**Emerging trends of Extremozymes in Industrial Biotechnology**

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| Dr. Prabhu ThangaduraiPSG Institute of ManagementPSG College of TechnologyCoimbatore, Tamil Nadu, India. Email: prabhuthangadurai@psgim.ac.in | Dr. Rachana D Sharma1BIRAC EYUVA CentrePSGR Krishnammal College for WomenCoimbatore, Tamil Nadu, India. Email: rachanasharma@psgrkcw.ac.in  |

**ABSTRACT**

Biocatalysis plays a pivotal role in achieving a green, sustainable, biobased economy, and extremozymes offer enhanced activity and stability under extreme conditions, making them valuable biocatalysts. These extremozymes have unique adaptations to cope with extreme environmental conditions, such as high and low temperatures, acidic or basic pH, high salinity, and high metal concentrations. Extremozymes are capable of catalysing reactions under harsh conditions, making them valuable alternatives for industrial processes previously thought unsuitable for enzymatic activity. Extremophiles, organisms thriving in extreme conditions, offer sustainable, efficient, and cost-effective alternatives to conventional methods in various industries. They produce extremolytes, extremozymes, biosurfactants, etc., which have applications in sustainable agriculture, food, cosmetics, pharmaceuticals, bioremediation, biofuels, biorefinery, and astrobiology. These organisms help us understand the boundaries of life, the origin and evolution of life on Earth, and research in astrobiology and space exploration. However, further investigation is needed to explore their structural and biochemical properties and long-term effects of their applications. Despite their potential, the availability of extremozymes is limited, partially due to challenges in cultivating extremophiles in the lab. This review provides an overview of extremozymes and their applications in various industrial markets. It also discusses the challenges in the mass production of extremozymes and explores future prospects and trends for their biotechnological applications. Extremozymes can contribute to the research and development of safe, healthy, and sustainable food products while minimizing waste generation.

**Keywords:** Extremozymes; industrial biocatalysts; thermophiles; acidophiles; halophiles; Psychrophilic enzymes.

1. **INTRODUCTION**

Extremophiles are microorganisms capable of surviving and thriving in extreme environmental conditions that are typically unsuitable for most life forms. These conditions include high and low temperatures, extreme pH levels, high pressure, high salinity, radiation, and lack of nutrients [1]. Extremophiles have developed various molecular strategies to adapt to these extreme conditions, such as the production of extremolytes, which are organic osmolytes that protect biological macromolecules and cells from damage caused by external stresses [2]. They produce specialized enzymes called extremozymes, which possess remarkable properties such as salt tolerance, thermostability, and cold adaptivity [3,4]. Enzymes from extremophiles, including amylases, proteases, lipases, cellulases, and many others, are of particular interest due to their stability and functionality under extreme conditions. The study of extremophiles and their enzymes has gained increasing attention in recent years, opening up new avenues for biotechnological and industrial advancements. These microorganisms play a vital role in various fields, including agriculture, biodegradation, chemical processing, food industry, pharmaceuticals, and bioremediation [5]. Extremophilic microbiomes have the ability to produce a wide range of bioactive compounds, secondary metabolites, and value-added products. They have applications in diverse areas such as white and green biotechnology, medicine, and food production [6]. The unique properties of extremophiles and their enzymes offer great potential for biotechnological advancements, contributing to the development of the economy and opening new avenues for research and innovation.

Extremophiles are characterized into different types based on their preferred extreme habitats, such as thermophiles (thriving in high temperatures), psychrophiles (thriving in low temperatures), halophiles (thriving in high salinity), and acidophiles (thriving in acidic environments) [7]. The demand for industrial enzymes is on a steady rise. The global enzymes market in 2021 was valued at $6.4 billion and is expected to reach $8.7 billion by 2026, with a compound annual growth rate (CAGR) of 6.3% from 2020 to 2026 [8] . However, present industrial biotechnology confronts a number of problems, including inadequate enzyme stability under harsh processing conditions, microbial contamination, poor biocatalyst recyclability, and limited capacity for synthetic processes [9]. Biosynthetic processes involving hydrolases frequently necessitate the presence of solvents, resulting in low water activity. This change in thermodynamic equilibrium promotes the reaction's synthetic direction and reduces aqueous side reactions [10]. Improving the characteristics of mesophilic enzymes via chemical or genetic modification and immobilization is time-consuming, costly, and frequently inefficient, as it can affect reaction rates, enzyme selectivity, and stability [11]. In light of these difficulties, extremozymes offer a natural alternative. These enzymes are designed to work in harsh environments and may provide viable solutions to overcome the limits of regular enzymes.

Extremophilic enzymes are highly adaptable and have numerous uses in the food and biotechnology industries. For example, thermostable enzymes are appropriate for high-temperature processes such as starch hydrolysis for glucose and fructose generation, as well as grain fermentation for distilled spirits. Psychrophilic enzymes, on the other hand, can be used in low-temperature food processing, whereas halophilic enzymes are effective in the fermentation of salty foods such as soy sauce, salted salmon, and sauerkraut [12]. Despite their enormous potential, the number of commercially available extremophilic enzymes is still very small, and their applications in the food business are not fully realized. Recent research has highlighted the importance of these enzymes in the breakdown of dietary toxins and polymers. This review aims to explore these unique features of extremophilic enzymes and shed light on their potential applications.

Extensive research has focused on identifying extremozymes that are relevant for industrial biocatalysis. Currently, researchers are looking for microorganisms that may produce new enzymes such as hydrolases, amylases, cellulases, peptidases, and lipases that have high activity at low temperatures [13]. Due to their biodegradability and extraordinary stability, extremophilic bacteria are significant sources of extremozymes, which have a wide range of commercial applications [14]. These extremozymes function as powerful biocatalysts, remaining active even in extreme environmental conditions that were previously thought to be incompatible with biological activity. The use of extremozymes has increased the possibility of using resistant biomolecules in a variety of industrial applications. Cold-tolerant extremozymes, acid-tolerant extremozymes, alkali-tolerant extremozymes, and salt-tolerant extremozymes, for example [15].

The number of extremophilic enzymes with commercial use is currently limited, recent research in the field has identified enzymes with industrial potential. These studies contribute to expanding our knowledge of extremophiles and their enzymes, paving the way for future developments in utilizing these unique biocatalysts. The ongoing objective in enzyme research is to explore enzymes with novel extreme activities and improved stability, which remains a priority [16]. This review specifically focuses on the industrial applications of enzymes derived from extremophilic microorganisms, highlighting their importance in various fields.

1. **UNIQUE ADAPTATION & FEATURES OF EXTREMOZYMES**

Extremophiles possess unique mechanisms to withstand harsh environmental conditions, necessitating genetic alterations that subsequently lead to changes in protein sequence and structure [17].

1. **Thermophiles**

Extreme temperatures can lead to irreversible protein folding and exposure of hydrophobic cores, resulting in protein aggregation [17]. To counteract this, thermophilic and hyperthermophilic proteins employ strategies such as oligomerization, a large hydrophobic core, an increased number of disulfide bonds, surface charges, and salt bridging for stabilization [18]. Heat-adapted extremozymes share structural similarities with mesophilic enzymes but exhibit differences in helix and beta sheet sizes, as well as ionic modifications in terminal portions [19]. A unique characteristic of heat-adapted extremozymes is their tightly packed protein structures, which control solvent interactions and contribute to stability by promoting proper folding, reducing protein unravelling, and controlling undesired solvent interactions. This tight packing, along with the presence of hydrophobic cores, increased levels of salt bridges and disulfide bonds, and decreased enzyme surface area relative to protein size, enhances the thermostability of the enzyme [20] .

Some heat-adapted extremozymes have a higher proportion of hydrophobic amino acids, a lower proportion of polar and charged amino acids, and a lower fraction of glycine [2]. Deep-sea thermophilic enzymes, in particular, have an excess of charged residues on their protein surfaces, which reflects the aquatic environment of the extremophiles expressing them. Heat-adapted extremozymes provide distinct benefits such as enhanced substrate solubility (particularly for polymers), lower contamination hazards, faster reaction speeds, and the capacity to maintain low solvent viscosity and miscibility [20]. Furthermore, several thermostable enzymes can maintain their catalytic characteristics in the presence of additional severe conditions such as high salinity, chemical solvents, and denaturing agents [20].

