our bioenzymes hence it shows presence of phenol. In Physio-Chemical Analysis and Applications of Bio-Enzymes Based on Fruit and Vegetable Peels documented by Lakra *et al* 2022 that having blue colour is the indication of absence of phenol.
Foam test (fig.19) conducted to know the foaming property of every bioenzymes. From the study we get to know that soap nut bioenzyme having highest amount of foaming property(table.3) of about 6.5cm.Documented by Ariharan *et al* 2015 in physico chemical studies on soap nut (*sapindus trifoliatus*) oil for source as biodiesel.
Bioenzyme made of Hibiscus having 1cm of foam study in *Hibiscus rosasinensis* A new analytical detection bio-enzyme method by Kapadi *et al*,2022 ,used for cleaning hair in ancient time .Tamarind enzyme having 1.5cm of foam. Kumar *et al* 2014 has documented in Phytochemical Studies of Seeds of *Tamarindus indica* , that it can also used for cleaning utensils.
Ginger, Neem, and Orange having foam production as 0.5cm, 0.7cm, 0.5cm respectively. Studied in production, extraction and uses of eco-enzyme using citrus fruit waste: wealth from waste study by Vama *et al* 2020 which are very less for any cleaning purpose so we use it as stain removing object.
In quantification of acetic acid it shows pink colour after titration. It show about how acetic a solution is after how much amount of the chemical added(table.4) to make it basic which shows by turning the liquid to pink colour(fig.16) documented by Lakra *et al* 2022 study in Synthesis, Physiochemical Analysis and Applications of Bio-Enzymes Based on Fruit and Vegetable Peels.
Nynhydrin test was performed to know about presence and absence of protein in bioenzyme . If protein present it changes colour and shows light to dark purple colour in the solution. In the present study it shows purple colour in orange, soap nut, ginger and neem bioenzyme. Which shows presence of protein in all 4 bioenzyme(fig.17). Study in Synthesis, Physiochemical Analysis and Applications of Bio-Enzymes Based on Fruit and Vegetable Peels documented by Lakra *et al* 2022 .
In biuret test a colour change from blue to greenish blue colour in all samples(fig.18) except soap nut bioenzyme was observed. It is inferred with the colour change that the above bioenzymes are acidic in nature. Lakra *et al* 2022 study in Synthesis, Physiochemical Analysis and Applications of Bio-Enzymes Based on Fruit and Vegetable Peels. That the enzymes which are tested using this method is shows acidic in the liquid.
The bioenzymes which were prepared used in plantlet formation from seeds like chickpea which shows growth and good amount of plantlets formation the size of the petiole is 3.5 cm approximately (fig.24). Documented by Win *et al* 2021 in Green synthesis and characterization of Fe3O4 nanoparticles using Chlorella-K01 extract for potential enhancement of plant growth stimulating and antifungal activity, which shows the growth of seeds by using bioenzyme.
The bioenzymes are used in making natural dishwash cleaner by mixing wood ash, neem, orange, tamarind and soap nut bioenzyme (fig.20) and then sundried after keeping in soap mould. Documented by Kora *et al* 2024 in Renewable, natural, traditional dish wash cleaning materials used in India, an overview detected that the wood ash is a natural cleaner used traditionally from ancient time for cleaning dishes. Infusion of bioenzymes which are acidic in nature will help in cleaning oil stains in dishes(fig.21) better than the market available dishwar bars.

Sl no	Name of bioenzymes	pH value
1	Orange	3.05
2	Neem	3.13

3	Soap nut	3.09
4	Hibiscus	3.20
5	Ginger	3.35
6	Tamarind	2.73

Table:1 Showing pH value of bioenzyme

Sl no	Name of bioenzymes	TDS values
1	Orange	136 ppm
2	Neem	149 ppm
3	Soap nut	236 ppm
4	Hibiscus	148 ppm
5	Ginger	139 ppm
6	Tamarind	153 ppm

