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Production of Eco-Friendly Liquid Detergent with Modified Microbial Enzymes

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Abstract
The environmental impact of conventional chemical detergents has increased the demand for sustainable and biodegradable alternatives. In the present study, microbial hydrolytic enzymes were explored for the development of an eco-friendly liquid detergent formulation. Bacterial strains producing amylase, protease, and lipase were isolated from environmentally stressed habitats and screened using substrate-specific media. Selected isolates were cultured for enzyme production, and crude enzymes were partially purified by ammonium sulphate fractionation. The enzymes were further modified through polysaccharide–aldehyde conjugation to improve stability and detergent compatibility. The modified enzymes exhibited enhanced stability and retained higher relative activity in the presence of detergent formulation components, including surfactants, builders, viscosity modifiers, and preservatives, compared to unmodified enzymes. An enzyme-based liquid detergent was formulated using the modified enzymes and evaluated for cleaning efficiency by visual stain removal analysis. The formulated detergent demonstrated effective removal of common household stains and showed performance comparable to a commercial detergent, while water served as a negative control. The findings indicate that modified microbial hydrolytic enzymes can be effectively utilized in eco-friendly liquid detergent formulations, offering a sustainable alternative to conventional detergents and contributing to environmentally responsible cleaning technologies.

Keywords: Microbial enzymes; Amylase; Protease; Lipase; Enzyme modification; Eco-friendly detergent; Liquid detergent formulation

1. Introduction

The extensive use of conventional chemical detergents has raised serious environmental and ecological concerns due to their poor biodegradability, aquatic toxicity, and high energy requirements during use. Surfactants, builders, and other chemical additives commonly present in detergents often persist in the environment and contribute to water pollution and ecosystem imbalance. These challenges have intensified the search for sustainable and eco-friendly alternatives capable of maintaining cleaning efficiency while reducing environmental impact.

Enzyme-based detergents have emerged as an effective and environmentally benign alternative to conventional formulations. Hydrolytic enzymes such as amylases, proteases, and lipases catalyze the breakdown of complex stains into simpler, water-soluble products, enabling efficient cleaning under mild conditions (Banerjee et al., 2014). The use of enzymes allows washing at lower temperatures and reduced chemical load, thereby conserving energy and minimizing environmental pollution. Among various enzyme sources, microorganisms are preferred due to their rapid growth, ease of cultivation, cost-effective production, and ability to produce extracellular enzymes with desirable industrial properties.

Microbial enzymes used in detergent formulations must exhibit stability under alkaline pH, elevated temperatures, and in the presence of surfactants, builders, and preservatives. However, many native enzymes suffer from reduced stability when incorporated into liquid detergent systems. To overcome these limitations, enzyme modification strategies such as chemical conjugation have been explored to enhance enzyme robustness, shelf life, and compatibility with detergent components. Polysaccharide–aldehyde conjugation is one such approach that improves enzyme stability by forming covalent linkages, thereby protecting the enzyme structure under harsh formulation conditions.

In the present study, microbial hydrolytic enzymes were produced from bacterial isolates obtained from environmentally stressed habitats. The enzymes were partially purified and chemically modified to improve their detergent compatibility. An eco-friendly liquid detergent formulation was developed using the modified enzymes, and its performance was evaluated in comparison with a commercial detergent. (Jaeger and Eggert, 2002). This integrated approach aims to demonstrate the feasibility of using modified microbial enzymes for the development of sustainable and effective liquid detergent formulations.

2. MATERIALS & METHODS

2.1 Sample Collection and Isolation of Enzyme-Producing Bacteria

Environmental samples were collected from hot water springs at Ganeshpuri (Virar), dhobi ghat water from Jogeshwari and Lower Parel, and compost samples from Andheri and Bhayender. These habitats were selected to isolate bacteria adapted to extreme and variable environmental conditions. Samples were enriched in Nutrient Broth and incubated at room temperature for 48 h, followed by streaking on Nutrient Agar plates supplemented with specific substrates for enzyme screening. (Elamary et al., 2020) (Saini et al., 2016)

2.2 Screening for Hydrolytic Enzyme Production

Primary screening for amylase, protease, and lipase production was carried out using Nutrient Agar supplemented with 1% starch, 10% skim milk, and 1% tributyrin, respectively. Plates were incubated at room temperature for 48 h and examined for zones of clearance around colonies, indicating enzymatic hydrolysis. Efficient enzyme-producing isolates were selected for further studies. (Elamary et al., 2020) (Saini et al., 2016)

