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Original Article

Bioconversion of Citrus Peel Waste into Bio-enzyme Cleaner for Sustainable Domestic Use

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Abstract

The Present research study focuses on developing a sustainable and eco-friendly" cleaning agent" using waste from citrus fruits through a fermentation process. The key objective is to minimize environmental pollution by recycling fruit and vegetable waste into a bio-enzyme cleaner. The fermentation process enables the extraction of enzymes and bioactive compounds, known for their potential cleaning properties, from the citrus waste. The physico-chemical properties of the fermented solution, including pH, acidity, and enzyme activity, are analyzed to assess its suitability as a cleaning agent. Furthermore, the effectiveness of enzymes and bioactive compounds in breaking down dirt and grease is examined to establish the solution's efficiency in various household applications. By utilizing waste material and reducing the reliance on chemical-based cleaners, this study aims to offer an environmentally responsible and economically viable cleaning alternative for everyday use. The research demonstrates how a simple, natural fermentation process can be leveraged to create a cleaner that aligns with sustainability goals while effectively meeting household cleaning needs.

Keywords: *Citrus, Bio-Enzyme, Amylase, Lipase, Cleaner, Environment, Peels, Fermentation*

Introduction

Ecological enzyme is an organic solution that varies in color from dark brown to yellow. It is made by fermenting fresh kitchen waste such as vegetable peels, fruit peels and flowers. Fermentation is a method of producing enzymes for commercial purposes. It involves the use of organisms such as bacteria and yeast to produce enzymes. Enzymes are proteins that function as catalysts. Enzymes reduce the energy, required for reactions to occur, but they are not used in the reaction itself. Many industries use enzymes to help improve their products. In recent years, enzymatic processes have found application in many industries because they are specific, fast-acting, and often save raw materials, energy, and chemicals. They are biodegradable and promote selective reactions. In some applications, such as tablecloths and cotton clothing, it is common to find many detergents formulated with some lipase and cellulase. The solution is a fruit juice obtained by fermenting waste food with jaggery and water. Citrus peels are often used in the preparation of ecological enzymes due to their special properties such as rich aroma, rich taste, high vitamin C content, high poly-phenol content, high medicinal value and high acidity. Citrus fruits are also rich in molecules such as flavanoids, alkaloids, coumarins, phenolic acids and essential oils [1].

The sugar or jaggery added during fermentation is used by bacteria and ferment produced as a result of the process has bactericidal properties. Eco-enzymes act as disinfectants, sanitizers, insecticides and can be used as detergents. The layers of this fruit, including the peel, rind and peel-like surface, are rich in poly-phenolic compounds. These compounds play an important role in protecting the fruit from ultraviolet and infrared radiation as well as microbial diseases.

Phenolic compounds in citrus peel extract include p-cinnamic acid, ferulic acid, isoferulic acid, 5-hydroxyvaleric acid, vanillic acid and 2-hydroxybenzoic acid. It is worth noting that lemon peel is rich in ferulic acid and sinapic acid [2].

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Flavonoids are the most abundant compounds in citrus fruits and are important bioactive compounds. They serve as precursors of many substances such as anthocyanins and play a role in the regulation of photosynthesis and redox reactions. Flavonoids are also known for their beneficial effects, including antioxidant, anti-inflammatory and anti-inflammatory activities, as well as their ability to induce lipid antioxidant effects [2]. In the last few years, the ecological problems caused by the use of synthetic pesticides that release toxins, unbalanced pH and environmental chemicals have attracted public attention. Since these synthetic pesticides are corrosive, toxic and slow to biodegrade, their widespread use can pollute the environment and lead to collapses. However, the latest cleaning technologies include enzymatic cleaners, which are effective, safe and specific in their functions, and are also easier and cheaper to prepare. Enzyme bio cleaning solutions are specifically designed to treat dirt and soil safely, cost-effectively and quickly, and to digest chemical and organic waste quickly and effectively without odor or pollution. Therefore, it is necessary to develop cleaners that are biodegradable, non-toxic, non-corrosive, and environmentally friendly with improved household applications.