Table:2 Showing TDS value of bioenzyme

Sl no.	**Bioenzyme name**	**Foam (in CM)**
1	Orange	0.5cm
2	Neem	0.7cm
3	Soap nut	6.5cm
4	Hibiscus	1cm
5	Ginger	0.5cm
6	Tamarind	1.5cm

Table:3 Showing Foaming quantity of bioenzyme

Sl no.	Name of bioenzymes	Initial amount	Required amount
1	Orange	50ml	1.2ml
2	Neem	50ml	1.4ml
3	Soap nut	50ml	3.4ml
4	Hibiscus	50ml	1.4ml
5	Ginger	50ml	1ml
6	Tamarind	50ml	1.2ml

Table: 4 Acetic acid test

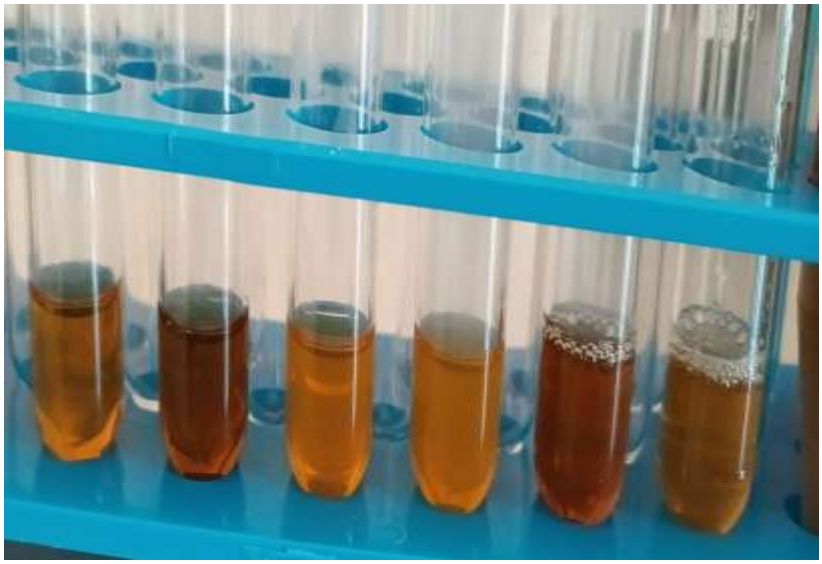

Fig.13 Before Alkaline reagent test

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

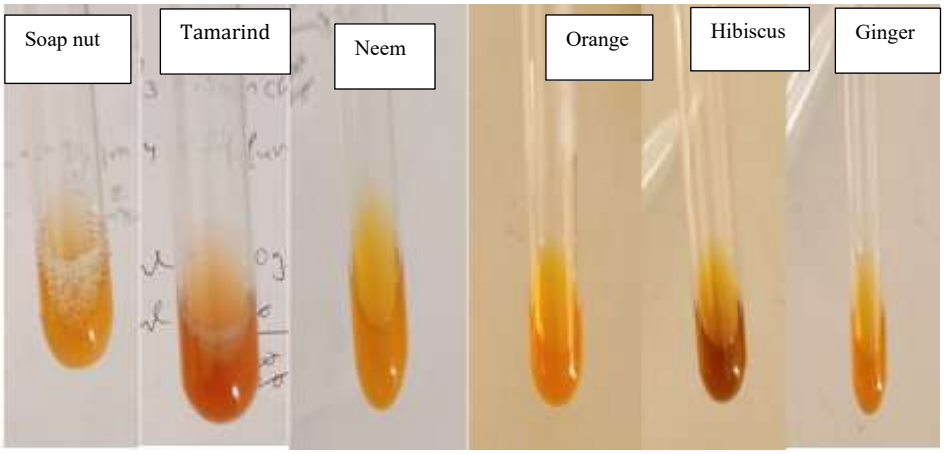

Fig.14 After Alkaline reagent test

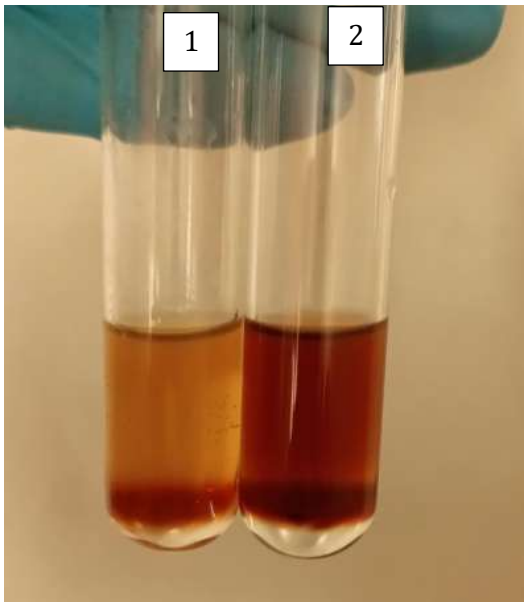

Fig.15 Killer test(1-Soap nut 2-Tamarind)

