Comparative studies on enzymatic efficacy and microbiological properties of Garbage Enzyme produced from various fruits and vegetables peels in biodegradation of cow dung

Swati Rose Toppo

Department of Microbiology and Bioinformatics, Atal Bihari Vajpayee Vishwavidyalaya Bilaspur Chhattisgarh - 495 009, INDIA E-mail: swatirosetoppo@bilaspuruniversity.ac.in

ORCID ID -0000-0002-1192-4247

ABSTRACT

The present work was conducted to study the enzymatic potentials and microbiological properties of Garbage Enzyme produced from various fruits and vegetables peels. Completely randomized designed experiment was framed with seven treatments each with fruits and vegetable peels separately. 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. Highest enzymatic efficacy was shown by T3- Pomegranate followed by T6- Banana. GE produced by vegetable wastes was less effective than fruit peels and maximum enzymatic activity exhibited by T1-Radish. A total of 27 amylase producing bacteria, 27 protease producing bacteria, 26 lipase producing bacteria, 26 cellulase producing bacteria 24 urease producing bacteria, 21 pectinase producing bacteria and 01 Amylase producing fungus was screened isolated from all the treatments. Garbage enzyme treatments in tray assay neutralized the alkaline pH of cow dung as garbage enzyme is acidic in nature. Highest Nitrogen (172 Kg/Hec) content was found when treated with garbage enzyme produced with banana peel. It is concluded that Garbage enzyme contains various enzymes, acid, and alcohol, can be utilized as a low cost alternative to biodegrade wastes to reduce pollution load of our environment.

Keywords: Garbage Enzyme, vegetable and fruit wastes, enzyme activity, biodegradation

INTRODUCTION

Dr. Rosukon Poompanvong, the founder of the Thai Organic Farming Association, conducted the initial study on eco-enzyme during the 1980s. Researcher in Naturopathy from Penang, Malaysia, Dr. Joean Oon, popularised the use of eco-enzymes. According to the Nusantara Eco Enzyme Community (KEEN) socialisation materials, 70% of the waste that is disposed of in Final Disposal Sites (TPA) is organic waste. This waste reduces the amount of plastic recycling, releases odours into the environment, and increases the risk of explosions and organic waste decomposition. generate gaseous methane. Given that 60% of Indonesia's trash is produced for home use, it is imperative to reprocess organic waste into eco-enzymes. This information comes from the Sustainable trash Indonesia research. (Setyaka, 2020; Adelliya, 2021). The term "garbage enzyme" (GE) refers to "kitchen waste ferments," an organic substance made from fruit and vegetable peels and sugar (molasses, brown sugar, or jaggery) simply fermented in water. Garbage enzymes have been reported to have use as a cleanser, deodorizer, insecticide, soil conditioner, and insect repellent (Sarabhai and Arya, 2019). 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). Liquid manure was prepared by fermenting the mixture of kitchen wastes in our own way i.e. potato peel (500g), carrot

peel (200 g), legume leaf (300 g), neem leaf (200g), tulsi leaf (100 g), cow dung (1.5 kg) in 6.0 liter of water, as a novel approach for the improvement of growth, yield, and quality of garlic (Allium sativum l.) cv. g-282 (Maji et al., 2015). Wastes from homes and industries alike produce enormous volumes of fruit and vegetable wastes, which might be better utilised in large-scale genetically engineered agriculture. Utilising GE, waste materials may be decomposed into organic fertiliser and applied again. In lieu of several other ineffective solid waste management techniques, this might offer a workable, affordable, and environmentally friendly substitute.

The present study therefore was carried out to investigate into the biochemical enzymatic efficacy and microbiological properties of Garbage Enzymes produced using different vegetables and fruits peels.

MATERIALS AND METHODS

The present study was aimed to produce garbage enzyme to study their biochemical and microbiological properties.

1. Production of garbage enzyme

Vegetable wastes and fruits peels were collected from local Vegetable and fruit market in Bilaspur. 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-VT7 (T1-Raddis, T2- cauliflower ,T3-cabbag,T4-carrot,T5-capsicum,T6-bottel guard, T7- Mix Vegetable peels). In second set of experiment 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, T7- Mixfruit peels). 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.