Objectives of The Present Study:

- To prepare the fermentation medium using the raw materials like fruit and vegetable wastes to minimize environmental pollution and recycle this waste.
- To analyze the physico-chemical properties of the cleaning agent.
- To study the effectiveness of enzymes and bioactive compounds present in the fermented solution.
- To study the activity of the solution as a cleaning agent in household applications

Review Of Literature

Swati Rose Toppo (2024) conducted a study on 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 treatments demonstrated a positive outcome for the Amylase Test, Lipase Test, Cellulase Test, Urease Test, Protease Test, MR Test, as well as for Alcohol and low pH. This indicates that the Garbage enzyme derived from fruit and vegetable waste contains a combination of enzymes, alcohol, and acid resulting from microbial fermentation. The highest enzymatic efficacy was observed in T3 - Pomegranate, followed by T6 - Banana. The Garbage enzyme produced from vegetable waste was less effective compared to that from fruit peels, with the maximum enzymatic activity recorded in 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 1 amylase-producing fungus were screened and isolated from all treatments. The Garbage enzyme treatments in the tray assay neutralized the alkaline pH of cow dung, as the Garbage enzyme is inherently acidic in nature. The highest nitrogen content, measured at 172 kg/hectare, was observed in samples treated with an enzyme derived from garbage that was produced using banana peels. 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 [9].

Suliestyah *et al.* (2022) conducted a study on the production of eco-enzymes. The growing amount of organic waste in landfill sites generates methane gas, a significant greenhouse gas contributing to global warming. By creating eco-enzymes from fruit peels and vegetable scraps, we can reduce organic waste while simultaneously producing useful products. In this study, eco-enzymes were derived from waste materials of papaya, dragon fruit, and orange peels, combined with water and molasses. The weight ratio of water, organic materials, and molasses was set at 10:3:1. The mixture underwent fermentation for a duration of three months. The enzyme activity was assessed using the DNS method; the bacteria-killing efficacy was evaluated through the ASTM 2315:2008 method; and the SNI 06-6989-3-2004 method was employed to determine the TSS levels in the liquid waste. The eco-enzyme produced in this research exhibited an amylase enzyme activity of 2.15 and a cellulase activity of 1.69. The effectiveness of the eco-enzyme in eliminating E. coli bacteria reached 99.95%, while it achieved a 99.90% reduction in P. aeruginosa bacteria, with only 20% eco-enzyme concentration within a contact time of 15 seconds. The findings from the liquid waste treatment using this eco-enzyme indicate a TSS reduction rate ranging from 65% to 88% based on an initial TSS concentration of 345 ppm [10].

Olgalizia galintin *et al.* (2021) conducted a study on the production of eco enzymes derived from vegetable and fruit waste. The significant proportion of food waste in municipal solid waste generation is a major environmental concern, as the decomposition process emits carbon dioxide and methane into the atmosphere, contributing to the greenhouse effect. Consequently, this research aimed to characterize the eco enzyme produced through the fermentation of fruit waste in order to reduce the amount of organic waste in landfills. Additionally, the study will assess the treatment of aquaculture sludge to evaluate its effectiveness. The efficacy of the eco enzyme in sludge treatment was tested using various dilution factors (5 %, 10 %, and 15 %) over a period of 10 days (batch process). The findings indicated that the eco enzyme contains protease, amylase, and lipase. Furthermore, the treatment results revealed that the enzyme solution at a concentration of 10 % was more effective and cost-efficient in treating aquaculture sludge, achieving reductions of 89 % in total suspended solids, 78 % in volatile suspended solids, 88 % in chemical oxygen demand, 94 % in total ammonia nitrogen, and 97 % in total phosphorus. The eco enzyme produced in this study has proven to be an environmentally sustainable solution for decreasing food waste in solid waste generation and holds promise for application in the wastewater sector [11]. Many scientists have worked on the production of bio-enzymes which had different applications. The present

