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Garbage Enzyme: Production and Characterization

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ABSTRACT

The garbage enzyme was prepared from Jaggery, fruit and vegetable dregs and water. Jaggery, fruit and vegetable peels and water were mixed in the ratio of 1:3:10. The mixing process was done in an air-tight plastic container. During the first month, gases produced were released as fermentation progressed. The container was placed in a cool, dry, and well-ventilated place. It was left to ferment for 3 months to produce enzyme. The fermentation yielded a brownish liquid, which was separated from the solids. The solution was filtered after 3 months to obtain enzyme solution which was light brownish yellow in color. All the treatments exhibited positive result for Amylase Test, Lipase Test, Cellulase Test, Urease Test, Protease Test, MR Test and Alcohol and low pH whereas negative for catalase Test, indicating that Garbage enzyme produced from fruit and vegetable wastes contained mixture of enzymes, alcohol and acid brought about by the process of microbial fermentation.

Keywords: Garbage Enzyme, vegetable, and fruit wastes.

INTRODUCTION

Nowadays, people have become more environmental conscious and try to recycle or reuse the waste they generate. Mostly, the typical wastes generated from the kitchen at home are fruit and vegetable wastes. These wastes such as fruit peel can be converted to a valuable product which known as “homemade enzyme” by undergoing fermentation process (Voet, 2012). Basically, this “homemade enzyme” solution is the fermentation product of fruit wastes in the mixture of brown sugar and water after a period of 3 months. It is the process of fermentation with the key ingredient of brown sugar that is metabolized by bacteria into alcohol which is subsequently reduced to acetic acid or vinegar.

Homemade enzyme is also known as garbage enzyme. This “homemade enzyme” solution has been claimed that it has a cleaning power which is as effective as chemical detergent in the market. There are several benefits of the “homemade enzyme” solution in daily life. One of the advantages is to save money by converting kitchen waste such as orange peel, pineapple peel and vegetable waste into multipurpose natural household cleaners instead of buying expensive and polluting chemical cleanser. The garbage enzyme production process requires three months. Sugar is used frequently as a substrate in fermentation processes; in the production of lactic acid, polyhydroxybutyrate, ethanol, pullulan, xanthan gum, and molasses has been widely used as a substrate in fermentation processes (Tang and Tong, 2011) The formula ‘garbage enzyme” was researched and popularised by the founder of the Organic Agriculture Association of Thailand, Dr. Rosukon Poompanvong who won an FAO award in 2003 for

her outstanding contribution to organic farming, through her work in using fermented organic waste as fertilizers, pesticides and livestock feed.

GE is nothing but a vinegar or alcohol derived from fermenting fresh kitchen waste such as veggie and fruit dregs (peels, cuttings, and bits), sugar (brown sugar, jaggery or molasses sugar) and water. The enzyme is derived after one filters and removes the residue after 3 months. The key ingredient is molasses, which the bacteria and microorganisms present in the waste metabolise into alcohol. This is reduced in its final form to acetic acid or vinegar. Vinegar with its acidic properties is well known as an all-round non-toxic cleaner.

Garbage enzyme can be classified as a complex organic substance of protein chains and mineral salt and juvenile hormones. This enzyme can function in four categoteries: decompose, compose, transform and catalysis. Garbage enzyme is produced by food waste such as fruit peeled through fermentation with brown sugar for 3 months' time. Then, after 3 months the enzyme are ready to use as a household cleaning liquid, to remove foul odours, toilet antibacterial anti-viral agent (Soo Poey Keat, 2011). The garbage enzyme functions differently in different concentrations (Tang and Tong, 2011)

