Research Article

Journal of Environmental Nanotechnology

Enhancing Shelf Life Storage Stability of Anaerobically Fermented Garbage Enzyme by Concentrating through Membrane Separation

A. Ashokkumar*, P. Mala, C. Arun and K. Geetha

Department of Biotechnology, Periyar Maniammai Institute of Science and Technology, Vallam, Thanjavur, TN, India

Received: 04.05.2024 Accepted: 19.06.2024 Published: 30.06.2024

*ashokkumar@pmu.edu

ABSTRACT

Enzyme solutions derived from garbage have many applications in the surfactant industry, industrial and municipal sludge stabilization, bio-fertilizer, bio-herbicide and bio-pesticide production, etc. Once the produced solution is taken into an aerobic environment, it induces microbial growth which may in turn produce desirable and undesirable biochemicals. To control the activation of biochemicals present in garbage-fermented solution, a membrane separation process was investigated. Fabricated Polyvinylidene Fluoride (PVDF) membrane separation set up with 0.45 µm pores produces a bifurcated yield of concentrate and water, which is a similar process as in a domestic water purification unit. As the water part is reduced by membrane separation from the enzyme solution the reaction with oxygen is controlled in a concentrated part which in turn reduces the hydrolysis reaction. The inactive condition was investigated by altering the pH of the system, which also reduced BOD and COD the main impact of increasing the stability of the enzyme. The experiment investigation is executed, discussed, and reported for the transport process parameters on the membrane process and control investigation by the parameters pH, BOD and COD. This is a trial attempt to check the perception of how far the membrane separation process works on concentrating the garbage-produced enzyme solution as well as reducing the rate of degradation while in contact with air. Also, the activity of protease, lipase, and amylase was increased in the concentrated garbage enzyme.

Keywords: Concentration; COD; BOD; Enzyme; Garbage; Membrane process; pH.

1. INTRODUCTION

The garbage enzymes derived from waste organic sources help in multiple directions such as the conversion of various value-added products, development of the rural technology and rural economy, and suppression of greenhouse gases production. However, bulk-produced enzymes cannot be utilized at a single time, nor are they always required by the same production unit for other applications. Garbage enzyme represents a circular economy approach, where it can be supplied as a raw material to various bioproduct industries. At this stage, maintaining the stability of the garbage enzyme is essential by preventing undesired conversions caused by exposure to water and oxygen.

Meanwhile, it is meaningless to spend higher costs on the design and fabrication of an exclusive storage tank for waste-derived garbage enzyme protection. Since the garbage enzyme is a raw source of many value-added products, it has significance in storage without biochemical activity with oxygen or further hydrolysis activity. But in general, garbage enzymes possess hydrolytic enzyme activity that leads to undesired biochemical activity during the normal storage period (Arun *et al.* 2017). The pH of garbage enzymes plays a vital role in enzyme activity at around 6 to 7 by bringing desired or undesired chemicals and fungus formation (Arun *et al.* 2015b). Previous studies have reported that garbage enzymes exhibit optimal biochemical activity at pH 6–7; therefore, reducing the pH can serve as an effective control strategy. Therefore, concentrating the garbage enzyme or removing its water content can help reduce the pH. The methods for concentrating garbage enzymes can be adapted from the following studies.

Fruit juice concentration by membrane process provides stability in microbial and shelf life packaging (Bhattacharjee *et al.* 2017). Ultrafiltration, microfiltration, nanofiltration, and reverse osmosis are effective alternatives for concentration processes in the juice and beverage industry. These membrane techniques help preserve nutritional value while enhancing shelf life, stability, and organoleptic properties during storage and packaging.

Enzyme membrane bioreactors are effective for the separation of products such as high-molecular-weight hydrolysis-derived carbohydrates, oligosaccharides, and for the bioconversion of compounds like insulin, lignin,

