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Evaluating the efficiency of Garbage enzymes in wastewater recycling: A pioneering study in environmental sustainability

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Abstract

Garbage enzyme is a complex solution produced by fermentation of fresh kitchen waste (fruit and vegetable peels), sugar (brown sugar, jaggery or molasses) and water. The use of garbage enzyme has been proven to be an effective method for wastewater treatment. In the present study, different concentrations of garbage enzymes 10% and 20%, and different treatment times (24 hours and 10 days), were used to treat wastewater after collection from treatment sites (Rustamia station) and before disposal into river water (Diyala River). Parameters such as pH, total dissolved solids, biological oxygen demand (BOD5), and chemical oxygen demand (COD) were analyzed for the present investigation. The results showed that the bio-enzyme could remove the properties of total dissolved solids (TDS, Mn, EC, Na, SO4, Cd NO3, .) from wastewater; the biological effectiveness of the bio-enzyme was also examined in inhibiting the growth of bacteria (Staphylococcus aureus (Gram-positive)) and bacteria (Pseudomonas aeruginosa (Gram-negative) as well as fungi (Aspergillus niger). No significant difference was observed in the pH properties; it remained acidic. Bio-enzyme can be used as an economical option to enhance the properties of wastewater by treating it with bio-enzymes and making it suitable for different purposes. Moreover, the use of bio-enzymes can also help in ensuring the removal of chemicals during wastewater treatment processes making it environmentally sustainable.

Keywords: Garbage enzyme; Waste water treatment; Fermentation process; Environmental sustainability

1. Introduction

Wastewater contains a variety of chemicals that can be harmful to the environment and public health. To minimize any potential harmful effects, it is highly recommended to treat wastewater before disposal or reuse. Many treatment and purification methods have made wastewater suitable for reuse. These treatment methods are based on three basic processes: physical, chemical, and biological [1,2]. The best option is biological treatment because it usually involves a large amount of organic matter. Many wastewater treatment studies seek to improve efficiency and enable the decomposition of environmental pollutants. There are many uses for wastewater enzymes, and some kind of reaction rate accelerator is needed. When it comes to biological wastewater treatment, enzymes may be the key to an effective and efficient solution. With the continuous advancement in using less money, time, and space [3]. Enzymes act as a catalyst, increasing the rate of reaction several times with less energy in the right direction to produce the desired result. Living cells create a biocatalyst to carry out various metabolic processes, which initiates a specific biochemical reaction essential for the existence of life. These biocatalysts are known as enzymes [4,5] According to [6], fruits and vegetables are fermented to produce an enzyme, a natural liquid formulation that is non-toxic, non-flammable and non-corrosive. Enzymes are superior to conventional chemical catalysts due to certain properties. Enzymes are substrate specific and catalyze one or a limited number of biological activities [7]. Most enzymes are composed of proteins and peptides. They can be easily removed without causing any disposal problems and can be biodegraded by bacteria. Anaerobic enzymes known as bio enzymes or garbage enzymes are produced by fermenting organic waste products, such as fruit and vegetable waste, in a specific ratio with brown sugar and water. [8]. Bioenzymes or garbage enzymes are different from

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fruit enzymes and other enzymes produced by living cells or microbes [9]. The use of bio enzymes is an alternative technology for biological recovery from organic waste, which also contributes to litter reduction and minimization, as organic waste constitutes the majority of municipal solid waste. It also reduces the burden on landfills and stops greenhouse gas emissions [10].

2. Materials and methods

The present study's methodology can be broken down into two primary steps:

- Preparation of bioenzymes
- Use of bioenzymes for wastewater treatment

2.1. Preparation of bio-enzyme

As organic material for the manufacture of enzymes, the required quantity of kitchen waste was collected. To ensure that no chemicals would enter the system and impede the operation, segregation was done after the rubbish was collected. The garbage was then broken up into little pieces to increase the surface area of the reaction. Accelerating the disintegration process is the aim. Molasses or brown sugar (carbon source) can be used to produce waste enzymes or bio-enzymes. water, and leftover fruit and vegetable peels were combined in a 1:3:10 ratio [11,12]. An airtight plastic container with screw clamps and the ability to stretch was used for the mixing process. The ideal way to release the gasses created during the generation of waste enzymes is to use a plastic bottle or container with screw caps. After that, the container was kept in a safe place to keep anyone from interfering with the digestion process. Bacteria break down food waste into smaller molecules and release gases during the fermentation process. To release the gases, the lid was raised once daily for approximately one minute. After that, it was returned to the dark and the procedure was carried out again the next day at least Gas production showed a little decrease in the third week. In the second month, the gas release significantly decreased .The container was once again kept in the dark after the first two weeks were over, and the lid was opened. The container was tightly sealed after the 45-day period and left to digest for an additional 45 days.. According to previous studies, the complete process took three months[13].After 90 days of digestion, a brown liquid with many small particles and some undigested residues formed in the container , After digestion, this brown liquid—the crude enzyme—had to be isolated from the remaining solids. Filtration was used to extract the waste enzyme or bioenzyme that was produced with improved structural and functional properties. The filtered bioenzyme solution was kept apart in a tightly sealed container. The bioenzyme was characterized immediately and again after 30 and 60 days of filtration in order to assess the stability of the enzyme solution [14] image (1)

Figure 1 Bioenzyme solution (Garbage enzyme)

2.2. Determination and assessment of enzyme activity in solutions containing fruit waste enzyme extract

The lipase, oxidase, catalase, protease, and amylase activity were assessed in the waste enzymes of the two different filtrate solutions. The activities of oxidase, lipase, amylase, catalase, and protease were tested using the procedure described in the literature. [15,16] Two to three drops of tetramethyl p-phenylenediamine dihydrochloride (TPDDC) were used to test the oxidase activity on two filter sheets that had been soaked with the two extracts of the waste enzyme solutions placed on clean slides. The test results suggest that oxidase absence is shown by no color (negative), whereas oxidase presence is indicated by a blue hue (positive).

2.2.1. Oxidase activity

Was measured by adding two to three drops of tetramethyl p-phenylenediamine dihydrochloride (TPDDC) to two filter sheets that had been soaked in the two extracts of the waste enzyme solutions placed on sterile slides. The test results suggest that oxidase absence is shown by no color (negative), whereas oxidase presence is indicated by a blue hue (positive).

2.2.2. Amylase activity:

Wwas verified using drops of iodine solution. 1% of a 1.5 cm^3 starch solution was put into a test tube marked A. After that, the fruit trash enzyme extract solution was added to the test tube solution and thoroughly mixed. Using a glass rod, two test tube drops .The tiles containing the iodine solution were covered with a mixture. The hue of the iodine solution shifted from dark blue to brown, which was identified as an indication of amylase.

2.2.3. Lipase activity:

Was investigated using the titrimetric method, which entailed filling a conical flask with 10 cm^3 of the waste enzyme extract solution and lowering the flask's pH to 6 using sodium phosphate buffer. Next, 1 cm^3 of the aliquot portion of the pH-adjusted sample was mixed with 2.5 cm^3 of distilled water, 1 cm^3 of Tris-HCl buffer, and 3 cm^3 of olive oil. The solution was properly mixed and then let to sit for fifteen minutes at room temperature. After adding 3 cm^3 of 95% ethanol solution and (3 drops) of phenolphthalein indicator, the solution was titrated against NaOH until a dark pink hue appeared.

2.2.4. Protease activity:

Was investigated using a titrimetric method, which involved filling a conical flask with 10 ml of trash enzyme extract solution with sodium phosphate buffer to lower the pH of the flask to 6. Next, 2.5 ml of distilled water was mixed with 1 ml of the aliquot fraction of the pH-adjusted sample, 1 ml of Tris-HCl buffer, and 3 ml of olive oil. The solution was properly mixed and then allowed to sit at room temperature for 15 minutes. Then, three centiliters of 95% ethanol solution and three drops of phenolphthalein indicator were added to the mixture, which was then titrated against NaOH until a dark pink hue developed.