FPJ is used in solutions for seed and soil treatments and plant nutrition. It consists of the young shoots of vigorously growing plants that are allowed to ferment for approximately 7 days with the aid of brown sugar. The brown sugar draws the juices out of the plant material via osmosis and serves as a food source for the microbes carrying out the fermentation process. The weak alcohol produced during fermentation extracts chlorophyll (soluble in ethanol) and other plant components. It is non-toxic and edible (Miller et al.,2013). Enzymes for industrial use are produced by growing bacteria and fungi in submerged or solid-state fermentation (Jegannathan et al.,2013). Rivas et.al., (2008) mentioned that orange peel also can be used for production of citric acid. Citric acid is a natural preservative mainly used to add an acidic flavour and more than a million tonnes are produced every year by fermentation. Orange peel has a potential as one of the wastes which can convert into something that is valuable. There are two common state of fermentation which is solid state fermentation (SSF) and submerged fermentation (SmF). Solid state fermentation is a process of fermentation involving solids without the presence of water. The substrate needs enough moisture to support growth of natural microorganism. Homemade solution from fermented orange peel has a high potential to be used as multipurpose cleaning agent. It was produced by undergoing fermentation process using a mixture of brown sugar, orange peel waste and water with correct proportion for at least 3 months (Othman, 2013). Garbage enzyme is different from fruit enzyme and is not for human consumption. Garbage enzyme is used as a natural household cleaner; air purifier; deodorizer; insecticide; detergent; body care; car care; organic fertilizer, etc. It removes odour and dissolve toxic air released from smoking, car exhaust, chemical residues from household products, etc. Enzyme that flows underground will eventually purify the river and the sea. It reduces mosquitoes, flies, rats, cockroaches etc. It is natural antiseptic for your home. The highlight of garbage/citrus enzyme is that it is organic and can be homemade at low costs, as compared to other products that contain synthetic chemicals (may be toxic to human health or environment) and consume high energy in their production.

Garbage enzyme can be utilized as a low-cost alternative to improve wastewater treatment processes. Garbage enzyme may be used effectively in the treatment of greywater (Nazim and Meera, 2013). Using and making your own garbage enzyme is a growing trend among those who know about it and care about the earth. Kitchen garbage can help to save Mother Earth and through routine daily activities at home, we can reduce global warming and protect the ozone layer. The garbage enzyme has been touted

in the Malaysian media recently as a multipurpose solution for a range of uses, including fertilizer and insect repellent in the garden, household cleaning and even as personal shampoo and detergent.

MATERIALS AND METHODS

The present study was aimed to produce fermented product from vegetable and fruit waste and to study their biochemical and microbiological properties.

1. Collection of wastes

Vegetable wastes and fruits peels were collected from local Vegetable and fruit market (Sanichurey Bazar) in Bilaspur and fruit juice corner in the month of January 2017.

2. Production of garbage enzyme

Two sets of Experiments was designed to produce Garbage enzyme for which Six treatments of Vegetable wastes such as cauliflower, cabbage, pea peel, bottle guard, Raddis and capsicum were taken single and combination of all 6 vegetables from VT1-VT8. (T1-Raddis,T2- cauliflower ,T3-cabbag,T4-carrot,T5-capsicum,T6-bottel guard) In second set fruit peel of papaya, orange, pineapple, banana, guava and pomegranate were taken single and combination of all 6 fruit peels from FT1-FT8 (T1 – papaya,T2-orange,T3- pomegranate,T4- guava, T5- pineapple,T6-banana.)Three replicates of all the treatments were taken along with control VT8 and FT8 (treatment without any wastes).A small batch of garbage enzyme had been produced for this study (Tang and Tong, 2011) **Brown sugar: residue of vegetable or fruit: water in the ratio 1: 3 : 10 was taken** in small bottles and kept air tight for three months in room temperature for production fermented juice. The fermentation yielded a brownish liquid, which was separated from the solids.

3. Biochemical Assays

The Fermented juice were sampled at the interval of 30 days for Biochemical analysis in different assays media such as starch Agar media, Tributyrin agar media, CMC agar media, Urea agar base ,Casein agar media by gel diffusion assay for Amylase Test, Lipase Test, Cellulase Test, Urease Test, Protease Test. Each plate containing different assay media with 5 mm diameter wells to which 05 ml of sampled ferment juice from each replicates of each treatment for vegetables and fruit peel was added. Enzyme assay were determined by presence of zone of clearance. For catalase test, a drop or two of fermentation broth was taken on a clean glass slide and emulsified with three to four drops of hydrogen peroxide. Presence of effervescence/air bubbles immediately indicated positive results.

4. Alcohol and acid Production Test:

Fermentation is a metabolic pathway by which cells cataboiize, or break down a carbon source to produce energy. Fermentation refers to catabolic processes where organic molecules, such as sugars or amino acids, are broken down to produce energy without the use of a membrane bound electron transport chain. Depending upon the organism, fermentation can occur in the presence (aerobic) and/or in the absence (anaerobic) of oxygen. Fermentation pathways produce by-products such as carbon dioxide, ethanol (alcohol), or organic acids (lactic acid or acetic acid, for example).