The existence of disulfide bridges, which diminish the entropy of the protein's unfolded form, has a significant impact on thermal stability in these enzymes. In several investigations [23, 24, 25], extremeozymes with longer disulfide bridges, particularly from the N-terminus, were reported to have improved thermostability. Aside from disulfide bridges, several other variables contribute to thermophilic extremozyme conformation maintenance. These include the compact barrel folding, shorter loops and helices, the existence of salt bridges, the distribution of surface charge, and interactions between inner hydrophobic amino acids [22]. Hyperthermostable enzymes have been found to include a high amount of charged amino acids, with ionic interactions playing an important role in their stability above 70°C [26]. In addition, the thermal adaptions in thermophilic enzymes are genetically encoded, as evidenced by their retention of thermostabilities even when cloned and expressed in mesophilic hosts [27] .

1. **Psychrophiles**

Cold Shock Proteins (CSPs) and Cold Acclimation Proteins (CAPs) are specific adaptations that allow psychrophiles to thrive in cold conditions. CSPs are overexpressed in reaction to mild cold shocks, whereas CAPs are overexpressed in response to extreme cold shocks. These proteins undergo structural changes, such as amino acid substitutions, that improve their functioning [28]. Because of reduced rigidity in the protein core and fewer connections between interdomains, the flexibility of enzymes in psychrophiles is improved, providing added stability and specificity at low temperatures [18].

Extremozymes adapted to cold conditions have garnered significant research attention due to their unique structural properties that determine their stability and activity. These enzymes find applications in various food technologies, such as milk pasteurization, lactose degradation, juice extraction, meat tenderization, and dough fermentation. The ability of cold-adapted enzymes to function under cold conditions reduces the enzyme requirements, saves energy costs, offers environmental advantages, and preserves heat-labile flavor compounds and nutrients [29] . Cold-adapted enzymes exhibit high flexibility and substrate promiscuity compared to mesophilic and thermostable enzymes [29]. They possess distinct structural features, including smaller buried amino acid moieties, higher hydrophobic amino acid content on the enzyme surface, lower arginine/lysine ratio, higher glycine levels, and reduced protein interactions. Additionally, the number of secondary structures and oligomerization is decreased, while the number and size of loops are increased [30]. These features, along with the high conformational entropy of the unfolded protein state, play crucial roles in determining the enzymatic activities of cold-adapted extremozymes [29].

1. **Halophiles**

Halophiles, or animals that flourish in highly saline environments, have evolved a variety of adaptations to cope with such harsh conditions. Because salt changes protein solubility, stability, and structure, halophiles devise ways to offset these effects [31]. They regulate osmotic pressure by preventing inorganic salts from entering and creating organic osmolytes [28]. Water availability to internal proteins is reduced in salty environments, leading to dehydration and enhanced interactions between hydrophobic amino acids, resulting in aggregation formation. Halophilic proteins have distinct properties such as a higher number of salt bridges and more acidic residues. Reduced hydrophobic residues, salt-dependent folding, and halophilic peptide insertion are examples of further adaptations [18].

Studies on the structural characterisation of halophilic enzymes have underlined the relevance of improving solvation for sustaining activity and solubility [32]. In water-stressed situations, stable hydration shells can form via hydrogen interactions between water molecules and negatively charged side chains [33]. Halophilic enzymes also have less hydrophobic surface patches, more ion-pair networks, and a greater number of ordered side chains [34]. Resistance to salt has been demonstrated in the structural basis of halophilic enzymes, such as a DNA ligase domain from *Haloferax volcanii* [35]. The effects of salt on the stability of halophilic enzymes are largely independent of total protein charge but are closely connected to the lowering of hydrophobicity of the exposed surface area [34]. Furthermore, experimental tests have demonstrated the importance of disulfide bonds in stabilizing enzymes in halophilic organisms [36].

1. **Acidophiles**

Protonation changes the charges of polar charged residues and proteins in acidic circumstances, reducing permeability and maintaining the proton gradient across the cell membrane. Acidophiles, which thrive in acidic conditions, use cytoplasm buffering to keep their intracellular pH balanced. The presence of an abnormally wide external loop in *Thiobacillus ferrooxidans* [37] exemplifies how these acidophiles lower membrane permeability by decreasing the size of their membrane pores [28]. Acidophiles enhance their negative surface charge as well [18].

The adaptation of acidophilic enzymes to low pH has not been fully investigated, however research on -amylases provides an explanation for this class of extremozymes' pH stability. On their surfaces, acid-stable -amylases have an abundance of glutamic acid (Glu) and aspartic acid (Asp) residues and less positively charged amino acids such as arginine (Arg), histidine (His), and lysine (Lys) [38]. The high concentration of positively charged amino acids on the enzyme surface may cause repulsion, which could lead to protein unfolding. However, negatively charged amino acid residues have a reduced negative charge at low pH, which helps to stabilize proteins in acidic conditions [38].

1. **Alkaliphiles:**

Phosphoserine aminotransferase (vitamin B6-dependent) is found in alkaliphiles and produces a homodimer [37]. These enzymes are structurally identical to their mesophilic counterparts but differ in some ways. They have more hydrogen bonds, more hydrophobic interactions at the dimer interface, and more negatively charged amino acid residues. These differences lead to alkaliphilic enzymes' increased stability and activity in very alkaline environments [37]. Alkaliphilic enzymes from Bacillus species, such as alkaline protease and alkaline cellulase, have been widely researched. The structural properties of alkaliphilic enzymes differ greatly from those of neutralophilic Bacillus species. In alkaliphilic enzymes, their ability to function under high-pH conditions necessitates a high isoelectric point. Consequently, these enzymes contain a higher number of amino acid residues with positively charged side chains, such as arginine (Arg) and histidine (His), while having fewer amino acid residues with low charged side chains like aspartic acid (Asp), glutamic acid (Glu), and lysine (Lys) [39]. Since the pKa of the arginine (Arg) side chain is 12.5, the Arg residues become negatively charged within the pH range of 9–12, which is optimal for the growth of alkaliphiles. In this pH range, Arg residues can easily form ion pairs with acidic amino acid residues [40]. The formation of Arg-Asp ion pairs is particularly crucial for the stability of alkaliphilic enzymes in high-alkaline environments [40].

1. **Piezophiles:**

Piezophilic proteins, which are specialized for functioning in high-pressure environments, exhibit several structural adaptations. These adaptations contribute to their stability and functionality under extreme pressure conditions. One prominent feature of piezophilic proteins is the presence of hydrophobic cores. These hydrophobic regions are composed of smaller amino acids, which helps to enhance protein stability by minimizing the exposure of hydrophobic residues to the surrounding water [28]. Additionally, piezophilic proteins tend to undergo multimerization, where protein subunits come together and form complexes through hydrogen bonding. This multimerization further enhances protein stability and provides structural support under high-pressure conditions.

Proline and glycine residues, which have unique properties, are found in lower quantities in piezophilic proteins. Proline residues can disrupt helical structures, while glycine residues confer flexibility due to their small size. By reducing the presence of these residues, piezophilic proteins maintain the integrity of their helical structures and reduce conformational flexibility. This reduction in flexibility helps to counteract the compressibility of proteins and ensures their functionality in high-pressure environments [18].

In the case of *Thermococcus barophilus*, a deep-sea organism, it has been observed that it accumulates a small organic osmolyte called mannosylglycerate. This osmolyte serves as a protective mechanism by minimizing the hydration layer around proteins, particularly at ambient pressure [41]. By reducing the hydration layer, mannosylglycerate contributes to protein stability and prevents the detrimental effects of high-pressure conditions [41]. Furthermore, bacteria residing in deep-sea hydrothermal vents have evolved a specialized pressure-sensing operon system. This system allows them to regulate their growth and physiological processes in response to changes in both temperature and pressure. The operon system enables these bacteria to adapt and thrive in the extreme conditions of deep-sea hydrothermal vents [28, 37].