and sugars. A key advantage of these systems is that the membrane separation process yields products with enhanced stability (Padhan *et al.* 2023). Another work reported that membrane filtration affects the enzymatic hydrolysis and biological activity of potato juice. In this report, the authors discussed how membrane separation influences hydrolysis and biological activity during product conversion; however, from a storage perspective, this property is referred to as stability (Kowalczewski *et al.* 2021). Microfiltration and Ultrafiltration are widely used membrane separation processes in the fruit juice industry due to the minimal pressure and temperature as well as the economical process with effective control stability (Katibi *et al.* 2023). As a membrane can separate the core particles from the liquid, it can be expected that it will break the unwanted biochemical activities due to hydrolysis (Servent *et al.* 2020). Hydrolysis in the enzyme makes multiple protein activities where the reducing water content declines or restricts the activity of hydrolysis. Hence, the basic process was chosen to remove the water content (Nongonierma *et al.* 2017). The enzymatic membrane reactor is widely used in various fields of biotechnology, agro, food, energy, and environment. The design and fabrication of an enzymatic bioreactor can be adapted for the membrane separation for the garbage enzyme application (Sitanggang *et al.* 2022). Fouling and microbial community growth at membrane pores are significant drawbacks of this system. Meanwhile, Polyvinylidene Fluoride (PVDF) membrane reported low fouling with the enzyme process (Schmidt *et al.* 2022). Hence, the same membrane system was integrated with the present system. Membrane reactors generally operate by maintaining contact between the enzyme and substrate to generate various products. In contrast, the present study is designed not to promote substrate interaction but to enable selective water separation, employing bifurcated outlets that yield concentrate and water streams (Prazeres *et al.* 1994). The mixed vegetable peal garbage-derived enzyme was used to suppress the Total Suspended Solids (TSS), BOD, and COD in dairy effluent for stabilizing purposes (Sambaraju *et al.* 2020). Hence, it proves that reducing pH, BOD and COD increases the stability of effluent means it is expected that it also increases the shelf-life stability of the enzyme. The advantage of this system is a simple continuous process that can execute effectively at room temperature and is economically affordable for the cottage industry level too. The shelf-life is extended well for the concentrated garbage enzyme obtained from the membrane separation process. The adapted membrane separation system applied to the concentrate obtained from the garbage enzyme process reduces the rate of deterioration, as demonstrated in the present study. In addition, the concentrated garbage enzyme being reduced in the total volume by an average of around 33% (in the present work from 20 liter of raw garbage enzyme yielded 13.4 liter concentrated garbage enzyme) helps ease compact packages for longer time storage and shipping in a protective environment.

2. MATERIALS AND METHODS

The garbage enzyme used in the present stabilization study was produced by a batch process using a weight ratio of 1:3:10 (molasses:citrus fruit peels:water) and incubated under optimal anaerobic conditions for a period of three months (normal cloth filtered supernatant part only). Upon relaxation of anaerobic incubation conditions, the generated garbage enzyme is exposed to air within 15–20 days, resulting in gradual deterioration due to the development of aerobic microbial communities. Since garbage enzyme has wide applications in various industrial production and waste treatment processes, bulk storage without alteration of its characteristics is essential. The water content present in the garbage enzyme is a primary factor contributing to the development of microbial communities and other forms of degradation, particularly through interaction with foreign particles present in air. Therefore, the removal of water is expected to limit biochemical interactions with air, thereby enhancing the stability and activity of the garbage enzyme. To achieve water removal, a PVDF bifurcated membrane separation system with a pore size of 0.45 µm was assembled and operated in continuous mode, as shown in Fig. 1.

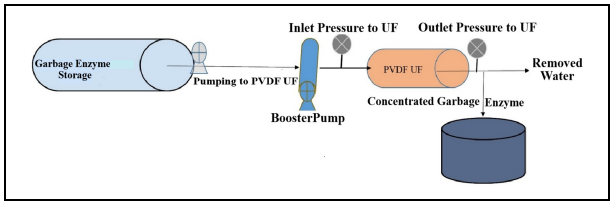


Fig. 1: Simple PVDF membrane UF setup for the enzyme concentrating process

In general, garbage enzyme is produced by adding ordinary tap water or recycled water to utilize waste within a circular economy framework. A PVDF UF system was assembled, which is capable of operating over a pH range of 2–10 and exhibits high mechanical strength, chemical resistance, and excellent stability at elevated temperatures.

