# Comparing the Efficacy of Homemade Bio-enzymes to Store-Bought Enzymes

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ABSTRACT

The cleaning industry has seen a surge in the use of manufactured enzymes for their effectiveness in breaking down organic stains and residues. Although commercial enzyme-based cleaning products have amassed popularity among the general public, concerns over their environmental consequences persist. This research study intends to comprehensively compare the efficacy of store-bought enzymes and homemade bio-enzymes in antimicrobial applications. We hypothesized that the bio-enzyme will exhibit comparable efficacy to the store-bought enzyme-based agent, while exhibiting more environmentally friendly properties. Bio-enzymes are synthesized through fermentation of jaggery and complex polyphenols (sourced from orange rinds) in water. Both types of enzymes are assessed on their antimicrobial effectiveness by observing activity in gelatin-based spread bacteria plates, streaked with E. coli. We found that the bioenzyme that we synthesized demonstrated greater levels of E. coli colony inhibition when compared to the store-bought enzyme-based cleaning agent. Finally, this study intends to address future research into the potential use cases of bio enzymes in industrial and medical settings as well as the possibility of creating recyclable cleaning agents through enzyme immobilization in polymer composites.

Keywords: antimicrobial, fermentation, complex polyphenols, bio-enzyme, bacteria, enzyme immobilization, polymer composites

## Introduction

In recent decades, concerns have risen regarding the potential dangers of store-bought cleaning products and their implications on the environment. Homemade bio-enzymes are a promising alternative to store-bought enzymes as they exhibit similar antimicrobial properties and are both easily synthesized and used.

Bio-enzymes are synthesized through anaerobic fermentation. The peels of various fruits and vegetables are collected and cut into smaller pieces to increase the surface area of the reaction. Jaggery, water, and peels are mixed in a plastic container, and a pinch of yeast is added. The container is then sealed and left undisturbed for one month to allow the fermentation reaction to proceed. The pH values of the different samples of bio-enzymes fall within the range of 5-6, indicating a weakly acidic nature.

Bio-enzymes also exhibit antimicrobial properties, making them effective in various applications. Tests for anti-fungal properties show that bio-enzymes resist the growth of fungi to varying degrees, with tests for antibacterial properties demonstrating bacterial inhibition. The zone of inhibition observed around the discs applied with diluted bio-enzymes indicates their effectiveness against bacteria.

Bio-enzymes have a wide range of applications. In contaminated water treatment, bio-enzymes can be applied to improve water quality by reducing total dissolved salts and adjusting pH levels. Finally, bio-enzymes find application in household cleaning as eco-friendly alternatives to chemical-based cleaning agents, which will be our primary field of discussion later in this research. (Lakra et al., 2022)

## Discussion of Environmental Impacts

Homemade bio-enzyme cleaners offer many environmental benefits when compared to their commercial counterparts. These products have a significantly lower environmental impact as they utilize fruit peels and organic sources of polyphenols. Bio-enzymes avoid resource-intensive manufacturing processes contributing to high energy usage and carbon emissions. Furthermore, their biodegradability aligns with sustainable waste-management

processes. It ensures that these cleaners break down naturally rather than residually posing threats to ecosystems in the long term through irresponsible chemical disposal. The reduced chemical usage in these cleaners minimizes the impact of harsh chemicals on the zone of application and the air quality of surrounding areas. By avoiding store-bought alternatives, these cleaners promote a healthier living environment while concurrently reducing the environmental burdens associated with using conventional products.

## Materials and Methods

In the synthesis of the bio-enzyme, 20g of powdered jaggery, which serves as the substrate of yeast, was added into a large plastic vessel. Subsequently, 60g of orange rinds and 200 mL of reverse-osmosis water were added to the container. The jaggery, orange rinds, and water must be approximately in a 1:3:10 ratio by mass. Finally, 1.5g of baker's yeast (Saccharomyces cerevisiae) was added to accelerate fermentation. The plastic vessel was closed and placed in a cool area. It was opened daily for a minute to release the gasses (mainly carbon dioxide) produced during anaerobic fermentation. This process was repeated for 90 days.

Filtration of the bio-enzyme was conducted using a funnel, filter paper, and beaker after the fermentation period of 90 days had concluded.

After the filtration process, a pH probe was introduced into the bio-enzyme to record the pH of the bio-enzyme. Similarly, the pH of Lysol was also recorded. Considering that the ideal pH of an enzyme-based cleaner is 6.5 to 8.5, the bio-enzyme and Lysol were categorized as having a lower than ideal pH, ideal pH, or above ideal pH.