2.3 Production of Crude Enzyme Extracts

Selected bacterial isolates were inoculated into Nutrient Broth (pH 7.0) supplemented with 10% starch (amylase), 10% skim milk (protease), or 5% tributyrin (lipase). Cultures were incubated for three days under controlled conditions. After incubation, cultures were centrifuged at 11,000 rpm for 7 min, and the cell-free supernatants were collected as crude enzyme extracts. (Elamary et al., 2020) (Saini et al., 2016)

2.4 Partial Purification of Enzymes

Partial purification of enzymes was carried out by ammonium sulfate fractionation. Amylase was precipitated between 30–70% saturation, protease between 40–80% saturation, and lipase between 50–90% saturation. The precipitated enzymes were collected by centrifugation at 8,000 rpm for 10 min and stored in 0.1 M phosphate buffer (pH 7) at 4 °C. (Meera Venugopal et al., 2007)

2.5 Enzyme Modification by Polysaccharide–Aldehyde Conjugation

All enzymes were chemically modified using polysaccharide–aldehyde conjugation. Carboxymethyl cellulose (1%) was reacted with glutaraldehyde (0.1%) in phosphate buffer (pH 7) and incubated for 1 h at room temperature. The enzyme solution was then added and incubated for 2 h to allow Schiff base formation. Sodium cyanoborohydride (100 mM) was added to stabilize the covalent linkage, and the reaction was incubated overnight at 4 °C. The resulting preparation was used as modified enzyme. (Kübelbeck, S., et al., 2018)

2.6 Enzyme Tolerance Studies

Tolerance of unmodified and modified enzymes was evaluated against detergent formulation components including 5% Tergitol, 3% sodium citrate, 2% sodium chloride, and 0.5% sodium benzoate. Enzyme samples incubated in phosphate buffer (pH 7) served as controls. Residual enzyme activity was measured, and relative activity was calculated using control activity as 100%. (David et al., 2019)

2.7 Formulation of Enzyme-Based Liquid Detergent

An eco-friendly liquid detergent was formulated using modified amylase and lipase along with Tergitol, sodium citrate, sodium chloride, and sodium benzoate. The pH was adjusted to approximately 7, and the formulation was stirred to ensure homogeneity. (Xie et al., 2019)

2.8 Evaluation of Detergent Performance

Cleaning efficiency was evaluated using a visual stain removal method. Fabric samples stained with common household stains were washed using the formulated detergent, a commercial detergent (positive control), and water (negative control). Washing experiments were performed in triplicate, and stain removal efficiency was assessed visually. (Xie et al., 2019)

RESULTS & DISCUSSION

3.1 Isolation and Morphological Characterization of Bacterial Isolates

Characteristics	GP1	GP2	DBJ	DBLP	CA	CB
Size (mm)	2	2	1	2	2	2
Shape	Circular	Circular	Circular	Circular	Circular	Circular
Color	White	White	White	White	White	White
Margin	Entire	Entire	Entire	Entire	Entire	Entire
Consistency	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Opacity	Opaque	Translucent	Opaque	Opaque	Transparent	Opaque
Gram Nature	Gram +	Gram +	Gram +	Gram +	Gram +	Gram +
Morphology	Rods	Rods	Rods	Rods	Rods	Rods

* GP1: - Ganeshpuri hot water spring sample 1
GP2: - Ganeshpuri hot water spring sample 2
DBJ: - Dhobhi Ghat water sample from Jogeshwari
DBLP: - Dhobhi Ghat water sample from Lower Parel
CA: - Compost sample from Andheri
CB: - Compost sample from Bhayander

A total of six bacterial isolates were obtained from hot water spring, compost, and Dhobi ghat water following enrichment and plating on Nutrient Agar. All isolates formed well-defined colonies that were circular with smooth margins and creamy white appearance. Microscopic examination revealed that all isolates were Gram-positive, rod-shaped bacteria, indicating their probable affiliation with Bacillus-like genera commonly associated with extracellular enzyme production. (Elamary et al., 2020) (Saini et al., 2016)

