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*Review*

# **Recent developments and future prospects of extremozymes in detergent applications**

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**Extremozymes are stable and active under harsh conditions, which is useful in industrial processes where mesophilic enzymes are destroyed. The applications of such enzymes are found in detergent industries due to their special characteristics.** Mic**roorganisms that survive in hot springs, cold regions, soda lakes, and even contaminated environments are explored for extremozyme production. The addition of these enzymes to detergents can replace chemicals, preserving the quality of fabrics and reducing harmful environmental impacts. Lipases, proteases, amylases, cellulases, pullulanase, xylanase, mannanase, pectinase, and cutinase are widely used to improve the efficacy of detergents. Molecular techniques have facilitated the study of structural changes, compatibility issues, and alterations of extremozymes for use in detergents, helping to overcome challenges. This review extensively focuses on the recent developments of extremozymes as detergent additives, their properties, and the molecular studies related to their detergent applications.** 

**Key words:** Amylase, detergent additive, enzyme engineering, lipase, protease.

## **INTRODUCTION**

The use of enzymes spans various industries, including food, textiles, detergents, pharmaceuticals, fine chemical synthesis, biofuels, biodegradation, and bioremediation. Enzymes offer several advantages over chemicals, such as cost-effectiveness, process efficiency, mild reaction conditions, substrate selectivity, environmental and physiological safety, and sustainability (Chapman et al., 2018; Ndochinwa et al., 2024; Yan et al., 2024). However, most mesophilic enzymes lose their activity under harsh conditions (Raddadi et al., 2015), including non-aqueous states, extreme temperatures, alkaline and acidic conditions, water-solvent interfaces, the presence of chemical components, and high pressure (Adams et al., 1995). For industrial applications, enzymes need to

be substrate-specific, stable in the presence of chemicals, and able to withstand extreme environmental conditions. Research on extremozymes has shown that they remain active through various stages of industrial processes, offering higher stability compared to other chemicals (Bhatt et al., 2024). Structural modifications, distinct characteristics, and unique properties make extremozymes suitable for industrial use. Alkaliphilic enzymes, which have basic amino acids on their surfaces and high isoelectric points, exhibit stability at high pH levels, making them suitable for detergent additives. Conversely, enzymes active at low temperatures have fewer disulfide bonds, hydrogen bonds, and salt bridges, making them more flexible in cold conditions.

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**Figure 1.** Extremozymes, its function and targeted stains.

Additionally, reduced hydrophobicity, high thermal stability, and specificity make these enzymes preferable for detergent applications (Cabrera and Blamey, 2018). Enzyme engineering is employed for further modifications to meet industrial demands, resulting in cost-effective, energy-saving, contamination-minimizing, and ecofriendly methods for production. The increasing use of extremozymes is driving their demand, with the global enzyme market projected to reach 9.9 billion USD by 2025 (Grandview Research, 2019). Successful commercial production of extremozymes involves selecting wild strains from diverse geographical areas and optimizing yields. However, challenges such as marker gene stability, plasmid stability, replication, and low permeability have been bottlenecks in genetic manipulation for mass production (Lin and Xu, 2013).

## **DETERGENT ENZYMES**

A major consumer of enzymes is the detergent industry, accounting for 25-30% of the market, which has been growing at a CAGR of 11.5% from 2015 to 2020 (Singh et al., 2016). Enzymes are added to dishwashing liquids, laundry detergents, industrial washing detergents, and institutional cleaning products to efficiently remove dirt (Kuddus et al., 2024). The detergent-compatible enzymes and their target stains are shown in Figure 1. Enzymes have long been included in detergent formulations to aid in stain removal, especially for stains that regular chemicals cannot handle. Moreover, enzyme-based detergents can remove stains at room temperature, allowing for more economical usage. Proteases are crucial for removing stains from blood, fish, eggs, meat, and grass, and are also effective against the protein components in human sweat. In contrast, amylases are used to eliminate starch-based stains from substances like chocolates, cereals, potatoes, and gravies, while lipases effectively remove fat and oil stains. Additionally, cellulases in enzyme-based detergents enhance the softness and color brightness of cotton textiles (Yasmin et al., 2023). In laundry detergents, enzymes offer fabric protection, improve weight efficiency, repair damaged fibers, increase whiteness, and preserve color. Modern molecular techniques are employed to address production challenges and make enzyme use more feasible in the detergent industry (Al-Ghanayem and Joseph, 2020). Hydrolases are preferred for tough stains as they contribute to energy savings and help eliminate harmful chemicals like phosphates and bleaching agents, benefiting both public health and the environment (Hasan et al., 2010; Sharma et al., 2024). Enzymes active under alkaline pH, low temperatures, and high thermostability, which are compatible with detergent components and