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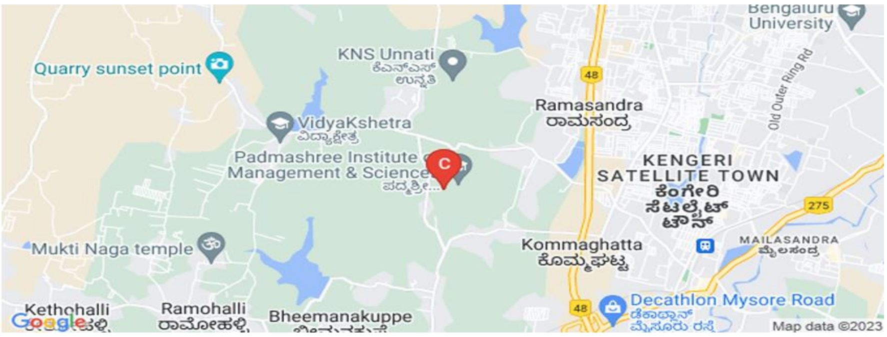
research study focuses on the production of a cleaning agent by green methods so as to provide an alternative to market available synthetic solutions. Materials and methods:

Materials And Methods:

The present study was conducted at Padmashree Institute of Management and Sciences, situated in Bengaluru, State of Karnataka.



Map 1-Location of the study – Padmashree Institute of Management and Sciences

The methodology for the current research study was divided into 4 phases as follows:

Phase 1: Preparation of Fermentation Medium.

Procurement of fruit peels i.e. Citrus and Banana from nearby fruit juice vendor located in Kengeri Satellite Town, Bengaluru. Fruit samples were cut into small pieces with a knife for further fermentation process. The above fruit waste was weighed to the required amount and mixed in the following way: -

Combine citrus fruit peels, jaggery and water in an air tight container in the ratio 3:1:10 respectively. Seal the container and let the mixture ferment for 3 months (the lid of the container should be opened every 24 hours in the initial 10 days for the produced gases to escape). After completion of the fermentation process, strain the fermented mixture and use the liquid as a cleaner. The remaining peels and fermented waste can be used as a culture for the production of the next batch of bio enzyme cleaner. Refer the following flowchart for further understanding of preparation process.

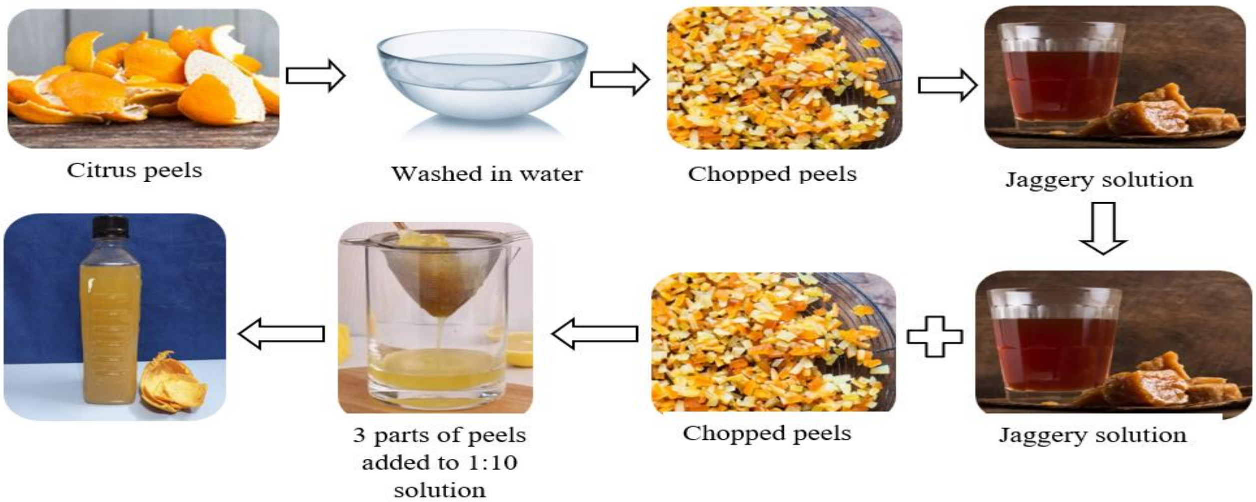


Fig.1: Preparation of Fermentation Medium

Phase 2: Physio-Chemical analysis

1. pH Identification: A pH Paper was dipped in undiluted cleaner and observed for color change.
2. Quantification of Acetic Acid: The 10 mL of sample was titrated against the titrant 1N NaOH. The few drops of phenolphthalein indicator were added and observed for the formation of pink color.

