International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Eco-Friendly Detergent Formulation Using Cellulolytic and Proteolytic Bacterial Enzymes from Waste Paper & Pistachio Shells

Afra Shaikh[1], Tanaya Mondal[2], Prof. (Dr.) S.V Raut[3]

[1,2,3]Department of Microbiology, M.M. College of Arts, N.M. Institute of Science, H.R.J. College of Commerce, Bhavans College, Munshi Nagar, Andheri West, Mumbai 400058, India.

Abstract

The increasing generation of paper waste has highlighted the need for sustainable waste management approaches. This study aimed to isolate and characterize cellulolytic and proteolytic bacteria from waste paper and evaluate their crude enzymes for use in enzyme-based detergent formulations. Waste paper samples were screened on Skim milk agar and Carboxymethyl cellulose (CMC) agar with Congo red staining. Selected isolates were characterized by morphological, Gram staining, and biochemical tests, while hydrolytic capacity and enzyme production were assessed using the Folin Ciocalteu and DNS methods. The most efficient isolates were incorporated individually and in combination into detergent formulations, and their cleaning performance was evaluated by stain removal and colorimetric analysis. Eight proteolytic and five cellulolytic bacterial isolates were obtained. Among these, P1 exhibited the highest protease production, while C5 showed the greatest cellulase production and hydrolytic efficiency. Enzyme-based formulations outperformed the enzyme-free control, with combined cellulase and protease producing the best cleaning performance due to synergistic action. These findings demonstrate that waste paper is a valuable source of hydrolytic enzyme-producing bacteria and highlight the potential of their crude enzymes for eco-friendly detergent formulations, sustainable waste valorization, and green biotechnological applications.

Keywords: Waste Paper, Cellulase, Protease, Enzyme Biotechnology, Eco-Friendly Detergent, Waste Valorization.

Introduction

The rapid growth of the global population, accelerated urbanization, and expanding industrial activities have resulted in a substantial increase in the generation of municipal solid waste worldwide. Among the various components of solid waste, paper waste constitutes a significant proportion because of its extensive use in educational institutions, offices, printing industries, packaging sectors, and households (Sulyman et al., 2020). Although paper is considered a biodegradable material and various recycling technologies have been developed, a considerable quantity of waste paper continues to be disposed of through landfilling or incineration. These disposal practices not only contribute to environmental pollution but also result in the loss of valuable renewable resources (Gupta et al., 2012). Consequently, the development of environmentally sustainable and economically feasible strategies for waste paper management has become an important area of scientific research (Sulyman et al., 2020).

Paper is primarily manufactured from plant-derived lignocellulosic biomass and mainly consists of cellulose fibers obtained from wood pulp and other plant sources (Sulyman et al., 2020). Besides cellulose, paper contains varying amounts of hemicellulose, traces of lignin, mineral fillers, pigments, and several chemical additives incorporated during manufacturing to improve strength, brightness, durability, water resistance, and printability (Gupta et al., 2012). Protein-based sizing agents, coatings, and adhesives may also be present in several paper products. Although cellulose is naturally biodegradable, these additional constituents often reduce the rate of natural degradation, leading to prolonged persistence of paper waste in the environment (Gupta et al., 2012).

Biological degradation of paper waste using microorganisms has emerged as an effective, eco-friendly, and sustainable alternative to conventional waste management methods. Numerous bacteria and fungi possess the ability to degrade complex organic polymers through the secretion of extracellular hydrolytic enzymes, converting them into simpler compounds that can subsequently be utilized as carbon and energy sources (Sulyman et al., 2020). Among these enzymes, cellulases and proteases play particularly important roles in the degradation of paper-based materials. Cellulases catalyze the hydrolysis of cellulose into soluble sugars, whereas proteases degrade proteinaceous compounds such as gelatin, casein, and other protein-based additives present in paper products (Gupta et al., 2012). The combined action of these enzymes accelerates the biodegradation process and facilitates the recycling of organic matter within natural ecosystems (Sulyman et al., 2020).

In recent years, microbial enzymes have attracted considerable attention because of their broad range of industrial applications. Owing to their high catalytic efficiency, substrate specificity, and environmentally friendly nature, these enzymes are widely utilized in food processing, textile manufacturing, pharmaceutical production, agriculture, biofuel generation, pulp and paper industries, and waste management technologies (Gupta et al., 2012; Sulyman et al., 2020). Their ability to function under relatively mild conditions significantly reduces energy consumption and minimizes the dependence on hazardous chemicals, making enzyme-based processes more sustainable than conventional chemical methods.

