

Experimental Study on Treatment of Wastewater using Garbage Enzyme

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ABSTRACT: Garbage enzyme is a fermentation product. It is prepared from organic waste, water and brown sugar. It is prepared by fermentation of jaggery, organic waste and water mixture with the ratio of 1:3:10. It takes a period of three months. Bio catalytic activity of enzyme shows that it contains protease, amylase and lipase activities. Enzyme is capable of degrading protein, starch and lipids present in wastewater. This study assesses the effectiveness of different types of garbage enzymes prepared with different raw materials such as fruit waste, eicchornia and bagasse. Garbage enzyme solution is added directly in wastewater with different proportions in order to treat it. However it is observed that, there is no reduction in the Chemical Oxygen Demand (COD) of the wastewater. Hence another method is used which is termed as enzyme immobilization method. This method is applied to treat synthetic wastewater prepared in the laboratory. Sodium alginate beads are used for enzyme immobilisation. The immobilised enzymes are used and tested for treatment of synthetic wastewater. Study revealed that, the enzymes prepared using fruit waste found to be most effective for reducing COD up to 80%. Enzyme prepared using eicchornia found to be most effective for reducing Biochemical Oxygen Demand (BOD) up to 48% and Ammonia Nitrogen reduction is up to 77%. Enzyme prepared using bagasse found to be most effective for reducing phosphate up to 82%.

KEYWORDS: Bagasse, Eicchornia, Enzyme Immobilization Method, Fermentation, Fruit Waste, Garbage Enzyme, Wastewater.

I. INTRODUCTION

Increasing water demand, disposal of sewage and waste management issues are becoming more and more important. There is an urgent need of developing low cost and efficient treatment methods to maintain environmental quality. There are many approaches attempted by many researchers on these kinds of issues. One of the approaches used by many researchers is to use garbage enzymes to treat wastewater. Literature surveys have shown such attempts but there are some areas where still the research needs to be done to develop a treatment method which will be reliable, efficient and economical. The garbage enzyme is a composite mixture developed by fruit peels, vegetable peels, sugar and water and according to Tang and Tong, (2011) the process requires the period of three months. The garbage enzyme is brown yellowish in colour, has sour test and fragmented. Garbage enzymes are mainly proteins of biological genesis that specially raise the rate of chemical reaction by reducing energy of activation without getting changed during the reaction and also does not disturb the equilibrium of reaction. Enzymes lower the energy of transition state and in the state all the species are less stable. The present study is aimed to (i) prepare garbage enzyme as per laboratory procedure and determine its characteristics, (ii) evaluate the wastewater quality at the selected station over a period of time and (iii) investigate potential of treatment of wastewater using garbage enzyme under different conditions.

II. MATERIAL AND METHODS

The garbage enzymes are prepared with the use of waste biomaterials and as per procedure given by Rosukon (2006) that is by fermentation / anaerobic oxidation. There are two methods used in the present study to treat wastewater using garbage enzyme namely, Method - 1: Treatment of domestic wastewater by direct garbage enzyme and Method - 2: Treatment of synthetic wastewater with immobilized garbage enzyme.

Raw Materials:

1. Vegetable and fruit dreg that mainly contain tomato, pineapple, lemon, orange all dregs taken in equal quantity.
2. Bagasse collected from sugarcane processing industry.
3. *Eicchornia* collected from Rankala Lake in Kolhapur city.
4. Jaggery.
5. Distilled water

b. Garbage Enzyme Production and Characterization:

The garbage enzymes are prepared using following procedure:

1. Three parts of wastes are combined with 1 part of jaggery, 10 parts of water in air tight containers. For preparing 6 L of garbage enzyme, 1.8 kg of vegetable and fruit biomass is fermented jointly with 1 kg jaggery and 6 L of sterile distilled water. Similarly 1.8 kg bagasse is used as biomass and prepared bagasse enzyme. 1.8 kg of *Eicchornia* is used to prepared *Eicchornia* enzyme.
2. Biomaterial derived from the plant *Eicchornia* examined for the absorption of heavy metals. It has been observed that in root, stem and leaves, Pd, Mn, Fe, Cu, Co, Cd ions get absorbed. In this study the root, stem and leaves samples of the plant are collected from the Rankala Lake in Kolhapur city. Then it is finely chopped and used as biomass for preparing enzyme.
3. Bagasse is a by product available from sugarcane industry. It is diversified fibrous surplus that remains after sugarcane juice extraction.
4. Peroxidase enzyme is isolated from sugarcane. The treatment with peroxidase enzyme leads to the lowering of total solids and BOD. Because of this it is used for preparing the bagasse enzyme.
5. As per the procedure given by Rosukon, (2006), water used for preparing enzyme must be chlorine free. Thus distilled water is used in enzyme preparation and jaggery is used without any chemical. These ingredients are added with the ratio of 1:3:10.