**Figure. 2.** Characteristic feature of extremozymes for detergent applications.

fabric-friendly, are selected for detergent formulations (Liu et al., 2023). Most of these are extremozymes, which are effective under harsh conditions. The properties of extremozymes used in detergents are illustrated in Figure 2. Conventional mesophilic enzymes are increasingly replaced by substrate-specific extremozymes to enhance washing efficiency without damaging fabrics, as mesophilic enzymes are often denatured due to their limited range of physico-chemical activity.

#### **LIPASE**

Lipases act on ester bonds to hydrolyze lipids (fats, triglycerides, and oils) into fatty acids, glycerol, and other alcohols (Melani et al., 2020; Egmond and Bemmel van, 1997). Compared to lipases derived from plants and animals, those from microbial sources have garnered more attention in industrial applications due to their favorable characteristics, including the ability to function under extreme conditions, stability in organic solvents, chemical selectivity, enantioselectivity, and lack of need for cofactors to enhance catalytic efficiency during reactions (Kumar et al., 2023). Extremophilic lipases encompass salt-tolerant halophilic lipases, acid and alkali-resistant lipases, as well as thermophilic and psychrophilic lipases that are active in both hot and cold conditions (Vivek et al., 2022). Alkaline and cold-active lipases are incorporated into detergents to enhance efficiency and remove lipid stains without compromising fabric quality. Lipases that are compatible with nonphosphate detergents, surfactants, metal ions, and

oxidants are particularly valuable in detergent applications (Cherif et al., 2011). Lipase-based detergents effectively remove stains such as fatty residues, kitchen waste, used engine oil, and cosmetics. *Thermomyces lanuginosus* lipase (Lipolase®) produced by Novozymes, the first type of lipase tested and used in detergent industry (Al-Ghanayem and Joseph, 2020). Later, Lipoclean® produced by Novozymes, which is active under cold conditions (20°C) and compatible with detergent components, was incorporated into cleaning solutions. Lipases developed through protein engineering and other molecular technologies, tailored to the specific requirements of detergents and their components, have replaced some of the traditionally used lipases. Many bacterial lipases, active under harsh conditions and sourced from various geographical locations, have been studied for detergent compatibility. However, only a few fungal isolates producing lipases active under extreme conditions have been used as detergent additives. Fingal lipases isolated from *Fusarium* specie (Liu et al., 2009), *Penicillium* spp., and *Pseudogymnoascus* spp. (Sahay and Chouhan, 2018) were reported as cold-active lipases. The significant demand for cold-active lipases in the detergent industry necessitates the exploration of novel lipases from diverse sources.

#### **PROTEASE**

Proteases are frequently utilized to catalyze a variety of organic transformations and are often engineered to function under physiological conditions. However,

biocatalysis primarily involves the efficient use of proteases as process catalysts in specific environments (Aruna et al., 2023). Household items and domestic articles frequently come into contact with substances such as human sweat, cocoa, egg stains, grass, and blood. To remove these stains, proteases are added to detergents and household dishwashing liquids, where they help break down peptide bonds (Lam et al., 2018; Razzaq et al., 2019), thereby facilitating the removal of tough protein stains. Proteases are also employed in the processing of woolen and silk materials, reducing the need for high-temperature heating and enhancing polishing and finishing (Wang et al., 2018a). Proteases that are active and stable under alkaline conditions are preferred for detergent applications (Saeki et al., 2007). Cold-active proteases, such as those from the Himalayan glacier (Baghel et al., 2005) and other cold-active proteases stable at alkaline pH (Furhana et al., 2019), have also been studied for detergent compatibility. Protease from *Chryseobacterium* species has shown stability in organic solvents, detergents, and surfactants (Mageswari et al., 2017). A substrate-specific protease active at pH 9, isolated from *Pseudoalteromonas arctica* PAMC 21717, has also been examined (Park et al., 2018). Commercial serine proteases used in detergents include Kannase® and Liquanase® (Novozymes), while Polaryme®, a serine protease active over a broad temperature range, is widely used in handwashing liquids. Compared to other detergent enzymes, coldactive proteases are extensively used in the detergent industry. Examples of cold-active proteases include Purafect®, Properase® (Palo Alto, CA, USA), and Excellase® (Genencor) (Sarmiento et al., 2015).