Microbial enzymes have become indispensable components of modern detergent formulations due to their ability to enhance cleaning efficiency while reducing the environmental impact associated with conventional detergents. Commercial detergents typically contain surfactants, builders, bleaching agents, fillers, stabilizers, and various performance-enhancing additives. Among these components, enzymes such as proteases, cellulases, lipases, and amylases play a crucial role in the targeted degradation of different types of stains (Gupta et al., 2012; Tilahun, 2020). Proteases hydrolyze peptide bonds present in protein-based stains, including blood, milk, egg, and food residues, thereby improving stain removal. Cellulases, on the other hand, hydrolyze microfibrils present on cotton fibers, enhancing fabric softness, brightness, and colour retention without damaging the fabric structure. The incorporation of these enzymes enables effective cleaning at lower washing temperatures and with reduced detergent concentrations, thereby conserving energy and minimizing the release of harmful chemicals into the environment (Tilahun, 2020; Ünlü-Yokuş et al., 2024).

Alongside microbial enzymes, agricultural residues have gained increasing attention as sustainable raw materials for various biotechnological applications. Large quantities of agro-industrial wastes are generated annually, many of which are rich in lignocellulosic biomass and can serve as economical substrates for microbial growth and enzyme production (Sulyman et al., 2020). Among these residues, pistachio shells represent an abundant agricultural by-product produced during the processing of pistachio

nuts. These shells primarily consist of cellulose, hemicellulose, and lignin, making them a valuable renewable resource for environmental and industrial applications (Anastopoulos et al., 2019; Igwegbe et al., 2023). Previous studies have demonstrated the potential use of pistachio shells as adsorbents for pollutants, substrates for microbial fermentation, and precursors for activated carbon production. Their effective utilization not only reduces agricultural waste accumulation but also promotes sustainable resource recovery and circular bioeconomy practices (Anastopoulos et al., 2019; Igwegbe et al., 2023).

Waste paper environments provide a unique ecological niche for microorganisms capable of degrading cellulose and other complex organic compounds. Continuous exposure to cellulose-rich substrates promotes the selection of microorganisms possessing efficient cellulolytic and proteolytic enzyme systems (Sulyman et al., 2020). Such microorganisms represent promising candidates for industrial enzyme production because of their ability to efficiently degrade lignocellulosic materials under diverse environmental conditions. Isolation and characterization of these naturally occurring microorganisms can therefore contribute to the discovery of novel enzyme producers with enhanced industrial potential (Gupta et al., 2012).

In view of these considerations, the present study was undertaken to isolate and characterize cellulolytic and proteolytic microorganisms from waste paper samples and to evaluate their potential for extracellular enzyme production. Waste paper was selected as the primary substrate because of its abundance, low cost, renewable nature, and high cellulose content, together with the presence of protein-based additives that support the growth of diverse enzyme-producing microorganisms (Gupta et al., 2012). The isolated microorganisms were screened for cellulase and protease production, followed by enzyme extraction and quantitative estimation of enzymatic activity. Furthermore, the extracted enzymes were incorporated into detergent formulations to evaluate their effectiveness in stain removal and fabric-care applications. The findings of this study are expected to contribute to sustainable waste paper management, promote the utilization of microbial enzymes in environmentally friendly detergent formulations, and provide valuable insights into the development of green biotechnological processes for industrial applications (Sulyman et al., 2020; Tilahun, 2020; Ünlü-Yokuş et al., 2024).

Materials and Methods

Materials

Waste paper samples comprising newspaper, cardboard, dictionary paper, scrap paper, and pistachio shells were collected from local sources and used for the isolation of cellulolytic and proteolytic bacteria. Analytical-grade chemicals and microbiological media were used throughout the study. Carboxymethyl cellulose (CMC) agar and broth were employed for the isolation and cultivation of cellulolytic bacteria, whereas skim milk agar was used for screening protease-producing isolates. Biochemical characterization was performed using standard microbiological media, including MR–VP broth, Simmons citrate agar, nitrate broth, tryptone broth, gelatin medium, lysine and ornithine decarboxylase broths, carbohydrate fermentation media, and egg yolk agar. Reagents used for enzyme assays included dinitrosalicylic acid (DNS), Folin–Ciocalteu reagent, bovine serum albumin (BSA), casein, tyrosine, Congo red, sodium chloride, trichloroacetic acid (TCA), and phosphate-buffered saline (PBS).

Methods:

Isolation of Protease-Producing Bacteria

Approximately 1 g of each waste sample was suspended in sterile physiological saline to obtain a homog-

eneous microbial suspension. Aliquots were streaked onto skim milk agar plates and incubated at room temperature for 24–48 h. Colonies surrounded by distinct zones of casein hydrolysis were considered potential protease producers. Morphologically distinct colonies were purified by repeated streaking and preserved for further characterization (Gupta et al., 2002), (Smibert & Krieg, 1994; Nguyen et al., 2013).

Isolation of Cellulase-Producing Bacteria

For enrichment of cellulolytic bacteria, 1 g of each sample was inoculated into sterile CMC broth and incubated on a rotary shaker at 30°C for seven days. Enriched cultures were streaked onto CMC agar plates and incubated for 48–72 h. Plates were subsequently flooded with 1% Congo red solution followed by destaining with 1 M sodium chloride. Colonies exhibiting clear halos against the stained background were selected as cellulase-producing isolates and purified for further investigation (Gupta et al., 2012; Teather & Wood, 1982; Levin et al., 2022).