Protease activity = Absorbance at 620 nm × proten concentration × time

2.3. Wastewater treatment using bioenzyme

Once the bioenzyme was completely described, the wastewater sample was collected from a garbage-free outflow. After gathering the raw sewage sample, its fundamental characteristics were investigated. After that, the sewage sample was poured into nine cups with different concentrations of bioenzyme solution or kitchen waste enzyme (10%, 20%, etc.) [7]. Three cups were made by combining the sewage sample with the same concentration of bioenzyme and allowing it to digest for an hour. The changed characteristics of the treated sewage sample were analyzed after a day of digestion and after ten days .

2.4. Parameter Tests Conducted

The tests that have been conducted are

- **pH**: The pH of the samples and solutions was measured using the MARTINI pH meter, which displays the pH measurement of the solution (between 1 and 14) on its digital display as soon as the sensor portion is dipped into the solution. investigated. And after ten days.
- **TDS**: The TDS of the samples/solutions was measured using a MARTINI TDS meter, which displays the TDS reading of the solution on its digital display as soon as the sensor component is dipped into the solution (the display value needs to be multiplied by 100 to get the real TDS reading in mg/L [17]

2.5. Microbial composition test

2.5.1. Bacterial composition

On a nutrition agar plate containing 100 µl of Garbag enzyme (GE), amphotericin B was employed as an antifungal agent. The plates were incubated at 37 °C for 24 hours in order to assess the growth [18]. Gram-positive and Gram-negative microorganisms were distinguished using the Gram stain. Biochemical assays including the catalase test [19]and the methyl red test [20] were used to identify the bacteria.. Fungal composition: Chloramphenicol, an antibacterial

agent, was used to create PDA agar plates [21]. After spreading 100 µl of GE onto the agar plate, it was incubated for three to five days at 27 °C. Lactophenol blue stain was used to identify fungal strains.

2.5.2. Antimicrobial assay :

To verify the waste enzyme's ability to kill pathogens, its antibacterial capability was examined. Garbage enzymes (10%, 20%) were used in the agar well diffusion method for the antimicrobial assay. Thirty milliliters of either PDA medium for fungus or NA media for bacteria were added to petri dishes. An agar plate that had solidified was infected with the test organism.

Bacteria and fungus were introduced to the medium's surfaces. The bacterial strains were *Pseudomonas aeruginosa* (gram negative) and *Staphylococcus aureus* (gram positive). *Aspergillus niger* was disseminated on potato dextrose agar, while *Pseudomonas aeruginosa* was spread on nutrient agar plates. using sterile filter paper that has been soaked in trash enzyme and labeled on the plate's reverse side. 15 µl of each sample was added to the corresponding wells that were labeled. The bacteria and fungal cultures were cultured for 24 and 48 hours, respectively, at 37°C and 27°C each sample underwent three examinations. The antibacterial activity of each extract was expressed as the mean diameter of the zone of inhibition [22].

3. Results and discussion

3.1. Physical and Biochemical characterization of garbage enzyme

Fruit and vegetable peels as well as other kitchen trash were used to make the waste enzyme used in this study, which fermented for three months while brown sugar was present. The physical properties of the GE preparations, including their color, turbidity, odor, and pH, were investigated. As shown in Table (1) above, the pH was found to be roughly 3.5–4, indicating an acidic character that might be connected to the high acetic acid content. The GE preparation was found to be less viscous than the GE preparation. Even though GE was typically dark brown, Depending on the type of raw material used, this physical characteristic might have varied noticeably. If more fruit peels than vegetables were included in the GE preparation, the aroma was potent and fragrant [17].

Table 1 Physical characterization of garbage enzyme

Physical characteristic	GE- Preparation
pH	3.5 - 4
Odour	Sweet sour
Color	Light brown
Turbidity	less viscous

3.2. Biochemical Analysis of Garbage Enzyme:

3.2.1. The trash enzymes' chemical characteristics and action.

- **Oxidase activity :** F In the outcome of such a test, blue color denotes oxidase presence (positive), and no color denotes oxidase absence (negative).
- **Oxidase activity**: Whereas the absence of catalase is shown by zero bubbles, the evolution of gas bubbles indicates the existence of catalase.
- **Amylase activity:** Iodine solution's color changed from dark blue to brown, which was noted as a sign of amylase..
- **Lipase activity** dark pink color appeared.
- **Protease activity**. The dark blue tint of the iodine solution turned brown, which was identified as an indication of amylase.