1. **Industrial applications of extremophiles**
2. **Agricultural industry**

Extremophiles have shown to play a crucial and indispensable role in supporting plant growth, development, and crop productivity, particularly in areas facing challenging environmental conditions such as low temperatures, high salinity, and drought [42,43]. They serve as valuable resources for biofertilizers, bioinoculants, and biocontrol agents. In the agricultural context, extremophiles offer a promising solution for improving water management in plants during times of water deficiency [44,45]. Additionally, their unique cold-active enzymes possess diverse applications in biotechnology and various industries [46]. Notably, extremophiles' biotechnological significance stems from their ability to produce enzymes that are useful in the development of commercial products, in industrial processes such as bioremediation of toxic contaminants from water and sediments, and in the production of essential biomolecules for medical and industrial applications. [47].

Biofertilizers and bioinoculants, which are microorganisms with a variety of functions, play critical roles in nutrient cycling, fixation, mineralization, and solubilization, making them viable alternatives to traditional agricultural technology. Furthermore, they have the ability to induce resistance, making them useful biocontrol agents [43,48]. The genetic variety of these microorganisms provides considerable prospects in the agro-industrial sector, allowing for the replacement of chemical-based goods as well as the development of cost-effective, environmentally friendly, and sustainable farming methods [49,50]. Soil salinity, defined by a high concentration of soluble sodium salts, is a major concern for the agricultural business, causing soil degradation and impeding plant growth [51].

Plant Growth Promoting Bacteria (PGPB) can be found in a variety of habitats, including epiphytic, endophytic, and rhizospheric niches. They help plants grow by creating phytohormones such as indole acetic acids (IAA), gibberellic acids (GA), and cytokinins, as well as biological nitrogen fixation and nutrient solubilization and binding (e.g., phosphorus, potassium, zinc). PGPB also exhibits 1-aminocyclopropa-ne-1-carboxylate (ACC) deaminase activity, which aids in the reduction of ethylene levels, a hormone that limits plant development and hence reduces salinity stress [42]. Extremophiles like *Enterobacter* and *Gluconacetobacter* play a role in nitrogen fixation, while *Methylobacterium*, *Microbacterium*, and *Ochrobactrum* produce phytohormones. Halophilic extremophiles, when faced with salinity stress, promote various aspects of plant growth, such as seedling germination, root and shoot length, biomass, yield, and chlorophyll content. Additionally, some halophilic extremophiles like *Haloarcula argentinensis* and *Haloferax alexandrinus* demonstrate phosphorus solubilization, increasing phosphorus availability in hypersaline soils [43].

Psychrophilic extremophiles are used as bio-inoculants because they aid in nutrient solubilization, nitrogen fixation, and phytohormone synthesis, hence promoting plant development under low-temperature circumstances and giving disease resistance [53].

Acidophilic extremophiles including *Azotobacter*, *Bacillus*, and *Flavobacterium* promote plant development, making them good alternatives for bio-inoculants and biocontrol agents in acidic soils. Furthermore, drought-tolerant and phosphorus-solubilizing extremophiles show promise as bio-inoculants, and exploiting their potential in agriculture within desert settings could assist greatly to ensuring global food security for the expanding human population [54].

Extremophiles are also powerful biocontrol agents, exploiting their ability to live in extreme environments through the expression of certain genes, which has piqued the interest of many industrial and biotechnological applications. Notably, one such application is using these microorganisms to control biological diseases [55]. Rhizobacteria, for example, play an important role in plant pathogen defense by creating a variety of defensive substances such as ammonia, hydrogen cyanide, siderophores (iron-chelating compounds), chitinases, and a variety of secondary metabolites [56]. These biocontrol agents remain active in a wide variety of harsh environments and effectively limit pathogen and nematode proliferation by disturbing their reproductive cycles and competing for resources. *Bacillus*, *Clavibacter*, *Microbacterium*, and *Pseudomonas* are examples of biocontrol agents that function as plant pathogen inhibitors [54].

1. **Food industry**

Extremophilic microbes are well known for their ability to create a diverse spectrum of bioactive chemicals, secondary metabolites, and value-added products, making them extremely important in the food and food processing sectors [57]. These compounds not only improve the nutritional value of food, but they also have health advantages and can help prevent some long-term disorders [58]. Carotenoids, one of the useful chemicals produced by extremophiles, serve an important role in the food business as additives, color intensifiers, and antioxidants [59]. Carotenoids offer numerous health benefits to customers, including improved nutrition content and oxidation stability in meat and poultry products [60]. They are high in provitamin A, have anti-aging qualities, boost the immune system, and protect against some cancers and other physiological diseases [6]. These visually appealing probiotic colorants are used in sauces, infant foods, processed cheese, milk products, morning cereals, and fruit and energy drinks. Extremophiles such as *Bradyrhizobium sp.* and *Haloferaxal exandrines* produce canthaxanthin, a colorant utilized in food and beverages as well as salmon flesh coloring [57]. Riboflavin in Ashbya Gossypii and carotene in *Blakeslea trispora* are used to make microbial food colors [57]. The microalgal biomass is also useful for extracting carotenoids, which are utilized in dietary supplements. Astaxanthin is a nutritional and feed supplement derived from the microalgae *H. pluvialis* and extremophiles found in Antarctica's red snow [61].

The two most important processes in enzymatic starch processing are liquefaction and saccharification. The liquefaction step involves dissolving insoluble starch in an aqueous solution and then partially hydrolyzing it with thermostable amylases. The following saccharification process uses glucoamylases to completely degrade oligomers into monomers. These reactions occur at temperatures ranging from 50 to 80°C, necessitating the employment of thermally stable enzymes. There are various thermophilic enzymes commercially accessible, including -amylases, pullulanases, glucoamylases, xylanases, and amylopullulanases [62]. Initially, α-amylases are used for liquefaction, while glucoamylases and pullulanases are employed for saccharification. Pullulanases and glucoamylases are utilized in the food industry for the production of glucose syrups, whereas ß-amylases are utilized in the pharmaceutical industry for maltose syrup production. Additionally, thermostable amylopullulanases are used for creating maltose and maltotriose syrups, offering the advantage of simultaneous debranching and liquefaction, which makes them highly desirable for applications in the food, beverage, and pharmaceutical sectors [63]. In submerged and solid-state fermentation processes, three thermophilic enzymes—glucoamylase, amylopullulanase, and α-amylase—effectively saccharify starch without the need for calcium or any supplementary enzymes [64].

A thermophilic *Bacillus* sp. isolated from an Indian hot spring was employed in a study by Rana et al. to manufacture -amylase for clarifying kiwi and apple juice as well as bun manufacturing [65]. Adding 1.25% (w/v) -amylase to kiwi juice during processing enhanced yield and greatly improved taste, color, flavor, and overall acceptability. Similarly, the same enzyme content of 1.25% (w/v) was found to be ideal for increasing taste, color, flavor, and overall acceptability in apple juice. The enzyme's capacity to breakdown polysaccharides resulted in reduced viscosity and cluster formation in the juices, which improved juice quality.In the context of bun making, α-amylase was added before mixing the ingredients (wheat flour, sugar, yeast, and oil). At an enzyme concentration of 0.75%, the authors reported a maximum leavening activity of 2.60 ml/hr, resulting in a considerable improvement in the quality of the buns [65].

