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Review Article

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Exploring Protease Enzymes from Extremophiles Novel Solutions for Sustainable Leather Processing

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A B S T R A C T

This review article highlights the industrial relevance of extremophile proteases and examines methods for isolating and characterizing them. Microorganisms known as extremophiles, which flourish in harsh conditions, have special enzymatic properties that may have important biotechnological uses. The methodical procedure includes gathering samples from extreme environments, isolating extremophiles, enrichment culture, and screening for protease activity. In order to predict the structure and function of proteins, promising strains go through gene sequencing, PCR amplification, genomic DNA extraction, and bioinformatics analysis. Together with protein extraction, purification, and characterization, the methodology described also makes use of biochemical assays to ascertain the enzymatic properties of proteins and methods such as SDS-PAGE. Stability, activity in harsh environments, and compatibility with industrial processes are all taken into account when evaluating industrial potential. Based on their strong and distinct characteristics, proteases derived from extremophiles are promising candidates for a range of industrial uses, according to the review. The systematic approach highlights the potential of proteases derived from extremophiles in the processing of leather and offers researchers a path forward. Because these enzymes can survive in harsh environments, there are potential for them to change and enhance sustainability in the leather sector. The study makes the case that more research into extremophile-derived proteases could lead to novel biotechnological approaches to leather processing as well as sustainable industrial practices.

Introduction

Leather is a fast rising worldwide business with an estimated $394.12 billion. It is applied in a variety of sectors, including footwear, accessories, clothing, and furniture. China is the world's largest leather manufacturer, with around 9000 tanneries, surpassing Brazil, Russia, and India. Italy, Russia, and other nations are also significant players in the leather industry.

The standard leather production process includes tanning, post-tanning, and finishing, all of which generate toxic

chemicals and polluting waste. Chromolithography (Cr) tanning is the primary process for tanning leather, although it creates harmful byproducts.

Water contamination is a major concern in the leather business, with industrial farms contributing to water pollution in the United States (Markets and Markets, 2022; Tatta *et al.,* 2022).

Enzymatic processes have been studied as a possible replacement to existing approaches. Proteases, (Research *et al.,* 2021) which are present in many living creatures, have shown promise in bioprocessing and can be employed at various phases of the leather-making process. They provide considerable advantages for improving the end product of the biocatalytic process (Atalah *et al.,* 2019). Proteases are categorized into three types microbial, plant, and animal proteases. They are utilized in a variety of industrial industries, including food and pharmaceuticals, although their employment in leather processing is uncommon.

Despite the various advantages of utilizing enzymes in industrial sectors, including environmental benefits, the use of proteases in leather production is uncommon.

Enzymes are protein catalysts found in animals, plants, and microorganisms that are employed in a variety of sectors such as food manufacturing, consumer products, and waste reduction (Delgado-García *et al.,* 2018).

They have also been used to wastewater treatment, recycling, and biofuel generation. However, their short shelf life, limited stability, and vulnerability to diverse process settings limit their use and broad use.

Enzyme immobilization methods have been developed since the 1960s to improve enzyme physicochemical properties and make them usable in a variety of applications (Gupta *et al.,* 2020). The effectiveness of these processes is dependent on the carrier material, which must have a big surface area, considerable porous characteristics, and be stable. The carrier material should also be easily changeable, affordable, abundant, and environmentally safe (Wanyonyi *et al.,* 2016).

Despite the bright future of enzyme technology, the FDA and EMA have only approved a few enzyme therapies due to limitations such as insufficient tissue selectivity, immunogenicity, and short half-life in live organisms. Off-target interactions between enzymes can cause undesirable side effects such as corneal opacification, retinopathy, optic nerve enlargement and atrophy, ocular hypertension, and glaucoma.

Enzymes have a long history of usage in a variety of sectors, but their short shelf life, limited stability, and vulnerability to different process conditions limit their broad use. Immobilizing enzymes can assist overcome these obstacles and increase their efficiency in a variety of applications.

Enzyme-based therapies can cause an immunological response in patients by introducing an exogenous recombinant enzyme. This can trigger antibody responses, including those against glucocerebrosidase in Gaucher's sickness, α-L-iduronidase in Hurler's syndrome, and anti-drug antibodies in Pompe's disease.

The immunogenicity of exogenous enzymes is modified by age, immunosuppressive medication, and immune system abnormalities. Patients with immune-system stimulating disorders, such as allergies or inflammation, may be more likely to experience severe immunological responses (Katsimpouras and Stephanopoulos, 2021). The method enzymes are administered to patients can potentially influence their immune systems' responses.