The hollow fiber membrane with a pore size of 0.45 µm was employed as a downstream processing unit to facilitate the separation of water from the core enzyme constituents, thereby enhancing the shelf life of the garbage enzyme. Approximately 20 L of citrus fruit peel-derived garbage enzyme, produced *via* a batch process, was stored in a horizontally oriented cylindrical holding tank. The setup was connected to a peristaltic pump via a 0.8 mm diameter tubing and subsequently coupled with a booster pump leading to a bifurcated PVDF membrane separation unit. Two bourdon tube pressure gauges were fixed at the inlet and outlet of the PVDF membrane

separation unit exhibiting 0.40-0.50 kg/cm^2 to flow 5.55 mL/s rate where within one hour it completed the separation process for 20 liters of feed. Recycle for further concentration was not preferred to compute the effect at minimum cost. The filtered concentrated stream (concentrated enzyme) is removed at one side and separated water content flows out another side for other usages with 0.5 mm dia of a tube. The characteristics of the enzyme before and after concentration are collected in Table 1.

Table 1. Characteristics of garbage enzyme before and after the concentration process

Properties	Raw Garbage Enzyme	Concentrated Garbage Enzyme
pH	5.4-5.8	2.9-3.3
TDS (mg/L)	868-1011	1029-1067
TSS (mg/L)	921-1055	1007-1092
BOD (mg/L)	61-66	57-61
COD (mg/L)	150-161	148-156
MPN (C.F.U/mL)	<3	<3
Acetic Acid (mg/L)	3.763-4.384	4.213-5.189
Density	1.07-1.13 g/cm^3	1.19-1.25 g/cm^3

Large-scale production (in this work approx.10 liter and above) varied with higher values in all above properties from the lower quantity or laboratory level production (below 10 liter) (Arun *et al.* 2015b; Sambaraju *et al.* 2020) is a significant result in the uncontrolled simple batch process of garbage enzyme. In Table 1, bulk production properties are listed as raw garbage enzyme. It is observed that though complete fermentation is obtained by producing acetic acid finally, the fungus forms once exposed to air after some days due to near neutral pH. Hence, both bulk raw and concentrated were characterized and tested the fungal formation (since the qualitative approach did not consider the types of fungal groups). All the characterization was performed under a standard procedure with standard instrumentation. At room temperature, pH of the raw garbage enzyme fell to 5.4–5.8 and the concentrated enzyme turned to a low value of 2.9-3.3 which is equal to laboratory-level production in a small unit (Arun *et al.* (2015b); Sambaraju *et al.* (2020). Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) increased in values in concentrated form than raw product, which could be the reason of loosing of water content. Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are determined at almost the same values for both the samples raw and concentrated. The presence of acetic acid is moderately inclined in concentrated than raw; the reason must be the same as happened for TDS and TSS. Most Probable Number (MPN) reports permissible limit values for both. The biocatalytic activity of garbage enzyme at raw and concentrated was investigated as per the procedure followed in Arun *et al.* (2015b), explained briefly in the result and discussion section. The removed water content has a pH of 6.8, TDS at 82 mg/L, TSS at 10 mg/L, and density meets with 0.98g/cm^3. Flow parameters were studied in the concentrating process of ultrafiltration in terms of density, viscosity, rheological characteristics, pressure, Reynolds number, percentage of permeation and fouling. Raw, concentrated enzymes and removed water content are shown in Fig. 2.

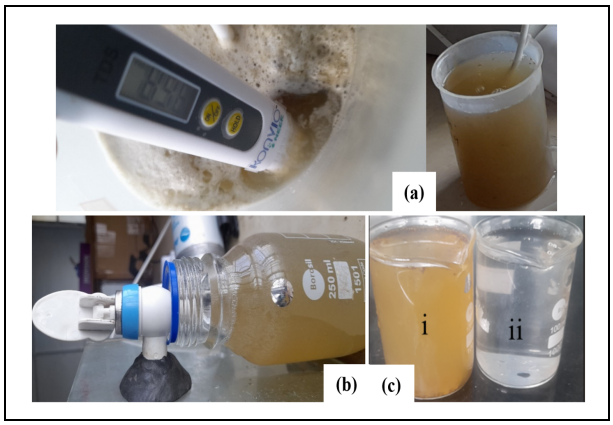


Fig. 2: (a) Raw garbage enzyme, (b), Concentrated garbage enzyme, (c) i-Concentrated enzyme and ii- Removed water