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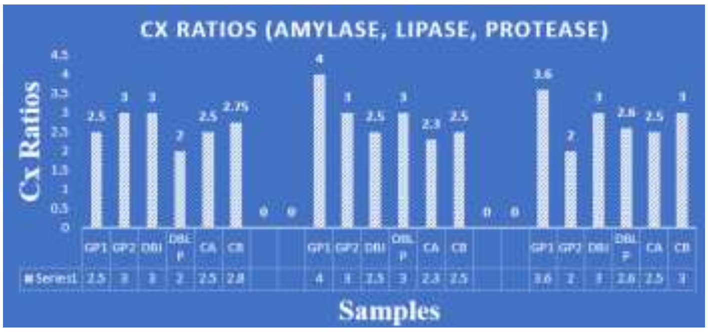
3.2 Screening for Hydrolytic Enzyme Production & Cx ratio analysis

Samples	1 % Starch Agar	1 % Tributyrin Agar	10 % Milk Agar
GP1	5 (2)	8 (2)	11 (3)
GP2	9 (3)	6 (2)	4 (2)
DBJ	6 (2)	5 (2)	9 (3)
DBLP	4 (2)	6 (2)	8 (3)
CA	10 (4)	7 (3)	5 (2)
CB	5.5 (2)	5 (2)	9 (3)

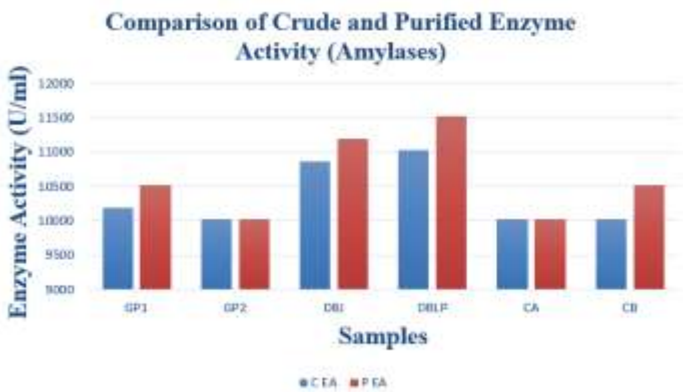
*Values in () represents size of colony

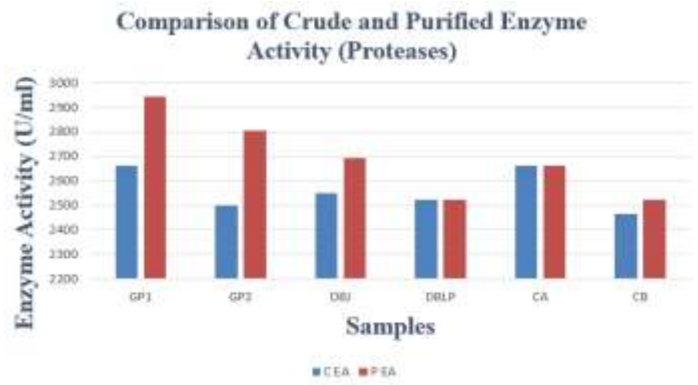
Samples	1 % Starch Agar	1 % Tributyrin Agar	10 % Milk Agar
GP1	2.5	4	3.6
GP2	3	3	2
DBJ	3	2.5	3
DBLP	2	3	2.6
CA	2.5	2.3	2.5
CB	2.75	2.5	3

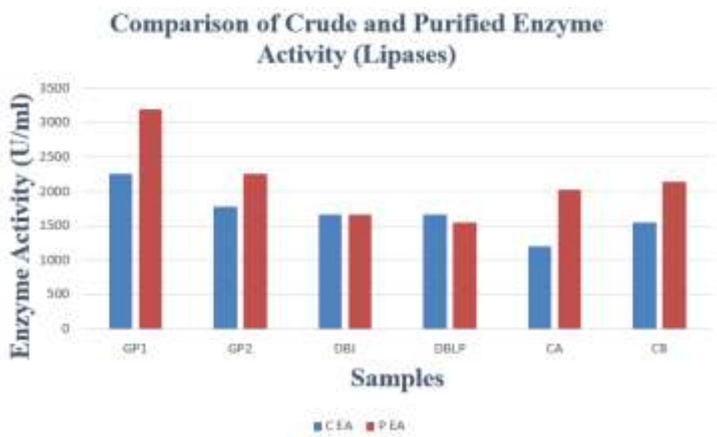


The C_x ratio analysis was calculated for caring out the primary screening and to check the hydrolytic capacity of bacteria from various sample, as seen in the above data and graph GP2 & DBJ samples exhibited highest C_x ratio and for protease and lipase it was GP1. C_x ratio was calculated by dividing the diameter of zone of clearance with that of the colonies. (Elamary et al., 2020) (Saini et al., 2016)