Morphological and Biochemical Characterization

Purified isolates were characterized based on colony morphology, Gram staining, and biochemical properties. Colony characteristics, including size, pigmentation, opacity, elevation, margin, and consistency, were recorded after incubation. Gram staining was performed using the standard crystal violet–iodine method followed by alcohol decolorization and safranin counterstaining.

Biochemical characterization included catalase, oxidase, citrate utilization, indole production, Voges–Proskauer reaction, nitrate reduction, gelatin liquefaction, lecithinase activity, lysine decarboxylase, ornithine decarboxylase, and carbohydrate fermentation tests using glucose, xylose, mannitol, sorbitol, xylitol, and arabinose. The identities of bacterial isolates were assigned by comparing the observed phenotypic characteristics with standard descriptions in Bergey's Manual of Systematic Bacteriology. Because molecular identification was not performed, all bacterial identifications were considered putative (Gupta et al., 2002; Nguyen et al., 2013; Gudeta & Krishna, 2019) (Cappuccino & Sherman, 2008).

Determination of Hydrolytic Capacity

Qualitative enzyme production was evaluated by measuring hydrolysis zones surrounding bacterial colonies on selective media. Colony diameter and halo diameter were measured in millimetres, and hydrolytic capacity (HC) was calculated using the following equation:

HC = Diameter of hydrolysis zone / Diameter of colony

Isolates exhibiting higher HC values were considered superior enzyme producers and selected for quantitative enzyme analysis (Smibert & Krieg, 1994; Nguyen et al., 2013).

Production and Extraction of Crude Enzymes

Selected bacterial isolates were cultivated in their respective production broths under shaking conditions for 48 h. Cultures were centrifuged at 5,000 rpm for 15 min at 4°C to remove bacterial cells. The resulting cell-free supernatants were collected and used as crude extracellular enzyme preparations for subsequent analyses (Gupta et al., 2002; Rao et al., 1998).

Quantitative Protease Assay

Protease activity was determined using the casein–Folin–Ciocalteu method. Casein served as the substrate, and enzyme activity was estimated by measuring the amount of tyrosine released following hydrolysis. After incubation, proteins were precipitated using trichloroacetic acid, and the soluble peptides reacted with alkaline copper reagent followed by Folin–Ciocalteu reagent. Absorbance was measured spectrophotometrically at 660 nm. Tyrosine standards were used to generate a calibration curve, and enzyme activity was calculated accordingly (Gupta et al., 2002; Nguyen et al., 2013).

Quantitative Cellulase Assay

Cellulase activity was determined using the dinitrosalicylic acid (DNS) assay. Crude enzyme preparations were incubated with 1% carboxymethyl cellulose substrate under standard assay conditions. Reducing sugars released during cellulose hydrolysis reacted with DNS reagent after boiling, and absorbance was recorded at 540 nm. Glucose was used as the standard for quantification (Gupta et al., 2012; Sulyman et al., 2020).

Determination of Protein Concentration and Specific Activity

Protein concentration of crude enzyme preparations was determined by the Lowry method using bovine serum albumin as the standard. Specific enzyme activity was calculated by dividing enzyme activity by the corresponding protein concentration and expressed as units per milligram of protein.

Preparation of Enzyme-Based Detergent Formulations

A detergent base containing sodium citrate, sodium carbonate, carboxymethyl cellulose, glycerol, calcium chloride, sodium formate, sodium benzoate, and tergitol was prepared. Crude cellulase and protease extracts obtained from the highest-performing isolates were incorporated individually at concentrations of 5% and 10% (v/v). Additional formulations containing combinations of selected cellulase and protease extracts were prepared to investigate synergistic cleaning performance.

Evaluation of Cleaning Performance

Cleaning efficiency was evaluated using standardized cotton fabric samples stained with flavoured milk. Stained fabrics were treated with enzyme-containing detergent formulations under identical washing conditions. Enzyme-free detergent served as the control. Following washing and drying, stain removal efficiency was evaluated using CIELAB colour parameters (L*, a*, and b*), and colour difference (ΔE) values were calculated to compare cleaning performance among formulations.

The combined enzyme formulations were further evaluated for dye degradation using Basic Fuchsin and a Tartrazine–Brilliant Blue FCF dye mixture. Following incubation, residual dye concentration was determined spectrophotometrically, and colourimetric parameters were calculated to assess the decolourization efficiency of each enzyme combination.

Results

Isolation and Screening of Enzyme-Producing Bacteria

A total of thirteen bacterial isolates exhibiting extracellular hydrolytic activity were recovered from waste paper and associated lignocellulosic samples. Of these, eight isolates (P1–P8) produced distinct zones of casein hydrolysis on skim milk agar and were designated as protease-producing bacteria (Fig 1). Five isolates (C1–C5) produced clear halos on carboxymethyl cellulose (CMC) agar following Congo red staining, confirming cellulolytic activity (Fig 2).