Intravenous medicines are often less immunogenic, and long-term treatment is more effective. Patients with endogenous anti-enzyme cross-reactive antibodies may suffer more severe symptoms. However, the intrinsic immunogenicity of therapeutic enzymes may result in adverse side effects (de la Fuente *et al.,* 2021).

Extremophiles, or bacteria that live in harsh settings, have received attention for their adaptation methods and physiological capabilities. They are very useful for bioremediation because of their capacity to withstand harsh environmental conditions. This chapter provides an overview of extremophiles and their possible uses in the bioremediation of ecological pollutants (Gallo *et al.,* 2021).

Extremophiles are creatures that live in severe settings, such as hydrothermal vents, under high pressure and temperatures (Jorquera *et al.,* 2019). Their specialized enzymes, known as "extremozymes," (Mutlu-Ingok *et al.,* 2022) allow them to function under these harsh circumstances, holding considerable potential for genetically modified pharmaceuticals, industrial chemicals, and processes.

In recent years, progress has been made in next-generation industrial biotechnology (NGIB), which employs extremophiles (Raddadi *et al.,* 2015) as chassis cells to construct open, non-sterile continuous fermentation manufacturing systems.

This technique offers several advantages, including non-sterility, energy saving, high product concentration, and easy separation operation.

Extremophiles provide economic and environmental benefits because their resistance in severe settings leads to more efficient industrial operations, (Schröder *et al.,* 2020) which reduce operating costs and environmental consequences. They also provide environmentally favourable (Vester *et al.,* 2015) alternatives to typical chemical processes, reducing the production of harmful by-products (Sharma *et al.,* 2012).

Thermophilic bacteria use distinct adaptation tactics, (Furhan, 2020) including enzyme stability, (Eppinger *et al.,* 2020) membrane adaptations, (Zhang *et al.,* 2020) osmotic management, (Puri *et al.,* 2021) DNA repair mechanisms, metabolic flexibility, spore growth, pH adjustment, (Jin, 2021) antioxidant defense mechanisms, and interactions with other microbes in communities (Bhalla, 2013). Extremophilic bacteria generate solvent-stable enzymes, which are of great relevance in a variety of industrial applications, particularly those involving organic solvents.

Extremophiles have developed a variety of adaption strategies, including enzyme stability, membrane adaptations, osmotic management, DNA repair systems, (Berg *et al.,* 2019) metabolic flexibility, (Gallo *et al.,* 2021; Wang *et al.,* 2020) and community interaction (Liu *et al.,* 2016). These adaptations have resulted in the formation of several enzymes, including amylase, lipase, xylanase, cellulase, and protease, which are required for their survival in hostile settings (Li *et al.,* 2019).

Esterases and Lipases

Extremophiles such as psychrophiles and thermophiles are the source.

Application The hydrolysis of lipids and ester groups is facilitated by these enzymes. In the process of producing biodiesel, solvent-stable lipases play a critical role by catalyzing transesterification processes in the presence of organic solvents (Lopez-Lopez *et al.,* 2014).

Proteases

Alkaliphiles, acidophiles, and thermophiles are the sources.

Application Solvent-stable proteases are used in the food industry, leather processing, detergent formulation, and other sectors where they aid in the hydrolysis and breakdown of proteins (Mokashe *et al.,* 2018).

Cellulases

Acidophiles and thermophiles are the sources.

Use In particular, solvent-stable cellulases (Beckham *et al.,* 2013) play a crucial role in the enzymatic hydrolysis of lignocellulosic biomass, which produces biofuel. They are resistant to the organic solvents that are utilized in several preparation procedures.

Peroxidases

Halophiles, acidophiles, and thermophiles are the sources.

Application Solvent-stable peroxidases are employed in a number of biotechnological procedures, (Zhu *et al.,* 2020) such as the elimination of contaminants, the decolorization of textile dyes, and solvent-assisted organic synthesis reactions.

Amylases

Extremophiles who are fond of thermophiles.

Application In the presence of organic solvents, starch and food industries use solvent-stable amylases for saccharification procedures (Parashar and Satyanarayana, 2018).

Polymerases

Thermophiles and extremophiles are the source.

Use Polymerase chain reaction (PCR) in the presence of organic solvents is one of the molecular biology applications where solvent-stable polymerases, especially DNA and RNA polymerases, are crucial (Mathur *et al.,* (1991).

Xylanases

Acidophiles and thermophiles are the sources.

Application Solvent-stable xylanases are used in the pulp and paper, biofuel, and food processing sectors. They contribute to the bioconversion of hemicellulose in lignocellulosic biomass (Bhardwaj *et al.,* 2019).