3.4 Enzyme Activity of Crude & Purified Extracts

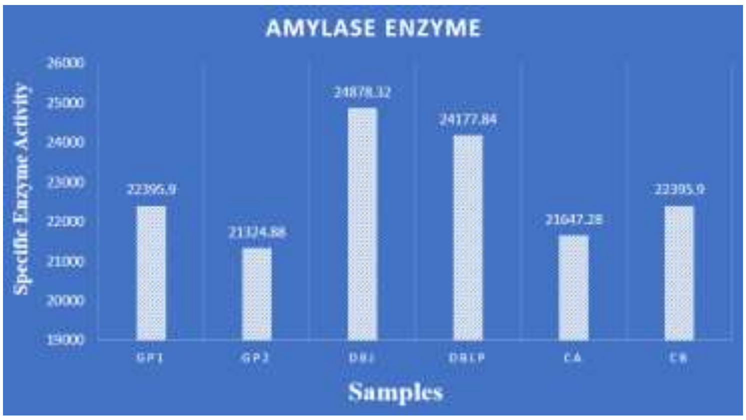


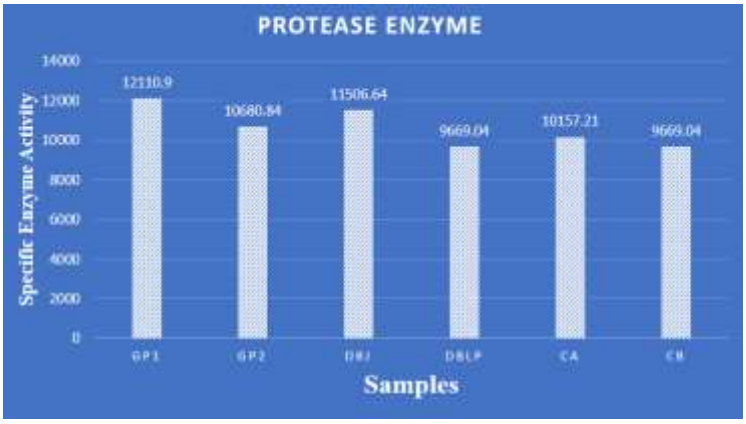


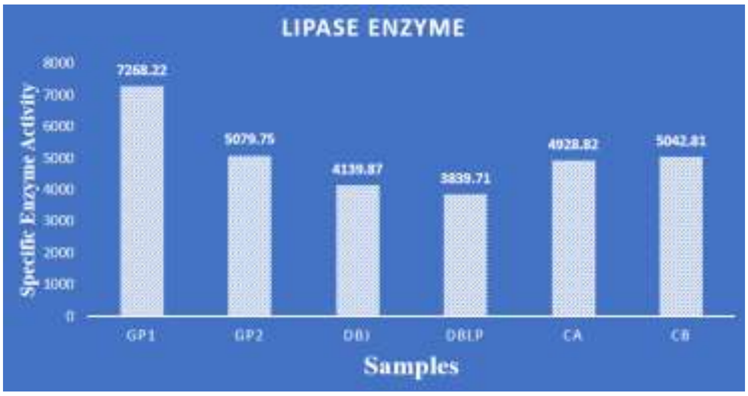


The above graph represents the comparison of the enzyme activities of crude and purified enzymes; The enzyme activity of purified enzyme was found to be significantly higher than the crude enzyme indicating the excellent effect of purification step which removed the unwanted proteins and salts it was calculated by plotting the standard graphs of 3,4 Di-Nitro Salicylic acid(DNSA) for amylase, Bovine Serum Albumin(BSA) for proteases & Phenol for Lipases. Few samples such as GP2 & CA in amylase, DBLP in protease and DBJ in Lipase showed no difference in the activity this may indicate that the crude extract already contained a high proportion of active enzyme with minimal inhibitory components. Additionally, possible enzyme loss during ammonium sulfate precipitation may have offset the expected increase in activity. Also, the activity of all the enzymes was as per the range of activity of enzyme used in commercial detergent. (Henkel AG & Co. KGaA, 2000)