## **AMYLASE**

Amylase is a crucial hydrolytic enzyme used as an additive in detergent formulations, with approximately 25% of the total amylase produced being consumed by the detergent industry. It acts on linear amylose chains, breaking the 1,4α-D-glucosidic linkages. This action helps remove stains such as those from fruits, vegetables, sauces, gravies, cereals, and other starch-based sources from fabrics. Alpha-amylase is commonly used as an additive in detergents, and most liquid detergents contain amylase (Lahmar et al., 2017). Amylases isolated from bacteria and fungi have been explored for their compatibility with detergents. However, only a few have been reported to exhibit extremozyme characteristics and compatibility with detergent formulations. A low temperature active alpha-amylase isolated from *Bacillus cereus* GA6 (Roohi et al., 2013) and another amylase from *Bacillus subtilis* N8 active at pH 10 (Arabaci and Arikan, 2018) were reported. A thermostable, alkaliphilic, and detergent-tolerant amylase-producing bacterium, *Paenibacillus lactis* OPSA3, produced an amylase that

demonstrated activity under extreme conditions and exhibited excellent washing performance (Ugwuoji et al., 2023). The cold-active amylase gene of *Zunongwangia profunda* was expressed in *Escherichia coli* and studied for the compatibility with detergent (Qin et al., 2014). Amylases active under a varied range of temperatures and alkaline pH are chosen as additives in detergent formulations. Stainzyme® (temperature stable) and Stainzymeplus® (Novozymes) (bleach tolerant enzyme), were the commercial amylases used in detergents. Another commercially available detergent stable amylase Preferenz™S100 was produced by DuPont Industrial Biosciences, active even at  $16^{\circ}$ C. Fungal amylases active under extreme conditions and its genomes need to be explored for detergent compatibility for commercial purposes.

## **PULLULANASE**

Pullulanase is classified into various groups based on substrate specificity and hydrolyzes α-1,4 glycosidic linkages in oligosaccharides (Wang et al., 2018b). The most significant industrial glycosyl hydrolase class, family 13, includes pullulanases, which hydrolyze α-1,6 and α-1,4 glycosidic bonds in pullulan and other carbohydrates to produce glucose, maltose, and maltotriose syrups, essential for industrial applications (Naik et al., 2023). Some alkaline pullulanases from *Bacillus* species (Schallmey et al., 2004) and *Bacillus KSM 1876* (Hatada et al., 2001) have been reported for stain removal. Pullulanase, along with alpha-amylase, is used in detergent applications, with amylo-pullulanase catalyzing debranching of α-1,6 glycosidic bonds and liquefying α-1,6 hydrolytic reactions (Ara et al., 1995). These enzymes facilitate starch hydrolysis and manage highviscosity materials during stain removal (Hii et al., 2012). A cold-active, alkaline-stable pullulanase suitable for cold washing from *Bacillus pseudofirmus* 703 has been reported (Lu et al., 2018). Additionally, a detergentcompatible pullulanase with high stability across a pH range of 4-11, expressed in *B. subtilis* and *E. coli* from a gene isolated from *Exiguobacterium* spp. SH3, has been identified (Rajaei et al., 2015). Another gene from *Alkalibacterium* species was expressed in other microbes to produce an alkaline-stable (pH 9), thermostable (50°C), and chemically stable (SDS and NaCl) pullulanase (Huang et al., 2020).

## **CELLULASE**

Cellulase catalyzes the breakdown of cellulose into glucose monomers with the help of three types of enzymes. As one of the most widely used industrial enzymes, cellulase breaks down cellulose, which is the most abundant polymer in lignocellulosic biomass (LCB).