The estimation method for ethanol is based on the complete oxidation of ethanol by dichromate in presence of sulphuric acid with the formation of acetic acid. The green colour produced during the reaction is due to the formation of chromate ions (Saxena et al., 2012).

$$2Cr_2O_7^{-2} + 3C_2H_5OH + 16\,H^+ \longrightarrow 4\,Cr^{+3} + 3CH_3COOH + 11\,H_2O$$

(yellow colour) (green colour)

Preparation of Potassium dichromate solution ($K_2Cr_2O_7$) :
Dissolve 34 g of $K_2Cr_2O_7$ in 325 ml of concentrated H_2SO_4 and make the volume up to 1000 with distilled water by stirring and keep the flask in ice bucket.
Ethanol estimation: To 1ml fermented broth sample 2ml of distilled water was added to make volume up to 3 ml. Then add 5 ml of $K_2Cr_2O_7$ solution and incubated the test samples at 60^0C for 20 min and observed for appearance of green colour.
Estimation of acid production: Estimation of acid production in fermentation broth was done by MR test. A drop of Methyl red indicator was added to 1ml fermentation broth and observed colour change from yellow to red. Due production of acid during fermentation pH of the broth falls to acidic range indicated by pH strip and at acidic pH methyl red indicator gives red colour.

RESULTS AND DISCUSSIONS

Garbage Enzyme was produced after 90 days of fermentation. The characteristics of pure garbage enzyme (after 3months fermentation) are shown in table 1-3. The preparation of garbage enzyme required three main materials that are easily obtained the cheap. The main materials were preparation was the food wastes such as peeled fruit skin and raw vegetables waste.

Table 1: Biochemical characteristics of Garbage enzyme of Fruit peels

S/no.	Treatments	Enzyme activity, acid and alcoholic fermentation of Garbage enzyme of Fruit peels								
		Amylase	Protease	Lipase	Urease	Cellulase	Catalase	Pectinase	MR	Alcohol
1.	FT_1	+++	+++	++	++	+	-	+++	+++	+++
2.	FT_2	+++	+++	+	+++	+++	-	+	+++	+++
3.	FT_3	+++	-	+++	+++	+++	-	+++	+++	+++
4.	FT_4	+++	+++	+++	++	-	-	++	+++	+++
5.	FT_5	+++	++	+++	++	+	-	++	+++	+++
6.	FT_6	+++	+++	-	+++	++	-	++	+++	+++
7.	FT_7	+	+++	+++	++	+++	-	+++	+++	+++

Note: Results in Table 1 represents biochemical activities of each treatment FT taken as average of three replications.

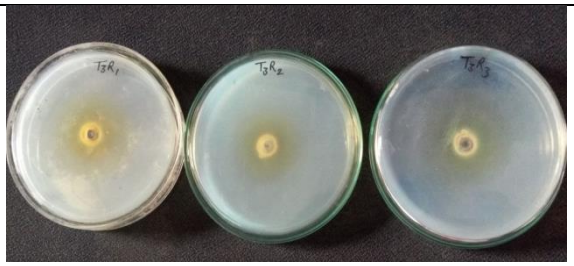
Table 2: Biochemical characteristics of Garbage Enzyme of raw vegetables waste

S/no.	Treatments	Enzyme activity, acid and alcoholic fermentation of Garbage enzyme of Vegetables waste								
		Amylase	Protease	Lipase	Urease	Cellulase	Catalase	Pectinase	MR	Alcohol
1.	VT_1	+++	++	++	++	++	-	++	+++	+++
2.	VT_2	++	+	+	+	++	-	++	+++	+++
3.	VT_3	++	+	-	+	+++	-	++	+++	+++
4.	VT_4	+	++	+	-	++	-	+++	+++	+++
5.	VT_5	+++	++	++	++	+	-	++	+++	+++
6.	VT_6	++	+	-	++	++	-	++	+++	+++
7.	VT_7	++	++	++	++	+++	-	++	+++	+++

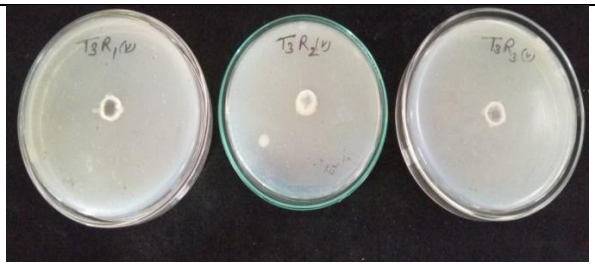
Note: result in Table 2 represents biochemical activities of each treatments taken as average of three replications