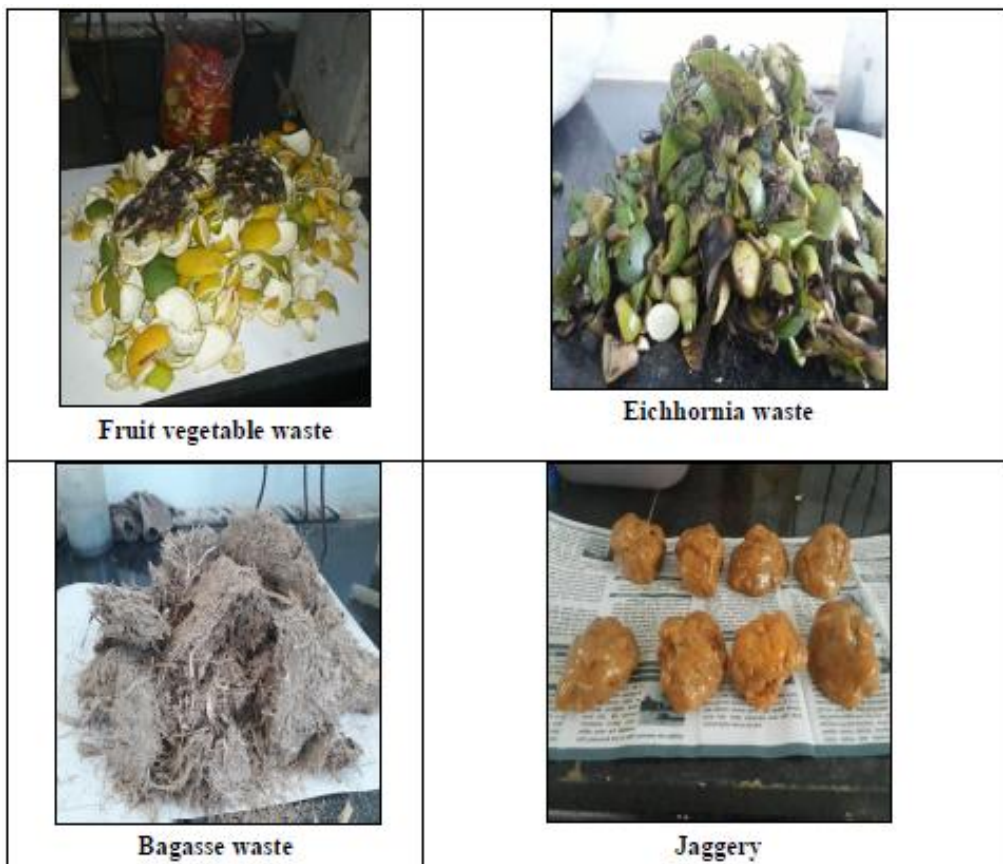


Figure 1: Raw Materials used for Preparation of Garbage Enzymes

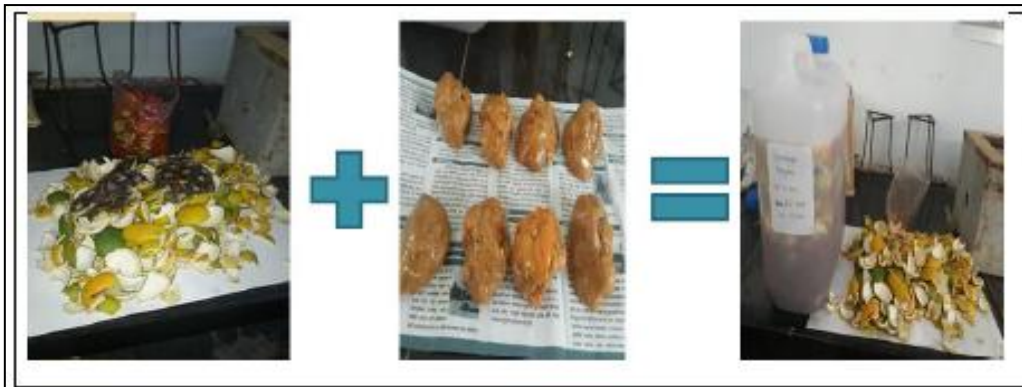


Figure 2: Garbage Enzyme Preparation



Figure 3: Containers used to Store the Material for Preparation of Garbage Enzymes

c. Treatment of Domestic Wastewater:

1. Batch test carried out in which 4 conical flask are filled with 250 ml wastewater sample in each.
2. 10 ml Enzyme solution is centrifuged at 4°C, 10000 rpm for 10 min. The supernatant is used for further process.
3. Firstly 25% of enzyme is added in each flask to avoid cross contamination. In one flask add 25% fruit vegetable enzyme (FE), second flask add 25% *Eicchornia* enzyme (EE), third flask add 25% bagasse enzyme (BE). Fourth flask is kept as blank without adding garbage enzyme (GE).
4. These flasks are covered with cotton plug and wrapped with Para film M sheet and kept for digestion of 5 days.
5. The parameters like pH, COD, BOD are examined in the laboratory for all the samples as per standard methods.

d. Preparation of Synthetic Wastewater:

The synthetic wastewater is prepared by adding the compounds in defined concentration in distilled water (Nasim, and Meera), 2013.



Table 1: List of Compounds used in Synthetic Wastewater

Sr. No.	Compounds	Quantity (mg/L)
1.	Glucose	300
2.	Sodium acetate tri hydrate	400
3.	Ammonium chloride	225
4.	Disodium hydrogen phosphate	150
5.	Potassium di hydrogen phosphate	75
6.	Magnesium sulphate	50
7.	Cow dung	225

e. Working Procedure for Treating Synthetic Wastewater:

1. Entrapment method for immobilization of garbage enzyme is used for treatment of synthetic wastewater.
2. 10 ml of partially purified (Ammonium Sulphate Purification) enzyme is immobilised by using gel entrapment method. Sodium Alginate gel is used for beads formation in 4% Sodium Alginate gel 10 ml of partially purified enzyme is mixed.
3. This mixture is added drop wise in chilled Calcium Chloride solution by using sterilized syringe.
4. Batch test is carried out in four conical flasks which are filled with 100 ml synthetic wastewater.
5. Sodium Alginate beads are added in the three flasks.
6. After 5 days the beads are separated and the solution is centrifuged for 10 min at 10000 rpm and used to determine the parameters pH, COD, BOD, Phosphate and Ammonia Nitrogen.

III. EXPERIMENTAL RESULTS

The results obtained by two methods, Method - 1: Treatment of domestic wastewater by garbage enzyme and Method - 2: Treatment of synthetic wastewater with immobilized garbage enzyme, are given below. It is observed that, Method – 2 shown good results.

a. Characteristics of Garbage Enzymes:

The characteristics of each type of garbage enzyme are checked after the fermentation process. All enzymes are acidic in nature and pH values are within the range of 2.4 - 2.7. This is because enzyme solution contains organic and volatile acid.