2. Biochemical Assays

Enzyme Production: The Fermented juice was 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 replicate of each treatment for vegetables and Fruit peel was added. Enzyme assay was determined by presence of zone of clearance (Emimol, 2012, Toppo and Maitry, 2024). 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.

Alcohol and acid Production Test:

Fermentation is a metabolic pathway by which cells catabolize 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).

$$\underset{\text{(yellow colour)}}{2Cr_2O_7^{-2} + 3C_2H_5OH + 16\,H^+} \longrightarrow \underset{\text{(green colour)}}{4\,Cr^{+3} + 3CH_3COOH + 11\,H_2O}$$

Preparation of Potassium dichromate solution (K2Cr2O7) : Dissolve 34 g of K2Cr2O7 in 325 ml of concentrated H2SO4 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 K2Cr2O7 solution and incubated the test samples at 600C 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.

3. Microbiological analysis

Screening of micro-organisms for enzyme activity in fermentation broth with reference to their proteolytic activity, amylase activity and lipase activity (Emimol, 2012 Toppo and Maitry, 2024,), urease activity, cellulose activity, and pectinase activity were done (Aneja, 2003, Toppo and Maitry, 2024).

4. In- vitro Biodegradation of Cow Dung

Fresh Cow Dung was collected from the Cowshed near of Mangala chauk Bilaspur. 200grams cow dung was weighed with an electronic balance and set of 7 treatments were made for biodegradation trials (3 replicates). In vitro tray was aligned from T1, T2, T3, T4, T5, T6, C. The content of the trays was mixed properly and then were left open for proper aeration as well as allowed to decompose for the period of 3-4 weeks and every alternate days 100ml of 60% garbage enzyme from fruit wastes was sprinkled in each treatments respectively. Samples pH and Nitogen content was measured at the interval of 7days.

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. 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** in plate assay in mm**,** acid, **and alcoholic fermentation of Garbage enzyme of Fruit peels**

		Amylase	Protease	Lipase	Urease	Cellulase	Pectinase	Catalase	MR	Alcohol
1.	FT_1	36	34	21	23	8	35	_	+++	+++
2.	FT_2	35	38	9	33	32	10	_	+++	+++
3.	FT_3	41	-	39	39	35	35	_	+++	+++
4.	FT_4	36	34	32	20	-	20	_	+++	+++
5.	FT_5	33	23	34	21	10	21	_	+++	+++
6.	FT_6	39	36	-	40	24	36	_	+++	+++
7.	FT_7	8	35	36	23	32	32	_	+++	+++

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

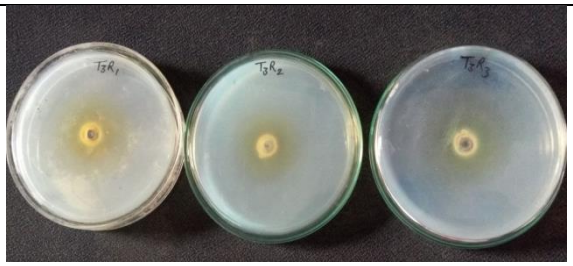
Table 2: Biochemical characteristics of Garbage Enzyme of raw vegetables waste

S/no.	Treatments	**Enzyme activity** in plate assay in mm**, acid and alcoholic fermentation** of Garbage **enzyme** of Vegetables waste								
		Amylase	Protease	Lipase	Urease	Cellulase	Pectinase	Catalase	MR	Alcohol
1.	VT_1	34	23	21	25	23	20	-	+++	+++
2.	VT_2	23	9	10	9	21	21	-	+++	+++
3.	VT_3	22	8	-	8	34	24	-	+++	+++
4.	VT_4	10	21	9	-	23	35	-	+++	+++
5.	VT_5	32	23	25	22	8	23	-	+++	+++
6.	VT_6	21	9	-	22	23	25	-	+++	+++
7.	VT_7	24	23	23	21	33	24	-	+++	+++

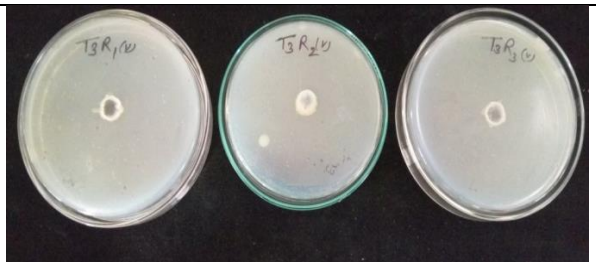
Note: result in Table 2 represents biochemical activities of each treatment VT taken as average of three replications

