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Synthesis, Physio-Chemical Analysis and Applications of Bio-Enzymes Based on Fruit and Vegetable Peels

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ABSTRACT

Consumption of harsh chemicals on daily basis and wastage of food are two huge problems in contemporary era which are needed to be addressed compulsory. Preparation of bio-enzymes can be helpful in curbing these problems efficiently as they are non-toxic, non-hazardous, non-corrosive, eco-friendly and completely natural liquids. They are easily synthesized by anaerobic fermentation of jaggery, fruit/vegetable/flower or plant waste in general and water with addition of micro-organisms in a plastic container. For checking their efficiency and usability in different sectors, six different samples were taken from different fruit and vegetable peels. The liquid part after filtration was used for characterization of bio-enzymes and solid residue was used for making of bio-compost. Different qualitative bio-chemical tests were carried out for detecting the presence of proteins, carbohydrates, metabolites and lipids in bio-enzymes. The antimicrobial properties of bio-enzymes were also checked to test their effectiveness. Bio-enzymes were also put in for different applications like soil stabilization, contaminated water treatment and usage at household cleaning. The bio-compost that was prepared from solid residue was tested to check the usability as a natural manure. All the six samples of bio-enzymes (1-6) and the bio-compost gave great results and showed that bio-enzymes are applicable in various sectors ranging from domestic household to agriculture. Their effectiveness showed that they have the potential to terminate the use of harsh chemicals. Today, Bio-enzymes are of great importance as they help in reduction of some waste and help in turning it into a useful product which is economical feasible to the people and the end product is sustainable to the nature which enhances the quality of life as a whole.

KEYWORDS- Bio-enzyme, Fermentation, bio-chemical test, anti- microbial properties, bio-compost, soil stabilisation, contaminated water treatment.

INTRODUCTION

Food Waste Index Report 2021 that was published by the United Nations Environment Programme shows that almost 50 kg of food is thrown away per person every year in Indian homes [1]. Almost 7.5 tonnes of food in India is wasted per day, it is thrown in the bins, sewage and all this adds to the amount of garbage production. There is a lack of consciousness around food waste. Food waste is usually dumped in the landfills, this increases the total amount of garbage stored in the landfills and also contributes to the increment in all types of pollution and greenhouse gas emission [2,3]. Also, there is a great usage of chemical fertilisers and pesticides in crop production because of this soil loses its binding capacity and its character deteriorates. Chemical fertilisers are effective but in the long run they are harmful this adds to soil acidification and soil crust, this affects plant growth and the pH of the soil is altered which leads to growing of pests, and even leading to the release of greenhouse gases [4].

Also, Benzalkonium chloride (BAC) is an essential component of disinfectants available in the market. Many studies have observed the harmful effects of BAC on the human body, such as skin irritation and many allergic effects [5,6,7]. These harsh chemicals like BACs and more human waste goes to the sewage and enters the different waterbodies. This leads to the pollution and deteriorated water quality which disturbs the ecosystem of aquatic life [5,8].

Bio enzymes are helpful in solving these problems as they are completely natural and help in minimizing the waste because its key ingredients are fruit and vegetable peels which are considered as waste and thrown in the garbage. They are non-hazardous, non-corrosive, non-toxic, eco-friendly and completely natural liquids. Bio-enzymes are simply

made from anaerobic fermentation of fruit and vegetable peels in the presence of water, jaggery and yeast [9,10]. They are a mixture of juvenile hormones and enzymes synthesised by the micro-organisms.

Bio-enzyme was first produced by Dr. Rosukon Poompanvong of Thailand [10]. Bio-enzymes are also called as Eco-enzyme, Garbage enzyme, Terrazyme, Fruit enzyme, Flower enzyme etc., [11,12]. Bio-enzymes are a mixture of enzymes as they catalyse large amounts of reactions within a short span of time [10]. Chemically, bio-enzymes are a mixture of proteins, carbohydrates, metabolites etc. which are produced naturally by bacteria or yeast used for preparation of bio-enzyme. The combination of jaggery/brown sugar/molasses, fruits and vegetable peels and water is involved in the fermentation of bio-enzymes [13].

It has been seen that bio enzymes have a varied usability ranging from domestic household to agriculture. Figure 1 depicts various applications of bio-enzyme. Usage of Bio enzymes is multifarious as it is completely natural and helps in reduction of waste. It is the best alternative to chemical based harsh, aggressive and non-biodegradable cleansing products which are accessible in the market. The cherry on top is it does not require fermenter or particular equipments for their preparation and anybody can prepare it at their homes and kitchen gardens [10].

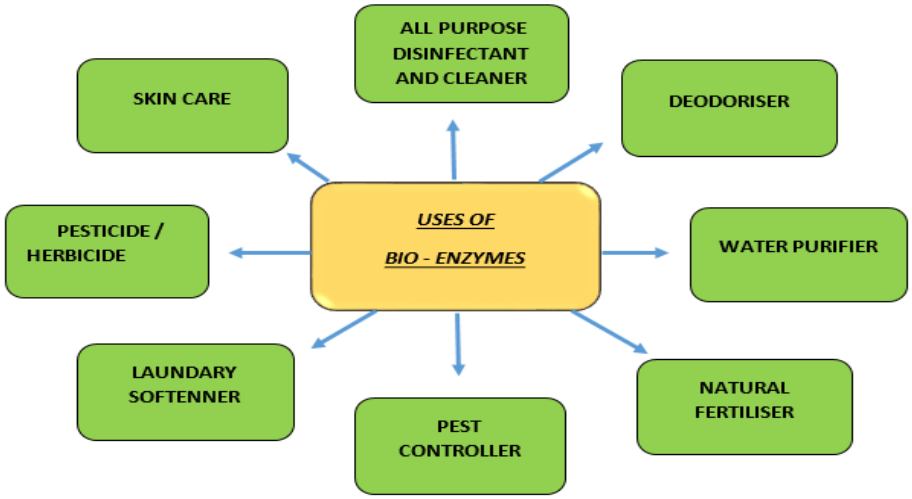

Figure 1: Uses of bio-enzymes

MATERIAL AND METHODOLOGY

Materials- In this study, six types of bio-enzymes were prepared for the analysis. The bio-enzymes are fermented from peels of banana, **(1)** beetroot, **(2)** orange, **(3)** pineapple, **(4)** raddish **(5)** and turnip **(6).** The materials used for preparation of enzymes are as follows-