β-glucosidase plays a crucial role in the processing of sugarcane bagasse, which is the dry pulpy fibrous residue left after juice extraction from sugarcane. A thermostable β-glucosidase produced by *Anoxybacillus flavithermus*, isolated from the Tengchong hot spring in Yunnan, China, has been shown to be highly effective in hydrolyzing cellulose. When this enzyme was used in combination with a commercial cellulase product (Celluclast®, Novozymes), the cellulose content of sugarcane bagasse decreased by 25%. However, when β-glucosidase (50 µg/g sugarcane bagasse) was used together with Celluclast®, a more significant reduction of 48% in cellulose content was observed [66]. These findings indicate the synergistic action of the thermostable β-glucosidase and Celluclast® in breaking down complex sugars present in the bagasse. Given its efficiency in degrading cellulose, this thermostable enzyme holds promise for treating food industry wastes that contain high levels of complex sugars.

α-glucosidase has found practical applications in converting maltose into isomalto oligosaccharides (IMO), which are well-known as low-calorie, high-fiber sweeteners and are sold as prebiotic fibers in China and Japan [67]. Moreover, the thermostable α-glucosidase derived from Thermococcus hydrothermalis has been effectively used in conjunction with α-amylase and pullulanase for starch processing, resulting in the production of glucose syrup [68]. Another commonly utilized enzyme in the starch industry is the glucosidase from *Aspergillus niger*, which has been widely employed for the production of glucose syrups and disaccharides [69].

An exceptionally heat-resistant glucoamylase isolated from the forest floor in China, derived from *Penicillium* *oxalicum*, shown extraordinary effectiveness in hydrolyzing raw starches from corn and cassava for ethanol manufacture [70]. Similarly, a thermostable glucoamylase generated by Bacillus licheniformis was used to hydrolyze potato starch, which is distinguished by big granules that are difficult for most enzymes to break down [71]. The use of fungal glucoamylases in conjunction with -amylase is common in the production of glucose and fructose syrups via starch hydrolysis [72]. These high-glucose syrups can be used to make crystalline D-glucose or as a starting material for the development of high-fructose syrups.

In bread manufacturing, a recently found extremophilic xylanase from *Aureobasidium* *pullulans* was used and compared to two commercially available xylanase preparations. The enzyme, at a concentration of 125 U/100 g flour, produced high-quality dough with increased water absorption. Notably, it resulted in a 30% increase in bread specific volume and a 30% decrease in crumb stiffness when compared to the effects of commercial enzymes [73]. Various microbial xylanases have been used in bread production, including those originating *from Bacillus subtilis, Aspergillus aculeatus, Aspergillus oryzae,* and *Trichoderma reesei* [74]. Among these, the xylanase from *A. oryzae* was shown to be the most effective bread improver, while the one from *Trichoderma reesei* demonstrated superior antistaling properties [75]. Additionally, enzymes like lipases and phospholipases have been utilized as in situ emulsifiers to improve dough stability and conditioning, while lipoxygenase and glucose oxidase have been used to strengthen dough and enhance bread whiteness [74].

Extremophilic lipases are widely used in the synthesis of structured lipids containing omega-3 fatty acids and in the concentration of omega-3 fatty acids from various sources. For example, *Candida antarctica (Cal-A)* thermostable lipase has been used to create very pure docosahexaenoic acid (DHA) concentrate, which is widely used in food and pharmaceutical goods, particularly those requiring high DHA levels, such as infant formula [76]. Another Candida antarctica lipase (Cal-B) has been used to create fat analogs for infant formula that are high in arachidonic acid (ARA) and DHA [77]. Extremophile lipases have been critical in the synthesis of a wide range of food additives, including antioxidants, tastes, coloring agents, phytosterol esters, sugar esters, and conjugates of bi-functional chemicals [76]. These enzymes have proven to be versatile tools in the food industry, enabling the creation of a wide range of functional lipids with diverse applications.

Microbial esterases offer a wide range of uses in the food and beverage sectors, as well as in the breakdown of synthetic materials. A p-coumaric esterase produced from *Rhizoctonia solani*, for example, has been used to valorize food processing wastes by releasing p-coumaric, caffeic, and ferulic acids from wheat bran, sugar beet pectin, and coffee pulp wastes [78]. Similarly, an esterase produced from *Candida parapsilosis*, a fungus yeast isolated from marine debris off China's East Sea coast, was used to synthesize taste esters such as n-propyl acetate, isobutyl acetate, and isoamyl acetate [79]. These microbial esterases have shown to be significant tools in a variety of industries, assisting in the efficient reuse of byproducts and waste materials, as well as the synthesis of desirable flavor compounds for the food and beverage industries. Additionally, their ability to degrade synthetic materials highlights their potential role in waste management and environmental applications.

Microbial proteases are important in the food sector for meat tenderization and peptide production. The unique thermostable aspartic protease developed from *Rhizomucor miehei*, which displayed outstanding efficacy as a meat tenderizer, is one notable enzyme in this area. Even at a modest dose (0.25 mg/100 g pork), this enzyme outperformed a commercial meat tenderizer called papain [80]. Furthermore, when used to generate angiotensin converting enzyme (ACE) inhibitory peptides from turtle meat, this aspartic protease produced a significant amount of short peptides (5,000 Da) with strong ACE-inhibitory activity[80]. These findings underscore the potential of the aspartic protease from *Rhizomucor miehei* as a highly valuable tool in the food industry for meat tenderization and the production of bioactive peptides with potential health benefits.

Numerous extremozymes with exceptional tolerance to high salinity have been documented for food-related applications. For instance, *Halobacterium sp.* Strain LBU50301, derived from salt-fermented fish (budu), exhibited the ability to produce a halophilic protease capable of withstanding 27.95% (w/v) NaCl [81]. Similarly, a thermo-solvent stable protease from *Halobacillus sp. CJ4*, isolated from the hypersaline Chott Eldjerid Lake in Tunisia, demonstrated remarkable stability and catalytic activity at 120 g/L NaCl (2 M)[82]. These extremozymes' remarkable high-salinity tolerance opens up exciting possibilities for their application in producing high-salt foods, such as soy sauce. By harnessing their unique properties, these enzymes hold the potential to enhance the processing and quality of food products in high-salt environments, providing valuable opportunities for the food industry.

1. **Extremozymes Applied in Paper & Pulp industry**

Pulp is a fibrous material made up of cellulose from wood, fiber crops, and waste paper. Traditionally, pulp is made using chemical and mechanical procedures that separate cellulose fibers from other components found in wood, such as hemicellulose and lignin. High temperatures (sometimes reaching 80°C), alkaline pH values, and the use of powerful chemicals such as sodium sulfide, sodium hydroxide, and chlorine are all used in these processes. This method has been linked to environmental concerns as well as significant operational expenses [83]. Enzymatic bio-pulping has attracted attention as an eco-friendly, safer, and economically viable alternative for the pulp and paper industry to supplement current pulping technologies. Stable hyperthermophilic/alkaline enzymes have emerged as beneficial complements to pulping operations, increasing efficiency while decreasing dependency on harmful chemicals. The enzyme market in the pulping and paper industry is expected to grow, reaching significant value, particularly in Europe [83]. Among the enzymes relevant to bio-pulping, xylanases play a crucial role as they can break down hemicellulose, facilitating the release of lignin in a process known as bio-bleaching. Hyperthermophilic xylanases have been identified in various microorganisms and have shown promise in bio-bleaching processes. However, there is a need for more xylanases in the market for pulp and paper applications [84]

Laccases are also used in bio-bleaching to decompose lignin and improve the end product's brightness. Several fungal laccases have been used in bio-bleaching, but more study is needed to develop enzymes appropriate for strong pulping conditions [85]. Pitch control is achieved by using hyperthermophilic lipases, which reduce sticky deposits that impact paper output. In addition, novel hyperthermophilic esterases are being investigated to improve pitch control and address other sticky substances. Cellulases improve the brightness and strength of paper sheets, as well as the general efficiency of the refining process. To improve ecologically friendly and efficient procedures in the pulp and paper business, efforts are being made to produce high-performance enzymes suitable to the pulping industry, such as hyperthermophilic pectinases and amylases [83]. Continued research and development in this field are necessary to achieve sustainable and improved practices in the industry.