Phase 3: Enzyme assay

The sample of the Bio enzyme was examined for different enzyme activities, including amylase and lipase.

Enzyme assay Amylase:

Bio enzyme sample was serially diluted 10 -1 to 10 -4 and 1 drop of sample was spread on starch nutrient agar plate. It was then incubated for 48 hours at approximately 37 C. 2-3 drops of 10 % iodine solution were added directly onto the edge of colonies and observed for zone of hydrolysis [3].

Enzyme assay Lipase: The cleaner was tested for potential Lipase and Surfactant activity by 2 methods of identification.

Drop Collapse method- A drop of water was placed on a watch glass, followed by a drop of oil on top. A drop of the cleaner was then added to the oil-water interface. The

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behavior of the oil droplet was observed; a collapse or spreading of the droplet indicated effective surfactant activity, demonstrating the cleaner's ability to reduce surface tension and enhance emulsification.

Oil- Water Emulsion- 5 ml of coconut oil was suspended in a test tube filled with distilled water and a clear demarcation between both phases was observed. 2-3 drops of undiluted cleaner were added and the contents were mixed slightly and observed after 5 minutes.

Phase 4: *Anti-microbial activity*

A swab was taken from a washroom basin surface and inoculated on Nutrient Agar (NA). After 24 hours of incubation, microbial growth was recorded. Also, bio enzyme cleaner was applied to the basin, allowed to dry for 10 minutes, and a second swab was taken and inoculated. A control was also conducted by washing the basin with water only, followed by swabbing and inoculation.

Results:

The effectiveness of bio enzymes can be assessed by analyzing various biochemical properties that contribute to their cleaning and antimicrobial capabilities. In this study, several key parameters have been evaluated: pH identification, acetic acid concentration, enzymatic activity (amylase and lipase), and anti-microbial efficacy. These factors provide insight into the bio enzyme's potential for applications in environmental and industrial cleaning. The results from these analyses are presented below, followed by a discussion on their implications.

- pH Identification: - The color change indicated a pH in the range of 2 - 3, thus acidic in nature, making it highly effective at removing tough stains, cleaning tiles and reduction in bacterial growth (Fig.2).
- Quantification of Acetic Acid: - Acetic acid is a key active component in many household cleaners due to its antibacterial properties and its ability to break down grease and grime. Quantifying the amount of acetic acid in the cleaner is crucial for determining its effectiveness and safety for use. In this case, 10 ml of cleaner contains 0.484 g of acetic acid, which translates to 24.2 g in 500 ml. This concentration helps ensure the cleaner is potent enough for cleaning tasks while not being overly corrosive to surfaces (Fig.3).

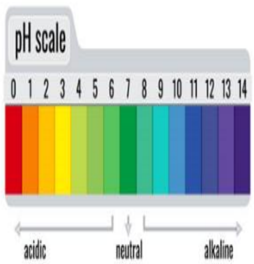

Fig.2: pH Paper color change

Fig.3: Pale Pink color obtained by titration

- Enzyme Assay Amylase: - Hydrolysis of starch was visualized as clear zones around the colonies against deep blue brown staining for starch (Fig.4). The presence of a clear zone of hydrolysis surrounding the application site indicated successful starch degradation, confirming the activity of amylase. The size of the hydrolysis zone correlates with the enzyme's efficacy, suggesting that the bio enzyme cleaner effectively breaks down starch-based substrates. These findings highlight the potential application of amylase-containing bio enzymes in cleaning formulations targeting starch-rich materials. Further investigations could optimize conditions for enhanced enzymatic activity and broaden the scope of application.