Table 2 Chemical Characteristics of of Garbage Enzyme

Enzymes activity	Estimation of Results
Oxidase activity	Blue color +
Oxidase activity	Gas bubbles (+)
Amylase activity	Dark blue to brown(+)
Lipase activity	Dark pink color(+)
Protease activity	16. 2 94 µ/l

3.3. Treatment of wastewater using 10% and 20% garbage enzymes after 24 hour and 10 th day :

Features of the residential wastewater samples: The Rustamiya station's outflow, where water is discharged into the Diyala River, is where the domestic wastewater samples used in this study were obtained. In various months of 2024, the samples were gathered in a sterile, impermeable plastic container. Analysis of the sample was done. Enzymes from household trash were later used to treat it.

3.3.1. Wastewater sample after 24 h treatment with Eco-enzyme 10%

Table 3 Treatment of wastewater by garbage enzyme (Concentration 10% (ppm) after 24 hour and 10th day .

Parameters (mg/L)	Control ppm	after 24 hour ppm	after 10th day ppm
EC	1937	1202	1290
TDS	1095	980	1092
TSS	12	10	8
PH	7.3	3.7	3. 11
COD	67	52	61
TOC	21	19	15
BOD_5	480	240	182
Na	236	229.5	134
SO_4	390.8	352	378
NO_3	52.65	42	39
Cd	0.025	0.009	0.007
Mn	0.16	0.10	0.035
oil	o.8	0.1	0.1

In table (3) Shows the vital parameters of the quality of the water of the Rustumiyah station, which is represented by the control. After adding 10% of the garbage enzymes to the wastewater, after 24 hours, the percentages decreased to EC(1937-1202), TDS(1095-980) TSS(12-10) PH(7.3-3.7) COD(67-52) TOC(21-19) BOD_5(480-240) Na(236-229.5) SO_4(390.8-352) NO_3(52.65-42) Cd(0.025-0.009) Mn(0.16-0.10) oil(o.8-0.1) the sample was left for 10 days, then the parameters were re-measured. There was a difference in the treated percentages, some of which increased and the other part decreased. EC(1937-1290), TDS(1095-1092) TSS(12-8) PH(7.3-3.11) COD(67-61) TOC(21-15) BOD_5(480-182) Na(236-134) SO_4(390.8.-378) NO_3(52.65-36) Cd(0.025-0.007) Mn(0.16-0.05) oil (o.8-0.1)

3.3.2. Wastewater sample after24 h treatment with Eco-enzyme 20%

Table 4 Treatment of wastewater by garbage enzyme (Concentration 20% (ppm) after 24 hour and 10thday

Parameter	Control ppm	after 24 hour	after 10 days
EC	1760	987	325
TDS	911	900	702
TSS	24	9	19
PH	7.6	4.5	6.5
COD	145	40	18
TOC	48	17	9.5
BOD_5	480	240	134
Na	179.2	102	73.04
SO_4	374	328	95
NO_3	58.66	38.2	12.9
Cd	0.025	0.007	0.005
Mn	0.126	0.08	0.001
oil	o.8	0.2	0.1

Table 5 shows the vital parameters of the quality of the water of the Rustumiyah station, which is represented by the control. After adding 20% of the garbage enzymes to the wastewater, after 24 hours, the percentages decreased to EC(1760-987), TDS(911-900) TSS(24-9) PH(7.6-4.5) COD(145-40) TOC(48-17) BOD_5(480-240) Na(179.2-102) SO_4(374-328) NO_3(58.66- 38.2) Cd(0.025-0.007) Mn(0.126-0.08) oil (0.8-0.0.2) .

