Research Article

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

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ABSTRACT

Enzyme solutions derived from garbage have many applications in the surfactant industry, industrial and municipal sludge stabilization, Bio-fertilizer, bio-herbicide & 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 by removing water content using 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, whichis a similar process as a water purification unit in the home. 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 parameter 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

In the era of start-up India, 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 cum rural economy, suppression of greenhouse gases production, and mitigation for environmental deployment. The garbage enzymes are derived from various fruit wastes at the pre-consuming stage through anaerobic fermentation at the hydrolysis process. It is well known that the produced garbage enzyme can be utilized for various product conversion. That is the systematic way of adding the garbage enzymes with the appropriate substrate in a controlled unit to produce the respective desired products. However, the bulk-produced enzymes cannot be utilized at a single time, or on the other side it does not need the same enzyme production company utilizing the enzymes for other product formation. The garbage enzyme is a circular economy that will be sold as raw materials for various other bio-product companies. In this stage, controlling the garbage enzyme's stability is a necessity by restricting the undesired product conversion through water-oxygen contact. 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). pH of the garbage enzymes plays a vital role in enzyme activity at around 6 to 7 by bringing desired, undesired chemicals and fungus formation (Arun *et al.* 2015b). It is recorded from the previous studies that the pH 6-7 of garbage enzymes actively performed in biochemical activity, hence, the reduction of pH could be an effective control unit. Hereby concentrating the garbage enzyme or separating of water content will reduce the pH. The concentrating methods for the garbage enzyme can be adapted from the following reports.

Fruit juice concentration by membrane process provides stability in microbial cum self-life packaging (Bhattacharjee *et al.* 2017). Filtration methods of Ultra, Micro, and Nano, and reverse osmosis are fine alternatives for the concentrate process in the juice and beverage industry, where these membrane processes

maintain the nutritional value meanwhile increasing the self-life stability organoleptic of the packaging. Enzyme membrane bioreactors work well on the separation of products such as large-molecule hydrolysis-derived carbohydrates, oligosaccharides, and bioconversion of insulin, lignin, and sugars. The separation process of enzyme membrane bioreactors yields products with stability is a highlight of the membrane separation unit (Padhan *et al.* 2023). Other work reported that membrane filtration affects the enzymatic hydrolysis and biological activity of potato juice. In this report, they discussed how the membrane separation affects the hydrolysis and biological activity over the product conversion, but other hand this property is called stability for storage purposes (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 from the 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, however, it is curable, and not dealt with in this presented work. Meanwhile Polyvinylidene Fluoride (PVDF) membrane reported low fouling with the enzyme process hence the same membrane system was integrated with the present system (Schmidt *et al.* 2022). Membrane reactors tend to enzyme and substrate in contact to produce various products, so, in this current study instead of making contact with the substrate only separation of water is framed in bifurcated outlets of concentrate and water (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. On the other side, the adapted membrane separation system on the concentrate process of garbage enzyme reduces the deterioration rate which is tested and reported in this 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 6.6 liter removed water content) helps ease compact packages for longer time storage and shipping in a protective environment.

2. MATERIALS AND METHODS

A 1:3:10 ratio in the weight percentage of molasses, citrus fruit peels, and water respectively produced garbage enzyme under optimum anaerobic incubation conditions for three months of the period by a batch process is taken for the present stabilization examine study (normal cloth filtered supernatant part only). Once the anaerobic incubation condition is relaxed, after 15-20 days the generated garbage enzyme makes contact with the air which turns to deterioration slowly due to the forming of aerobic microbial communities. Since the garbage enzyme has wide applications in various industries' production and waste treatment units it is required for bulk storage of garbage enzyme without changing its characteristics. The water content present in the garbage enzyme is the core reason for the development of microbial communities or other kinds of deformation that happen by collaborating with foreign particle content in the air. Hence, the removal of water is expected to control the action between biochemical and air which would increase the stability and activity of the garbage enzyme. To reject the water content from the enzyme the PVDF bifurcated 0.45 µm membrane separation process continuous mode is assembled as shown in Fig. 1.

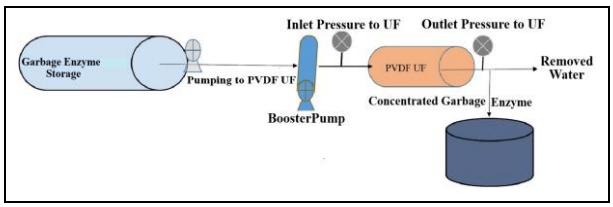

Fig. 1: Simple PVDF membrane UF setup for the enzyme concentrating process

In general, the garbage enzyme is produced by adding ordinary tap water or recycled water to utilize the waste as a circular economy. PVDF membrane-based Ultra Filtration (UF) is assembled which has the potential to operate between the range of 2 and 10 pH, high physical strength cum chemical resistance, and excellent stability at higher temperatures. The hollow fiber 0.45 µm pores membrane functions as a downstream process to separate the core enzyme molecules from the water which could extend the shelf life period of that garbage enzyme. Around 20 liters of the citrus fruit peels derived garbage enzyme through the batch process is stored at a horizontal hold cylindrical tank connected with a peristaltic pump and 0.8 mm dia tube joined via another one booster pump 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 tableted 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) (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 approx10liter and above) varied with higher values in all above properties from the lower quantity or laboratory level production (below 10liter) (Arun *et al.* 2015b; Sambaraju *et al.* 2020) is a significant notified 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 the pH comfortably close to neutral with nominal water content. 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 of Arun *et al.* (2015b) and 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. Reported despite the significance of this present work objective. 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 & fouling. Raw, concentrated enzymes and removed water content are shown in Fig. 2, the results are presented and discussed in the next section.