Hydratases of Nitrile

Alkaliphiles and acidophiles are the source.

Use Solvent-stable nitrile hydratases (Grötzinger *et al.,* 2020) are useful in industrial synthesis procedures because they may be used to produce carboxylic acids and amides in the presence of organic solvents.

The utilization of solvent-stable enzymes derived from extremophilic microbes in industrial processes has several benefits, including heightened stability in extreme environments, greater substrate selectivity, and higher reaction speeds. These enzymes aid in the creation of biocatalytic processes that are both economically and ecologically sustainable across a range of industries (Renganathan *et al.,* 2017).

A sustainable and environmentally friendly method for producing fine compounds is biocatalysis (Lozano *et al.,* 2015). In order to fine-tune enzymes to have characteristics appropriate for commercial biocatalysis, (Reshmy *et al.,* 2021) enzyme engineering is crucial. Apart from the widely recognized directed evolution approach, rational-design and semi-rational-design protein engineering techniques (Xue *et al.,* 2018) have shown promise in enhancing enzyme stability, catalytic efficiency, selectivity, and substrate range. These techniques can be carried out using a small-scale library for screening (Grötzinger, *et al.,* 2020). We have seen an increase in computational tools (Knapic, 2012) and the application of mechanistic data to direct the creation of enzymes (Wang *et al.,* 2012). With less resources and smaller libraries, this information may be used to improve the performance of enzymes, which should enable a larger population to conduct fruitful enzyme engineering projects (Martínez and Schwaneberg, 2013).

Enzyme-assisted biotransformation significantly increases the rate and stereospecificity of organic chemistry processes (Elend *et al.,* 2007). However, enzyme deactivation or a sharp decline in catalytic activity is frequently associated with the use of organic solvents and severe conditions in biotechnological applications. Through precise manipulation of the solvent, the support, or the enzyme's active site, we may fine-tune catalytic activity and overcome such constraints thanks to our comprehensive molecular understanding of protein structure and conformational dynamics. In addition to physico-chemical techniques for stabilizing enzymes, such as chemical modification, immobilization, and additive approach, DNA recombination-based methodologies for enzyme engineering can be employed to improve the efficiency of biocatalysts. As effective synthetic and industrial uses of biocatalysts necessitate systems that are not only stable and active but also reusable in a continuous flow process, lowering the cost of production, this chapter aims to present the reader with the wide range of methods available for improving enzymes, emphasizing both their advantages and disadvantages for practical technological applications (Hübscher *et al.,* 2000).

Industry-wide competitive sustainable production necessitates the development of improved biocatalysts, streamlined bioprocesses, and economical product recovery. From the identification of novel enzymes and their related genes to the other stages towards these aims, this overview provides insight into the progress accomplished. The enzymes are then modified to enhance their functionality, incorporated into whole cells to create the ideal environment for bioconversion, and joined in reaction cascades to broaden the scope of the process. Reaction design optimizes the circumstances of the reaction, downstream processing guarantees the effective recovery of economically viable chemicals, and strain engineering via synthetic biology techniques adjusts the host for production (Buchholz and Bornscheuer, 2017). A few selected examples show how future-focused applications ranging from the bioproduction of bulk, specialty, and fine chemicals, active pharmaceutical ingredients, (Prasad and Roy, 2018) and carbohydrates, to the recycling of synthetic polymers and the recovery of precious metals, can be revolutionized by modified enzymes. Other applications include the conversion of greenhouse gas CO2 into valuable products and biocontrol in agriculture (Wiltschi *et al.,* 2020). Molecular techniques and methodologies for improving catalysis and stability of solvent-tolerant enzymes (Kimura, 2018; Beaufils *et al.,* 2021).

Improving the catalysis and stability of solvent-tolerant enzymes is critical for their effective use in a variety of industrial applications. Researchers use molecular

techniques and methodologies to improve the efficiency of these enzymes. Here are a few major strategies Protein Engineering Directed Evolution Using methods like PCR or DNA shuffling, researchers may create enzyme variations with higher catalytic activity and stability in solvents. Mutagenesis is followed by screening or selection to discover enzyme mutants with the required features. Site-Directed Mutagenesis Enzyme mutants are rationally designed by altering particular amino acids to increase stability or substrate affinity. This strategy requires a thorough grasp of the enzyme's structure and activity (Sun, 2024; Petri and Schmidt-Dannert, 2004).