Experimental Set 1 Fruit Waste

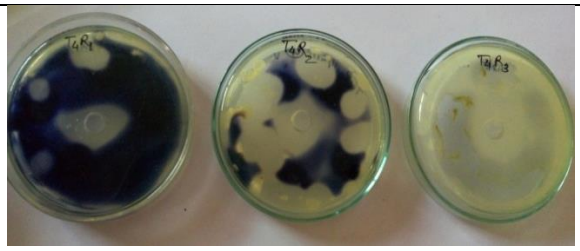
Experimental Set 2 Vegetables Waste



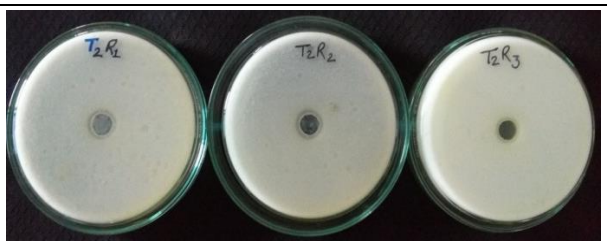
Cellulase Activity



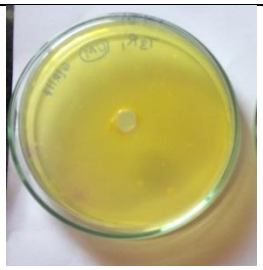
Lipase Activity

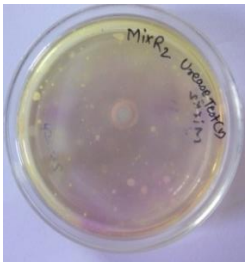


Amylase Activity



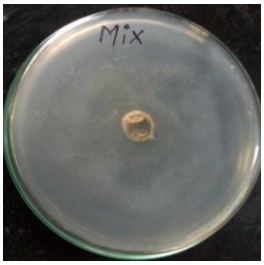
Protease Activity

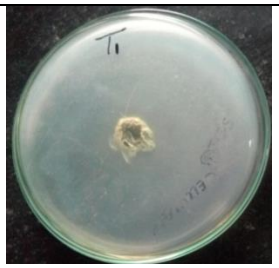




Negative **Positive**

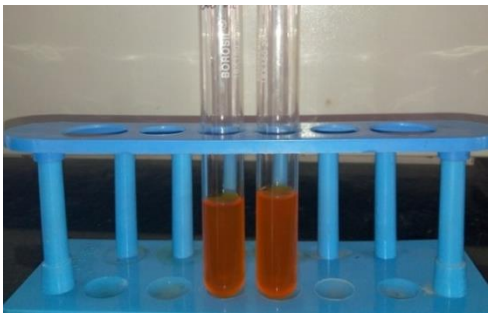
Urease Activity

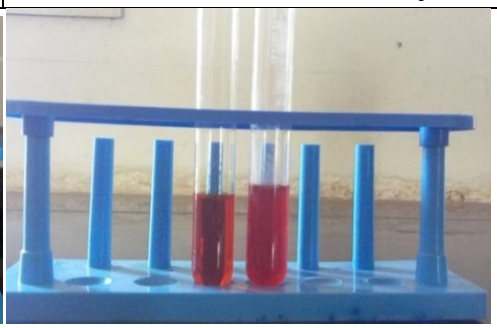




Positive **Negative**

Pectinase Actvity





Negative **Positive**

Acid Production test

Picture :1 "Garbage Enzyme" Enzyme assay

Results of Enzyme activity of garbage enzyme are depicted in Table 1 and Table 2. The garbage enzyme was prepared from sugar, fruit or vegetable dregs and water. Jaggery, fruit/vegetable peels and water were mixed together in the ratio of 1:3:10. The mixing process was done in an air-tight plastic container which was able to expand. During the first month, gases were released during fermentation process. The container was placed in a cool, dry and well-ventilated place. It was left to ferment for 3 months to produce enzyme.

The fermentation yielded a brownish liquid, which was separated from the solids. The solution was filtered after 3 months to obtain enzyme solution which was light brownish yellow in colour. All the treatments exhibited positive result for Amylase Test, Lipase Test, Cellulase Test, Urease Test, Protease Test, MR Test and Alcohol and low pH, indicating that Garbage enzyme produced from fruit and vegetable wastes contained mixture of enzymes, alcohol and acid brought about by microbial fermentation.