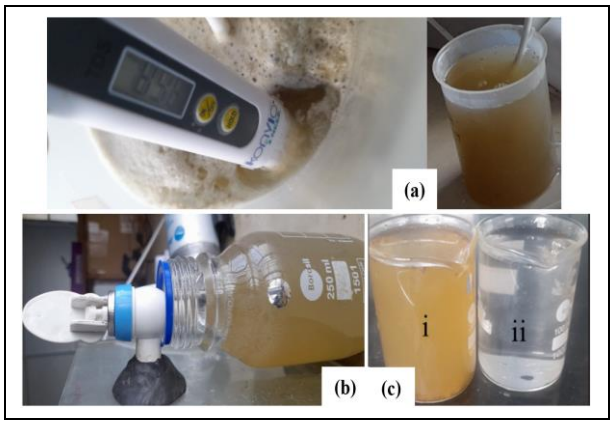

Fig. 2: (a) Raw Garbage Enzyme, (b), Concentrated Garbage Enzyme, (c) i-Concentrated Enzyme and ii- Removed water

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 the 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

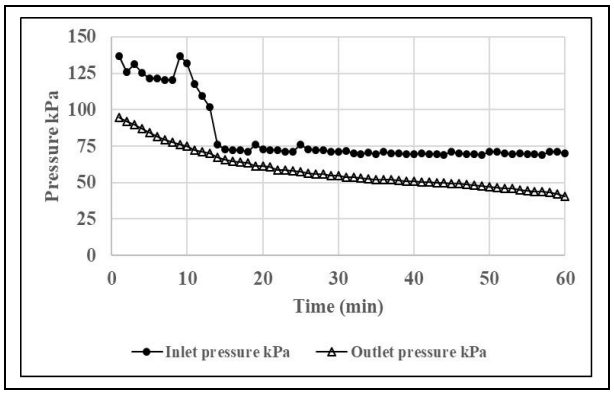

Fig. 3: Pressure behaviour between inlet and outlet

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 & Fig. 5.

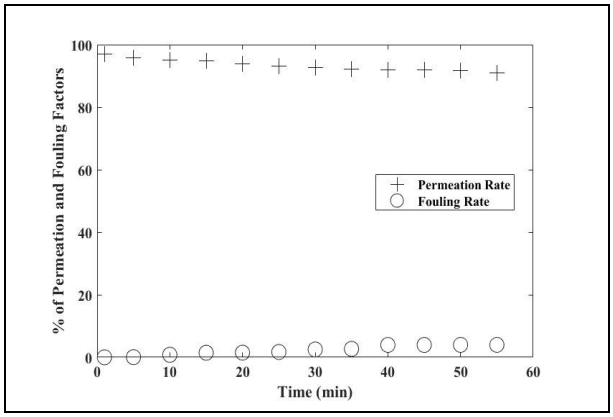

Fig. 4: Percentage of permeation and fouling factors of garbage enzymes

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

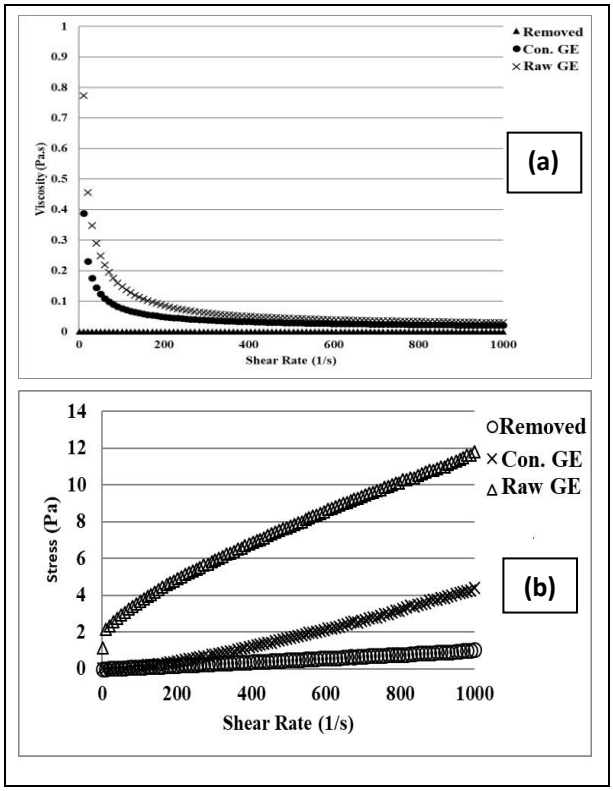

Fig. 5: Flow behavior at shear rate against (a) viscosity, (b) Stress

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, as a result of this, to perform with a uniform pH for checking the activity at the same stage the raw and concentrated were diluted with phosphate until pH 7 where concentrated produced larger activity, meanwhile raw activity rest at lower. The reason for higher activity in concentrated garbage enzymes can be more purest form at this stage shown in Fig. 7.