- Jaggery (used at homes)
- Water (normal drinking water)
- Plastic container with a screw cap
- Fruit peels and vegetable peels (mentioned above)
- Yeast (available at local market)

Methodology- The present analysis and characterisation can be divided into five main steps-

1. Preparation of Bio- Enzyme
2. Filtration of Bio-enzymes
3. Characterisation of Bio-Enzymes
4.Qualitative analysis for Biochemical analysis
5. Test for microbial properties

1. Preparation of Bio-Enzymes

The peels of consumed fruits and vegetables at home were collected for the analysis. The peels were further divided into smaller pieces to increase the surface area of the reaction. Jaggery (10g), peels (30g) and water (100 mL) were taken in the ratio of 1:3:10 into an air tight plastic container and mixed thoroughly. (Figure 2) Then a pinch of yeast was added. The yeast used is baker's yeast or *Saccharomyces cerevisiae*. This procedure was repeated for all the six samples. Gases will be produced in this process of fermentation. So, we choose plastic containers because they can expand otherwise glass bottles would have exploded. Then the containers were kept undisturbed at a safer place for 1 month for the fermentation reaction to proceed. Gases are required to be released at different time intervals from the containers. To the gases to be released, the lid of the plastic containers was opened once in a day for a minute and the lid was closed again. After some days the gases will considerably decrease and after one month a coloured liquid will be produced along with the small particles and some solid residue [9, 10]. The liquid part is the raw bio-enzymes and it is needed to be separated out by filtration as shown in figure 4.

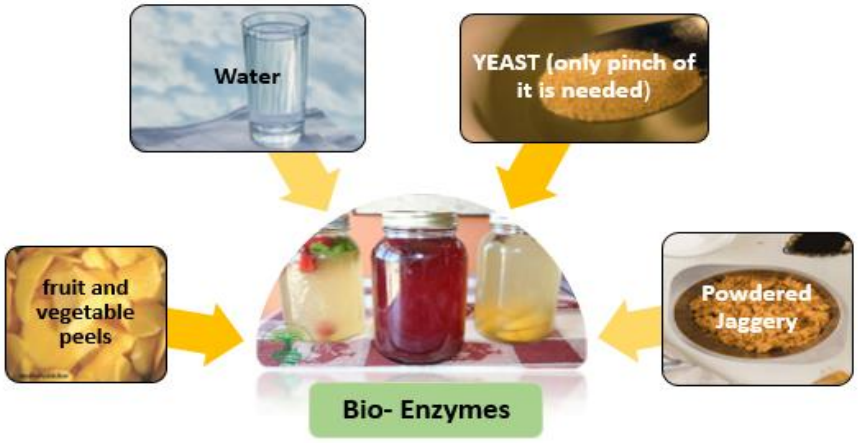

Figure 2 Procedure of preparation of Bio-Enzymes

Figure 3 Samples of different Bio-Enzymes (**1-6**) prepared after 1 month of fermentation.

2.Filtration of Bio-enzymes

Figure 4 Filtration of bio-enzymes (**1-6**)

Filtration of Bio-Enzymes was done after 1.5 months to obtain the raw liquid sample, see Figure 5 Filtered bio-enzyme solution was stored separately in the bottle. And the solid residue left at the last is collected and dried for the preparation of bio-compost as shown in figure 11. The characterisation of the bio-enzymes was done with the liquid part collected.

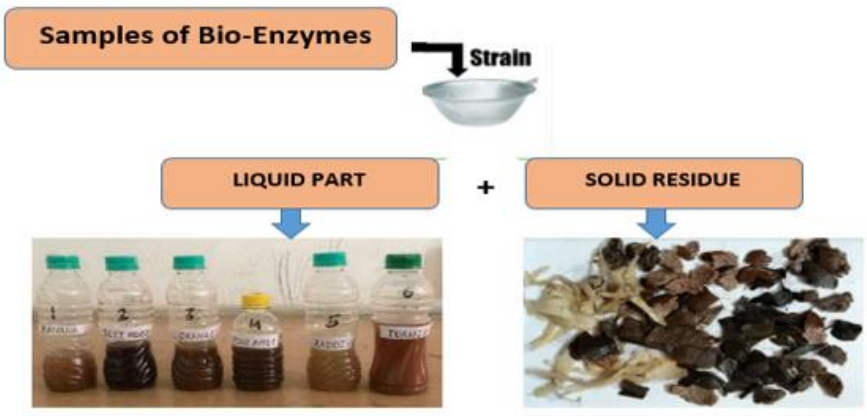

Figure 5 Products obtained after filtration

3. Characterisation of Bio-Enzymes

The filtered liquid part is used for the characterisation of bio-enzymes. Before finding usability of bio-enzymes it is necessary to find out the physical characteristics of bio-enzymes (**1-6**). The parameters like pH, density viscosity, and TDS (total dissolved salts) were found out. The physical characteristics of the different bio-enzymes extracted are given in the table 1 below.

Table- 1 Physical Characteristics of Bio-enzymes prepared

Sample	Source	Colour	Odour	Yield (mL)
1	Banana peels	Light yellow	Alcoholic	98 ml
2	Beetroot peels	Wine red	Alcoholic	100 ml
3	Orange peels	Orange	Alcoholic	99 ml
4	Pineapple peels	Bright yellow	Alcoholic	98 ml
5	Raddish peels	Yellowish white	Alcoholic	100 ml
6	Turnip peels	Dark Orange	Alcoholic	100 ml

The different parameters used for performing characterisation of Bio-enzymes are given below and displayed in table 2.