Enzymes will never expire when transferred to a plastic bottle was suggested. The longer it is kept, the stronger it becomes. The power of the enzyme was enhanced when water is added to it. Garbage enzyme is only for external use. Garbage enzyme is at its best after 06 months of fermentation[1]. According to

[1] Pencinta Alam, newsletter of the Malaysian Nature Soceity, http://reviews.ebay.com.sg / GarbageEnzyme-DIY

research 45% household waste is organic waste such as fruit peeled. Thus, these proved the main material of the garbage enzyme preparation was easily obtained daily. This food was waste was fermented in a bottle for 3 months along with brown sugar and water rasio 1:3:10 for example 100 ml of prepare garbage enzyme:30 gm of food waste 10gm of brown sugers,100 ml of water is required. Precaution have to be taken, if the container used is completely air tight make sure the container was released at least once a day for the few weeks to let out build-up gas of fermentation in order to avoid any explosion due to high pressure exeration from the fermentation gas is released (Soo poey keat, 2011). It should not be stored in a refrigerator is suggested (Nazim and Meera, 2013). Garbage enzyme is a complex solution produced by the fermentation of fresh kitchen waste (fruit and vegetable peel), sugar (brown sugar, jaggery or molasses) and water. It is dark brown and has a strong sweet sour fermented scent. Garbage enzyme is a multipurpose liquid and its applications covers household, agriculture, animal husbandry, etc. It is a complex organic substance of protein chains and mineral salts and juvenile hormones. The functions of Garbage enzyme is to resolve (decompose), transform (change), and catalyse the reactions (Voet 2012; Nazim and Meera, 2013).

Garbage enzyme is one such value added product produced by fermentation of organic solid waste. Arun and Sivashanmugam, (2015) studied the enzyme activity and disinfectant potential of garbage enzyme was evaluated and its influence on reduction of total solids, suspended solids and pathogens in dairy waste activated sludge. Their investigation of biocatalytic potential of garbage enzyme, showed that garbage enzyme possesses protease, amylase and lipase activity and reduced 37.2% of total solids, 38.6% of suspended solids and 99% of pathogens in dairy waste activated sludge. In 2017, Arun and Sivashanmugam, studied the effect of fruit peel composition and sonication time on enzyme activity were investigated. Garbage enzyme was produced from 6g pineapple peels: 4g citrus peels pre-treated with ultrasound for 20min shows higher hydrolytic enzymes activity. They used statistical optimization tools to model garbage enzyme production with higher activity of amylase, lipase and protease. The maximum activity of amylase, lipase and protease were 56.409, 44.039, 74.990U/ml respectively at optimal conditions (pH (6), temperature (37°C), agitation (218 RPM) and fermentation duration (3days).. Main findings of Sarabhai and Arya, (2019) indicated that GE was acidic in nature, acetic acid being a major component. Biochemical analysis of GE preparations revealed the presence of acetic acid, sugars, proteins, alcohol, enzyme activities like protease, amylase, lipase and papain. Heterogeneous microbial flora was found to be present.

Reference

1. Aneja K.R. (2003) microbiology,plant pathology and biotechnology(fourth revised edition) Kurukshetra University , Kurukshetra (Haryana).
2. Arun C and Sivashanmugam P (2015). **Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge**. Process Safety and Environmental Protection. Vol. 94, Pages 471–478.
3. Arun, C. and Sivashanmugam, P. (2017). Study on optimization of process parameters for enhancing the multi-hydrolytic enzyme activity in garbage enzyme produced from pre-consumer organic waste. Bioresource Technology. 226: 200-210