3. RESULTS AND DISCUSSION

3.1. Process and Flow Conditions

In this work, a simple continuous process is proposed to concentrate the anaerobically produced garbage enzyme. It passes through the 0.45 µm PVDF membrane built with online detectors of TDS, TSS, and pH ultrafiltration process. The concentrating stream was rich in organic compounds and the removed water content was obtained with close to drinking water quality. The ultrafiltration process was operated at only one cycle not tent to recycle to set the process at low energy cost. For 60 minutes of the process run by continuously adding raw garbage enzyme, there was no significant change in TDS and TSS for the entire run period in the respective concentrated and raw enzyme. However, the variations in TDS and TSS were obtained between the concentrated and removed streams. The variations occurred in inlet and outlet pressure indirectly help to calculate the apparent transmembrane pressure, which gives the strength of membrane at continuous operating load. Inlet flow entered at the rate of 5.5 mL/s for one hour of operating period for 20 liter. Hence, this small assembled setup can operate at about 480 liter capacity per day in room temperature. The approximate garbage enzyme fluid transport properties were investigated based on the existing conditions during the operation. The inlet and outlet connected pressure gauge measured at kg/cm^2 and then converted to kPa. Inlet and outlet circumstances are only considered to probe flow parameters. The indirectly explored values may deviate quantitatively from the original in a slight manner but qualitatively it exhibits a perfect outcome of transmembrane pressure and also it supports anticipating

the fouling rate. The estimated transport operation parameters are listed in Table 2. The behavior of pressure variations qualitatively appeared with predicted values in Fig. 3. The transmembrane pressure was calculated from the standard formula by applying inlet pressure as feed, outlet pressure as permeate, and for retentate pressure value, the sum of inlet and outlet divided by 2 was taken (Pimentel *et al.* 2017).

The influence of fouling factors on the membrane is unavoidable. Despite the potential of membrane stability and flexibility, the new membrane was kept under laminar flow conditions (explained in section 3.2) as well for one-hour running process was not obtained worthy of attention level effect which is qualitatively interpreted in Fig. 4.

Table 2. Computed transport operation properties as Reynolds number

Process Streams	Re @ 0.8 mm D	Re @ 0.5 mm D	Re @ 0.000045 µm D MBR Membrane
Raw GE	890.1	562	0.05941
Concentrated GE	859.1	524.71	0.0006085
Removed Water	33.10	20.485	0.00005565

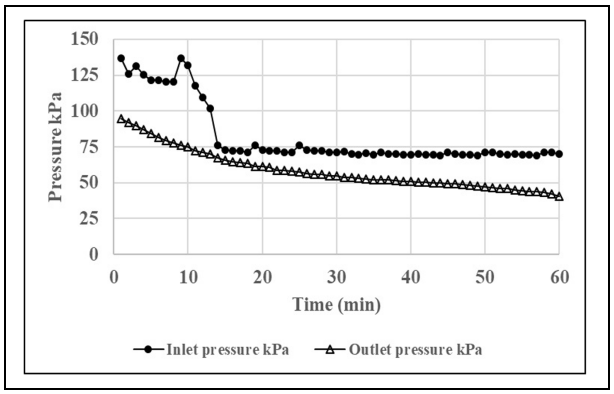


Fig. 3: Pressure behaviour between inlet and outlet

3.2. Study on Rheological Properties

The viscous flow behavior of garbage enzyme and its concentrated and removed water content products assist in the appropriate membrane selection and process flow. In addition, the behavior of the flow pattern against the shear force obliges in the design and selection of pumps, and pipelines followed by valves for the respective membrane processes. Since the nature of the garbage enzyme has a moderate density almost equal to the water the rheological measurements were executed in a 1.12 mm measuring gap (inner radius 13.331 mm, outer radius 14.450 mm) at Anton Paar MCR 302 Rheometer. The results are reported on viscosity against varying shear as well as stress in Table 3 and Fig. 5.

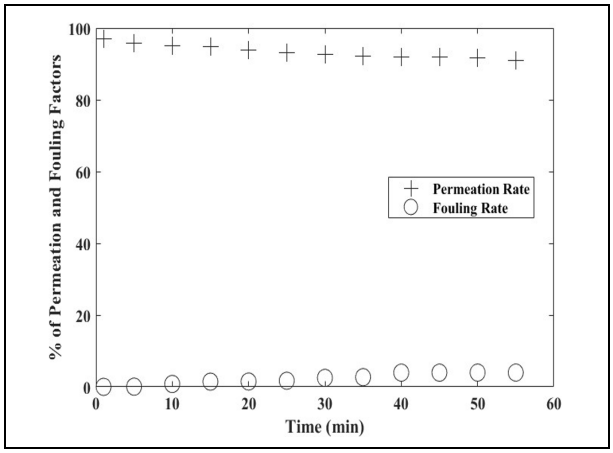


Fig. 4: Percentage of permeation and fouling factors of garbage enzymes

Table 3. Rheological study data on three different process streams

Process Streams	Viscosity (Pa.s)	Shear Stress (Pa) Average
Raw GE	0.0026-0.0029	5.4
Concentrated GE	0.0030-0.0033	3.2
Removed Water	0.0010-0.0012	1.0