1. **Extremozymes used in textile industry**

Extremozymes, which are noted for their eco-friendliness and versatility in dealing with a wide range of substrates, are increasingly being used in the textile sector to improve fabric quality and address numerous processing issues [86]. These extremozymes serve an important function in increasing fabric appeal throughout the finishing process by resisting stressors such as bending and tension that cause thread degradation. They operate well in moderate circumstances, are biodegradable, and speed up reactions by lowering activation energy through substrate specificity [87].

Desizing, a technique used to remove the protective starch layer placed during weaving that may interfere with later operations, is one key application of extremozymes [88]. Extremophiles such as *Thermus thermophilus HB8*, *Euplotes focardi*, *Alkalibacillus sp. NM-Da2*, and *Geomyces sp*. produce enzymes such as -amylase, lipase, and proteases that aid in the desizing of woven fabric, notably denim and cotton fabrics [89].

Extremozymes are also used in bioscouring to remove non-cellulosic contaminants from fabric surfaces, such as pectin and waxes. To remove impurities, pectinase, xylanase, protease, lipase, and their combinations are utilized, and alkali-thermophilic thermozymes have demonstrated substantial efficacy due to their capacity to endure alkaline pH and high temperatures [90]. Notably, extremophiles such as Bacillus sp. and Pseudomonas sp. produce alkaline pectinases, which are good for cotton bioscouring to preserve cellulose and fiber damage [91]. Extremozymes such as glucose oxidase, catalase, and laccase are used in bleaching operations to generate pure white cotton fibers by removing natural colors and eliminating residual hydrogen peroxide in an environmentally benign manner [92]. Bacterial species such as *Geobacillus thermopakistaniensis* and *Brevibacillus agri* have been identified as sources of catalase and laccase enzymes, respectively, providing effective options for denim bleaching and decolorizing agents [93]. Furthermore, extremophiles such as *Vibrio sp*. and *Chromobacterium violaceum* have been studied for their ability to produce bio-dyes like as prodigiosin and violacein, which offer sustainable options for coloring wool, acrylics, silk, and other fabrics [94]. Continued research in this area holds the prospect of discovering even more efficient extremozymes that will transform the textile industry, making it more sustainable and environmentally friendly.

1. **Extremozymes in Detergent Market**

Cold-water detergents, which function as well as standard detergents but at lower temperatures, are the current trend in the detergent industry. Using cold-water detergents can result in decreased energy use, lower CO2 emissions, and better fabric protection. Despite the benefits, the use of cold-water detergents has been slow to expand, as hot water is still the favored method for cleaning garments. Nonetheless, recent efforts to identify and develop novel enzymes that perform successfully at cold temperatures have increased awareness in the cleaning sector, creating an ideal opportunity for the use of cold-wash detergents. [95].

Several cold-adapted enzymes are being researched to improve the efficacy of cold-water home and industrial laundry and dishwasher detergents. Among the most important cold-adapted enzymes are- **Lipases:** these enzymes hydrolyze fats (lipids) and remove fatty stains from fabrics. Lipoclean® is a cold-adapted lipase created by Novozymes that targets triglyceride stains and remains active at low temperatures (20°C). They have also created new lipases, such as Lipex® and Lipolase® Ultra, which work best at low to moderate temperatures [96]. **Proteases:** they catalyze the hydrolysis of peptide bonds in proteins, which aids in the breakdown of protein stains such as blood, egg, grass, chocolate, and perspiration [97]. Kannase® and Polarzyme® are cold-adapted proteases developed by Novozymes and Genencor for laundry detergents. **Amylases:** These enzymes degrade starch-based stains from foods such as cereals, fruits, and pasta[98]. Stainzyme® and Stainzyme® Plus, both from Novozymes, are effective at moderate/low-temperature washing. **Cellulases:** These enzymes degrade cellulose in cotton fibers, which reduces fuzz and pills. Celluzyme®, developed by Novozymes, is generated from the fungus Humicola insolens and is active at low temperatures (15°C) [99]. In addition to the regularly used dishwashing enzymes, the industry is actively researching alternative cold-active enzymes such as mannanases and pectinases, which target specific types of stains in food and personal care products [95]. Despite advances in cold-active enzyme development for the detergent business, there is still a need for new psychrophilic/psychrotolerant enzymes that can work optimally under current cold-washing techniques. Enzymes that function successfully at low temperatures, stay active over a wide temperature range, and are compatible with surfactants and alkaline pH hold a lot of potential for the future [95].

Apart from laundry detergents, extremozymes are used in cleaning-in-place operations in the food, brewing, and dairy industries. Enzymes are used in the beverage sector to clean equipment and deblock filters. Lipases, proteases, amylases, and pullulanases are very good at degrading molds and biofilms found in filters and building surfaces, allowing for improved cleaning performance without the use of chemical detergents, surfactants, or organic solvents [100].

1. **Biofuel Production**

Through enzyme-catalyzed fermentations, biofuels are produced directly from biomass. First-generation biofuels, such as corn, sugar beets, and wheat, encountered issues as they competed with global food supplies, resulting in higher food prices (Bhalla et al., 2013). Second-generation biofuels, on the other hand, have emerged as a more viable option by exploiting lignocellulose, which is abundant, cheap, and frequently obtained from agricultural and forestry waste. Lignocellulose is made up of three components: lignin, cellulose, and hemicellulose, with cellulose and hemicellulose serving as the key sources for second-generation biofuel production. Because of lignocellulose's stiff and compact structure, pretreatment is required to make cellulose and hemicellulose accessible to enzymes [101]. Higher temperatures (over 50°C) are frequently used in successful pretreatment procedures to break the lignocellulose structure and improve enzyme penetration [101].

The destruction of lignocellulose necessitates the use of cellulase and xylanase enzymes, which work together to hydrolyze cellulose and hemicellulose, respectively. Cellulases are made up of three enzymes: endoglucanase, ß-glucosidase, and exoglucanase, which all work together to completely hydrolyze cellulose into glucose [102]. In contrast, xylanases are a class of enzymes that breakdown xylan in hemicellulose into monosaccharides and xylo-oligosaccharides. The degradation of lignocellulose necessitates the use of enzyme combinations that can be structured in many ways, including the utilization of a multienzyme complex known as the cellulosome and multifunctional megazymes [103].

Multifunctional megazymes are enzymes that have at least two distinct catalytic modules, and are frequently bifunctional. *Caldicellulosiruptor bescii* has a hyperthermophilic cellulase/hemicellulase system that degrades xylan, microcrystalline cellulose, and non-pretreated grass and rice straw at increased temperatures and specified pH levels [104]. *Clostridium thermocellum cellulosomes* and thermophilic ß-glucosidase from *Thermoanaerobacter brockii* demonstrated high glucan conversion from pre-treated rice straw with significant economic benefits [105]. When compared to commercial enzymes, *C. thermocellum's cellulosome* accomplished full glucan conversion of microcrystalline cellulose [106]. Lipases, for example, play an important part in biotechnological applications and help to produce biofuels such as biodiesel. Cold-active lipases from psychrophilic extremophiles are very useful for industrial biodiesel synthesis because they accelerate reactions at extremely low temperatures [107].

Lignocellulose-degrading enzymes are required for the breakdown of lignocellulose, a refractory biomass composed of cellulose, hemicellulose, and lignin. These hydrolytic enzymes break down cellulose to produce fermentable sugars, which can then be turned into ethanol. Cellulases, xylanases, lignases, lignin peroxidases, and manganese peroxidases are known to be produced by thermophilic microbes such as *Geobacillus sp. R7*, *Phanerochaete chrysosporium*, and *Sporotrichum thermophile* [108]. *Caldicellulosiruptor bescii*, an anaerobic bacterium, efficiently destroys untreated biomass and crystalline cellulose, promising bioconversion of lignocellulose to ethanol without pretreatment [109]. Another important enzyme is -amylase, which is essential for starch breakdown. Extremozymes from thermophilic organisms, such as Bacillus licheniformis, are ideal for isolating -amylase because they function best at high temperatures[110]. These enzymes have significant relevance in the production of ethanol through the processes of liquification, saccharification, and fermentation.