4. Azian Bt Othman (2013). investigation on the potential of orange peel waste in the production of useful homemade solution. Faculty of Chemical and Natural Resources Engineering university malaysia pahang**.**
5. Bakar K.B.B (2010). Garbage enzyme as an alternative method in treatment of sullage", Master thesis, University Technology Malaysia.
6. Bhavani Prakash (2011). How to make and use of garbage enzyme**.**
7. Buzzini, P. and Martini, A. (2002): Extracellular enzymatic activity profiles in yeast and yeast like strains isolated from tropical environments. Brazilian Journal of Microbiology (2011) 42: 394-409, ISSN 1517-8382 *J.*Appl. Microbial., 93:1020-1025.
8. Chandrakant, S. Karigar. and Shwetha S. Rao. **(**2011). Role of Microbial Enzymes in the Bioremediation of Pollutants: Enzyme *R*, 11: 1-11.
9. Colin, Scott, Steve, E. Lewis, Rob, Milla, Matthew, C. Taylor, Andrew, J.W. Rodgers, Geoff, Dumsday, Jon, E. Brodie, John, G. Oakeshott, Robyn, J. Russell. (2010). A free-enzyme catalyst for the bioremediation of environmental atrazine contamination. J Environ Manage, 91: 2075-2078.
10. Dr. Rosukon (2013). use of garbage enzyme, reduce greenhouse effect founder of organic Agriculture association of Thailand.
11. Emimol .A, Ganga.G, Parvathy R, Radhika.G, Nair G.M (2012). Screening of Microbes producing extracellular hydrolytic enzyme from corporation waste dumpin site and house hold waste for the enhancement of bioremediation methods Journal of Pharmacy and Biological Sciences Volume 4, Issue PP 54-60.
12. Fazna Nazim and V. Meera (2013). Treatment of Synthetic Greywater Using 5% and 10% Garbage Enzyme Solution. Bonfring International Journal of Industrial Engineering and Management Science, Vol. 3.
13. Fu E. Tang, and Chung W. Tong (2011). A Study of the Garbage Enzyme's Effects in Domestic Wastewater .World Academy of Science, Engineering and Technology International Journal of Environmental, Chemical, Ecological, Geological and Geophysical Engineering Vol:5
14. Gupta, S., Krishna, M., Prasad, R.K., Gupta, S., Kansal, A. (1998). Solid waste management in India: options and opportunities. Resour Conservat Recycl ,24: 137–154.
15. Ho Yuek Ming , Ling Leong Kee and Manaf Latifah Abd (2013). Garbage Enzyme as a Solution to Waste Minimization.
16. Kelly,C.T.,Fogarty,W.M. (1983) Microbial glucidase *P Biochem*, 18:315-320.
17. Kenthorai R Jegannathan , Per Henning Nielsen (2013). Journal of Cleaner Production 42: 228-240.
18. Lal ,R., Saxena, D. M. (1982) Accumulation, metabolism andeffects of organochlorine insecticides on microorganisms, Microbiol Rev, 46: 95–127.
19. Li H. Dang H, T, Zhu _ J. Wang (2009) Extracellular hydrolytic enzyme screening of culturable heterotrophic bacteria from deep-sea sediment of the Southern Okinawa Trough World J. Microbiol. Biotechnol, 25:71–79
20. Mosib B. Tagotong, Onofre S. Corpuz (2015)**.** Bio-organic Fertilizer on Pechay Homegarden in Cotabato.
21. Pinang, P.S. "Garbage enzyme-Change climate formula is in your kitchen waste", unpublished.
22. Pencinta Alam, newsletter of the Malaysian Nature Soceity, http://reviews.ebay.com.sg / GarbageEnzyme-DIY.

23. Rathi, S. (2006). Alternative approaches for better municipal solid waste management in Mumbai, India. *J Waste Manage,* 26: 1192–1200.
24. Rivas, B.; Torrado, A.; Torre, P.; Converti, A.; Domínguez, J.M. (2008). Submerged citric acid fermentation on orange peel autohydrolysate. *J. Agric. Food Chem.* 56, 2380–2387.
25. Sanchez-Porro, C., Mart´ın, S., Mellado, E., Ventosa, A. (2003). Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes, J Applied Microbiolo, 94: 295–300.
26. Sarabhai, S.S. and Arti Arya, A. 2019 Garbage enzyme: A study on compositional analysis of kitchen waste ferments The Pharma Innovation Journal 2019; 8(4): 1193-1197
27. Soo Poey Keat (2011) Determination of Acetic acid in garbage enzyme properties Associated with improving water quality of recreational lake. School of Arts and science Tunku Abdul Rahman College Kualalumpur**.**
28. Saxena J, Baunthiyal M and Ravi I. (2012). Laboratory manual of microbiology, Biochemistry and Molecular biology. pp 111
29. Sharma, S., Shah, K.W. (2005). Generation and disposal of solid waste in Hoshangabad. In: Book of Proceedings of the Second International Congress of Chemistry and Environment, Indore, India, pp. 749–751.
30. Torrado Ana María; Cortés Sandra; José Manuel Salgado; Belén Max; Noelia Rodríguez; Belinda P. Bibbins; Attilio Converti; José Manuel Domínguez. (2011). Citric acid production from orange peel wastes by solid-state fermentation. Brazilian Journal of Microbiology 42: 394-409, ISSN 1517-8382
31. Voet V (2012). Biochemistry, 2nd. New York: John Wiley & Sons, ".Ways to Save Energy" (2008). Retrieved, 2012, from http://way stosaveenergy.net/