The sample was left for 10 days, then the parameters were re-measured. There was difference in the treated percentages, some of which increased and the other part decreased EC(1760-325), TDS(911-702) TSS(24-19) PH(7.6-6.5) COD(145-18) TOC(48-9.5) BOD_5(480-134) Na(179.2-73.04) SO_4(390.8-352) NO_3(58. 12.9) Cd(0.025-0.005) Mn(0.126-0.001) oil(o.8-0.1).

10% and 20% dilution levels of garbage enzyme were used to remediate the wastewater. TSS G and COD were among the metrics that were examined. The changes in the parameters over time for various garbage enzyme to wastewater dilution ratios are displayed in Tables (4, 5).

The tables demonstrate that TSS might be decreased from the start of the trial until its conclusion. Within a day, TSS decreased at all dilution levels. TSS at 10% dilution was found to be substantially lower than TSS at 20% dilution. When lipase was used to pre-treat wastewater, the amount of organic matter and suspended particles was lowered [23,24]

Additionally the study by Parmar *et al*. 2001 shown that the treatment of sewage sludge with lipase and protease was capable of reducing about 80% of the biosolids in the sample.

For all dilution levels of 10% and 20%, it was shown that the COD concentration dropped between days 0 and 24. According to a prior study, the reduction in COD was only noticeable after 27 days of digestion. This might be because the litter enzyme produced a high initial concentration of COD [25] According to a research, one way to determine the overall amount of organic matter is by COD. Organic debris made up the majority of the suspended particles in wastewater [26]. The COD may be decreased because the lipase and protease in the enzyme solution were able to eliminate all of the suspended particles. It demonstrates unequivocally that the litter enzyme could break down oil at various dilution levels. at a 10% dilution level, the oil concentration steadily dropped from time 0 to 24 hours. But on day 10, it drastically dropped. According to a prior study by [27], lipase could eliminate scale and oil from wastewater. During hydrolysis, lipases break the ester bonds of triacylglycerol in oil, releasing all forms of acyl chains that aid in lowering oil concentration and generating short-chain volatile fatty acids. (VFA) [27].

3.4. Anti microbial Assay:

Table 6 displays the litter enzyme's antibacterial activity against bacteria and fungi. The bacterial and fungal inhibition zone of 150 µl of litter enzyme solution is larger than that of the positive control. It was shown that the inhibitory zones for *S. aureus* and *Pseudomonas aeruginosa* were 10 mm and 13 mm, respectively, when 150 µl of 10% litter enzyme solution (pH 3.6) was utilized. 22 mm was the *Aspergillus niger* inhibitory zone.

Because of its acidic character, which aids in the extraction of extracellular enzymes from organic waste materials into the solution during fermentation, this observation unequivocally shows that litter enzyme has the strongest capacity to decrease or inhibit infections [14]. The degradative activity on pathogens frequently seen in trash is probably caused by these extracellular enzymes [28]The trash enzyme's ability to destroy or inhibit infections is confirmed by this observation. [29], noticed that raising the pH increased the trash enzyme's antibacterial activity. The garbage enzyme has the ability to biostimulate and suppress infections, according to the study's findings. Therefore, by eliminating particulates and reducing microbial activity in the sludge, it may improve sludge stability.

Table 5 Anti-microbial activity of fermented garbage enzyme extract on bacterial sp. and fungi

Microorganism(bacteria & fungi)	inhibition zone (mm)
S. aureus and	10 mm
Pseudomonas aeruginosa	13 mm
Aspergillus niger	22mm