1. **Biomining**

Biomining, also known as mineral extraction through bioleaching and bio-oxidation, is a low-cost and environmentally beneficial approach. The oxidation of metal sulfides in an acidic environment by thermophiles and sulfur-oxidizing chemolithotrophs results in sulfur compounds or metal ions [111]. Bio-oxidation, on the other hand, degrades the mineral matrix surrounding the target metal, exposing it to oxidation slag. Extreme circumstances, such as high salt concentrations, high and low temperatures, organic solvents, low pH, and high metal concentrations due to acid mine drainage, are frequently encountered during biomining. Polyextremophiles such as *Acidithiobacillus ferrooxidans, Sulfobacillus sp.,* and *Ferroplasma sp.* are often employed for bio-mining of metals such as copper, nickel, and uranium in such conditions. Acidophiles such as *Acidihalobacter prosperus* and *Acidihalobacter ferrooxidans* are also employed in bio-metallurgy [112]. Archaebacteria like *Metallosphaera* and *Sulfolobus* are effective at very high temperatures in biomining. These microorganisms can fix carbon dioxide and grow under aerated conditions [113].

Biomining, which uses techniques such as roasting and smelting, is more energy-efficient than traditional mining processes and does not emit hazardous gases such as sulfur dioxide. It has advantages for leaching both low and high-grade ores economically. Furthermore, it aids in the reduction of acid mine drainage, hence lowering environmental contamination [114]. For commercial-scale extraction, several technologies such as bio-reactors, piles, and dumps are utilized, whereas in-situ mining and vats are used for low-grade ores. By eliminating excess iron, sulfate, and other impurities from hydro-metallurgical solvents, acidophilic iron-oxidizing extremophiles play an important role in creating and recycling reagents used as lixiviants. By enhancing tolerance to variable environments, genetically modified (GM) microorganisms have improved the efficiency and management of biomining [114]. OMICs technologies (genomics, proteomics, transcriptomics, and metabolomics) have made significant contributions to a better understanding of the internal growth mechanisms that help extremophiles adapt. Continued research is needed to identify microorganisms with higher metal tolerances, better attachment to minerals, and high growth rates at elevated metal concentrations. Furthermore, the potential of bioleaching and biomining for asteroid and planetary deployment can be explored using synthetic biology [114].

1. **Bioremediation, biodegradation & pollutant revomal**

Bioremediation has become an urgent and crucial need since it is so important in the repair of contaminated and degraded landscapes. Microorganisms, particularly extremophiles, contribute significantly to this process by digesting heavy metals and organic pollutants, detoxifying polluted soil, waste water, and radioactive waste, and even assisting in the decomposition of plastic, a major environmental contaminant [115]. Numerous industrial activities result in the release of heavy metals and radioactive contaminants into the environment, endangering both human health and the ecology. Extremophiles have extraordinary capacity to convert, immobilize, or degrade these hazardous contaminants into non-toxic chemicals via a variety of mechanisms including biodegradation, biosorption, bioreduction, and bioemulsification [116]. Extremozymes, produced by these remarkable microorganisms, act as efficient biocatalysts, facilitating the catalysis, insolubilization, and precipitation of pollutants through redox reactions while minimizing the generation of secondary pollutants [117]. This bioremediation process offers a promising solution for addressing environmental contamination and preserving the health and balance of the ecosystem [117].

Acid mine drainage (AMD) occurs when sulfide minerals in the Earth are exposed during mining or big construction operations. Most sulfide minerals oxidize when they come into contact with water and oxygen, resulting in the creation of sulfuric acid, which then enters surface and groundwater. AMD contributes significantly to water contamination and the release of heavy metals into the environment. AMD typically has a pH range of 2 to 8 and contains high quantities of metals and sulfides. Conventional AMD treatment procedures include alkalinization of the acidic effluent above the ideal pH needs of iron-oxidizing bacteria or the use of crushed limestone to minimize acid production. However, these methods are inefficient, costly to operate, and generate large amounts of solid sludge that require proper disposal [118].

Acidophilic microbes, primarily *Acidiphilum, Acidithiobacillus, Acidisphaera,* and *Leptospirillum*, inhabit AMD settings [119]. These microorganisms not only survive in acidic environments, but they can also oxidize and decrease iron and sulfur. They are also resistant to hazardous elements such as cadmium, chromium, nickel, and arsenic. AMD bioremediation solutions use bioreactors containing acidophilic iron-oxidizing bacteria (e.g., Leptospirillum ferroxidans) and sulfate-reducing bacteria (e.g., *Acidithiobacillus ferroxidans, A. ferrivorans*) rather than specific enzymes. Extracellular oxidoreductases are secreted by these bacteria and are stable at pH levels significantly lower than their cytoplasmic pH (about pH 5) [120]. AMD goes via a fermentation process in the bioreactors, which generates conditions conducive for sulfate reduction and metal precipitation [121]. Sulfate reduction leads to the generation of alkalinity by converting sulfate to sulfide. The dissolved metals then bind with sulfide to form insoluble metal sulfides. The treated, neutralized drainage, free from metals, is then released from the bioreactor. Several successful examples of AMD treatment bioreactors achieving over 90% removal of heavy metals have been reported [122]. Because of their amazing capacity to survive high temperatures and decrease metals, thermophiles can be used efficiently in heavy metal bioremediation (Mn, U, Tc, Cr, Co, Mo, Au, and Hg). *Geobacillus thermantarcticus* and *Anoxybacillus amylolyticus*, for example, have a high biosorption ability, allowing them to bond with heavy metals such as Cr, V, and Co and hence facilitate their removal from polluted settings [123]. These thermophiles convert contaminants into non-toxic compounds. Bacillus sp. has also been shown to be successful in eliminating aliphatic and aromatic hydrocarbons as well as synthetic colors from diverse industries [124].

Petroleum industries and oil spills often lead to soil and groundwater contamination with harmful substances, such as poly-cyclic aromatic hydrocarbons and long-chain alkanes (C10 to C32). Certain extremophiles, including *Bacillus, Thermus*, and *Geobacillus*, are capable of decontaminating such pollutants [125]. Geobacillus SH-1 strain, for example, is capable of degrading saturated alkanes ranging from C12 to C33 and naphthalene, whereas thermophiles such as *Geobacillus thermoparaffinivorans IR2*, *Geobacillus stearothermophilus IR4*, and *Bacillus licheniformis* can transform long alkyl (C32 and C40) hydrocarbons into non-toxic substances [125]. Thermophiles such as *Thermus scotoductus*, *Thermoterrabacterium ferrireducens, Pyrobaculumis landicum,* and *Thermoanaerobacter sp.* have also showed potential in radioactive waste treatment by reducing certain radioactive chemicals such as enzymatic uranium and technetium [126]. Furthermore, hyperthermophilic species such as *Pyrobaculum sp*. are implicated in uranium reduction [127]. Extremozymes derived from thermophilic molds, such as *Talaromyces emersonii, Rhizopus sp.,* and *Thermomucorindicae seudaticae*, are highly effective and persistent at high temperatures, making them useful for decomposing hazardous and organic pollutants from industrial processes [126].

Psychrophiles, which flourish in cold environments, are excellent in utilizing organic pollutants, particularly hydrocarbon mixtures and halogen compounds, rendering them non-toxic [124]. *Pseudoalteromonas sp. P29* and *Oleispira antarctica RB-8T*, for example, exhibit modifications that allow them to breakdown crude oil, jet fuel, and other contaminants [125]. Furthermore, psychrophiles play an important role in plastic breakdown, particularly when building biofilms on plastic waste [128].

Extracellular polymeric substances (EPS) produced by halophiles aid in their attachment to surfaces and the creation of biofilms, boosting their efficacy in the treatment of harmful organic contaminants [129]. These microbes are capable of decomposing hydrocarbons prevalent in hypersaline environments, such as alkanes, benzene, biphenyl, anthracene, and naphthalene [125]. They may also remove hazardous chemicals such as tributyltin, phenol, hydrocarbons, and azo dyes from diverse industrial effluents [125].