3.1 The pH of Bio-enzymes

In order to determine the pH of the bio-enzymes extracted, laboratory pH meter (Labtronics: MODEL LT10) was used. The calibration of the pH meter was done by using buffer solution of pH =7 and pH = 4, already prepared by using Fischer Scientific qualigens tablets. The following table 2 shows the pH of different samples of Bio-enzymes extracted from different samples.

Table 2 Parameters of Bio-enzymes (**1-6**) synthesised

Sample	Source	pH	Density (g/mL)	Viscosity (centipoise)	TDS (ppm)
1	Banana	5.25	1.004	1.0210	2840
2	Beet root	5.43	1.001	0.7581	3580
3	Orange	5.31	0.999	0.7076	1980
4	Pineapple	5.28	1.000	1.0413	2150
5	Raddish	5.15	1.003	0.7743	3020
6	Turnip	5.01	1.035	1.3699	1980

3.2. Determination of Density of Bio-enzymes

To find out the density of the samples, specific gravity bottles were used and were filled with the samples one by one and weighed on analytical balance. Density of water at 40˚Celsius was 0.992 g/mL which was used as reference for determination of density of six samples.

3.3 Viscosity of the bio-enzymes samples

To find out this interfacial property of samples of different bio-enzymes Ostwald viscometer was used to find out the viscosity. The liquid was sucked till the two point and the distance travelled by the liquid in time (sec) was noted. Using reference values for water, viscosity of bio-enzymes was calculated shown in table 2.

Temperature during observation – 40˚Celsius

Viscosity of water at 40˚Celsius- 0.6526 centipoise

4. Determination of TDS (Total dissolved Salts)

To check the TDS of bio-enzymes (**1-6**), contaminated water sample collected and for various applications, a market available hand held TDS meter (Ionix) with working range of 0-9990 ppm was used.

4. Bio-Chemical Analysis of bio-enzymes

Preliminary tests for qualitative analysis of bio-enzymes were carried out in order to test the presence of different biochemical constituents. This was done because bio-enzymes are prepared by fermentation of fruit and vegetable peels, which shows that there should be presence of organic compounds. Alcohol and carboxylic group were already present in the samples because that was a fermentation process [13]. All the samples (**1-6**) were tested for carbohydrates, metabolites, lipids, proteins with chemicals and solutions available in laboratory [13,14].

4.1 Identification of metabolites (Phytochemicals)

Tests (**T1-T6**) were carried out to confirm the presence of flavonoids, phenols/ tannins, alkaloids, cardenolides, quinones and saponins, respectively.

Test 1 (T1)- For Flavonoids – Alkaline reagent test

The 2 ml of sample was taken in a test tube and few drops of dilute 10% NaOH solution were added to it. Then dilute HCl was added to the solution and yellow colour formed and after addition of base it was changed to colourless.

Test 2 (T2)-For Phenols (Tannins) – Ferric chloride test

Dilute 5% ferric chloride ($FeCl_3$) was added to the 2 ml of the sample and the deep blue colour was noted.

Test- 3 (T3)- For Cardenolides- Keller test

Few drops of acetic acid were added to 2 ml of sample in a test tube and few drops of dilute 5% $FeCl_3$ solution was added to it. Then conc. H_2SO_4 was added carefully to the walls of the test tube and formation of brown rings was done.

Test-4 (T4)- For Quinones- Acid test

Concentrated HCl was added to 2ml of sample till yellow precipitate was seen.

Test -5 (T5)- For Alkaloids- Wanger's reagent

3-5 drops of Wanger's reagent (1.27 g of iodine and 2g of KI in 100 ml of distilled water) was added to the 2ml samples in test tubes and brown/reddish precipitate was obtained.

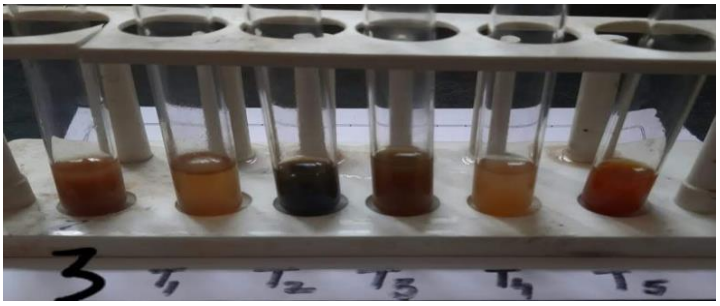

Figure 6 Phytochemicals qualitative analysis (**T1-T5**) of bio-enzymes **3.**

Test-6 (T6)-For Saponins – Foam test (Figure7)

2 ml of sample was added to 6 ml of water and was shaken vigorously. Formation of foam was seen which did not get dissolved after addition of dilute HCl.

Figure 7 Foam test of bio-enzymes for presence of saponins.

4.2 Identification of Carbohydrates

In the fermentation process jaggery was added to the solution, so carbohydrates should be present in the samples of bio-enzymes. To test the presence of carbohydrates two tests were performed and the reference was taken as market available sugar.

- **Molisch's test**- 1 ml of sample solution was added to α-naphthol and mixed well. Then conc. H_2SO_4 was added along the sides of the test tube to form the purple rings at the interface of the two layers.
- **Benedict's test** – This test was performed to check the presence of reducing sugars in the samples. Commercially available 2 ml Benedict's reagent (mixture of sodium citrate, sodium carbonate, and the pentahydrate of copper(II) sulphate) was added to the samples and was added to a water bath for 3-5 minutes. Change of clear blue to greenish blue or yellow-orange colour precipitate was seen.

4.3 Identification of Proteins

Yeast is used for making of solution of bio-enzymes, it produces enzymes to complete metabolic activities for growth and later on, it dies because of lack of nutrients but enzymes are left behind in the solution. As enzymes are complex proteins so the solution of bio- enzymes also contains proteins. Two tests were performed to confirm the presence of proteins and the reference was taken as Amul Full fat milk from the market.