The successful recovery of both proteolytic and cellulolytic bacteria demonstrates that waste paper provides a suitable ecological niche for microorganisms capable of producing industrially relevant hydrolytic enzymes.

Fig 1: Isolated colonies of protease producers on Skim milk agar plate.

Fig 2: Isolated colonies of cellulase producers on CMC agar plate flooded with Congo red dye.

Morphological and Biochemical Characterization

The bacterial isolates displayed considerable variation in colony morphology, pigmentation, colony size, and biochemical characteristics. Most protease-producing isolates were Gram-positive rod-shaped bacteria, whereas cellulase-producing isolates were predominantly Gram-negative rods.

Based on colony morphology, Gram reaction, and biochemical characteristics, the isolates were putatively assigned to different bacterial genera. The character exhibited by each isolates are enlisted in Table 1. The proteolytic isolates showed characteristics consistent with members of the genera *Bacillus* and *Serratia*, while the cellulolytic isolates were putatively identified as *Flavobacterium*, *Cellvibrio*, *Sphingomonas*, *Pseudomonas*, and *Enterobacter*. The probable genera of the isolates were determined by biochemical identification. Since identification was based solely on phenotypic characteristics, these assignments should be regarded as tentative pending molecular confirmation.

Hydrolytic Capacity of the Isolates

Hydrolytic capacity (HC) was determined by measuring the ratio between the diameter of the hydrolysis zone and colony diameter.

Among the protease-producing isolates, P1 exhibited the highest HC ratio (3.0), followed by P3 (2.8) and P7 (2.5), indicating superior extracellular protease production. In contrast, P6 recorded the lowest HC ratio (1.3).

Among cellulase-producing isolates, C5 demonstrated the highest HC ratio (2.1), followed by C4 (1.8) and C1 (1.7), whereas C2 exhibited the lowest cellulolytic activity.

These findings identified P1 and C5 as the most promising enzyme-producing isolates for subsequent quantitative enzyme analysis.

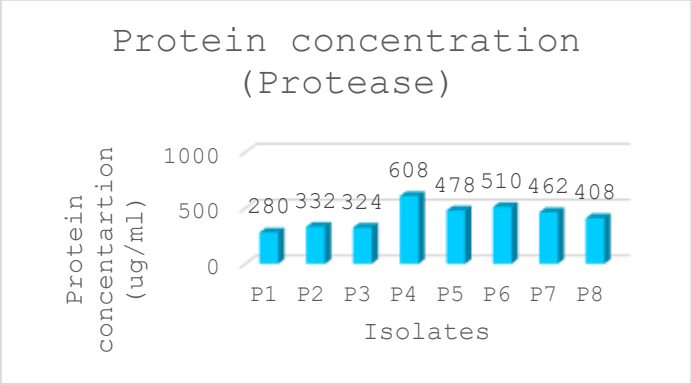
Protein Concentration of Crude Enzyme Extracts

Protein concentration varied considerably among the isolates, indicating differences in extracellular enzyme secretion.

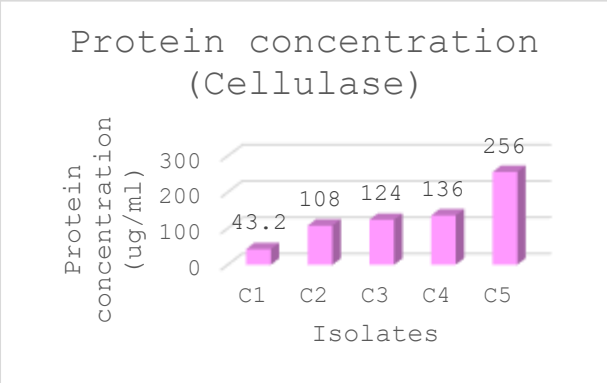
Among protease-producing bacteria, P4 exhibited the highest extracellular protein concentration (608 µg mL^{-1}), followed by P6 (510 µg mL^{-1}) and P5 (478 µg mL^{-1}).

Among cellulase-producing isolates, C5 produced the highest protein concentration (256 µg mL^{-1}), while C1 produced the lowest (43.2 µg mL^{-1}). Graph 1 and 2.

These observations indicate variability in protein secretion among the recovered bacterial isolates.



Graph 1: Protein concentration of crude protease enzyme.

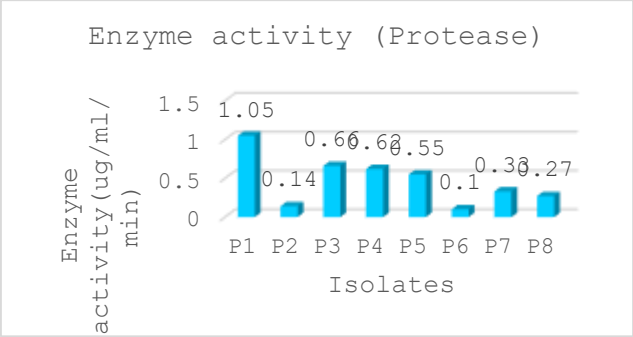


Graph 2: Protein concentration of crude cellulase enzyme.