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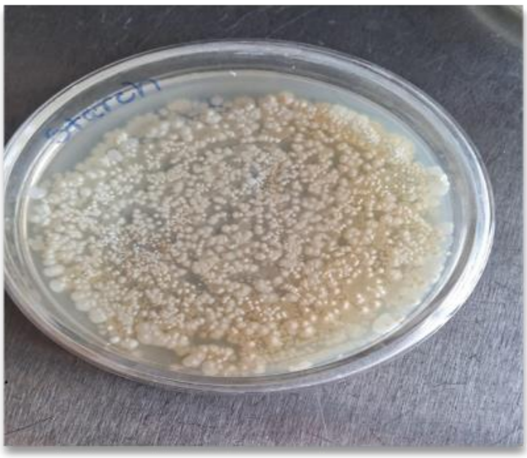
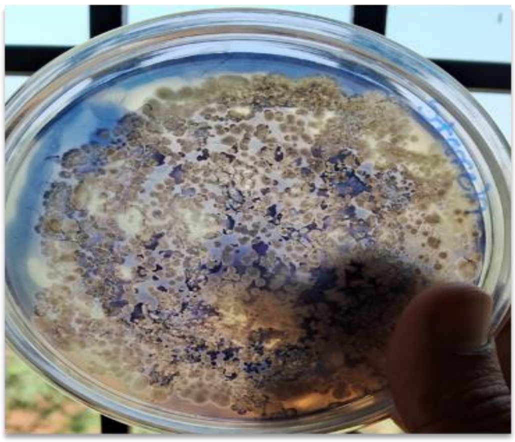


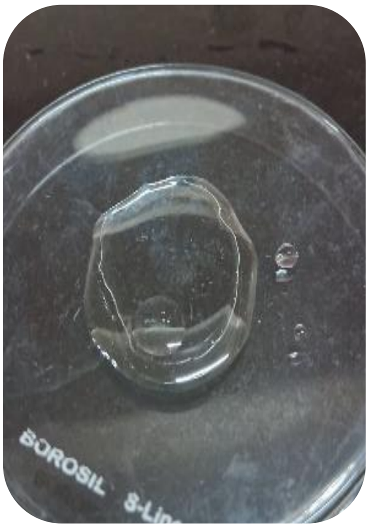
(a) NA starch plate



(b) Observed zone of hydrolysis around colonies upon iodine staining

Fig.4: - Hydrolysis of starch

- Enzyme Assay Lipase: - To assess the lipolytic activity of the bio enzyme cleaner, two experimental methods were employed: the drop collapse method and the oil-in-water emulsion test. A control test was conducted using a 1% Sodium Dodecyl Sulphate (SDS) solution in both methods to establish baseline activity levels. The results demonstrated that the bio-enzyme cleaner effectively exhibited lipase activity, indicated by significant collapse in the drop test and stable emulsion formation. Lipase facilitates the hydrolysis of triglycerides, resulting in the formation of glycerol and free fatty acids. This enzymatic process disrupts the hydrophobic nature of fats, reducing interfacial tension and enabling the dispersion of oil droplets in the aqueous phase, leading to emulsification. The appearance of bubbles suggests potential surfactant production or the release of fatty acid byproducts, further supporting enzymatic lipid breakdown.



Oil on Water Droplet

1% SDS solution Drop collapse

Bioenzyme solution Drop collapse

Drop collapse Method

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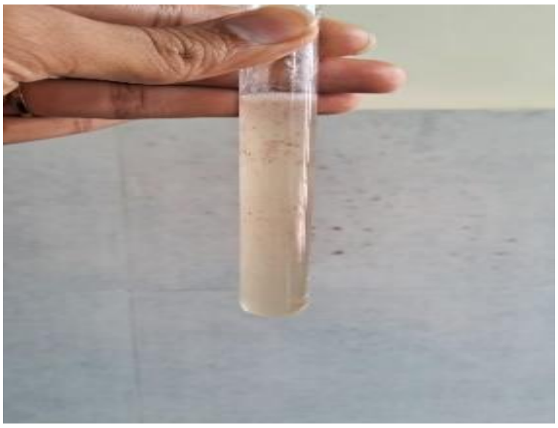
Oil-Water emulsion treated with 1% SDS solution



Oil-Water Emulsion treated with Bio enzyme solution

Water-Oil Emulsion Method

Fig.5: Enzyme Assay Lipase

- Anti-Microbial Activity: - The Wash-basin swab inoculated on NA plate showed growth of microbial colonies after 24 hours. Upon microscopic analysis by gram staining method, we were able to observe both gram-positive and gram-negative Streptobacillus's (Fig: - 6) based upon their morphological characteristics. As water alone is able to wash out microbes present on the sink surface, we kept a control plate of the sink surface washed with water only, followed by swabbing and inoculation, and growth was still observed after 24 hours. The plate inoculated with the bio enzyme swabbed surface showed no growth after 24 hours, thus exhibiting antimicrobial properties. The cleaner can be thus used for washing bathroom tiles, washrooms or used to disinfect any surface.