In each trial, approximately 1 mL of both our bio-enzyme or Lysol was applied to an agar spread plate containing E. coli live culture, resulting in the formation of an inhibition zone. The radius of the inhibition zone was measured using a digital caliper for each trial after 5 minutes of introduction of bio-enzyme, serving as a quantitative indicator of the effectiveness of both Bio-enzyme and Store-bought Lysol in controlling E. coli growth.

**Figure 1.** Primary Ingredients: Orange Rinds, Powdered Jaggery, and Reverse Osmosis Water

**Figure 2.** Plastic Vessel Containing Bioenzyme After 90 Days.

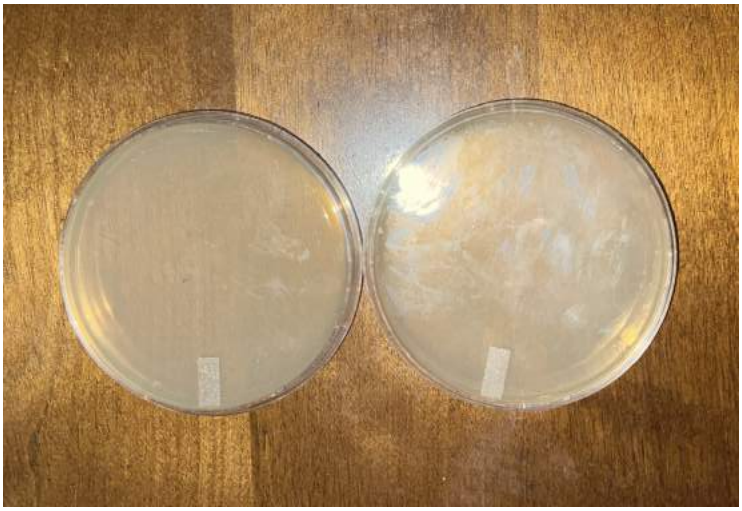

**Figure 3.** Agar Spread Plates Streaked with E. Coli Bacteria Prior to Application of Enzymes

## Results and Analysis

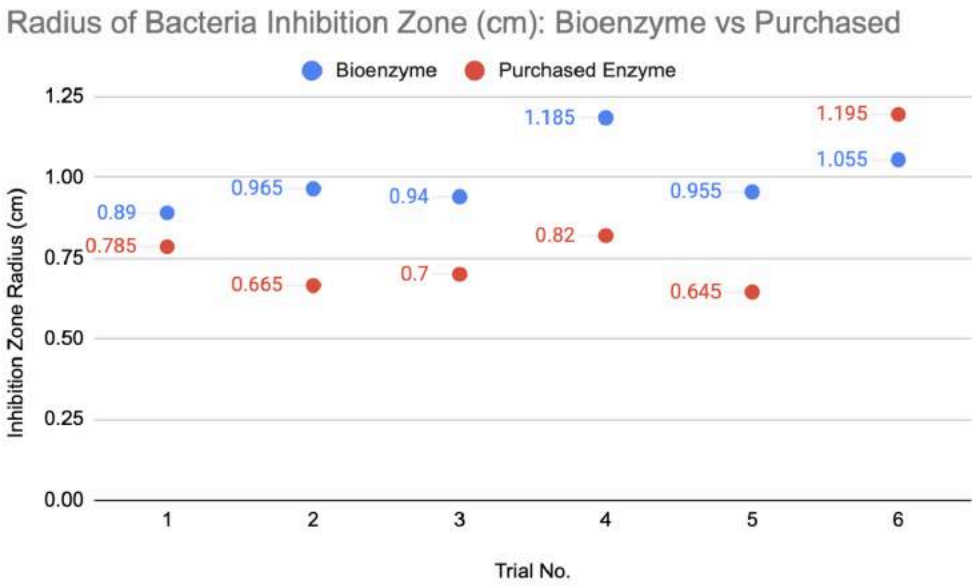


**Figure 4.** Radius of Bacteria Inhibition Zone in Centimeters for Bioenzyme vs. Purchased Enzyme, 5 minutes after application

The data clearly indicates that the bio-enzyme exhibited greater effectiveness in inhibiting bacterial growth compared to Lysol. Across all trials, the average radius of the bacterial inhibition zone for the bioenzyme was notably larger than that of Lysol. Specifically, the average radius for the bio-enzyme trials was approximately 0.9983 cm, while for Lysol, it was around 0.8016 cm. This demonstrates the superior antimicrobial activity of the bio-enzyme, suggesting its potential as a more potent alternative for controlling bacterial growth.