Table 2: Characteristics of Garbage Enzyme Solution

Sr. No.	Parameters	Fruit Enzyme	<i>Eicchornia</i> Enzyme	Bagasse Enzyme
1.	pH	2.55	2.40	2.70
2.	TDS(mg/l)	13000.00	5333.33	11000.00
3.	TSS(mg/l)	12333.33	10000.00	1333.40
4.	COD(mg/l)	56000.00	40000.00	38000.00

b. Effluent Characteristics after Treatment of Domestic Wastewater:

The domestic wastewater is treated using 25% of garbage enzyme. Then it is left for digestion period of 5 days and every day COD analysis is carried out. For first cycle of 5 days digestion period, the wastewater has initial COD as 120 mg/L and for second cycle of 28 days digestion period, the wastewater has initial COD as 180 mg/L. After digestion period it is observed that, COD values increased drastically. The result indicated that, garbage enzyme contains high amount of organic matter. This happened because garbage enzyme is not purified and domestic wastewater also contains organisms which inhibit the enzyme activity.

Table 3: COD Values for First Cycle of 5 Days Digestion Period

Duration (Days)	COD Values (mg/L)		
	Fruit Enzyme	Eicchornia Enzyme	Bagasse Enzyme
0 th	-	-	-
1 st	32000	26000	34000
2 nd	12400	19200	18000
3 rd	28800	26000	18000
4 th	26000	9200	8800
5 th	8400	8000	8100

Table 4: COD Values for Second Cycle of 28 Days Digestion Period

Duration (Days)	COD Values (mg/L)		
	Fruit Enzyme	Eicchornia Enzyme	Bagasse Enzyme
0 th	-	-	-
1 st	11000	10000	20000
2 nd	19000	20000	22000
3 rd	9000	3600	4000
4 th	2000	2400	3600
5 th	1100	2200	2000
6 th	900	1800	2200
7 th	850	1200	1100
8 th	750	900	850
9 th	700	850	1100
10 th	900	2000	2000
11 th	1200	2200	3600
25 th	1100	3600	4000
26 th	18000	12400	8800
27 th	19000	15000	11000
28 th	20000	16000	10000

c. Characteristics of Synthetic Wastewater:

The synthetic wastewater is prepared by adding the compounds in defined concentration. The pH of synthetic wastewater is alkaline in nature.

Table 5: Characteristics of Synthetic Wastewater

Sr. No.	Parameters	Value (mg/L)
1.	pH	8.21
2.	TDS(mg/L)	560.00
3.	Chemical oxygen demand (mg/L)	666.00
4.	Biochemical oxygen demand (mg/L)	167.00
5.	Ammonia Nitrogen (mg/L)	10.600
6.	Phosphate (mg/L)	12.00

d. Effluent Parameters after Treatment of Synthetic Wastewater using Immobilization of Garbage Enzyme:

From the analysis it is observed that, when garbage enzyme is in direct contact with domestic wastewater the treatment is not efficient. This may be due to the fact that, the garbage enzyme used for treatment of domestic wastewater is not purified, percentage of addition of enzyme is more and also in domestic wastewater organisms are more because of which enzyme catalytic activity inhibited. Therefore, to overcome this problem, enzyme immobilization technique is used in the present study. Instead of domestic wastewater the synthetic wastewater is treated using partially purified garbage enzyme. Entrapment method is used and Sodium Alginate gel is used for entrapment. With the help of beads, synthetic wastewater is treated as shown Tables 6, 7 and 8.