- **Ninhydrin test-** 1 ml of Ninhydrin solution was added to 1 ml of sample and was shaken and was kept on the water bath for 5-10 minutes till boiling. Then the dark purple to light purple colour was observed in all the samples taken as shown in figure 8.

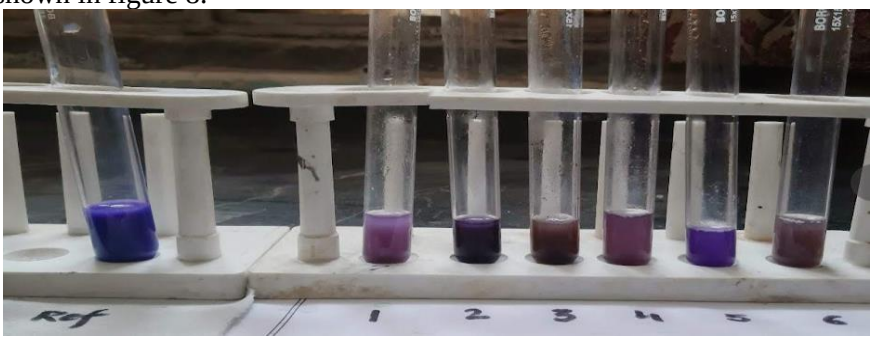

Figure 8- Ninhydrin test for all samples

- **Xanthoproteic test with HNO_3-** A few drops of concentrated nitric acid was added to the samples and were heated. Formation of yellow precipitate was seen.
- **Biuret test-** A few drops of copper sulphate solution was added to 2 ml of dilute sodium hydroxide and then there were mixed with the diluted solution of the sample. Blue to Greenish blue colour was observed in all the samples.

4.4 Identification of Lipids

The 2 ml of sample was taken in a test tube and 2 ml ethanol and water was added to the samples. Formation of cloudy solution was seen in all the samples.

5. Test for microbial properties

(a) **Anti-fungal properties** - To check the anti-fungal properties of bio-enzymes that were extracted they were applied to different bread pieces and tap water was used as a reference solution. Water was applied to the bread to do the differentiation and analyse the better anti- fungal agent among 6 samples. All the samples were packed in the plastic bag to intact the moisture content in the breads pieces till 7 days. And on the eighth day the bread pieces were taken out of the plastic bags and checked for fungal growth [12].

(b) **Anti-Bacterial properties-** To check the anti-bacterial properties of bio-enzymes, agar disc diffusion method was performed for both Gram positive and Gram-negative bacteria [11,13,14,15,16].

Preparation of Discs- Whatmann paper number-1 was taken and cut into circular shapes and were sterilised using autoclave. Then they were impregnated with 20 microlitres of the bio-enzymes extract. Three solutions were prepared for impregnation one was concentrated bio-enzyme sample (the master solution), other two were dilutions- 10^{-1} and 10^{-2} concentrations. And were allowed to dry under a laminar flow cabinet for 30 minutes.

Disc Diffusion Test- Petri plates of Mueller-Hinton agar were prepared. The bacteria used was *E. coli* and *staph-aureus.* They were inoculated one-day prior in an incubator at 37^0 C in broth. Then the bacteria were spread on the agar plates using a sterilised swab. Then the impregnated discs were placed on the agar plates containing bacteria using sterilised forceps. All the petri plates were sealed and were incubated for 24 hours. The results were recorded after incoming zone of inhibition around the discs.

Applications of bio-enzymes

(1) Preparation of bio-compost - The solid residue that was left behind after filtration of the bio-enzymes can be used for organic compost making after drying [17]. The solid pulp was collected and air dried for 15 days and then was grinded in a mixer. The weight of the raw samples was 60 g and the yield of the compost is 12.25 g.

To check the properties of bio-compost following procedure were carried out-

Variation in pH and TDS- This was done in order to check the activity of the bio-compost made from solid residue of bio-enzymes**.** To check its efficiency two solutions were prepared-

- **Solution 1**- The compost was mixed in tap water and its pH was determined using laboratory pHmeter. The solution was kept for 5 days and its pH was calculated immediately after 5 min, on day1, day2 and day 5.
  pH of tap water in which bio-compost mixed was 7.21.

Table 3- Variation of pH of solution 1 (compost + tap water)

Solution 1	**After 5 min**	**Day 1**	**Day 2**	**Day 5**
pH	5.00	6.25	7.00	6.79
TDS (ppm)	658	694	742	1000

- **Solution 2** -The bio-compost was mixed with diluted KOH solution (1%) and pH metery was done in order to check its efficiency. The solution was kept for 5 days and its pH was calculated immediately after 5 min, on day1, day2 and day 5.

Table 4 – Variation of pH of solution 2 (compost + diluted KOH solution)

Solution 2	**pH**
1% KOH	12.80
KOH+ Compost (After 5 min)	12.10
KOH+ Compost (Day 1)	10.42
KOH+ Compost (Day 2)	7.65
KOH+ Compost (Day 5)	7.42

(2) Soil Stabilisation –Soil stabilisation is important as due to extreme temperatures and cropping techniques soil loses its binding ability and its activity is disturbed. Bio-enzymes help in soil stabilisation due to presence of all natural raw materials within it. It activates the biology and helps in stabilisation of the pH of the soil and enhances its activity for plant growth [18,19]. In order to test the efficiency of Bio-Enzymes and its activity on soil the following experiment was carried out—

The soil sample was collected from the institution's park and its pH was determined by mixing the soil sample with tap water. To check the efficiency of bio-enzymes, 1% of bio- enzymes were added to the 20 ml soil solution (10 g) and the pH was recorded after 10 minutes (Table 5).