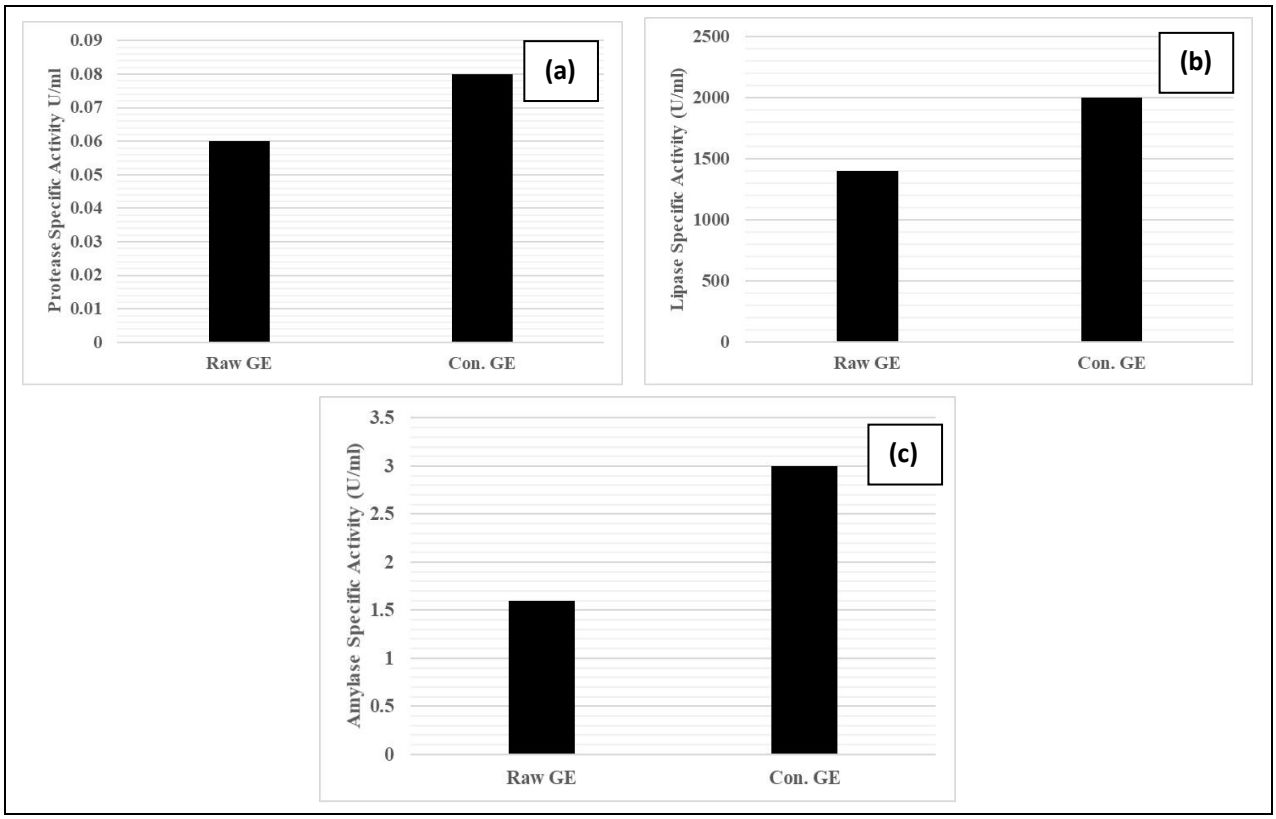

Fig. 7: Activity of (a) Protease, (b) Lipase, (c) Amylase for Raw GE and Concentrated GE at pH7

4. CONCLUSION

The concentration of garbage enzyme by simple low-cost ultrafiltration method solved the problem simultaneously in the environment and startup production industries. That is, in the municipal solid waste treatment, the veg and fruit market wastes as well as household wet wastes are disposed of in biogas plants and compost manufacturing yards. But especially fruit and veg peels are extreme barriers in biogas production and compost formation. Hereby, the segregated fruit and veg peels can be taken for simple enzyme production by batch process followed by concentration process through simple ultrafiltration. The present concentration process

leads to large-scale production as well as a long period of shelf life potential. So, it can be utilized for the various startup production industries in bulk and industrial/ municipal effluent & sludge treatment. Other hand, the present work supports and ensures the circular economy of fruit &veg peel wastes, to meet the bulk demand and also indirectly helps to enhance biogas production and compost conversion by peels-free wet feed.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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