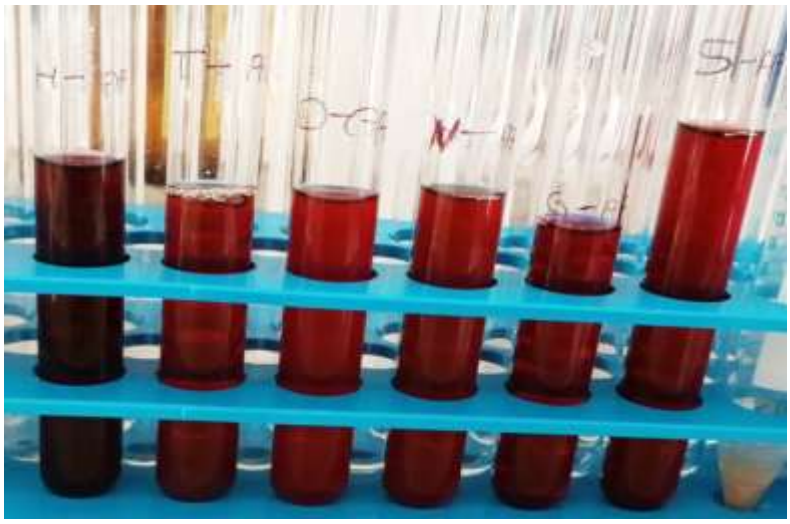

Fig.16 Acetic acid test

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

Fig.17 Ninhydrin test

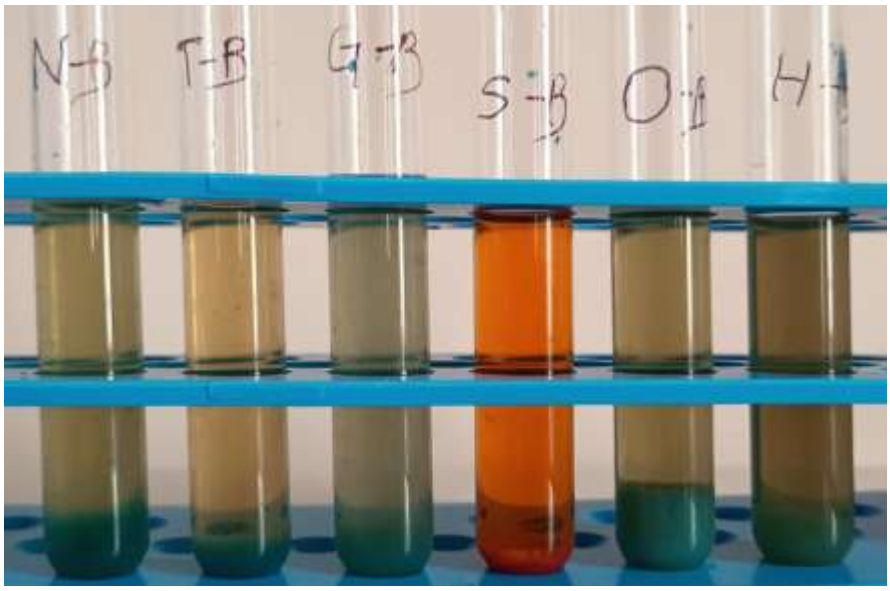

Fig.18 Biuret test

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

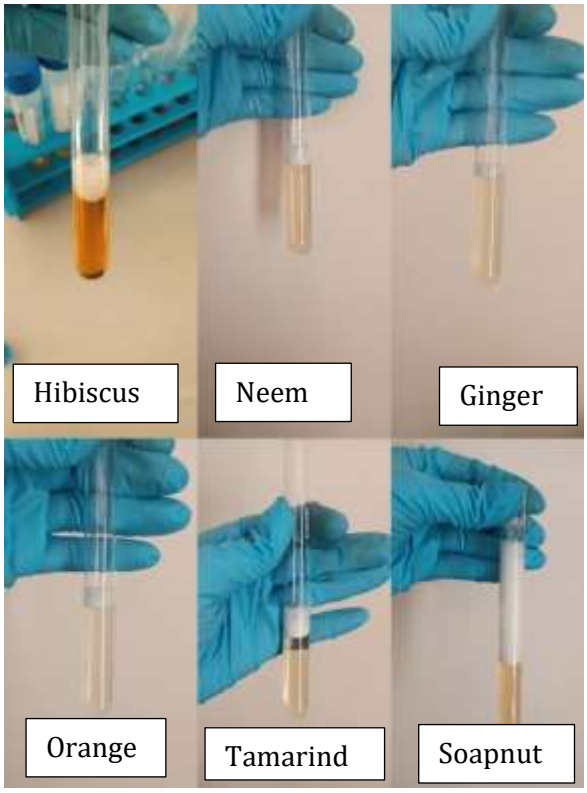

Fig.19 Foam Test

Fig.20 Used Orange of Orange Bioenzyme

Fig.21 Dish wash soap with and with out Multani mitti

Fig.22 showing result of bioenzyme infused with wood ash

Fig .23 Before and after result of the soap containing bioenzymes, Multani mitti and wood ash

Fig.24 Showing result of soap nut bioenzyme

Fig.25 Bioenzyme used for plantlets growth

CONCLUSION

The present study documented the preparation of bioenzyme which are prepared from kitchen waste like fruit peals, flowers, leaves etc. For preparation of these bioenzyme a specific amount of water and jaggery mixed with kitchen waste and fermented it. The amount of bioenzyme contains one part of jaggery, three parts of kitchen waste and ten parts of water in a fifteen times big container and fermented for minimum three months. After three months, a perfectly fermented golden liquid was obtained and mostly every enzyme have a sour smell because of its acidic nature.

However the bioenzyme 6 to 7 months old and the enzyme has a special character if we store in proper way it increases its productivity as time increases because it ferment further after three months also. As we show in the results it helps in gardening because it packed with proteins and other micronutrients, these have antimicrobial property. The bioenzymes are used for cleaning which were made in the form of soap using wood ash, multani mitti, and other four types of bioenzymes. It is concluded from this study that this bioenzyme based products may be a good alternative for chemical products and safer for environment as well as our health, and more interesting fact is that it can be easily made with the products available at home and more effective than market made chemical dish wash bar.