Finally, radiophiles contribute to radionuclide treatment in soils and aquatic mediums via biomineralization, biotransformation, and biosorption processes. Some radiophilic strains, such as *Shewanella* and *Geobacter* species, can reduce uranium and other radioactive contaminants, making them helpful for decontamination [125]. Extremophiles, which include thermophiles, acidophiles, psychrophiles, halophiles, and radiophiles, provide excellent solutions for the bioremediation of diverse pollutants in a variety of environmental situations.

1. **Cosmetic industry**

Mycosporines and mycosporine-like amino acids (MAAs) find applications in the cosmetic industry due to their ability to protect the skin from harmful UV radiation. UV exposure can lead to sunburn, premature aging, and even skin cancer over prolonged periods [130]. MAAs have been demonstrated to be more effective in protecting the skin than synthetic sunscreens comprising organic filters (oxybenzone, avobenzone, aminobenzoic acid, etc.) or inorganic filters (titanium dioxide, zinc oxide, etc.) or both. They provide anti-aging protection by acting as antioxidants, defend against microorganisms, and effectively absorb UV radiation while having less negative impacts on marine life and causing few side effects in people, such as allergic reactions, phototoxicity, and endocrine abnormalities. They are also more environmentally friendly [130, 131]. When exposed to UV light, lichens, fungi, and cyanobacteria naturally create MAAs, and their stability at high pH and temperatures makes them suitable natural bioactive components in cosmetic products [132]. However, they provide less protection against UVB radiation than UVA radiation [130]. Because synthetic UV filters in sunscreens absorb photons rather than reflecting them, the inclusion of antioxidant-rich compounds is critical to improving sunscreen efficiency [132]. Extremophiles exhibit a variety of biochemical adaptations, including the creation of carotenoid pigments (e.g., lycopene, astaxanthin), which act as natural antioxidants, protecting against UV radiation and lowering skin photodamage [133].

Biosurfactants have acquired popularity in the cosmetic sector due to their positive features such as foaming, emulsification, and water binding, which make them preferable to other surface-active chemicals [134]. The output of monoglyceride, a frequently used surfactant, can be increased by treating glycerol tallow with the psychrophile Pseudomonas fluorescens lipase [132]. *Arthrobacter, Pseudomonas, Halomonas*, and *Bacillus* are examples of marine organisms that could be used to produce biosurfactants and bioemulsifiers [132].

1. **Medical applications of extremophiles and their products**

Extremophiles, which flourish in harsh settings, are important sources of antibiotics, antifungals, and anticancer chemicals. They produce antimicrobial peptides, such as halocins, which are found in *Halobacteriaceae* and *Sulfolobus* species and are effective at killing archaeal cells while causing no harm to harmful germs in humans [135]. Furthermore, extremophiles produce diketopiperazines, which are derived from halophiles such as *Naloterrigena hispanica* and *Natronococcus occultus* and have antibacterial, antifungal, antiviral, and anticancer activities. These diketopiperazines also have an effect on human blood coagulation, making them possible candidates for alternate treatments against drug-resistant *Pseudomonas aeruginosa* infections via regulating quorum-sensing pathways [135]. Furthermore, extremophiles may synthesize Polyhydroxyalkanoates (PHAs), which act as carbon storage for microbial cells and provide a biodegradable and biocompatible alternative to petroleum-based plastics. Because PHAs are non-cytotoxic, they are highly adaptable as biopolymers and have uses in implants, drug delivery systems, and other areas [135]. Recombinant gas-filled vesicles produced by extremophiles such as *Halobacterium sp. NRC-1, Halobacterium salinarum, Haloferax terranei,* and *Holoquadratum walsbyi* are another fascinating breakthrough. These vesicles are largely non-toxic while provoking a strong immunological response. As a result, they have demonstrated good outcomes in mouse studies, making them prospective candidates for alternate vaccine delivery systems [135].

DNA polymerases derived from thermophiles have proven to be useful in medicine and biotechnology. Taq from Thermus aquaticus, Pfu from *Pyrococcus furiosus*, and Vent from *Thermococcus litoralis* are a few examples. Taq polymerase from Thermus aquaticus is particularly relevant since it is frequently used in PCR, a fundamental technique in molecular biology [135, 136]. *Aeropyrum pernix K1* is a hyperthermophilic bacterium that produces a remarkable extremozyme called nucleoside phosphorylase, which has uses in antiviral therapy by helping in the manufacture of nucleoside analogs. Because of their capacity to act at high temperatures and with minimal substrate viscosity, thermozymes like this one has a wide range of clinical applications [130,135].

Pyochelin, a Pseudomonas sp.-derived chemical, has antifungal and iron-binding characteristics, making it effective against *Candida* and *Aspergillus* species. Dried Dunaliella, a halophile, has antioxidant and cryoprotectant properties due to antifreeze proteins [137]. Furthermore, Dunaliella generates -carotene, which is used as a colorant in the pharmaceutical industry and as a food supplement [138]. Furthermore, two benzoxazine glycosides (arcticoside and C-1027 chromophore-V) isolated from a marine *Streptomyces* strain inhibit *Candida albicans* isocitrate lyase as well as breast and colorectal cancer cells [139]. Because of their anti-proliferative, anti-inflammatory, and chemo-preventive effects, extremeolytes have therapeutic potential. Extremophiles create a variety of metabolites, including biosurfactants, biopolymers, and peptides, which have a wide range of applications in the pharmaceutical sector [138].

Biosurfactants have therapeutic applications and are frequently used in the pharmaceutical and medical industries. These surface-active compounds are useful for a variety of therapeutic uses due to their antibacterial, antiviral, anticancer, and antifungal activities [138]. Biosurfactants have a wide range of applications in the pharmaceutical business, demonstrating antibacterial, antiviral, anticancer, antifungal, and even antitumor characteristics. *Bacillus circulans*, for example, produces a biosurfactant with significant antibacterial action against multidrug-resistant (MDR) and other pathogenic and semi-pathogenic microbial strains [140]. Biosurfactants are used in pharmaceutical formulations to enhance the creation of new collagen fibers via the action of purified lactone sophorolipid [141]. They also aid in the recovery of intracellular products by assisting in cell lysis after fermentation [141].

Biosurfactants, by their antiadhesive and antibiofilm properties, also play an important role in fighting pathogenic microbe attachment and colonization. Biosurfactants derived from *Lactobacillus fermentum*, a thermo-acidophilic lactic acid bacteria, have been shown to suppress Staphylococcus aureus infection on surgical implants. Similarly, surfactant-treated PVC plates and vinyl urethral catheters inhibit *E. coli* biofilm development [141]. Furthermore, biosurfactants, particularly liposome-based biosurfactants, have the potential to be used in gene transfection as alternate gene delivery methods and immunological adjuvants. Because of their low toxicity and pyrogenicity, they are ideal candidates for such applications [141].