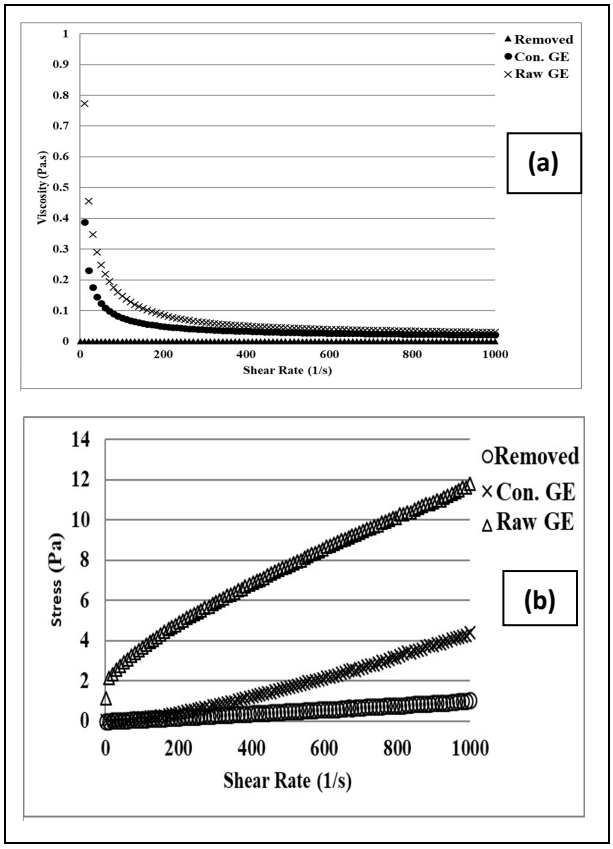


Fig. 5: Flow behavior at shear rate against (a) viscosity, (b) stress

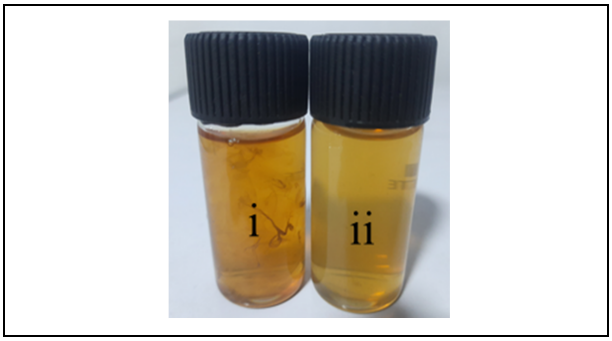


Fig. 6: After 15th day (i) Raw (ii) Concentrated garbage enzyme

3.3 Shelf-life Storage Test Against Fungal Formation in Garbage Enzymes

A straightforward test was performed to evaluate the shelf life of raw and concentrated garbage enzymes kept in an open container for monitoring for some days. Before that, both raw and concentrated were diluted with phosphate to make pH 7. By the progress of days to around the 15th day a white fungal started to form at raw garbage enzyme but even after 20 days nothing happened in concentrated garbage enzyme, as displayed in Fig. 6.

3.4 Biocatalytic Activity between Raw and Concentrated Garbage Enzyme

To investigate this activity, the same system followed by Arun *et al.* (2015b) was adapted for the protease, lipase, and amylase performance. As per the report of the above article, the protease, lipase, and amylase exhibited extreme activity at a pH of around 6.5, and minor activity at a lower pH around 3.5. In the present work, raw garbage enzyme has a pH of around 5.8, other side concentrated has 3.5. To ensure a uniform pH during activity measurement, both the raw and concentrated samples were diluted with phosphate buffer to pH 7. Under these conditions, the concentrated sample exhibited higher activity, whereas the raw sample showed comparatively lower activity. The enhanced activity of the concentrated garbage enzyme can be attributed to its higher purity at this stage (Fig. 7).