REFERENCE

- Akusu MO, Mohammed BA, Sule S. Performance Evaluation of Biogas Yield from Organic Fraction of Municipal Solid Waste using Plastic Digester. Indian Journal of Engineering, 2019, 16, 93-101
- Ashraf M.A., Maah M.J., Yusoff I, Soil contamination, risk assessment and remediation, In: Hernandez-Soriano MC (ed) Environmental risk assessment of soil contamination. INTECH, Rijeka, 3-56 (2014).
- Dhavale, V., & Shimpi, T. (2020). Bio Enzymes from Organic Waste. Journal of Emerging Technologies and Innovative Research. Retrieved November 8, 2022, from https://www.jetir.org/papers/JETIR2008330.pdf
- Ding, M., Zhang, Y., Li, J., & Pu, K. (2022). Bioenzyme-based nanomedicines for enhanced cancer therapy. *Nano convergence*, *9*(1), 7.
- Forbes, H. (2021). Food waste index report 2021.
- Jabhade, S. B., & Bhosale, A. C. (2022). A Study of Garbage Enzyme Solution. IJRASET. Retrieved November 8, 2022, from https://www.ijraset.com/research-paper/garbage-enzymesolution
- Krogsrud N.E. and Larsen A.I., Airborne irritant contact dermatitis from benzalkonium chloride, Contact Dermatitis.,36(2), 112 (1997).
- Kumar S, Smith SR, Fowler G, Velis C, Kumar, SJ, Arya S, Kumar R, Cheeseman C., " Challenges and opportunities associated with waste management in India," in Royal Society open science, 2017, pp. 60764.
- Kurniawan, A., Mustikasari, D., Kurniawan, A., Muntoro, M., Setiadi, J., & Kholishah, N. A. (2024). The Preliminary Study about Physico-Chemical Property of Bio-Enzyme Produced from Orange Fruits Waste Treated with Different Concentrations of Probiotic. *Jurnal Ilmu Lingkungan*, *22*(4), 861-867.
- Lakra, P., Saini, S. K., & Saini, A. (2022). Synthesis, Physio-Chemical Analysis and Applications of Bio-Enzymes Based on Fruit and Vegetable Peels. *Journal of Emerging Technologies and Innovative Research*, *9*(9), a670-a680.
- Medina-Ramón M, Zock JP, Kogevinas M, Sunyer J, Torralba Y, Borrell A, Burgos F, Antó JM., " Asthma, chronic bronchitis, and exposure to irritant agents in occupational domestic cleaning: a nested case-control study," in Occupational & environmental medicine, 2005, pp. 598-606.

- Monney, I., Tiimub, B.M., and Bagah, H.C. 2013. Characteristics and management of household solid waste in urban areas in Ghana: the case of WA. Civil and Environmental Research, 3 (9): 10-22.
- N. Muliarta and I. K. Darmawan, "Processing Household Organic Waste into Eco-Enzyme as an Effort to Realize Zero Waste," Agriwar Journal, vol. 1, no. 1, pp. 6–11, 2021.
- Oiso N., Fukai K. and Ishii M, Irritant contact dermatitis from benzalkonium chloride in shampoo, Contact Dermatitis., 52(1),54 (2005).
- Ominski et al., "Utilization of by-products and food waste in livestock production systems: a Canadian perspective," Animal Frontiers, vol. 11, no. 2, pp. 55–63, May 2021, doi: 10.1093/af/vfab004.
- Parul Lakra , Dr. Sunil Kumar Saini and Dr. Ankita Saini*Synthesis, Physio-Chemical Analysis and Applications of Bio-Enzymes Based on Fruit and Vegetable Peels. Journal of Emerging Technologies and Innovative Research (JETIR) 2022.(9);670-681.
- Patolia, A. (2021, April 28). Review Article. International Journal of Recent Scientific Research. Retrieved November 8, 2022, from http://www.recentscientific.com/sites/default/files/17583-A2021.pdf
- Prerna S Kapadi, Vedita Hegde Desai et,all.,Estimation of Antimicrobial Efficacy of Bio-enzyme Extracts,Indian Journal of Applied Microbiology 2022,vol.24;21-31.
- Samriti, Sajal Sarabhai and Arti Arya. Garbage enzyme: A study on compositional analysis of kitchen waste ferments. The Pharma Innovation International Journal. 2019:8: 1193-1197.
- Sethi S.K., Soni K., Dhingra N. and Batra G.B., Bringing Lab to Our Home: Bio-Enzyme and its Multi-utility in Everyday Life, International Research Journal of Engineering and Technology (IRJET)., 8(3), 1461-1476 (2021).
- Vama, L. A. P. S. I. A., & Cherekar, M. N. (2020). Production, extraction and uses of eco-enzyme using citrus fruit waste: wealth from waste. *Asian Jr. of Microbiol. Biotech. Env. Sc*, *22*(2), 346-351.
- Yadav H, Singh VP. An approach for risk index assessment due to direct contact to the municipal solid Waste dumps. Discovery, 2021, 57(302), 135-144