Table 5- Variation of pH of soil sample on addition of Bio-enzymes (**1-6**)

S. NO	SAMPLE	pH (after 10 minutes
1	Soil solution	7.51
2	Banana bio-enzyme (**1**)+ Soil solution	7.09
3	Beetroot bio-enzyme (**2**)+ Soil solution	6.76
4	Orange bio-enzyme (**3**)+ Soil solution	6.65
5	Pineapple bio-enzyme (**4**)+ Soil solution	6.92
6	Raddish bio-enzyme (**5**) + Soil solution	7.01
7	Turnip bio-enzyme (**6**) + Soil solution	6.21

Treatment of contaminated water- There is a huge problem of sewage and industrial waste water disposal problem everywhere. Nearly 70-80% water of rivers is contaminated. There is a need of treatment of sewage water. Bio- enzymes are made up of natural components, their usage in treatment of waste water can be useful [20-25]. For testing this, the following experiment was carried out-- The 20 ml raw contaminated water sample was collected from village pond and was treated with 1% of bio-enzymes. These samples were left for 1, 2 and 5 days. The variation in TDS and pH was observed for different concentrated samples of bio-enzymes solution.

Variation in pH –After treatment with bio-enzymes (**1-6**) the variation in pH of contaminated water was seen and recorded and the corresponding variation in the characteristics of contaminated water sample which was recorded immediately after 5 minutes, after 1 day, 2 days and 5 days as given below in table 6.

The calibration was done by buffer solution of pH=4 and pH=7. pH of tap water and contaminated water was found to be 7.21 and 7.84, respectively.

Table 6 Variation of pH of contaminated water when treated with bio- enzymes

Sample	pH of bio- Enzymes used	Bio-Enzymes + contaminated water solution			
		After 5 min	After Day1	After Day2	After Day5
1	5.25	6.66	8.35	8.19	7.38
2	5.43	6.60	8.33	8.22	7.44
3	5.31	5.95	8.25	7.97	7.28
4	5.28	6.58	8.39	8.13	7.28
5	5.15	6.66	8.37	8.29	7.38
6	5.01	5.85	7.72	7.49	7.22

Variation in TDS –After treatment of contaminated water with bio-enzymes the variations were observed in TDS content and the characteristics of contaminated water sample which was recorded immediately after 5 minutes, after 1 day, 2days and 5 days are given below (Table 7 and 8)-

Table 7 TDS of Reference samples

Sample	**TDS (ppm)**
Contaminated water	1650
Tap water	157
RO water	91
RO discarded water	648

Table 8 Variation of TDS of contaminated water when treated with bio- enzymes

Sample	TDS of bio-Enzymes, ppm	Bio-Enzymes + contaminated water solution, ppm			
		Immediately	After Day1	After Day2	After Day5
1	2840	1680	1580	1400	1330
2	3590	1670	1500	1390	1280
3	1980	1680	1500	1380	1260
4	2150	1680	1500	1390	1270
5	3020	1680	1520	1300	1250
6	1980	1660	1590	1450	1330

(3) In household application – Bio-enzymes are acidic in nature as it contains alcohols and carboxylic acids. Also, they show antimicrobial properties. So they were used at different places of cleaning as disinfectants, hand sanitisers, floor cleaners and dishwashers for cleaning of utensils at our home. Usability of bio-enzymes is depended on its source. From different sources different bio-enzymes can be prepared. The amount used is different for different applications and the dilution factor should be considered [26].

RESULT AND DISCUSSION

The following results can be found out by the different experiments carried out to test the efficiency, characteristics and properties of bio-enzymes.

(1) Yield- All the samples extracted gave economical yield.

(2) Cost effectiveness- The cost in making of bio-enzymes is comparatively very less than the cleaning agents like Lizol, Dettol, Lifebuoy and Dishwashers etc.

Table 9 Cost effectiveness of Bio-enzymes prepared

Sample	Volume	Cost
Fruit/vegetable peels	30 g	Free (from Kitchen waste) (30*6= 360g)
Jaggery	10*6g	Rupees 3.6 (1kg = Rupees 60)
Yeast	A pinch (0.5 g)*6	Rupees 3.75 (40 g of Rupees 50)
Water	600 ml	Free
Total	~600 ml	Rupees 7.35
pH is within the range of 5-6 which matches to the skin		

Table 10 Different cleaning agents available in market

Sample	Volume	Cost	pH
Dettol hand wash	500 ml	Rupees 80	4.40
Lifebuoy hand wash	500 ml	Rupees 99	9.90
Lizol	500 ml	Rupees 74	1.19
Vim liquid wash	500 ml	Rupees 115	7.1
pH does not match to the skin.			

(3) pH – The different bio-enzymes (**1-6**) extracted were found to have weakly acidic character. All of them have the pH in the range of 5-6. Amongst all the six samples, Bio- Enzyme from Turnip peels (**6**) is more acidic and bio-enzyme from beetroot peels (**2**) is least acidic. (Table 2) The decreasing order of pH all the samples of bio-enzymes (**1-6**) used is as follows- **6>5>1>4>3>2**

(4) Density- All the samples that were extracted, shows the following decreasing trend of density.

6>1>5>2>4>3 or Turnip>Banana>Raddish>Beetroot>Pineapple>Orange

Bio-enzyme from Turnip peels is denser among all the samples and orange is least dense. It may be attributed due to the presence of more amount of fibres and metabolites present in the turnip bio-enzyme. (Table- 3)

(5) Viscosity- Samples shows the following decreasing order of viscosity attributed to the density and the presence of fibrous content or organic metabolites.

6>4>1>5>2>3 or Turnip>Pineapple>Banana>Raddish>Beetroot>Orange

(6) TDS – The Total Dissolved salts of all the samples were recorded and found to be in the particular order-

2>5>1>4>3=6 or Beetroot>Raddish>Banana>Pineapple>Turnip-Orange

It was found that Beetroot sample (**2**) had large amounts of TDS within it and Orange and turnip samples (**3** and **6**) had least amounts of TDS in it (Figure 9) Bio- Enzymes had high amounts of TDS, this may be because of presence of high amounts of organic content within them.