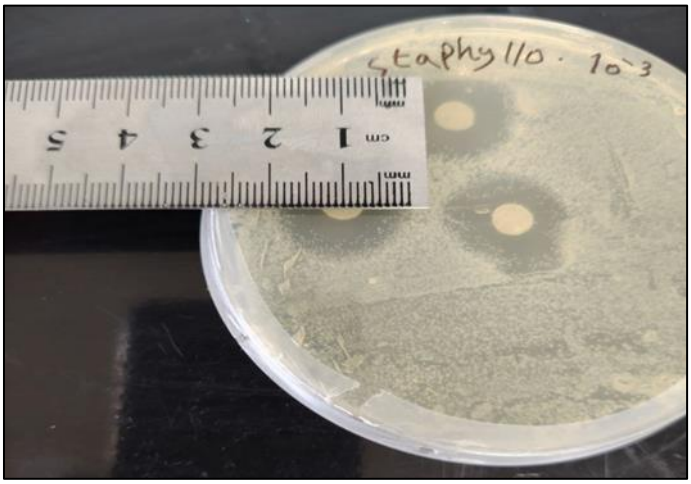

Figure 2 Inhibition zone of the antibacterial activity of fermented garbaenzyme extract on bacterial sps

4. Conclusions

The bioenzyme in this study was constructed using organic waste materials, from fruit and vegetable peel residues. Due to the large amount of organic content, the bioenzyme showed acidic properties and high initial BOD requirement. The results show that the 10% bioenzyme solution can successfully eliminate the BOD, total dissolved solids, and some chemical components properties. It also had an effective role in inhibiting the activity of Gram-positive *Staphylococcus aureus*, Gram-negative *Pseudomonas aeruginosa*, and some fungi *Aspergillus niger.* Since the bioenzyme is made from waste, it is inexpensive and economical. Therefore, bioenzymes are a cost-effective way to improve the quality of wastewater so that it can be used again

Recommendation

Wastewater samples should be used in future studies to ascertain whether time control is suitable for favorable outcomes and whether higher waste enzyme concentrations are managed to enhance treatment. . In addition, research can be carried out to identify suitable enhancers, activators, or additives for enzyme function.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Anshal Kumar, Himanshu Kumar Sadhya, Dr. Esar Ahmad, Shashivendra Dulawat . International Research Journal of Engineering and Technology (IRJET) 2020 Volume: 07 Issue: 05 .

[2] Yatin Shivaliks ., "Domestic wastewater sources and its characteristics," TUHH interactive Web Server, 2017. pg_1066.

[3] Tang F. E. and C. W. Tong, "A Study of the Garbage Enzyme's Effects in Domestic Wastewater," Int. J. Environemntal, , 2011, vol. 5, no. 12, pp. 887–892

[4] Adrio J. L. and A. L. Demain, "Microbial Cells and Enzymes A Century of Progress," resarch gate, 2005,. vol. 1, no. May, pp. 1–27,

[5] Rahman, S., Haque, I., Goswami, R.C.D., Barooah, P., Sood, K., Choudhury, B.,. Characterization and FPLC Analysis of Garbage Enzyme: Biocatalytic and Antimicrobial Activity. Waste Biomass-. 2021 ,Valoriz. 12, 293–302.

[6] Balvantbhai S., U. Patel, and S. K. S. Published, "Improvement of Strength Characteristics of Bio enzyme-Terrazyme treated Expansive soil by Gypsum as an Additive," Int. J. Adv. Res. Innov. Ideas Educ., 2018,vol. 4, no. 2018, pp. 1–3,.

[7] Nazim F.and V. Meera, "Comparison of Treatment of Greywater Using," Bonfring Int. J. Ind. Eng. Manag. Sci. 2017., vol. 6, no. 4, pp. 49–54,

[8] Arun and P. Sivashanmugam, "Identification and optimization of parameters for the semi-continuous production of garbage enzyme from pre-consumer organic waste by green RP-HPLC method," Waste Manag., 2015 .vol. 44, no. 1, pp. 28–33,

[9] Rasit N.and F. S. Mohammad, "Production and characterization of bio catalytic enzyme produced from fermentation of fruit and vegetable wastes and its influence," 2018, vol.4,no.2,pp.12–26,

[10] Rajasulochana P.and V. Preethy, "Comparison on efficiency of various techniques in treatment of waste and sewage water – A comprehensive review," Resour. Technol., 2016 ,vol. 2, no. 4, pp. 175–184,

[11] Neupane K.and R. Khadka, "Production of Garbage Enzyme from Different Fruit and Vegetable Wastes and Evaluation of its Enzymatic and Antimicrobial Efficacy," Tribhuvan Univ. J. Microbiol., 2019 ,vol. 6, no. 1, pp. 113–118.