**Table 1** gives a comprehensive list of extremozymes, their role played in various industries.

**Table1: Extremozymes and their applications in different industries.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Industries** | **Name of enzymes** | **Microbe** | **application** | **reference** |
| **Agricultural industry** | Cold active enzymes | Arthrobacter and Bacillus | Aid in low-temperature farming | 54 |
| Cellulase | Acidothermus cellulolyticus | Hydrolyzing cellulose | 1 |
| Urease | Bacillus sp. strain TB-90 | Breakdown of urea | 1 |
| **Pharmaceutical and****medical industry** | Thermolysin | Bacillus thermoproteolyticus | Synthesis of dipeptidesproduction of NAOS | 110 |
| Prolidase | Pyrococcus furiosus | Cleavage of dipeptide | 110 |
| Monoacylglycerol lipase (GMGL) | Geobacillus sp. 12AMOR1 | Acts on monoacylglycerol substrate | 110 |
| β-agarase AgaP4383 | Flammeovirga pacifica WPAGA1 | Hydrolysis of agar and recovery of DNA from agar gels, production of NAOS | 110 |
| β-agarase Aga4436 | Flammeovirga sp. OC4 | Hydrolysis of agar, recovery of DNA from agar gel, | 110 |
| Est11 esterase | Psychrobacter pacificensis | Act as catalysts  | 110 |
| EstO esterase | Pseudoalteromonas arctica | enhance the solubility ofanti-inflammatory drugs | 110 |
| Mercuric reductase | Archaeon SCGC-AAA261G05 | Bacterial detoxification | 113 |
| DNA polymerase | *Thermus aquaticus*, *Thermococcus litoralis*, *Pyrococcus furiosus*, Archaeon SCGC-AAA261G05,  | PCR | 135 |
| Nucleoside phosphorylase | *Aeropyrum pernix* K1 | Synthesis of nucleoside analogs | 135 |
| **Textile industry** | Amylase, lipase, and proteasesα- Amylase | *Thermus thermophilus* HB8, *Euplotes**focardi*, *Alkalibacillus* sp. NM-Da2, and*Geomyces sp*.*Geobacillus stearothermophilus,* *Halothermothrix orenii**Streptomyces sp*. TO1 | Increase the lubricity of the yarn in cases of cotton fabrics & denim | 8989 |
| β-glucosidase | *Martelella mediterranea* | Degradation of cellulose | 16 |
| Cellulase Puradax HA | *Bacillus sp.* and *Paenibacillus tarimensis* | Remove stains and protects color fromfabric | 16 |
| OptisizeVR COOL and Optisize NEXT | Geomyces sp. P7 | Desizing of woven fabric | 90 |
| Pectinase | Tetracladium sp., Bacillus sp. andPseudomonas sp. | Bioscouring: Removal of pectin from fabrics | 90 |
| Lipase | *Penicillium canesense,**Pseudogymnoascus roseus* | Bioscouring: Removal of fats from fabric | 90 |
| Xylanase | *Flammeovirga pacifica* WPAGA1 | Bioscouring | 90 |
| Catalase | *Geobacillus thermo pakistaniensis* | Bleaching | 90 |
| Laccase | *Geobacillus thermo pakistaniensis* | Artificial dyes | 90 |
| Laccase | *Streptomyces psammoticus* and*Stenotrophomonas maltophilia* | Decolourising agents | 90 |
| Laccase LacT | *Brevibacillus agri* | Biobleaching: helps indepigmentation of azo dyes | 6 |
| **Bioremediation and****biodegradation** | Hydrolytic enzymes,oxidoreductases | Anoxybacillus sp. | Reduce and degrade pollutants like polyaromatic hydrocarbons dyes, phenol, heavymetals, phosphates and antibiotic residues, from waste water | 6 |
|  | *Talaromyces emersonii*, Rhizopus sp.,and *Thermomucorindicae seudaticae* | Degrade organic contaminants & toxic from effluents | 126 |
|  | *Acidothiobacillus ferrooxidans* and *Acidothiobacillus ferrivorans* | Help in precipitation of Copper | 125 |
|  | *Acidocella aromatica* PFBC | Reduce vanadium ions | 125 |
|  | *Acidiphilium symbioticum* H8 | Biosorption of Cd cations | 125 |
| Nitrile hydratase/amidase | *Pseudomonas putida*, and Rhodococcussp. | Elimination of acetonitrile from organic pollutants | 126 |
| **Bioenergy, biofuels****and biorefinery** | Cellulases, xylanases, lignases,lignin peroxidases and manganeseperoxidases (lignocellulosedegrading enzymes)α-Amylase | *Caldicellulosiruptor bescii*, Geobacillussp. R7, *Phanerochaete chrysosporium*, and *Sporotrichum thermophile**Bacillus licheniformis*, *Pyrococcus**furiosus*, *Bacillus acidocaldarius* , &*Bacillus stereothermophilus*, and Alteromonassp. | Degrades untreated biomass, crystalline cellulose and helps in bioconversion of lignocellulose to ethanol without pretreatmentProduction of ethanol | 108108 |
| Glucoamylase | *Aspergillus niger* | Helps in saccharification | 108 |
| **Food industry** | Lipase | *Bacillus* stearothermophilus *Acinetobacter calcoaceticus* LP009Moraxella sp. | Dairy industryBaking industryDairy industry | 111 |
| Protease | *Bacillus brevis**Bacillus HUTBS62**Bacillus HUTBS71**Alteromonas sp.**Bacillus sp.**Bacillus sp.* 158 | Baking industry,Brewing industry,Dairy industryBaking industry,Brewing industry,Dairy industry | 1 |
|  | Xylanase | *Actinomadura sp. strain Cpt20**Clostridium strain PXYL1**Paenibacillus curdlanolyticus B6* | Baking industry,Baking industryFruit juice processing | 1 |
| α- Amylase | *Bacillus sp. isolate A3-15**Aeromonas veronii NS07* | Bakery industryBakery industry | 1 |
| β- Glucosidase | *Fervidobacterium islandicum**Bacillus sp.* | Brewing industry,Brewing industry, | 1 |
| Esterase | *Anoxybacillus gonensis A4**Oleispira antarctica* | Food industryFood industry, | 1 |
| Cellulase | *Flavobacterium sp.**Geomyces sp.**Shewanella sp. G5* | Brewing industry,Fruit juice processing, Wine industry | 1 |
| **Detergent industry** | Lipase | *Geobacillus sp. SBS-4S* | Stain removal | 1 |
| Protease | *Bacillus sp. JB-99* | Stain removal | 1 |
| Xylanase | *Bacillus sp.* | Stain removal | 1 |
| **Paper and pulp industry** | Lipase | *Pseudomonas aeruginosa BTS-2* | Paper industry | 1 |
| Xylanase | *Caldocellum saccharolyticum**Dictyoglomus thermophilum* | break down hemicellulose and bio-bleaching  | 1 |
| Esterase | *Pseudoalteromonas arctica* | improve pitch control and address other sticky compounds | 1 |
| **Pharmaceutical industry** | Lipase | *Thermosyntropha lipolytica* | Transesterification of oils and fats | 1 |
| Protease | *Chaetomium thermophilum**Geobacillus collagenovorans MO-1**Paenibacillus tezpurensis**sp. nov. AS-S24-II**Pyrodictium sp.**Thermococcus onnurineus NA1**Thermus aquaticus YT-1**Clostridium schirmacherense**Flavobacterium YS-80**Halomonas sp.**Pseudoalteromonas sp. NJ276**Pseudomonas strain DY-A**Pseudomonas fluorescens 114**Streptomyces sp.**Rheinheimera sp.* | Flavor modification, optically active esters;Cleavage of proteins | 11 |
|  | Esterase | *Pseudomonas sp. B11-1**Psychrobacter sp. Ant 300**Streptomyces coelicolor A3* | Increases the solubility ofanti-inflammatory drugs | 1 |

1. **Conclusions and Prospects:**

Extremozymes generated from extremophilic microbes have piqued the interest of researchers due to their stability and capacity to persist in harsh environments. Despite their enormous promise, the number of extremozymes available now is restricted. Because of their resilience to severe temperatures, chemicals, organic solvents, and pH levels, thermophilic enzymes are very important in biotechnology. Agriculture, food and drinks, medicines, detergents, textiles, leather, pulp and paper, and biomining are all businesses that use extremozymes. Exploring commercial applications for extremozymes and satisfying the growing need for innovative biocatalysts in biotech industries remains a top priority in extremophile research.

The discovery and utilization of extremophiles and their products have already demonstrated their superiority over conventional methods. With further research and development, their potential applications can extend to various industries that have yet to be explored. Advancements in nanotechnology can enhance the specificity of extremozymes, benefiting sectors like bioremediation, nuclear power, and textiles. Additionally, genetic modification of extremophiles can boost biofuel production and aid in the disposal of radioactive waste. The economic potential of extremophiles is significant, and further studies should focus on their genetic modification, biochemical properties, and long-term effects. Government funding and collaboration between institutions are essential for driving research and realizing the sustainable and efficient applications of extremophiles in various industries.

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