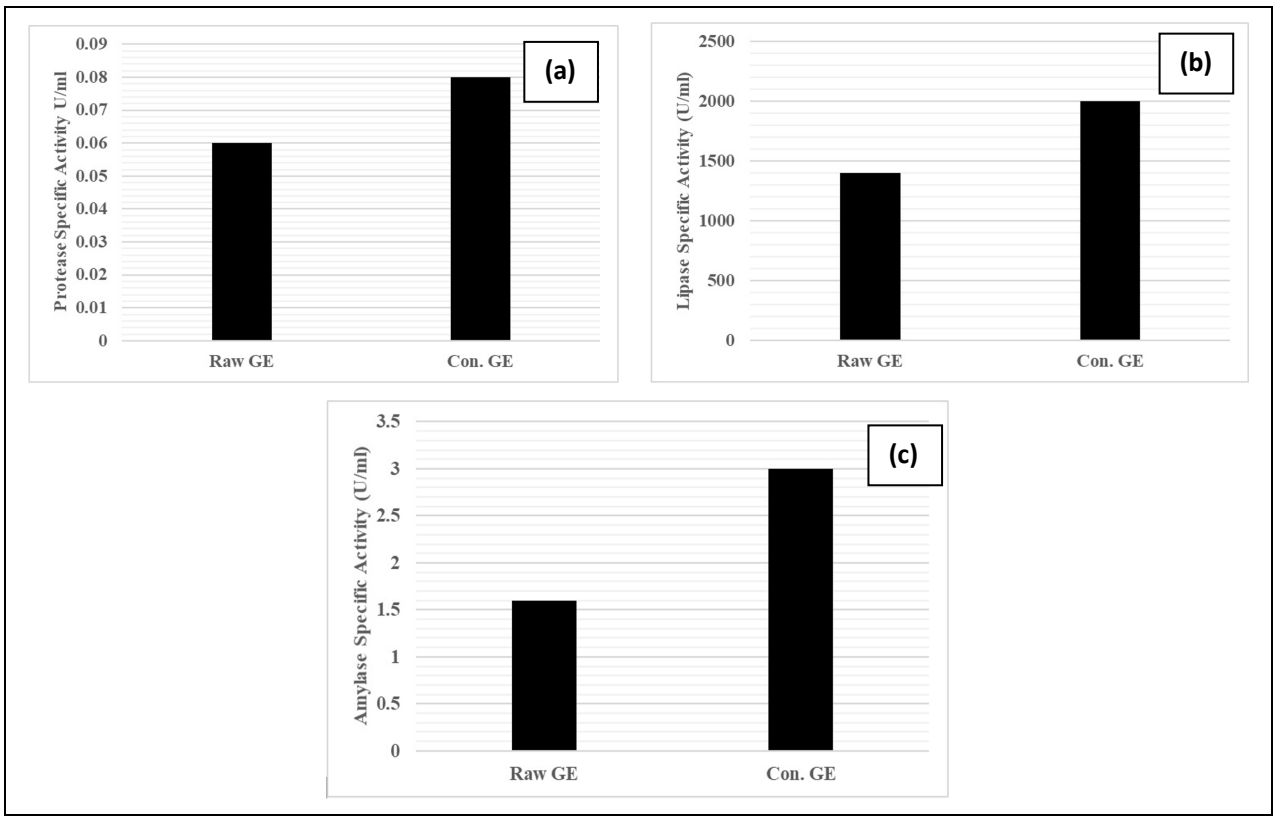


Fig. 7: Activity of (a) Protease, (b) Lipase and (c) Amylase for Raw GE and concentrated GE at pH7

4. CONCLUSION

The concentration of garbage enzyme by simple low-cost ultrafiltration method solved the problem simultaneously in the environment and startup production industries. The proposed membrane separation using a PVDF system effectively concentrates garbage-derived enzyme solutions while minimizing oxygen-induced degradation. By reducing water content, the process limits hydrolysis and microbial overgrowth, thereby enhancing stability. Adjustment of pH further contributes to decreased BOD and COD, indicating

improved biochemical control. The concentrated fraction exhibited higher protease, lipase, and amylase activities compared to the raw solution. These findings demonstrate that membrane processing is an efficient approach for stabilizing and improving the functional performance of garbage-derived enzymes. Overall, this method offers a promising strategy for extending shelf life and enhancing applicability in industrial, environmental, and agricultural sectors.