Experimental Set 1 Fruit Waste

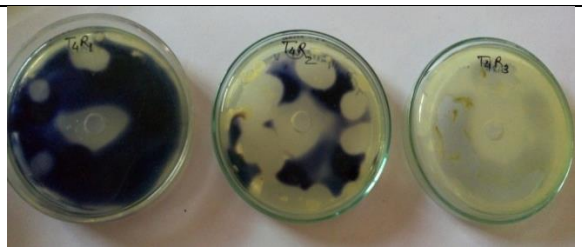
Experimental Set 2 Vegetables Waste



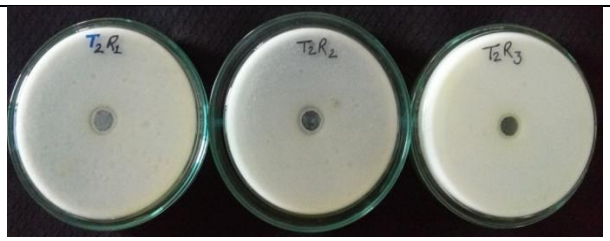
Cellulase Activity



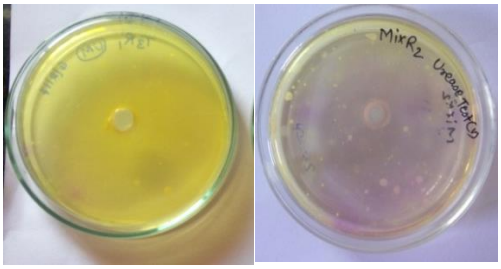
Lipase Activity



Amylase Activity

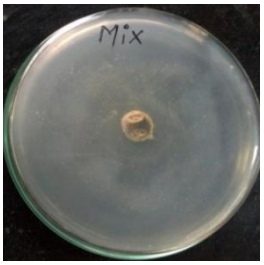


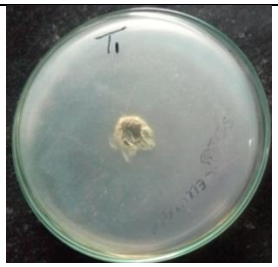
Protease Activity



Negative **Positive**

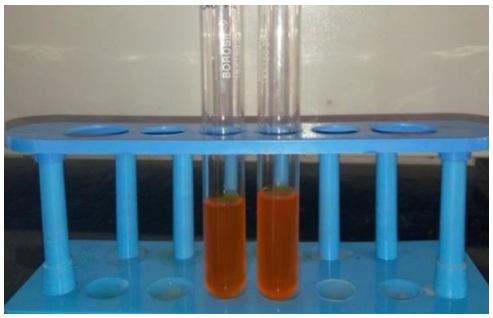
Urease Activity

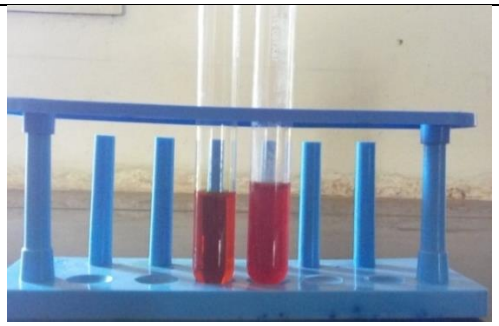




Positive **Negative**

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. Highest enzymatic efficacy was shown by T3- Pomegranate followed by T6- Banana. GE produced by vegetable wastes was less effective than fruit peels and maximum enzymatic activity exhibited by T1-Radish.