3.5 Specific Activity of Purified Enzymes

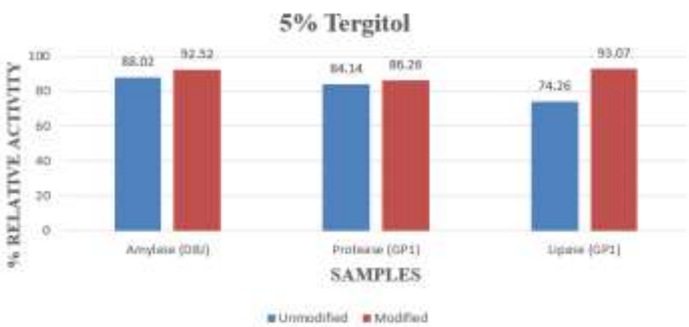


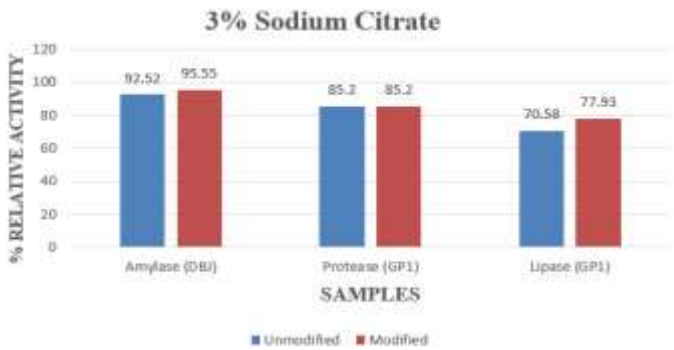


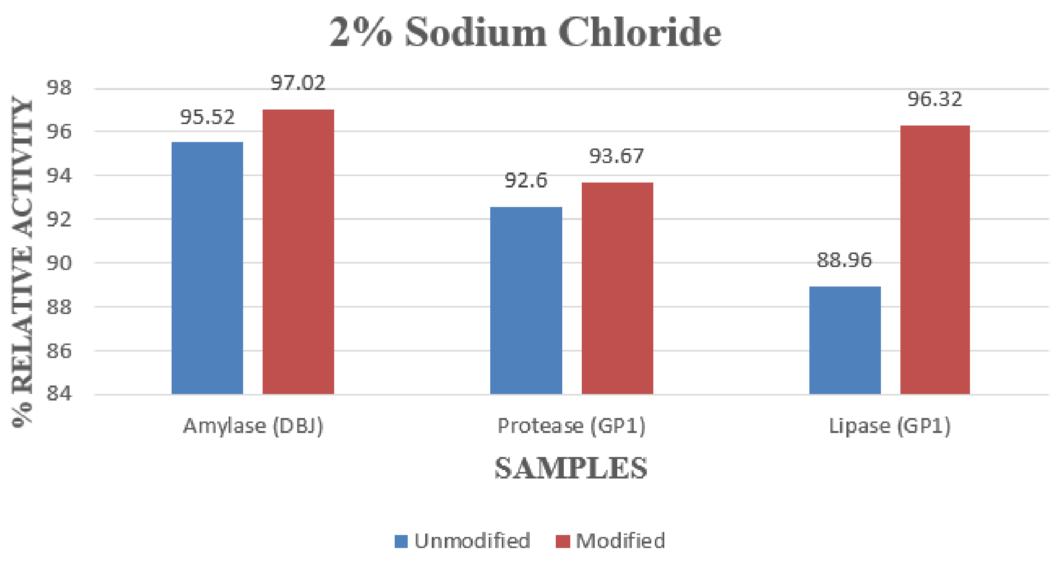


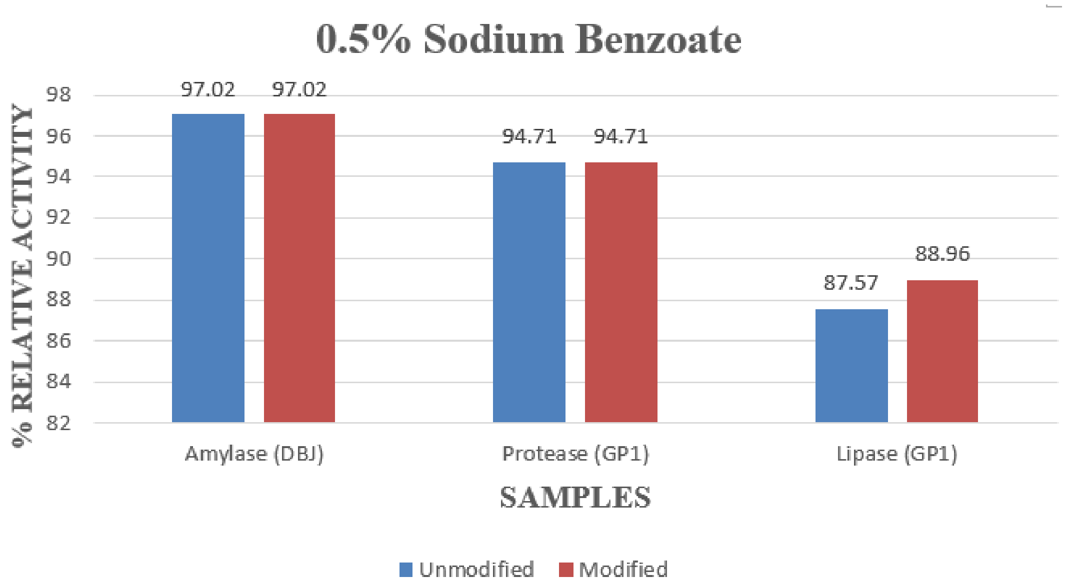
The above graph represents the Specific activity of enzyme calculated by dividing the enzyme activity obtained by the protein concentration to evaluate enzyme purity & catalytic efficiency an increase in specific activity after purification indicates successful removal of non-enzymatic proteins and enrichment of the active enzyme fraction.

3.6 Tolerance of Enzymes to Detergent Formulation Components of modified & unmodified









Enzymes with the highest specific activity DBJ (Amylase) & GP1 for Protease and Lipase were selected for chemical modification to enhance the activity and there was a significant increase found indicating the measurable effect of the modification the specific activity for modified amylase was 27221.10, for modified protease was 14712.62, and for lipase was 8498.7. (Kübelbeck, S., et al., 2018) Further these enzymes were subjected to tolerance checking with the main components of the detergent 5% Tergitol used as surfactant, 3% Sodium citrate as builder, 2% Sodium chloride as viscosity adjuster and 0.5% Sodium benzoate as preservative and was compared with the unmodified one. The relative activity calculated showed a significant increase after modification and effect of modification was highest for lipase. (Henkel AG & Co. KGaA, 2000) (Xie et al., 2019)

3.7 Evaluation of Cleaning Efficiency of Formulated Detergent

Stains	Stained Cloth	Cloth Washed with Water (Negative Control)	Cloth Washed with Commercial Detergent (Positive Control)	Cloth Washed with Formulated Eco-Friendly Detergent
Oil				
Turmeric				
Tea				
Chocolate				
Blood				

The Modified enzymes were used to formulate the liquid detergent (100ml) and enzyme specific and common house hold stains were washed using the formulated detergent and visually compared with the commercial detergent and water, the visual comparison showed efficient cleaning compared to water and equivalent or more efficient then the commercial one, which states that this formulated eco-friendly liquid detergent could replace the commercial ones. (Xie et al., 2019)