Table 6: Characteristics of Treated Synthetic Wastewater by using Immobilized Fruit Enzyme (FE)

Parameter	1 day	3 days	5 days
pH	4.42	5.22	6.34
COD(mg/L)	600.00	333.00	133.00
BOD(mg/L)	260.00	190.00	110.00
Ammonia Nitrogen(mg/L)	5.20	4.60	3.30
Phosphate (mg/L)	5.70	4.80	3.40

10 ml of partially purified fruit enzyme is used for formation of beads for the treatment of synthetic wastewater. The pH value of garbage enzyme is acidic when it is mixed with synthetic wastewater. The pH is increased from 4.42 to 6.34 over the period of 5 days. The BOD and COD values are decreased to some extent. It is observed that among all enzymes, COD removal efficiency of fruit enzyme is more.

Table 7: Characteristics of Treated Synthetic Wastewater by using Immobilized Eicchornia Enzyme (EE)

Parameter	1 day	3 days	5 days
pH	4.20	5.80	6.70
COD(mg/l)	533.00	400.00	200.00
BOD(mg/l)	150.00	130.20	87.00
Ammonia Nitrogen(mg/l)	4.20	3.80	2.50
Phosphate (mg/l)	4.40	3.60	2.70

10 ml partially purified *Eicchornia* enzyme is used in the form of beads for the treatment of synthetic wastewater. The pH value of garbage enzyme is acidic when it is mixed with synthetic wastewater. The pH value is increased from 4.20 to 6.70 over the period of 5 days. The *Eicchornia* enzyme shows more removal efficiency for BOD and Ammonia Nitrogen.

Table 8: Characteristics of Treated Synthetic Wastewater by using Immobilized Bagasse Enzyme (BE)

Parameter	1 day	3 days	5 days
pH	4.70	5.21	6.50
COD(mg/l)	466.00	400.00	266.00
BOD(mg/l)	190.00	120.00	96.00
Ammonia Nitrogen(mg/l)	4.60	3.90	2.80
Phosphate (mg/l)	3.80	3.50	2.20

10 ml partially purified bagasse enzyme is used in the form of beads for the treatment of synthetic wastewater. The pH value of garbage enzyme is acidic when it is mixed with synthetic wastewater. The pH is increased from 4.70 to 6.50 over the period of 5 days. The value in the Table 8 shows that, phosphate removal efficiency of bagasse enzyme is increased over the period of 5 days.

e. Removal Efficiencies:

- 1. Removal Efficiency for COD from Synthetic Wastewater using Garbage Enzymes:** In fruit enzyme COD concentration has been significantly decreased during the 5 days digestion period. It is observed that, the COD removal rate is less in case of *Eicchornia* and bagasse enzymes as compared to fruit enzyme. It is observed that, fruit enzyme found to be the most effective in removing COD and is up to 80%.
- 2. Removal Efficiency for BOD from Synthetic Wastewater using Garbage Enzymes:** The BOD removal rate of garbage enzyme is less as compared to removal rate of Ammonia Nitrogen and Phosphate. It is observed that, the *Eicchornia* enzyme found to be the most effective in removing BOD and is up to 48%.
- 3. Removal Efficiency for Ammonia Nitrogen from Synthetic Wastewater using Garbage Enzymes:** The garbage enzymes show good efficiency for the removal of Ammonia Nitrogen from synthetic wastewater. It is observed that, the *Eicchornia* enzyme found to be the most effective in removing Ammonia Nitrogen and is up to 77%.

4. **Removal Efficiency for Phosphate from Synthetic Wastewater using Garbage Enzymes:**The garbage enzymes show good efficiency for the removal of phosphate from synthetic wastewater. It is observed that the bagasse enzyme found to be the most effective in removing Phosphate and is up to 82%.

IV. CONCLUSIONS

The study emphasizes on the preparation of garbage enzyme from fruits, *Eicchornia*, and bagasse waste to treat the wastewater. From fermentation process enzymes are prepared and tested for bio catalytic activity. From results it is found that enzyme exhibit bio catalytic activity. It can be used to decay the carbohydrates, lipids and proteins which are present within wastewater. Based on results of parameters such as BOD, COD, Ammonia Nitrogen, and Phosphate, the study revealed that, garbage enzymes show a significant evidence of effectiveness and efficiency. The findings of this study show that, garbage enzyme with partial purification and immobilization proved to be the efficient for the treatment of wastewater. The present study has been conducted on synthetic wastewater. Further research can be carried out by using different characteristics of wastewater. The efficiency of immobilization depends on various factors that include immobilization method, reaction time and mixing condition. Hence further research work can be carried out on efficiency of immobilization technique.