Microbiological analysis of Garbage enzyme:

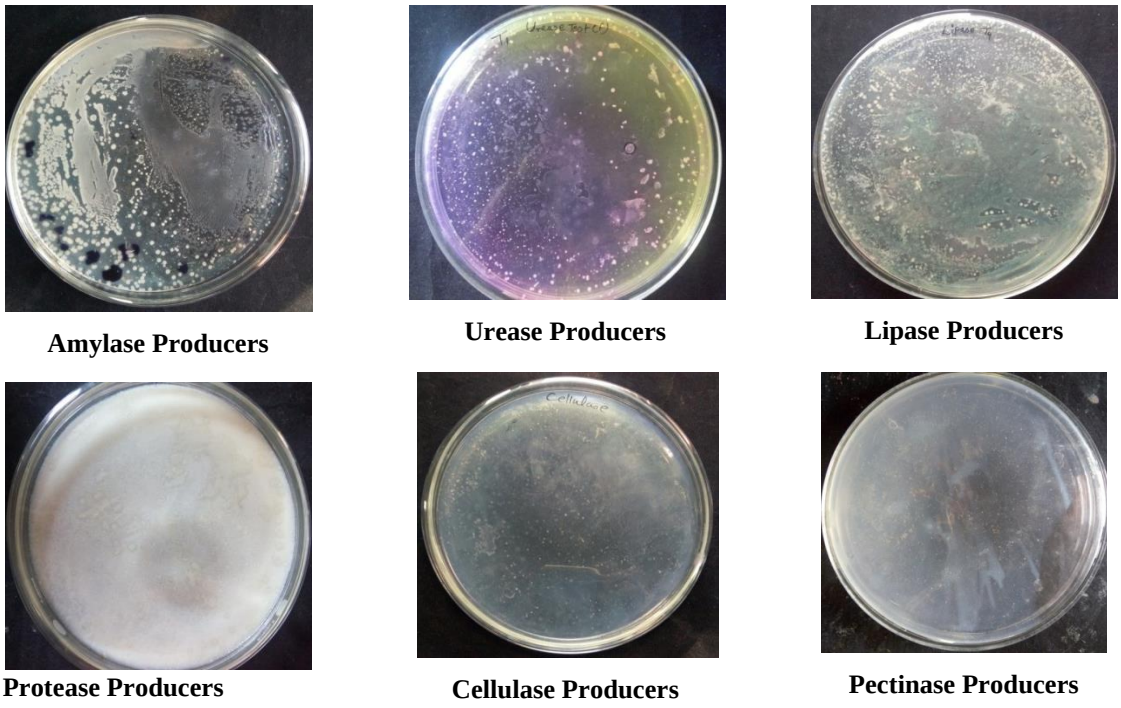
It is evident from results in table 3 that raw materials for production of garbage enzyme harbour various microorganisms that brought about degradation of organic compounds. Their growth and hydrolysis zone were monitored at 24 hours. Zones of hydrolysis varied from 2-35 mm in diameter. The isolates were screened for lipid, starch, protein, cellulose, pectin and urea degradation. The extracellular enzyme activities were visualised as opaque halo around the colonies for lipase production, Starch utilisation was visualised as clear zones against dark blue brown staining for starch when flooded with iodine solution, deep pink coloration for urease activity, and zone of clearance around colonies for proteolytic activity, lipase and cellulose and pectinase when flooded with 1% HDTMAB solution. Similar results were obtained by the experiments conducted for production of Garbage enzyme by Toppo and Maitry, 2024.

Table 3: Microbiological analysis of Garbage enzyme:

Experimental set 1 Treatments –FT	Microbial Isolates from Garbage enzyme of Fruit peels					
	Amylase Activity	Protease Activity	Lipase Activity	Urease Activity	Cellulase Activity	Pectinase Activity
FT_1	2 B	2 B	2 B	2 B	2 B	1B
FT_2	2 B	2 B	2 B	2 B	2 B	1B
FT_3	2 B	2 B	2 B	2 B	2 B	2B
FT_4	2 B	2 B	2 B	1B	2 B	1B
FT_5	2 B	2 B	2 B	2 B	2 B	2B
FT_6	1 Fungus+	2 B	2 B	2 B	2 B	2B
FT_7	2 B	2 B	2 B	1 B	2 B	2B

Total isolates	**13 bacterial & 1 fungal isolates**	**14 bacterial isolates**	**14 bacterial isolates**	**12 bacterial isolates**	**14 bacterial isolates**	**11 bacterial isolates**
Experimental set 2 Treatments VT	**Microbial Isolates from Garbage enzyme of raw vegetables wastes**					
VT_1	**2 B**	**2 B**	**2 B**	**2 B**	**2 B**	**2B**
VT_2	**2 B**	**2 B**	**2 B**	**2 B**	**2 B**	**1B**
VT_3	**2 B**	**2 B**	**2 B**	**2 B**	**2 B**	**2B**
VT_4	**2 B**	**2 B**	**2 B**	**2 B**	**2 B**	**2B**
VT_5	**1B**	**2 B**	**2 B**	**2 B**	**2 B**	**1B**
VT_6	**2 B**	**1B**	**1B**	**2 B**	**1B**	**1B**
VT_7	**2 B**	**2B**	**1B**	**1B**	**1B**	**1B**
Total isolates	**13 bacterial isolates**	**13 bacterial isolates**	**12 bacterial isolates**	**13 bacterial isolates**	**12 bacterial isolates**	**10 bacterial isolates**