CONCLUSION

The present study demonstrated the successful isolation, production, modification, and application of microbial hydrolytic enzymes for the development of an eco-friendly liquid detergent formulation. Bacterial isolates obtained from environmentally stressed habitats proved to be efficient producers of amylase, protease, and lipase, highlighting the suitability of such environments as sources of detergent-compatible enzymes. Partial purification through ammonium sulfate fractionation enhanced enzyme activity and specific activity, indicating improved catalytic efficiency with minimal processing. Chemical modification of the enzymes using polysaccharide–aldehyde conjugation significantly improved their stability and tolerance toward detergent formulation components. Modified enzymes retained higher relative activity in the presence of surfactants, builders, viscosity modifiers, and preservatives, demonstrating improved compatibility with liquid detergent systems. The enzyme-based liquid detergent formulated using the modified enzymes exhibited effective stain removal and performance comparable to a commercial detergent during visual assessment. Overall, the findings confirm that modified microbial hydrolytic enzymes can serve as effective and sustainable alternatives to conventional chemical detergents. The integrated approach adopted in this study supports the development of environmentally friendly cleaning formulations with reduced chemical load and potential industrial applicability. Statistical analysis using one-way ANOVA demonstrated significant variation in tolerance levels across different chemical agents ($p = 0.008$). Furthermore, a significant difference was observed between modified and unmodified samples ($p = 0.007$), confirming that modification enhanced/altered tolerance efficiency.

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