### Derivation of Inhibition Area Equations:

$A = \pi r^2$

$A = \pi(t \cdot dr/dt)^2$

$dr/dt_{avg}$ for bio enzyme = $\frac{0.9983cm}{5min} = 0.1996cm/min$

$dr/dt_{avg}$ for store-bought enzyme = $\frac{0.8016cm}{5min} = 0.1603cm/min$

Using our data from the trials of the bacteria inhibition zone radii, we constructed equations modeling the inhibition zone area as a function of time for both our bioenzyme and the purchased enzyme. This allowed us to better understand the efficacy of each enzyme as a function of time in minutes. We found the equations for the area to be $A_{bioenzyme} = \pi(0.1996t)^2$ and $A_{store-bought} = \pi(0.1603t)^2$; this further depicts the bio-enzyme's faster and more prevalent inhibition of the bacteria.

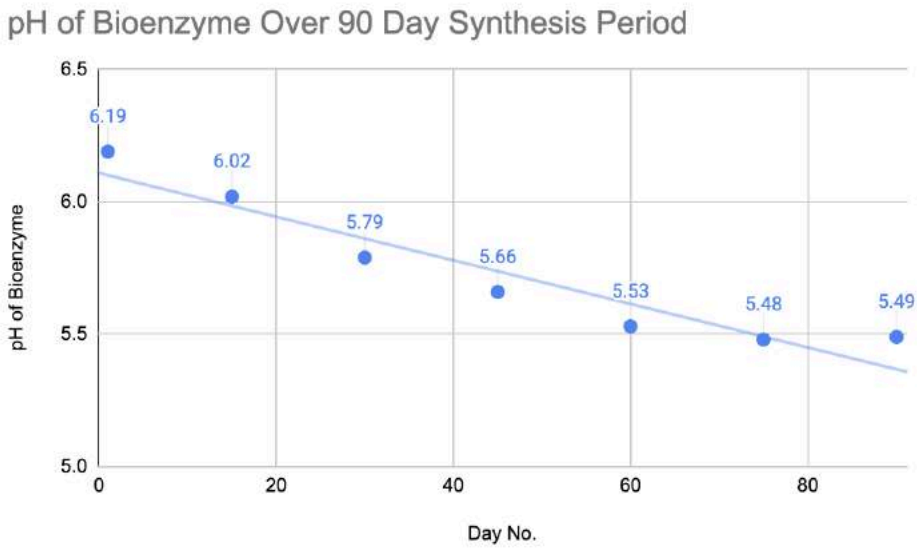


**Figure 5.** pH of the Bio-enzyme over the 90-day synthesis period

The pH of the bio-enzyme underwent a notable decrease during the synthesis period, starting at 6.19 on day 1 and gradually declining to 5.49 by day 90. This significant pH shift could influence the bio-enzyme's antibacterial efficacy, as enzymatic activity is often pH-dependent, with optimal activity occurring within specific ranges. Therefore, the observed decrease in pH suggests potential alterations in enzymatic activity over time, likely impacting the bio-enzyme's ability to inhibit bacterial growth.

On the other hand, the pH of the store-bought enzyme was measured to be 10.71, indicating a far more basic nature. A substance of pH 10.71 can be extremely corrosive and can harm the texture and area of enzyme application.

## Future Research

Future research should focus on improving the portability and reusability of the bio-enzyme through the polymer fixation of the bio-enzyme into a photopolymer. Essentially, various methods of fixing the bio-enzyme through the process of photopolymerization to reduce the bio-enzyme into a polymer need to be explored. This will allow the sample of bio-enzyme to be used repetitively over a few days after which the efficacy of the polymer would decrease due to the leaching of bioenzyme.

Furthermore, the amounts of different ingredients that make up the bio-enzyme should be analyzed to figure out what the optimal quantity measurement for each ingredient is in order to maximize the efficacy of the bio-enzyme. Possible variables that could be tested are pH, solubility, concentration of solution, etcetera.

## Limitations

Some of the limitations of our research lie intrinsically in the resources we had access to. Though the synthesis of our bio-enzyme was largely successful, it is important to note that the anaerobic fermentation synthesis was done in a home environment and was subject to temperature and lighting changes. Furthermore, we were only able to conduct five trials per enzyme type as the agar plates were difficult to synthesize and successfully streak with the E. coli culture. The manually operated digital caliper may have also introduced inaccuracy in our inhibition zone radii measurements.

## Acknowledgements

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## References

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