Note: Isolation was made based on highest hydrolysis zone and coloration on assay media by microbes. B- Bacteria, F-Fungi



Picture :2 Bacterial isolates from Garbage Enzyme

A total of 27 amylase producing bacteria, 27 protease producing bacteria, 26 lipase producing bacteria, 26 cellulase producing bacteria 24 urease producing bacteria, 21 pectinase producing bacteria and 1Amylase producing fungus was screened isolated from both the sets of experiment. Acetic acid is a key component of GE, which has an acidic character. Acetic acid, sugars, proteins, alcohol, and enzyme activity such as protease, amylase, lipase, and papain were detected by biochemical analysis of GE preparations, with pomegranate GE with highest potential (Sarabhai and Arya, 2019). Mosambi (Citrus limetta), Pomegranate (Punica granatum), Pineapple (Ananas comosus), Papaya (Carica papaya) and mixed fruits collected from fresh fruit stall and vegetable peels was collected from college's hostel. After fermentation, enzyme activity (amylase, protease, caseinase, cellulase and lipase) and antimicrobial effi cacy (S. aureus, S. aureus (ATCC 25923), Bacillus spp, Salmonella Typhi, E. coli, E. coli (ATCC 25922), Shigella spp, Pseudomonas aeruginosa) were analyzed. All the samples showed amylase and caseinase enzyme activity, only Pineapple (Ananascomosus), Papaya (Carica papaya) and Mixed fruit showed protease enzyme activity while only Pomegranate (Punicagranatum) showed lipase enzyme activity Neupane and Khadka, (2019).

Picture: 3 In vitro tray assays for Biodegradation of cow Dung

Table 4: The pH values

Treatments	1st week	2nd week	3rd week	4th week
FT1	8.80	8.77	8.04	6.95
FT2	8.59	8.32	7.76	6.87
FT3	8.44	8.33	7.71	6.76
FT4	8.59	8.37	8.14	6.88
FT5	8.4	8.32	7.80	6.50
FT6	8.47	8.41	7.98	6.62
C	8.30	8.38	7.86	6.78

Table 5: The Nitrogen content Kg/Hec

Sample	1st week	2nd week	3rd week	4th week
FT1	122.00	141.00	163.00	129.00
FT2	129.00	148.00	166.00	110.00
FT3	112.00	162.00	156.00	105.00
FT4	105.00	161.00	152.00	100.00
FT5	100.00	155.00	164.00	118.00
FT6	108.00	150.00	172.00	180.00
Control	141.00	154.00	122.00	114.00

Cow dung is a type of delayed-acting fertilizer because of its fine texture, higher water content, slow breakdown, and low calorific value. Cow dung can be processed using a variety of techniques and tools, particularly compost making technology to create a range of products with added value that are safe, resource-efficient, and beneficial to society while also preventing and controlling environmental pollution. The garbage enzyme especially fruit based fermented enzyme was used as biodegrading enzyme to easy and speed up its decomposition in in-vitro trial in tray assay. The pH values vary at the beginning of bio-composting in tray

assay and declined in the period of 4 weeks in all the treatments, ranged from 8.8 to 6.5 as the biodegradation proceeded. Table 4 indicates that treatment with garbage enzyme neutralized the alkaline pH of cow dung as garbage enzyme is acidic in nature. Nitrogen content increased as the biodegradation process proceed and found high in all the treatments in the third week and declined in the 4th week (Table 5). Highest Nitrogen (172 Kg/Hec) content was found when treated with garbage enzyme produced with banana peel.

https://compost-turner.net/composting-technologies/how-to-compost-cow-dung-manure.html

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]. Precaution must 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 to avoid any explosion due to high pressure exertion from the fermentation gas is released (Soo poey keat, 2011). It should not be stored in a refrigerator is suggested (Nazim and Meera, 2013). Mamma *et al*., (2007) mentioned that orange peel is a raw material which can be direct utilized in daily life such as animal feed and organic fertilizer. The use of orange peel waste as an organic fertilizer seems to be low cost if compared with chemical fertilizer which can pollute the soil. According to Rivas et al. (2008), orange peel composed of 16.9% soluble sugar, 9.21% cellulose, 10.5% hemicelluloses and 42.5% pectin. Orange peel has a potential valuable composition that can be developed into high quality products (Othman, 2013).