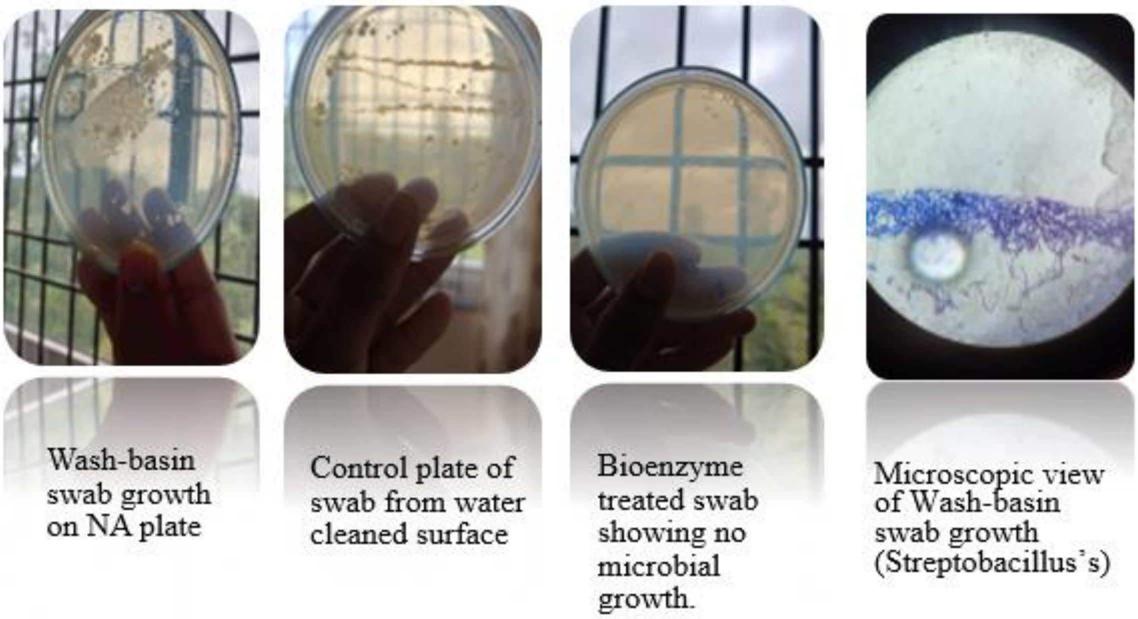


Fig: 6 Anti-Microbial Activity

- **Cost-Efficiency of Bio-enzyme**: -

  The Production of Citrus Bio enzyme is very cheap as it requires only 3-4 ingredients while citrus fruit peels are a waste product and mostly free of cost when acquired through a fruit juice vendor [Table 1]. When Bio-Enzyme production cost was compared with commercially available products, it was found to be very cheap and thus cost efficient [Table 2].

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Table 1: - Cost and Ingredients/Components of Bio-enzyme cleaner

Sl. No.	Ingredients	Cost
1	Citrus fruit peels	0 Rs
2	Water	0 Rs
3	Container	30 Rs
4	Jaggery	20 Rs

Table 2: - Price Comparison of Commercially available products with Bio enzyme Cleaner

Sl. No	Commercial products	Online market Cost/100 ml	Bio enzyme cost/100 ml
1	Dettol	47 Rs	10 Rs
2	Vim	14 Rs	10 Rs
3	Colin	53.8 Rs	10 Rs
4	Savlon	26.9 Rs	10 Rs

Discussion:

Bio enzyme cleaners, in addition to producing enzymes like amylase and lipase, also generate cellulase and protease [4]. The cellulase enzymes produced by microbes have significant industrial and biotechnological potential, making them highly sought after [4]. The lipase activity, identified through methods such as the drop collapse technique and oil-water emulsions, aligns with findings from current literature. In these studies, zones of clearance were observed on lipid agar plates inoculated with bio enzymes, confirming lipase production [3]. This makes bio enzyme cleaners a viable alternative to commercially available products for the removal of oil, grease, and other household cleaning tasks. Additionally, bio enzymes contain secondary metabolites, including alkaloids, flavonoids, quinones, saponins, tannins, and cardenolides, which exhibit insecticidal, anti-feedant, antimicrobial, antioxidant, and foaming properties [5]. Citrus - based bio enzymes are especially effective cleaners due to the presence of organic acids like citric acid, malic acid, oxalic acid, succinic acid, and glutaric acid [5]. Citric acid, a key active ingredient, functions as a chelating agent, aiding in the removal of lime scale from boilers, taps, and tiles, dissolving rust from steel, and eliminating hard water stains on glass surfaces [5]. Furthermore, bio enzymes possess antifungal properties attributed to the presence of propionic acid, acetic acid, and alcohol. Mold, which thrives in warm, moist environments, is inhibited by these components. Alcohol, in particular, prevents mold growth by increasing cell membrane permeability, which leads to solute leakage and eventual cell lysis [5]. Bio enzyme cleaners also exhibit antimicrobial properties. In our current study the cleaner was found to inhibit Bacillus species isolated from a wash basin swab, but the current literature also demonstrated potential pathogen-inhibiting potential against harmful bacteria like E. coli, Staphylococcus, Streptococcus, Pseudomonas, and Salmonella. These findings suggest that bio enzymes not only possess biocatalytic properties but also suppress pathogenic microbial activity, making them a promising tool for enhancing sludge stability by reducing solids and microbial growth [6]. Another notable application of bio enzyme cleaners is their positive impact on plant growth. By promoting natural soil microbiology, bio enzymes help boost plant development and yield, which contrasts with the negative effects of chemical cleaning agents [3]. Bio enzymes have also been tested in fish culture wastewater treatment, where citrus peel-based bio enzymes were found to effectively improve water quality [7]. Additionally, they have proven to be an economical and efficient solution for treating dairy wastewater [8]. Thus, bio enzyme cleaners are versatile, cost-effective solutions, suitable for household cleaning, industrial waste treatment, and other environmentally-friendly applications.

Conclusion:

The bio enzyme cleaner demonstrated strong enzymatic activity, with both amylase and lipase contributing to its effectiveness. The clear zones of hydrolysis around the starch plates confirmed amylase activity, indicating the breakdown of starch into simpler sugars. Additionally, the presence of lipase activity would enable the cleaner to break down fats and oils into fatty acids and glycerol. This dual enzymatic action makes the bio enzyme cleaner highly versatile, capable of addressing various organic residues commonly found in household environments, such as food stains, grease, and oils. A key advantage of the bio enzyme cleaner is its cost-effectiveness. The production process relies on basic raw materials and waste ingredients, which are readily available at minimal cost. Compared to commercial cleaning products, the bio enzyme cleaner offers a cheaper yet highly efficient alternative. The combined action of amylase and lipase allows it to be used in a wide range of household applications, from cleaning kitchen surfaces and appliances to breaking down organic waste and grease in drains. This affordability, combined with its efficiency, makes the bio enzyme cleaner an attractive option for consumers seeking reliable, economical, and multipurpose solutions. Beyond its effectiveness and low cost, the bio enzyme cleaner meets the growing demand for environmentally friendly cleaning solutions. As a natural, biodegradable product, it offers a sustainable alternative to traditional chemical cleaners. The enzymatic breakdown of organic matter reduces the reliance on harmful chemicals, making the cleaner safer for both users and the environment. This contributes to reducing pollution and promoting healthier ecosystems. The bio enzyme cleaner is a vital tool for promoting a greener future. As research and innovation in bio enzyme technology continue to advance, the potential for bio enzymes to revolutionize cleaning industries and contribute to a healthier planet will only expand. By adopting and promoting bio enzyme cleaners, we take significant steps toward reducing our environmental footprint and creating a more sustainable and eco-friendlier world for future generations. With further optimization, this product holds great potential for diverse cleaning applications while contributing to environmental sustainability and the reduction of harmful chemical use.

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Conflicts of interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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