Protein structure stability factors include additives and co-solvents. Polyols or osmolytes can be added to the reaction medium to improve enzyme stability and solvent tolerance. These co-solvents can help to avoid enzyme denaturation and aggregation in the presence of organic solvents (Beaufils *et al.,* 2021). Chemical cross-linking agents can be used to create covalent bonds between amino acid residues, therefore strengthening the enzyme structure. This method can enhance enzyme stability in adverse conditions (Bharmoria *et al.,* 2024). Enzyme immobilization on solid supports or encapsulation in matrices may enhance stability by reducing aggregation and denaturation. Immobilized enzymes usually exhibit higher solvent tolerance and recyclability (Homaei *et al.,* 2013).

Mutagenesis can modify an enzyme's surface charge distribution, affecting its interaction with solvents and other chemicals. This may increase enzyme stability in the presence of organic solvents (Appukuttan *et al.,* 2006). The addition of charged molecules to the enzyme's surface, such as surfactants or polyelectrolytes, may alter its interaction with solvents, resulting in enhanced stability. Co-factor Engineering Improving Co-factor Binding. Increasing enzyme affinity for cofactors can improve catalytic efficiency.

This approach keeps the enzyme active even in the presence of solvents that may interfere with cofactor binding Villarino (2020). Inspired by extremeophiles, we want to mimic natural adaptations. Extremophilic bacteria's enzymes, which thrive in solvent-rich environments, can assist researchers comprehend molecular strategies for solvent tolerance. Mimicking natural adaptations can result in the production of stronger enzymes. Using computational tools to model enzyme structures and predict the impact of mutations or changes can aid in rational enzyme design. This strategy accelerates the selection of probable candidates for experimental validation (Sun, 2024; Petri and Schmidt-Dannert, 2004).

Metagenomic analysis of hostile environments may uncover enzymes with specific solvent tolerance. Screening diverse genetic material from environmental samples can result in enzymes suitable for certain industrial applications (Distaso, 2019). The combination of these molecular approaches and procedures allows researchers to tailor solvent-tolerant enzymes to specific commercial needs, improving catalytic efficiency and stability in the presence of organic solvents. These advancements contribute to the development of long-lasting and effective biocatalytic processes in a number of industries (Rodrigues, 2022).

Enzyme immobilization to improve solvent stability and reusability of the Extremozymes

Enzyme immobilization has been a captivating research topic since the 1960s. Immobilization technologies have developed progressively but reached a plateau recently. The recent expansion of biotechnological/nanotechnology advances has revitalized interest in the immobilization strategies of enzymes to a great extent. Immobilization strategies entail fixing or entrapping enzymes within solid support materials. Researchers have suggested several support materials, besides many beneficial approaches for the immobilization of enzymes. The main function of the support is to stabilize the structures of enzymes and accordingly preserve their efficacy to a great extent by rendering them more resistant to the surrounding environments. Enzyme immobilization permits an easy recovery of both the used enzymes and their support materials, and this is particularly beneficial in the food, medical, and pharmaceutical applications (Mesbah, 2019). Enzymes in their immobilized forms possess much higher stability and are also easier to handle when compared to their free forms. Additionally, the enzymatic reaction can occur in a nonaqueous medium where the solid supports preserve the enzyme’s constituents and make them stronger, which enhances their catalytic activity and renders them reusable for several times. Another benefit of the immobilization process is that the catalysts can alter from homogeneous to heterogeneous forms after the enzymatic binding, which assists in separating the enzymes, producing products with high purity. Various immobilization methods have been applied so far including encapsulation, cross-linking,

covalent binding, adsorption, and entrapment, (Movahedi *et al.,* 2021) as displayed in Fig. 1.