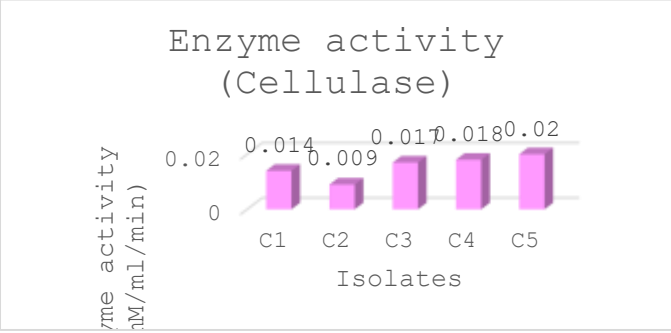
Enzyme Activity

Quantitative enzyme assays demonstrated substantial differences in catalytic activity among the isolates. Protease activity ranged from 0.10 to 1.05 µg mL^{-1} min^{-1}. Isolate P1 exhibited the highest enzyme activity (1.05 µg mL^{-1} min^{-1}), followed by P3 (0.66 µg mL^{-1} min^{-1}) and P4 (0.62 µg mL^{-1} min^{-1}).

Cellulase activity ranged from 0.009 to 0.020 mM mL^{-1} min^{-1}, with isolate C5 showing the greatest activity (0.020 mM mL^{-1} min^{-1}), followed by C4 (0.018 mM mL^{-1} min^{-1}) and C3 (0.017 mM mL^{-1} min^{-1}). From the graph 3 and 4 it is confirmed that P1 and C5 were the most efficient producers of protease and cellulase, respectively.



Graph 3: Enzyme activity of crude protease.



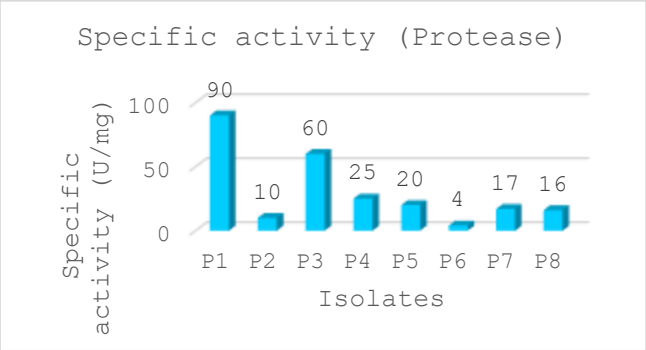
Graph 4: Enzyme activity of crude cellulase.

Specific Enzyme Activity

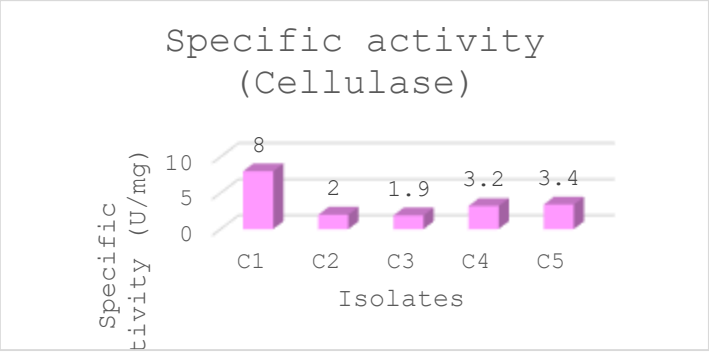
Specific activity was calculated to evaluate enzyme efficiency relative to protein concentration, Graph 5 and 6 demonstrates the calculated specific activity of the isolates obtained.

Among protease-producing isolates, P1 exhibited the highest specific activity (90 U mg^{-1}), followed by P3 (60 U mg^{-1}). The remaining isolates showed considerably lower catalytic efficiency.

For cellulase-producing isolates, C1 recorded the highest specific activity (8 U mg^{-1}), followed by C5 (3.4 U mg^{-1}) and C4 (3.2 U mg^{-1}). Although C1 exhibited the greatest specific activity, C5 was selected for detergent formulation because it demonstrated superior hydrolytic capacity, protein production, and overall cellulase activity.



Graph 5: Specific activity of crude protease



Graph 6: Specific activity of crude cellulase

Evaluation of Enzyme-Based Detergent Formulations

Detergent formulations containing crude microbial enzymes exhibited improved cleaning efficiency compared with the enzyme-free detergent base.

Increasing enzyme concentration from 5% to 10% generally enhanced stain removal performance. The detergent formulation containing 10% crude protease from isolate P1 produced one of the highest brightness values, indicating efficient degradation of proteinaceous stains.