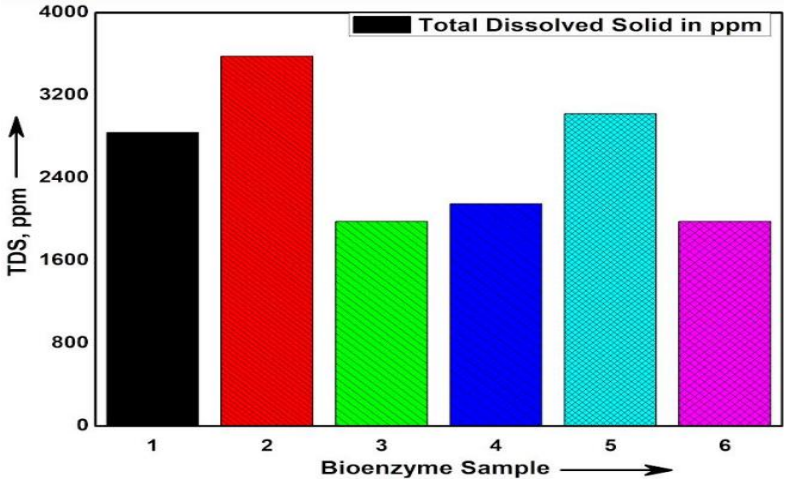

Figure 9 TDS of different bio-enzymes sample **(1-6)**

(7) Anti-microbial properties- Following tests showed the antimicrobial properties of bio-enzymes-

Test for Anti-Fungal properties- All the samples were found to show antimicrobial properties as seen a week later of its application. It resists the growth of fungus in moderate to high degree where it was applied. (Figure 10) All the samples showed resistance to fungus but Bio-enzymes from Orange peels (**3**) showed the best results and pineapple bio-enzyme (**4**) was least resistant to fungus.

Figure 10 Validation of Antifungal property after 7 days. (Left: Day 1, Right: Day 8)

All the samples had no growth till day 3, after that the fungus started growing. The order that was observed for the anti-fungal resistivity is as follows-

3>5>6>1>2>4 or Orange>Raddish>Turnip>Banana>Beetroot>Pineapple

Orange showed best resistance to fungus, they may be because of the fact that citrus fruits have a sharp flavour which keeps the fungus away.

Test for Anti-bacterial properties-

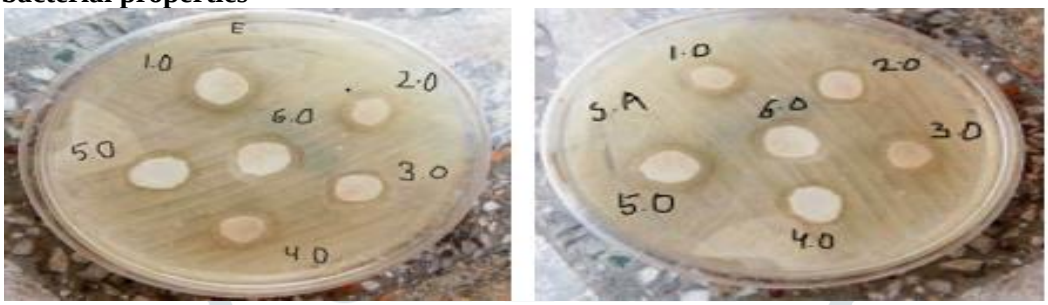

Figure 11 Validation of Antibacterial properties of bio-enzymes (Left- *E. coli*, Right- *Staph-aureus*)

All the samples showed antibacterial properties till 10^{-2} dilution of samples in water and showed resistance towards both Gram positive and Gram negative bacteria. All samples have shown formation of a ring around the disc clearly showing the zone of inhibition. The diameter of the circle around the disc provided information about the degree of inhibition of growth by the bio-enzymes.

(8) Biochemical Tests- These following tests indicated the presence of different groups within the samples of bio-enzymes which are-

(1) Metabolites (Phytochemicals)- All the tests **T1-T6** for different metabolites confirmed the presence of flavonoids, alkaloids, quinones, cardenolides, phenols and saponins.

(2) Identification of Carbohydrates- The Molisch's Test and Benedict's Test that were carried out confirmed the presence of carbohydrates and reducing sugars in the different samples of bio-enzymes.

(3) Identification of Proteins- Ninhydrin test, Xanthoproteic Test and biuret test gave positive results and confirmed the presence of proteins in the bio-enzymes.

(4) Identification of Lipids- All the samples contain Lipids as formation of cloudy solution confirmed the presence of lipids in all the samples.

(9) Applications of Bio-enzymes

Following tests validate the use of bio-enzymes at different places.

(1) Use of Bio-Compost- The compost that was prepared from the solid residue of bio-enzymes gave satisfactory yield of 12.25 g and was prepared from 60g of raw peels. It found to be acidic in nature and was effective as it changed the pH of the solution from basic to neutral as it was acidic in nature. This could be useful in helping alkaline soil for maintenance of its pH. Also this would lead to reduction of usage of chemical fertilisers and promote natural farming.

- The increased number of TDS on the 2 day showed that nutrients and organic content have started diffusing into the solution and this is a good sign for usage of this bio-enzyme in farming for providing nutrients to the crops.
- Lastly, addition of this compost to KOH confirms the fact that bio-enzymes are capable of lowering the pH of the solutions as the pH of the solution in which KOH was present was lowered down at Day-2 and 5.

(2) Soil stabilisation- For soil stabilisation bio-enzymes proves to be an effective method of application as bio-enzymes were capable of lowering the pH of the soil solution within 10 minutes that was taken. Different bio-enzymes samples were used for testing of stabilisation of soil, the decreasing order of action of samples of different bio-enzymes is as follows- **6>3>2>4>5>1** or Turnip>Orange>Beetroot>Pineapple>Raddish>Banana. The results show that sample from Turnip peels is a good soil stabiliser owing to the presence of metabolites and enzymes present in it.