ACKNOWLEDGMENT

This work is supported by Periyar Maniammai Institute of Science and Technology, Thanjavur, Tmilnadu-613403 Under Seed Money Project Proposal of Project Number Lr.No.1019/Registrar/2022-23/Date:10.10.2022

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

COPYRIGHT

This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).



REFERENCES

Arun, C., Sivashanmugam, P., Solubilization of waste activated sludge using a garbage enzyme produced from different pre-consumer organic waste, *RSC Adv.* 5(63), 51421–51427 (2015a). https://doi.org/10.1039/C5RA07959D

Arun, C., Sivashanmugam, P., Study on optimization of process parameters for enhancing the multi-hydrolytic enzyme activity in garbage enzyme produced from preconsumer organic waste, *Bioresour. Technol.* 226, 200–210 (2017). https://doi.org/10.1016/j.biortech.2016.12.029

Arun, C., Sivashanmugam, P., Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge, *Process Saf. Environ. Prot.* 94, 471–478 (2015b). https://doi.org/10.1016/j.psep.2014.10.008

Bhattacharjee, C., Saxena, V. K., Dutta, S., Fruit juice processing using membrane technology: A review, *Innov. Food Sci. Emerg. Technol.* 43, 136–153 (2017). https://doi.org/10.1016/j.ifset.2017.08.002

Katibi, K. K., Mohd Nor, M. Z., Yunos, K. F. M., Jaafar, J., Show, P. L., Strategies to Enhance the Membrane-Based Processing Performance for Fruit Juice Production: A Review, *Membranes (Basel).* 13(7), 679 (2023). https://doi.org/10.3390/membranes13070679

Kowalczewski, P. Ł., Olejnik, A., Rybicka, I., Zielińska-Dawidziak, M., Białas, W., Lewandowicz, G., Membrane Filtration-Assisted Enzymatic Hydrolysis Affects the Biological Activity of Potato Juice, *Molecules* 26(4), 852 (2021). https://doi.org/10.3390/molecules26040852

Nongonierma, A. B., FitzGerald, R. J., Unlocking the biological potential of proteins from edible insects through enzymatic hydrolysis: A review, *Innov. Food Sci. Emerg. Technol.* 43, 239–252 (2017). https://doi.org/10.1016/j.ifset.2017.08.014

Padhan, B., Ray, M., Patel, M., Patel, R., Production and Bioconversion Efficiency of Enzyme Membrane Bioreactors in the Synthesis of Valuable Products, *Membranes (Basel).* 13(7), 673 (2023). https://doi.org/10.3390/membranes13070673

Pimentel, G. A., Almeida, P., Hantson, A.-L., Rapaport, A., Vande Wouwer, A., Experimental validation of a simple dynamic model of a laboratory scale recirculating aquaculture system fitted with a submerged membrane bioreactor, *Biochem. Eng. J.* 122, 1–12 (2017). https://doi.org/10.1016/j.bej.2017.02.005

Prazeres, D. M. F., Cabral, J. M. S., Enzymatic membrane bioreactors and their applications, *Enzyme Microb. Technol.* 16(9), 738–750 (1994). https://doi.org/10.1016/0141-0229(94)90030-2

Sambaraju, S., Sree Lakshmi, V., Eco-friendly treatment of dairy wastewater using garbage enzyme, *Mater. Today Proc.* 33, 650–653 (2020). https://doi.org/10.1016/j.matpr.2020.05.719

Schmidt, M., Prager, A., Schönherr, N., Gläser, R., Schulze, A., Reagent-Free Immobilization of Industrial Lipases to Develop Lipolytic Membranes with Self-Cleaning Surfaces, *Membranes (Basel).* 12(6), 599 (2022). https://doi.org/10.3390/membranes12060599

Servent, A., Abreu, F. A. P., Dhuique-Mayer, C., Belleville, M.-P., Dornier, M., Concentration and purification by crossflow microfiltration with diafiltration of carotenoids from a by-product of cashew apple juice processing, *Innov. Food Sci. Emerg. Technol.* 66, 102519 (2020). https://doi.org/10.1016/j.ifset.2020.102519

Sitanggang, A. B., Drews, A., Kraume, M., Enzymatic membrane reactors: Designs, applications, limitations and outlook, *Chem. Eng. Process. - Process Intensif.* 180, 108729 (2022). https://doi.org/10.1016/j.cep.2021.108729