REFERENCES

- [1] Arun, C., & 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*, 94, 471-478.
- [2] Arun, C., & Sivashanmugam, P. (2015). Identification and optimization of parameters for the semi-continuous production of garbage enzyme from pre-consumer organic waste by green RP-HPLC method. *Waste Management*, 44, 28-33.
- [3] Anamika D.P., Govind M Suresh, Litty G. Das, Dhanyalekshmi C.S. (2019). Organic Wastewater Treatment using Enzyme Immobilization. *International Research Journal of Engineering and Technology*, 6 (3), PP. 5534-5539.
- [4] Dwevedi, A. (2016). Enzyme Immobilization: Solution Towards Various Environmental Issues. In *Enzyme Immobilization* (pp. 87-106). Springer, Cham.
- [5] Gouri J Pilla, Duithy George. 2017. Fruit Peel Enzyme Solution for Synthetic Sewage Treatment. *International Journal for Research in Technological Studies*, Vol. 4, Issue 6, PP. 70-73.
- [6] <http://www.enzymesos.com/what-is-eco-enzyme/how-to-make-eco-enzyme>
- [7] Jung-Hoo Min, Young-Joon Kim, Ian Fong, Low Yu Teng, Hae-In Seo, Chia Hui Peng. (Undated). Using Enzymes from Recycled Fruit Wastes for Water Treatment.
- [8] Krishna, P. N. (2011). Enzyme technology: pacemaker of biotechnology. PHI Learning Pvt. Ltd.
- [9] Nazim, F., & Meera, V. (2013). Treatment of greywater using Garbage Enzymes for reuse applications. In *Proceedings of International Conference on Materials for the Future-Innovative Materials, Processes, Products and Applications*.
- [10] Nazim, F., & Meera, V. (2017). Comparison of treatment of greywater using garbage and citrus enzymes. *International Journal of Innovative Research in Science, Engineering and Technology*, 6(4).
- [11] Neupane, K., & Khadka, R. (2019). Production of Garbage Enzyme from Different Fruit and Vegetable Wastes and Evaluation of Its Enzymatic and Antimicrobial Efficacy. *Tribhuvan University Journal of Microbiology*, 6, 113-118.
- [12] Penmatsa, B., Sekhar, D. C., Diwakar, B. S., & Nagalakshmi, T. V. (2019). Effect of Bio-Enzyme in the Treatment of Fresh Water Bodies. *International Journal of Recent Technology and Engineering*, Volume- 8 Issue-1S3, PP. 308-310.
- [13] Rasit, N., & Mohammad, F. S. (2018). Production and Characterization of Bio Catalytic Enzyme Produced from Fermentation of Fruit and Vegetable Wastes and its Influence on Aquaculture Sludge. *Matter: International Journal of Science and Technology*, 4(2).
- [14] Robinson, Gary. (2010). *Methods in biotechnology—immobilization of enzymes and cells*; Edited by G F Bickerstaff. pp 367. Humana Press, New Jersey. 1996 ISBN 0-89603-386-4. *Biochemical Education*. 25. 232 - 232.
- [15] S. Grewal and P. Mutha, *Enzyme technology*, Vedams eBook Pvt. Ltd, 2010.
- [16] Tang, F. E., & Tong, C. W. (2011). A study of the garbage enzyme's effects in domestic wastewater. *World Acad Sci Eng Technol*, 60, 1146-8.
- [17] Whiteley, C. G., & Lee, D. J. (2006). Enzyme technology and biological remediation. *Enzyme and Microbial Technology*, 38(3-4), 291-316.