(3) Treatment of contaminated water- After the treatment of contaminated water with 1% Bio-enzymes, variation was observed in the pH and TDS of the solution which was observed for 5 days (Figure 12 and 13).

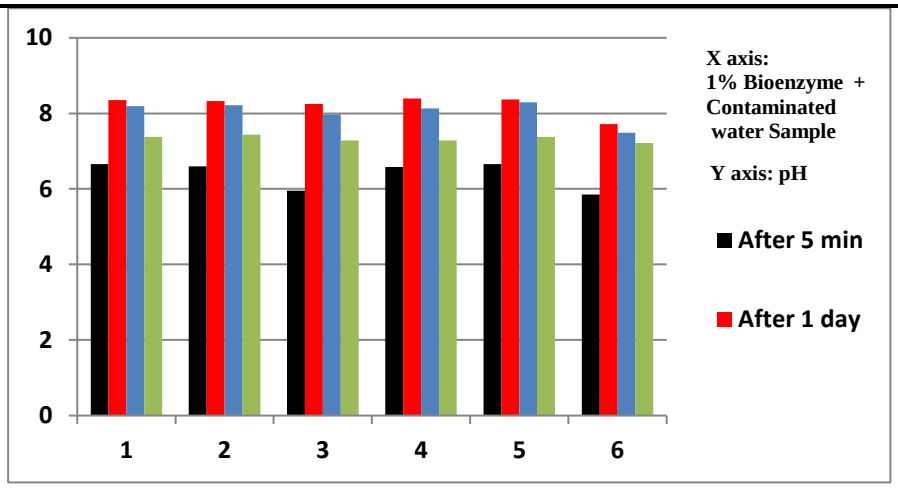

Figure 12 Variation of pH of contaminated water when treated with bio-enzymes (**1-6**)

The order of the activity for the variation of pH in the contaminated water after addition of various bio-enzymes on the day 5 was-

6>3=4>5=1>2 or Turnip>Orange=Pineapple>Raddish=Banana>Beetroot

The change in pH suggested that the proteins and metabolites present in the bio-enzymes were able to digest the dissolved solids in contaminated water. During the initial days, pH of the solution increases which could be due to the digestion of contaminants present in water result in basic components, but with the passage of time as seen on day 3 and then on day 5, pH decreases and reached to neutrality (Figure 12). This shows the self-biodegradable property of bio-enzymes.

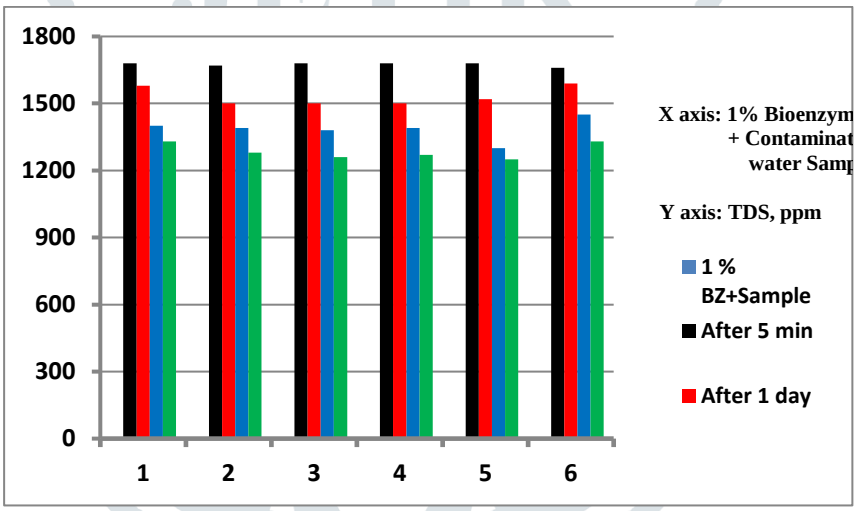

Figure 13 Variation of TDS of contaminated water when treated with bio- enzymes (**1-6**)

The order of the activity for the variation of TDS in the contaminated water after addition of various bio-enzymes on the day 5 was-

5>3>4>2>6=1 or Raddish>Orange>Pineapple>Beetroot>Turnip=Banana

The change in TDS of contaminated water showed that the acidic pH and presence of protein and metabolites in the bio-enzymes were able to reduce the dissolved solid in contaminated water. During the 5 days of digestion, TDS of the solution gradually decreases by 20%-25% on addition of 1% bio-enzymes to the solution (Figure 13).

(4) Usage of Bio-enzymes for household use- Using bio-enzymes at different places at our homes gave satisfactory results. The amount used is different for particular application so dilution should be done. As the bio-enzymes show acidic property it has to be diluted before usage because the acidic character may spoil the texture of the things on which they will be applied. For cleaning of dark stains, it can be used directly but while using on human body or pets or for sanitising purposes it is needed to be diluted first.

CONCLUSIONS

It is concluded that the bio-enzymes are biological catalysts and are made up of proteins, they also contain metabolites, lipids and carbohydrates. In the present study, all the samples of bio-enzymes found to be cost effective, anti- microbial and eco-friendly. All the bio-enzymes showed acidic character and high TDS values due to the presence of high amounts of organic content. They can be used in household purposes due to their acidic character and has the potential of replacing harsh chemical cleaning agents used in our households. Bio-enzymes can act as good soil stabilizers. The results showed that only 1% bio-enzymes was sufficient to change the pH of soil solution There are various applications of bio-enzymes such as to improve the consistency and binding property of soil. They can treat wastewater very easily as the results showed that only 1 ml of bio-enzymes was sufficient to increase the pH and decrease the TDS of the 20 ml contaminated water sample. Anyone can follow simple methodology for the preparation of the bio-enzymes and can very easily make at their homes. Bio-enzymes can be useful in making compost from the

solid residue of the mixture bio-enzymes. The compost is capable of stabilising the pH of alkaline soils as it is acidic in nature. Thus, the bio enzymes can be used for various purposes since it is organic and it does not have any side effects. Bio-enzymes helps to reduction of some waste and turn into a useful substance to the society which is economical and cheaply available and the end product can be completely useful and enhances the quality of life as a whole [11]. More investigations could be done in order to find out the action of bio-enzymes on treatment of wastewater by using other methods like D.O, C.O.D, B.O.D, amounts of phosphates, chlorides and alkalinity. Enzymes concentration and activity of different enzymes can be carried out on growth of microbes. We can also check the effect of bio-enzymes on plant's growth by adding them to the soil and their time of maturity and health. Bio-enzymes from different sources could be used to check different applicability in multiple sectors and can be helpful removal of usage of harsh chemicals from the market.