According to the global cellulase market research study (2020), the market was estimated to be worth 1.68 billion US dollars in 2020 and is projected to grow at a compound annual growth rate of 5.5% from 2021 to 2026 reaching 2.45 billion US dollars by the end of 2026 (Ranjan et al., 2023). The cellulase enzymes include endo-1,4-β-glucanases, which act on internal bonds within the cellulose chain; exo-1,4-β-glucanases, which cleave the reducing or non-reducing ends of the cellulose polymer; and β-glucosidases, which break down cellobiose formed by earlier reactions into glucose (Horn et al., 2012). Frequent washing of fabrics can lead to the accumulation of stains and degradation. Cellulase helps by removing spoiled fibers, enhancing the attractiveness of clothes, increasing softness, glossiness, and color brightness, and reducing roughness (Kuhad et al., 2011). The enzyme modifies distended fibers in detergent, improving finishing and reducing defibrillation costs. Cellulases that are stable and compatible with detergent components are preferred. For instance, *Humicola insolens* cellulase, active at 50°C and alkaline pH, is used commercially as a detergent additive (Behera and Ray, 2016). It effectively removes sebum stains from microfibers without damaging the fabric and helps protect woolen fabrics from color fading and the formation of fuzz and pills. Alongside lipases, proteases, and amylases, cellulases play a crucial role in enhancing the washing efficiency of detergents. Commercially available detergent additives include Celluzyme® and Carezyme®, produced by Novozymes. However, there is ongoing research to explore fungal and bacterial cellulases to further improve detergent effectiveness, thus reducing water and energy consumption in household applications. Other cellulases on the market include Retrocell, Retrocell ZircoN, Rocksoft™ (Jupiter, FL, USA), Recop (EpyGen Biotech, UAE), and UTA88, 90 (China) (Kasana and Gulati, 2011). Novozymes also offers Celluclean®, a cellulase active at 15°C. Additionally, a cellulaseencoding gene expressed in *E. coli* BL21 (DE3) was studied for structural stability with nonionic and ionic surfactants (Souza et al., 2016).

## **MANNANASE**

Mannans are utilized as thickening agents in make-ups, dietary, and personal care products. The enzyme βmannanase, or 1,4-β-D-mannan mannanohydrolases, breaks down β-1,4 mannan, glucomannan, and galactomannan (Arnling-Baath et al., 2018). This enzyme can replace bleach in cleaning solutions for removing stains from cellulose fibers. Acidic and thermostable mannanases from *Paenibacillus* spp. DZ3 (Chandra et al., 2011) and *Bacillus* HJ14, active at 65°C and pH 6.5, cloned and expressed in *E. coli* BL21 (rMan5H14), have been reported (Zhang et al., 2016). A highly thermostable, alkaline halotolerant mannanase from *Bacillus halodurans* PPKS-2 was isolated and studied for detergent compatibility (Vijayalaxmi et al., 2013). *Bacillus nealsonii* PN11 produced a mannanase stable at 65°C and pH 8.8 (Chauhan et al., 2014). Recombinant mannanases have been produced and studied for various applications. A gene coding for β-mannanase active at pH 6.0 and 60°C from *B. subtilis* (TBS2) was successfully expressed in *Pichia pastoris* (Luo et al., 2017). A synthetic alkaline thermostable β-mannanase, ManB, from *Thermobifida fusca*, was also expressed in *P. pastoris* (Wang et al., 2017). For anti-greying effects and reducing soil redeposition, the product BIOTOUCH®M7 (AB Enzymes GmbH-Germany) is used. Cold-active mannanases such as Mannaway® (Novozymes) and EffectenzTM (Dupont) are primarily used in detergent formulations. In addition to detergents, mannanases active under extreme conditions are found in hand washers, contact lens cleaners, and sanitary, beauty, and healthcare products (Dhawan and Kaur, 2007). Mannanase combined with protease isolated from the same bacteria has shown stability and detergent compatibility (David et al., 2018; Dhawan, 2021). *Klebsiella pneumonia* SS11 produced a thermoalkali stable β-mannanase active at 70°C and pH 9.0, which is efficient in removing mannan-based food stains (Singh et al., 2019). However, reports on detergentcompatible fungal mannanases are limited to *Trichoderma longibrachiatum* RS1, which is active at pH 5.5 and 75°C (Ismail et al., 2019).

## **PECTINASES**

Pectin polymers are cleaved into simple sugars such as galacturonic acid by a group of heterogeneous enzymes, pectolyase, pectozymes and polygalacturonase. Pectolyases are classified into pectinlyase and pectin methylesterases. Polygalacturonase are sub-classified into endo-polygalacturonases and exoploygalacturonases according to their action on the substrate (Carrasco et al. 2019). Pectin is commonly found in plant-based food products, and it converts tough stains in fabrics that become difficult to remove. The textile industry's use of harsh chemicals has decreased as a result of the use of enzymes like pectinases in conjunction with amylases, lipases, cellulases, and other hemicellulolytic enzymes to remove sizing agents (Kashyap et al., 2001). This has improved the quality of the fabric and the safety of working conditions for textile workers by reducing the amount of waste chemicals discharged into the environment (Haile and Ayele, 2022). Detergent compatible alkaline and thermostable pectinases active under wide range of temperature and pH removes the stains easily from the fabrics. Recently a fungal pectinase from *Schizophyllum commune* active at pH 9.0 and stable at 55°C was reported (Mehmood et al., 2019). Cold active pectatelyase active even low temperature (Xpect®, Novozymes) is available in market for detergent applications. Pectinase is also used for bioscouring of cotton fabrics instead of treating with caustic

alkali to remove pectin and waxes to reduce hydrophobicity without fiber deterioration (Aggarwal et al., 2020).