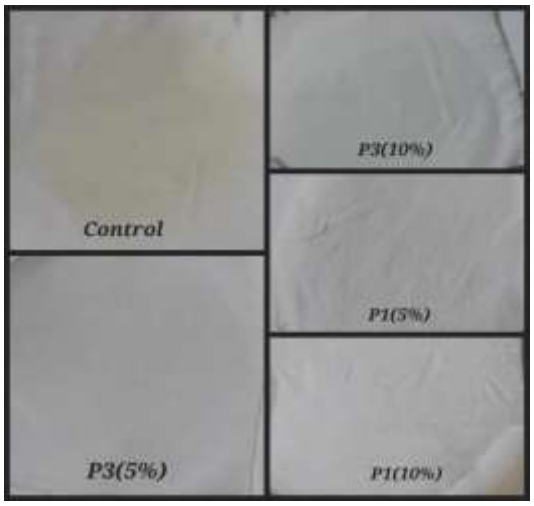


Fig 3: Stain removal on fabric after 30 mins of stain exposure.

Colourimetric analysis using the CIELAB system demonstrated a reduction in colour difference (ΔE) following treatment with enzyme-containing detergents, confirming effective stain removal.

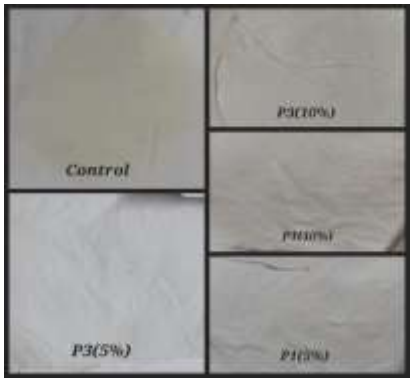


Fig 4: Stain removal on fabric after 1 hour of stain exposure.

Fig 5: Stain removal on fabric using combined enzyme formulations.

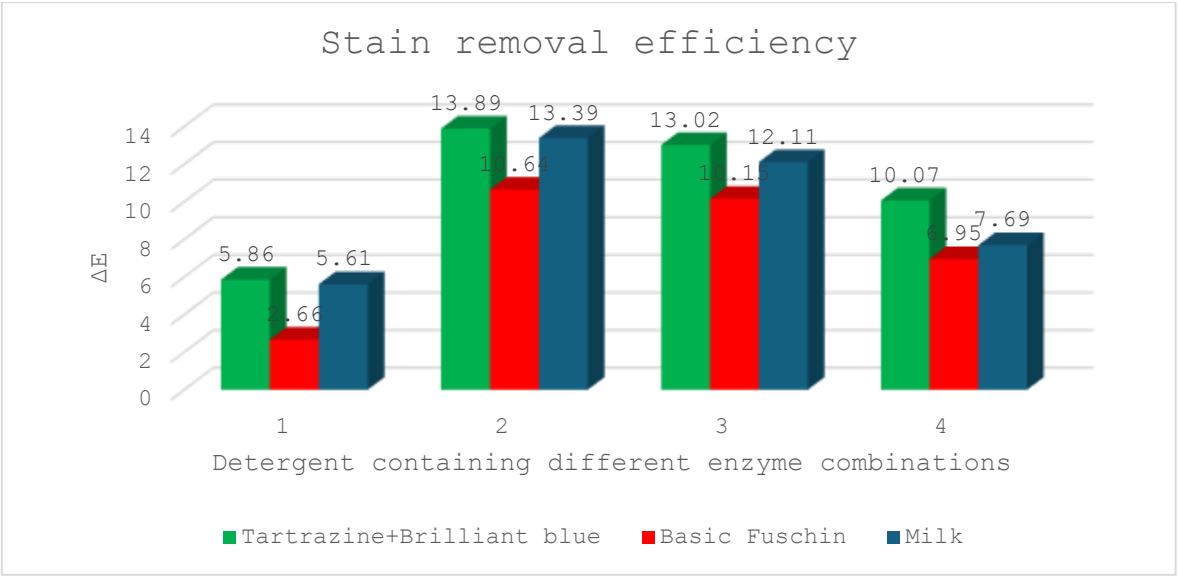
Performance of Combined Enzyme Formulations

Combined detergent formulations containing cellulase and protease produced superior cleaning performance compared with formulations containing individual enzymes.

Several enzyme combinations demonstrated enhanced removal of milk stains and synthetic dyes, suggesting synergistic interactions between cellulase and protease during stain degradation.

Spectrophotometric analysis of Basic Fuchsin and Tartrazine–Brilliant Blue dye solutions further confirmed the decolourization ability of the combined enzyme formulations. Changes in colourimetric parameters (L*, a*, b*, and ΔE) indicated that enzyme combinations improved dye degradation efficiency relative to the enzyme-free control.