REFERENCES

[1] UNEP Food Waste Index Report 2021
[2] M, S., Khan. Environmental Impact of Paper industry. *International Journal of Engineering Research and Technology*, 2278:018 **(2015**).
[3] Arora P.K., Kumar M., Chauhan A.,*et al*. OxDBase: a database of oxygenases involved in bio-degradation, BMC Res NMotes **(2009**).
[4] 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**).
[5] Krogsrud N.E. and Larsen A.I., Airborne irritant contact dermatitis from benzalkonium chloride, *Contact Dermatitis.*,**36(2**), 112 (**1997)**.
[6] Mauleón C., Mauleón P., Chavarría E., de la Cueva P., Suárez R., and Lázaro P. Airborne contact dermatitis from n-alkyl dimethylbenzylammonium chloride and n-alkyl dimethylethyl-benzylammonium chloride in a detergent, *Contact Dermatitis.*, **55(5**),311-312 (**2006)**.
[7] Oiso N., Fukai K. and Ishii M, Irritant contact dermatitis from benzalkonium chloride in shampoo, *Contact Dermatitis*., **52(1)**,54 **(2005**).
[8] Krogsrud N.E. and Larsen A.I., Airborne irritant contact dermatitis from benzalkonium chloride, *Contact Dermatitis.*,**36(2),** 112 (**1997)**.
[9] *What is ECO enzyme?* Enzyme SOS. (n.d.), from https://www.enzymesos.com/what-is-eco-enzyme.
[10] 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).**
[11] Khadka R. and Neupane K., Production of garbage enzyme from different fruit and vegetable wastes and evaluation of its enzymatic and antimicrobial efficiency, *Tribhuvan University Journal of Microbiology***., 6**, 113-118 **(2019**).
[12] Singh H., Prasad A. and Nal K.V., Study and analysis of bio-enzymes based natural product, *International Journal of Engineering Applied Sciences and Technology***., 4(8***)*, 205-207 **(2019**).
[13] Vama L. and Cherekar M. N., Production, Extraction and uses of eco-enzymes using citrus fruit waste:Wealth from waste, *Asian Jr. of Microbial, Biotech***., 22(2),** 346-351 (2020).
[14] Samriti., Arya A. and Sarabhai S., Garbage Enzyme: A study on compositional analysis of Kitchen waste ferments, *The Pharma Innovation Journal.*, **8(4),**1193-1197 **(2019).**
[15] Teh H., Chan A., Kamal N.F., Sahidan N.I., Abdul W., Anti-Microbial Activity of Bio-enzymes Extract from Garcinia mangostana Peel, Morinda citrifolia fruit and Hibiscus sabdariffa Petal, *International Conference on Food and Nutrition Technology.*,**72(4***)*,16-20 (**2014)**.
[16] Arya A., Antibacterial property of laboratory preparations of garbage enzymes, *International Journal of Applied Research***., 5(4)**, 109-112 (**2019**).
[17] Agarwal P. and Kaur S., Effect of Bio-Enzyme on unconfined compressive strength of expansive soil, *International Journal of Research in Engineering and Technology***., 3**, 30-33 **(2014).**
[18] Rohith M. S. R., Sankriti R. and Kumar R. S., Review paper on use of Bio-enzyme, *Global Journal of Engineering Science and Researches.*, **4**, 187-194 **(2017**).
[19] Jadhav G., Panchal G. and Mane R., A study on investigation of Bio-enzyme stabilized expansive soil, *International Journal of Innovative Research in Science & Engineering***., 2**, 311-320 **(2016**).
[20] Javalkar S.D., Shinde S.C., Savalkar S., Pawar S. E., Dhamdhere A. H. and Patil S.T., Use of Eco-enzymes in Domestic waste water treatment, *International Journal of Innovative Science and Research technology.*, **4(2***)*,568-570 **(2019**).
[21] N S., M S., J A., and T N., Biological Treatment of Dairy Wastewater using Bio Enzyme from Citrus Fruit Peels, *International Journal of Recent Technology and Engineering (IJRTE)***, 9(1)**, 292-295 **(2020**).
[22] Kumar A., Sandhya H., Ahmad E. and Dulawat S., Application of bio-enzyme in waste water(Greywater) treatment, *International Research Journal of Engineering and Technology (IRJET.),* **8(3),** 2886-2890 (**2020)**.
[23] F N. and V M., Treatment of synthetic greywater using 5% and 10% Garbage enzyme solution Bonfring, *International Journal of Industrial Engineering and Management Science.*, **3**, 111-117 (**2013**).
[24] Tang F.E and Tong C.W., A Study of the Garbage Enzyme's Effects in Domestic Wastewater, *International Scholarly and Scientific Research & Innovation***., 5**, 887-892 **(2011).**

[25] Kumar N., Rajshree Y., Yadav A., Malhotra H., Gupta N. and Pushp P., Validation of eco-enzyme for improved water quality effect during large public gathering at river bank, *International Journal of Human Capital in Urban Management*., **4(3)**,181-188 **(2019**).

[26] Naik V.V., Studies on preparation of bio-enzymes at home using fruits and vegetables waste and their different applications, *The Official Journal of Scientist R Academy.*,**1(1)**,146-154 **(2022).**