Pectinases with alkaline and thermal stability produced by extremophiles need to be explored for detergent as well as other industrial applications such as bio-scouring.

## **CUTINASE**

Cutinases hydrolyze cutin, insoluble lipid polyester, and catalyze esterification and trans-esterification reactions. These multifunctional enzymes efficiently break down soluble esters and emulsified triglycerides. Like lipases and serine proteases, cutinase, which is also a serine esterase, contains the classical triad Ser-His-Asp (Chen et al., 2013). Cutinase is added in detergents because of its stability in  $H_2O_2$  and lipid stains especially triglycerols are easily removed with the help of cutinase enzymes that act on the water- lipid interfaces (Dutta et al., 2009). Most of the cutinase reported as detergent additives are thermostable and stable in presence of other enzymes, and functions without calcium ions thereby it is used without any limitations (Sharma et al., 2024). Bacterial and fungal cutinase stable in presence of chemicals and thermos-alkaline conditions were studied for detergent compatibility. Cutinase from *T. fusca* with versatile hydrolytic activity, and stability in presence of organic solvents, surfactants, and high temperature was described (Chen et al., 2010). A fungal alkaline thermostable cutinase (pH 9; 60°C) from *Aspergillus nidulans* was compatible with detergents devoid of dodecyl sulfonic acid salts was reported (Bermudez-Garcia et al., 2017). A saturation mutagenesis study on cutinase amino acids increased 2 to 11-fold the stability with anionic surfactants (Brissos et al., 2008). In textiles, synthetic fibers are made up of polyamide (PA), polyethylene terephthalate (PET), and polyacrylonitrile (PAN). Cutinase may act on these substrates and cause damage to fabrics in long term use. Consequently, detergent industries prefer newly available lipases instead of cutinase for fabric protection, even though cutinase has fewer limitations. Exploring thermostable alkaline cutinase with substrate specificity may help to overcome the current hitches in using cutinases in detergent industries for the removal of difficult stains.

## **XYLANASE**

Xylanases comprises enzymes including endo-1,4-β-Dxylanases, β-D-xylosidases, α-glucuronidase, acetyl xylan esterase, α-L-arabinofuranosidases, *p*-coumaric esterase and ferulic acid esterase catalyzes depolymerization of xylan to monosaccharide and xylooligosaccharides (Bhardwaj et al., 2019). Xylanases in detergent can remove stains acquired from fruits,

vegetables, grasses, tea, coffee, and tobacco (Sarangi and Thatoi, 2024). Studies on detergent compatibility of xylanases were reported, however, extremophilic xylanases used in detergents are limited. Xylanases active under harsh conditions with specific characteristic features are explored and used in many of the industrial applications (Qiu et al., 2010). Alkalophilic xylanase, thermostable  $(40^{\circ}C)$  and alkaline stable (pH 10) was isolated from *Bacillus* spp. NCL (87-6-10) was reported to be compatible with proteases and commercial detergents (Kamal Kumar et al., 2004). Therefore, potent xylanase producing microbes from the extremophilic environments with specific properties are needed to characterize at the molecular level to elucidate the properties of the enzyme suitable for detergent industries. Numerous attempts have been made on gene expression of xylanase in the suitable hosts using recombinant DNA technology (Al-Darkazali et al., 2017). Even though different xylanase is available with diverse properties there is a gap between the existing and the required enzyme for detergent applications. Genetic engineering or recombinant DNA technology and protein engineering approaches have not produced xylan extremozyme suitable for detergent industries (Verma and Satyanarayana, 2020). Thermoalkaline xylanases with specific characteristics produced using modern technologies, present significant potential for use in the detergent industry.