[12] Bulai, I.S., Adamu, H., Umar, Y.A., Sabo, A.,. Biocatalytic remediation of used motor oil-contaminated soil by fruit garbage enzymes.J.Environ.Chem.Eng.2021,9,10,5465 .

[13] Salvi S., "Application of Eco-Enzyme for Domestic Waste Water Treatment," Int. J. Res. Eng. Appl. & Manag. 2020, vol. 05, no. 11, pp. 114–116, ,

[14] Bhavani Prakash,. How to make and use garbage enzyme 2011 .http://www.ecowalkthetalk.com (accessed 17.12.13.).

[15] Arun, P. Sivashanmugam, Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge, Process Saf. Environ. Prot. 2015, 94, 471-478

[16] Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd Edn., Cambridge University Press, 2006, Cambridge, UK, 2005, pp. 135-162. ISBN-13: 9781139449298.

[17] Bharvi S. Patel , Bhanu R. Solanki , and Archana U. Mankad , .Effect of eco-enzymes prepared from selected organic waste on domestic waste water treatment . World Journal of Advanced Research and Reviews, 2021, 10(01), 323–333

[18] Cooper KE. The theory of antibiotic inhibition zones. Analytical Microbiology.. *Technol* 1963; page 1 - 86

[19] Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd Edn., Cambridge University Press, 2006, Cambridge, UK., ISBN-13: 9781139449298

[20] Olutiola PO, Famurewa O and Sonntag HG. Introduction to General Microbiology: A Practical Approach. 2nd Edn., Bolabay Publications, Ikeja, Nigeria, 2000.

[21] Mathur A, Singh R, Yousuf S, Bhardwaj A, Verma SK, Babu P et al. Antifungal activity of some plant extracts against clinical pathogens. Advances in Applied Science Research. 2011; 2(2):260-264.

[22] Geetha Saramanda. Antimicrobial Activity of Fermented Citrus Fruit Peel Extract Int. Journal of Engineering Research and Application www.ijera.com 2017 :, Vol. 7, Issue 11, pp.25-28

[23] *Leal*, *D.M.G. Freire*, *M.C. Cammarota*, and *G.L.*. *Sant'Anna Jr*. Effect of enzymatic hydrolysis on anaerobic treatment of dairy wastewater. *ProcessBiochem.* 2006 .41 (5) 1173-1178.

[24] L. Masse., K.J.Kennedy and S.P. Chou, "The effect of an enzymatic pretreatment on the hydrolysis and size reduction of fat particles in slaughterhouse wastewater", Journal of Chemical Technology and Biotechnology, vol. 76, pp. 629 – 635, 2001.

[25] Nazim. F and V. Meera, "Treatment of Synthetic Greywater Using 5 % and 10 % Garbage Enzyme Solution," Bonfring Int. J. Ind. Eng. Manag. Sci. , 2013 ,vol. 3, no. 4, pp. 111–117.

[26] Nawawai Mohd Mg, Othman N, Sadikin An, Khalid Ss, Yusof N , Development Of Empty Fruit Bunch Filter With Addition Of Chitosan For Pre-Treatment Of Palm Oil Mill Effluent, Journal ofChemical and Natural Resources Engineering 2008 : 8-16

[27] Jeganathan J, Bassi A, Nakhla G. Pre-treatment of high oil and grease pet food industrial wastewaters using immobilized lipase hydrolyzationJournal of Hazardous Materials October 2006 ,137(1):121-8

[28] Straub, T.M., Pepper, I.L., Gerba, C.P.,. Hazards frompathogenic micro-organisms in land-disposed sewage sludge.Rev. Environ. Contam. Toxicol ,1993. 132, 55–91.

[29] Puupponen-Pimia, R., Nohynek, L., Ammann, S.,Oksman-Caldentey, K.M., Buchert, J.,. Enzyme-assisted processing increases antimicrobial and antioxidant activity ofbilberry. J. Agric. Food Chem2008. 56,681–688.