Overall, the combined enzyme formulations exhibited greater cleaning efficiency than formulations containing individual crude enzymes as exhibited by Graph 8, highlighting the potential application of mixed microbial enzyme systems in environmentally friendly detergent formulations.



Graph 8: Comparative analysis of stain removal efficiency of different enzyme based detergent formulation based on ΔE values

Fabric softening activity of Cellulase:

The fabric softening activity of cellulase enzyme based detergent formulation was evaluated using a sensory assessment method and compared with a commercially available fabric softener used as the control. Two cellulase-producing isolates, C4 and C5, were tested at concentrations of 5% and 10% on fabric samples. The treated fabrics were assessed manually for softness and texture after washing. The results indicated that both enzyme extracts exhibited noticeable fabric softening activity compared to untreated samples. Among the tested concentrations, the 10% enzyme solutions demonstrated relatively better softening effects than the 5% concentrations for both C4 and C5 extracts. However, the degree of softness achieved with the enzyme treatments was lower than that observed with the commercial fabric softener control. These findings suggest that cellulase enzymes produced by the isolates possess fabric softening potential, although their effectiveness was comparatively lower than that of the commercial product.

Discussion

The present study demonstrates that waste paper serves as an effective reservoir of cellulolytic and proteolytic bacteria capable of producing extracellular hydrolytic enzymes with potential industrial applications. The successful isolation of thirteen enzyme-producing bacterial isolates confirms that cellulose-rich waste materials support diverse microbial communities adapted to degrade complex organic substrates. These findings reinforce the concept that paper waste is not merely an environmental pollutant but also a valuable source of industrially important microorganisms that can be exploited for sustainable biotechnology.

Among the protease-producing isolates, P1 exhibited the highest hydrolytic capacity, enzyme activity, and specific activity, indicating superior extracellular protease production. Members of the genus *Bacillus*, to which P1 was putatively assigned, are among the most extensively studied industrial producers of alkaline proteases because of their rapid growth, high enzyme yield, and ability to secrete enzymes directly into the extracellular environment. Similar observations have been reported in previous studies, where *Bacillus species* consistently demonstrated high proteolytic activity and suitability for detergent applications. The superior performance of P1 may therefore be attributed to its efficient secretion of extracellular proteases and its adaptation to protein-rich environmental substrates.

The cellulase-producing isolates also exhibited considerable variation in enzyme production. Isolate C5 demonstrated the highest hydrolytic capacity, protein concentration, and cellulase activity, indicating its superior ability to degrade cellulose. Although C1 showed the highest specific activity, C5 was selected for detergent formulation because its overall enzyme production and cellulolytic performance were substantially greater. This observation illustrates that selection of industrial strains should be based on overall production efficiency rather than a single enzymatic parameter. Similar trends have been reported in cellulase-screening studies, where isolates exhibiting high enzyme yield often outperform strains with comparatively higher specific activity during practical applications.

The observed differences in enzyme production among the isolates are likely due to species-specific metabolic capabilities and differences in extracellular enzyme secretion. Genetic variation, regulatory mechanisms controlling enzyme synthesis, substrate utilization efficiency, and environmental adaptation all influence the production of hydrolytic enzymes. Waste paper contains cellulose together with starch, proteins, adhesives, and other organic compounds that may selectively enrich microorganisms possessing

multiple degradative enzyme systems. Such environmental adaptation probably contributed to the diversity of enzyme-producing bacteria recovered during the present investigation.

Detergent formulations containing crude microbial enzymes exhibited improved stain removal compared with the enzyme-free detergent base. Increasing enzyme concentration from 5% to 10% generally enhanced cleaning efficiency, demonstrating that enzyme dosage influences detergent performance. Proteases facilitate the degradation of protein-based stains such as milk by hydrolysing peptide bonds into smaller soluble fragments that are more readily removed during washing. This finding agrees with previous reports describing microbial proteases as the most important class of enzymes used in commercial laundry detergents.

The incorporation of cellulase into detergent formulations provided additional benefits beyond stain removal. Cellulases hydrolyse damaged cellulose microfibrils present on cotton fibres, thereby reducing surface fuzz, improving fabric softness, restoring brightness, and enhancing colour retention. These characteristics explain the widespread incorporation of cellulases into modern fabric-care detergents. The improved colourimetric values observed in the present study suggest that crude cellulase preparations were capable of modifying the fibre surface sufficiently to enhance the overall appearance of washed fabrics.

An important finding of this study was the improved performance of detergent formulations containing both cellulase and protease. Combined enzyme formulations generally exhibited greater stain removal and dye decolourization than formulations containing individual enzymes, indicating a synergistic interaction between the two enzyme systems. Proteases remove proteinaceous deposits that may hinder access to fibre surfaces, whereas cellulases act directly on exposed cellulose microfibrils. The complementary action of these enzymes likely contributed to the enhanced cleaning efficiency observed in the combined formulations.