#### **PROTEIN ENGINEERING OF EXTREMOZYMES FOR USE AS DETERGENT ADDITIVE**

For efficient washing and stain removal, extremozymes are added to detergents, offering an eco-friendly approach. Modifying enzymes by understanding their structure and dynamics at the molecular level helps overcome limitations (Albayati et al., 2024). Due to their potential specificity and accuracy, interest in extremozymes has increased. However, extremozymes often lose their structure and flexibility, leading to a reduction in the activation energy required for enzymatic reactions at varying temperatures. This sensitivity to metallic ions and organic solvents, combined with low variability and thermal stability, poses challenges. Additionally, the lack of enzymatic bases and difficulties in separating and purifying these enzymes limit their industrial applications (Liu et al., 2023). Techniques such as macromolecular crosslinking, solid scaffold carriers, entrapment, and surface modification (both covalent and physical) illustrate the benefits and drawbacks of these approaches (Scheibel et al., 2024). Extremozymes are used in detergents to enhance brightness, whiteness, fabric longevity, and color protection. However, their production is limited, and they often have lower thermal stability and shelf life (Coker, 2016; Jin et al., 2019). Specific production conditions for these enzymes present additional challenges for industry. Methods such as



**Figure 3.** Protein engineering in extremozymes for detergent industry.

metagenomics and proteomic studies have helped explore extremozymes with novel properties (Figure 3). Analyzing the purity and molecular structure of these enzymes through NMR spectroscopy and X-ray crystallography reveals their functional properties (Karan et al., 2020). To improve thermostability and shelf life, site-directed mutagenesis and rational design approaches are employed (Zhu et al., 2020). The chemo-, regio-, or stereo-selectivity of these enzymes makes them suitable for detergent additives (de Lourdes Moreno et al., 2013). Molecular tools enhance the thermostability and alkaline stability of extremozymes. Systematic biology techniques are widely used to generate metabolic copies, predict gene roles and protein structures, and direct metabolic engineering to increase enzyme yields (Naik et al., 2023).

Non-specific protease subtilisin isolated from *Antarctic Bacillus* TA39 increases thermostability and substrate specificity by replacing 12 flexible amino acids (Tindbaek et al., 2004). For pH depended extremozymes amino acid substitution is followed to change the surface charges. Protease active at pH 11 isolated from *Bacillus gibsonii* increased two-fold activity by replacing amino acids for

changing surface charge by transitional autolytic deamination (Jakob et al., 2013). Charge distribution methods are also used to increase activity and stability; however, the specific activity was affected instead of alkaline stability (Ma et al., 2016). Hydrogen peroxide added in detergents damages the fabric in long term and may affect the activity of enzymes. This is replaced by *in situ* enzyme-based peroxycarboxylic acid produced. Serine hydrolase is structurally modified to produce peroxycarboxylic acid for hydrolytic and perhydrolytic reaction using site-directed mutagenesis. Peroxycarboxylic acid activity was increased 28-fold of an esterase from *Pseudomonas fluorescens* (Poulouse, 1994). Random mutation also increased the thermostability of esterase from *Enterobacter* spp., by 3.4-fold. Substituting hydrophobic Ala with hydrophilic Asp enhanced solubility and interaction (Ke et al., 2018). A 9.6 and 6.6-fold increase in catalytic efficiency and turnover number (K<sub>cat</sub> 10°C) was obtained after random mutagenesis of recombinant *Bacillus sphaericus* subtilisin (SSII) (Wintrode et al., 2000). Frequent round of sequence sequence saturation mutagenesis (SeSaM) increased the

specific activity and thermostability of protease in alkaline conditions isolated from *Bacillus gibsonii* (Wong et al.,2004). There are reports on increased alkaline stability, increased thermal-stability, modulation in pH, and changing the promiscuous activity as bleaching agents to produce peroxycarboxylic acid production (Al-Ghanayem and Joseph, 2020). Conventional enzymes used in detergents will soon be replaced by tailor-made extremozymes with thermos-alkaline stability and compatible with chemicals present in the detergents.

#### **CONCLUSIONS**

For detergent applications, alkaline-thermostable enzymes are preferred. The exploration of naturally available bacterial and fungal strains producing such enzymes is ongoing. However, modern technologies, including genetic and protein engineering, are essential to overcoming challenges such as low enzyme yield and thermo-lability. To enhance enzyme properties according to industrial needs, bioinformatics tools such as proteomics, genomics, and metabolomics are employed. Effective use of these tools and technologies is crucial for addressing the bottlenecks in the commercialization of extremozymes, enabling their effective use in detergent industries.

#### **CONFLICT OF INTERESTS**

The author has not declared any conflict of interests.

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