A study was conducted (Yuek Ming Ho *et al.,* 2013) to assess the implementation of garbage enzyme making and usage as an initiative to reduce the amount of municipal solid waste generated by a hawker community. They found that practical values, communal spirit, and awareness of environmental consequences were among the factors that encourage the practice of garbage enzyme making, while ignorance; time and convenience factors hinder garbage enzyme making and usage. They suggested that garbage enzyme could be used for biological recovery of organic waste and may provide a solution to waste minimization and reduction since a large proportion of municipal solid waste consists of food waste. 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). Pre-consumer waste from supermarkets, such as vegetables and fruits dreg are always discarded as solid waste and disposed to landfill. Implementing waste recovery method as a form of waste management strategy will reduce the amount of waste disposed. This study has been conducted by Rasit and Kuan, (2018) to produce and characterize biocatalytic garbage

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

enzyme and to evaluate its influence on palm oil mill effluent as a pretreatment process before further biological process takes place. the characterization of enzyme was conducted based on pH, total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), and enzyme activities. The influence of produced enzyme was evaluated on oil & grease (O&G), TSS and COD of palm oil mill effluent (POME). Different levels of dilution of garbage enzyme to POME samples (5%, 10%, 15%) were explored as pretreatment (duration of six days) and the results showed that the garbage enzyme contained biocatalytic enzyme such as amylase, protease, and lipase. The pre-treatment showed removal of 90% of O&G in 15% dilution of garbage enzyme. Meanwhile, reduction of TSS and COD in dilution of 10% garbage enzyme were measured at 50% and 25% respectively. The findings of this study are important to analyse the effectiveness of pre-treatment for further improvement of anaerobic treatment process of POME, especially during hydrolysis stage.

Tong and Liu, (2020) selected Apple peel, dragon peel and eggplant peel as to ferment for 6 months. Many active enzymes and microbiology flora were reported by them in GE. They used diluted (ratio of 1:800) self-made garbage enzyme to research its effect to improve soil nutrient. Soil was irrigated by dilution once in two days for four weeks. Their results illustrated that garbage enzyme gradually increased soil total nitrogen and organic matter with the increase irrigation time. Soil sample's total nitrogen of apple peel, dragon peel and eggplant begin to increase gradually and respectively peaked at 3.17 g/kg, 4.13 g/kg and 4.27 g/kg after 4 weeks irrigation. The content of total nitrogen is classified as the first level (>0.20 g/kg). After 4 weeks irrigation of dilution of garbage enzyme made from eggplant peel, the sample's organic matter begins to increase gradually and peaked at 49.33 g/kg after 4 weeks. The content of organic matter is higher than background (24.32g/kg) and classified as the first level (>40 g/kg). Sitong Gu et al., (2021), investigated the properties and application of Chinese honeylocust garbage enzyme (CHGE), which is produced when equal amounts of Chinese honeylocust fruits and fresh wastes are mixed. The results showed that CHGE had lesser microbial communities and lower surface tension than GE. CHGE also had higher viscosity, foam stability and emulsion stability than GE. Compared with GE, CHGE induced higher enzymatic amylase, cellulase, lipase and protease activities. CHGE had stronger detergency than GE and a 100× dilution of CHGE could significantly remove pesticide residues after a 30 min soaking treatment.

Conclusion: Garbage enzyme can be utilized as a low-cost alternative to enhance the composting processes of solid waste like cattle dung. 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 is a multipurpose solution for a range of uses, including fertilizer. Using and making our own garbage enzyme can reduce pollution load of our environment. Potential bacteria exhibiting biodegradation activity isolated from garbage enzyme can be identified and consortium of these bacteria can be prepared and applied on cow dung composting pile to enhance degradation process.

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