The colourimetric analysis using the CIELAB colour system provided an objective method for evaluating detergent performance. Increases in L* values and corresponding changes in ΔE confirmed that enzyme-containing formulations produced greater stain removal than the enzyme-free control. The use of instrumental colour analysis minimizes observer bias and provides quantitative evidence supporting the cleaning efficacy of microbial enzyme formulations.

From an environmental perspective, the study supports the concept of waste valorization by converting paper waste into a source of industrially useful microorganisms. The production of crude enzymes from naturally occurring bacteria offers an environmentally sustainable alternative to chemically intensive detergent additives. Furthermore, the use of crude enzyme preparations without extensive purification may reduce production costs and improve the commercial feasibility of enzyme-based detergents.

Despite these promising findings, several limitations should be acknowledged. Bacterial identification was based solely on morphological and biochemical characteristics; therefore, the identities of the isolates remain putative. Molecular characterization using 16S rRNA gene sequencing would provide more reliable taxonomic identification. In addition, enzyme purification, optimization of fermentation conditions, determination of enzyme stability over a wide range of pH and temperatures, compatibility studies with commercial detergent ingredients, and pilot-scale washing trials should be undertaken before large-scale industrial application. Future investigations should also evaluate storage stability and shelf life of the formulated enzyme detergents.

Overall, the findings demonstrate that waste paper is a promising source of cellulolytic and proteolytic bacteria capable of producing industrially relevant enzymes. The enhanced cleaning performance achieved by combined crude enzyme formulations highlights their potential as sustainable alternatives to

conventional detergent additives and supports the development of environmentally friendly biotechnological products.

Conclusion

The present study demonstrated that waste paper serves as a valuable source of cellulolytic and proteolytic bacteria capable of producing extracellular hydrolytic enzymes with significant industrial potential. Thirteen bacterial isolates exhibiting cellulase and protease activities were successfully recovered and characterized using morphological and biochemical methods. Among the isolates, P1 and C5 exhibited superior enzymatic performance based on hydrolytic capacity, enzyme activity, and protein production, making them suitable candidates for detergent formulation.

Crude enzyme preparations incorporated into detergent formulations enhanced stain removal efficiency compared with the enzyme-free detergent base. Furthermore, formulations containing both cellulase and protease exhibited improved cleaning performance, suggesting a synergistic interaction between the two enzymes. Colorimetric evaluation using the CIELAB system further confirmed the effectiveness of the enzyme-based formulations.

These findings highlight the potential of utilizing waste paper as a renewable source of industrially relevant microorganisms while supporting sustainable waste valorization. The use of crude microbial enzymes represents an environmentally friendly alternative to conventional detergent additives and may contribute to the development of greener cleaning products. Future studies should focus on molecular identification of the isolates, optimization of enzyme production, purification and characterization of the enzymes, compatibility with commercial detergent formulations, and large-scale industrial evaluation to facilitate commercial application.

References:

1. Gupta, P., et al. (n.d.). Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. [Complete journal name, volume, issue, pages, and DOI required].
2. Gupta, R., Beg, Q. K., & Lorenz, P. (2002). Bacterial alkaline proteases: Molecular approaches and industrial applications. Applied Microbiology and Biotechnology, 59(1), 15–32. https://doi.org/10.1007/s00253-002-0975-y
3. Gudeta, K., & Krishna, C. (2019). Cellulolytic microorganisms can be used as probiotics in ruminant diets to improve digestion of fibrous components. Journal of Biochemicals and Microbial Biotechnology, 2(1), 15–24.
4. Igwegbe, C. A., Onukwuli, O. D., Ighalo, J. O., & Umembamalu, C. J. (2023). Environmental applications of pistachio waste: A review. Environmental Pollution, 317, 120771. https://doi.org/10.1016/j.envpol.2022.120771
5. Nguyen, T. H., Quyen, D. T., & Le, H. T. (n.d.). Screening and characterization of protease-producing bacteria isolated from shrimp waste. [Journal details required].
6. Rao, M. B., Tanksale, A. M., Ghatge, M. S., & Deshpande, V. V. (1998). Molecular and biotechnological aspects of microbial proteases. Microbiology and Molecular Biology Reviews, 62(3), 597–635.
7. Smibert, R. M., & Krieg, N. R. (1994). Phenotypic characterization. In P. Gerhardt, R. G. E. Murray, W. A. Wood, & N. R. Krieg (Eds.), Methods for General and Molecular Bacteriology (pp. 607–654). American Society for Microbiology.

8. Teather, R. M., & Wood, P. J. (1982). Use of Congo red–polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Applied and Environmental Microbiology, 43(4), 777–780.
9. Tilahun, R. (2020). Production and characterization of protease enzyme and its application in detergent industry. International Journal of Microbiology and Biotechnology, 5(4), 193–202. https://doi.org/10.11648/j.ijmb.20200504.15
10. Ünlü-Yokuş, Y., et al. (n.d.). Amylolytic and proteolytic bacteria in deteriorated paper-based historical manuscripts. [Complete journal details required].