Enzyme immobilization offers various techniques to lock enzymes in place, improving their stability and reusability. Covalent binding creates strong chemical links between enzymes and support materials, enhancing stability but requiring complex methods. Cross-linking directly connects enzymes for improved stability, with variations like spray-drying and enzyme aggregates. Adsorption is a simple and inexpensive technique using physical attraction to carriers, but the bonds are weak. Ionic bonding is reversible but can affect enzyme structure and activity. Entrapment uses a polymeric network to trap enzymes, preventing leakage but potentially limiting diffusion. Encapsulation surrounds enzymes with a permeable membrane, protecting them from harsh environments but requiring careful control to avoid diffusion limitations. Each technique has advantages and limitations, making the choice dependent on the specific enzyme and application (Berenguer-Murcia *et al.,* 2019). Extremophile-derived proteases hold immense potential for various industries due to their unique properties. Protein engineering methods like directed evolution and site-directed mutagenesis help create improved enzyme variants with higher activity and stability (Beaufils *et al.,* 2021). Additionally, techniques like cross-linking, immobilization, and surface charge alteration further enhance enzyme resilience in harsh environments (Beaufils *et al.,* 2021). Mimicking natural adaptations of extremophiles and using computational tools for enzyme structure simulation are valuable approaches (Beaufils *et al.,* 2021). Metagenomics allows for exploring environmental DNA to discover novel enzymes with solvent tolerance (Beaufils *et al.,* 2021). Thermophiles, thriving in high temperatures, offer thermostable enzymes ideal for industrial processes. For instance, thermostable cellulases from thermophiles can significantly improve biorefining efficiency (Piroozmand, 2024). Psychrophiles and psychrotolerant microbes, functioning in cold environments, have great potential for applications like biofuel production due to their cold-adapted enzymes (psychrozymes) (Cavicchioli, 2016; Salwan & Sharma, 2020). These enzymes offer advantages in processes like lignocellulose breakdown and cold bioremediation (Cavicchioli, 2016; Salwan & Sharma, 2020). Acidophiles and alkaliphiles, thriving in extreme pH environments, provide enzymes valuable for industrial operations. For example, cellulase from alkaliphiles shows promise in lignin valorisation processes (Kim, 2020). Halophiles and halotolerant bacteria, found in saline environments, produce highly stable enzymes with applications in biofuel production (Malviya *et al.,* 2021). However, challenges remain in all these areas, such as improving enzyme production and thermostability (Piroozmand, 2024; Kim, 2020; Jitendra *et al.,* 2021). Overcoming these hurdles will unlock the full potential of extremophiles and their enzymes in various industries.

Extremoxymes

Thermophilic Enzymes

Conducting reactions at elevated temperatures offers numerous benefits, such as reduced susceptibility to microbial and fungal contamination, enhanced solubility of substrates, and accelerated hydrolysis rates. The optimal operating temperature range for thermophilic extremozymes is between 50 and 125°C (Han *et al.,* 2019). Disulfide bridges are of significant importance in determining the thermal stability of proteins, as they reduce the entropy of the unfolded state. Several enzymes exhibited improved thermostability when disulfide bridges were extended, specifically from the N-terminal side (Mallick *et al.,* 2002; Liu *et al.,* 2016; Tang *et al.,* 2017; Landeta *et al.,* 2018; Han *et al.,* 2019). Additional elements that contribute to the conformational stability of thermophilic extremozymes include compact (β/α)8 barrel folding, shortened loops and helices, salt bridges, surface charge, inner hydrophobic amino acids, and interactions (Han *et al.,* 2019). A significant portion of the stabilization mechanism of hyperthermostable enzymes with maximum activity above 70°C consists of charged amino acids, for which ionic interactions are crucial (Karshikoff *et al.,* 2015). The thermostabilities of thermophilic enzymes are maintained even when they are cloned and expressed in mesophilic hosts; this suggests that the genetic code controls their thermal adaptations (Vieille and Zeikus, 2001).

Among extremozymes, thermophilic extremozymes are the most extensively utilized. The initial application of a thermophilic extremozyme on a large scale was the DNA polymerase (Taq polymerase) derived from thermophilic *Thermus aquaticus*, which was isolated from hydrothermal vents in the United States' Yellowstone National Park (Chien *et al.,* 1976). DNA polymerase, derived from *Pyrococcus furiosus*, is an additional well-known instance of a thermostable enzyme (Pfu DNA polymerase) (Mathur *et al.,* 1991). Without these extremozymes, the tremendous advances in molecular

biology and genetic engineering over the past four decades would not have been possible.

The proliferation of thermophilic extremozymes has provided the industrial sector with novel opportunities. The most prevalent applications of thermophilic enzymes are in the production of fine compounds and drug intermediates, polysaccharide processing, biofuel manufacturing, pulp and paper manufacturing, and biofuel production.

Thermophilic Enzymes in the Processing of Polysaccharides

Saccharification, in which glucoamylases completely degrade oligomers to monomers, and liquefaction, which is the dispersion of insoluble starch in an aqueous solution followed by partial hydrolysis with thermostable amylases, are the two processes involved in enzymatic starch processing (Li *et al.,* 2015). Liquefaction and saccharification transpire within the temperature range of 50 to 80°C, thereby requiring enzymes that are thermostable. A wide range of thermophilic amylases, including pullulanases, glucoamylases, xylanases, and amylopullulanases, are readily available in the marketplace (Sarmiento *et al.,* 2015). Typically, alpha-amylases are employed during the preliminary liquefaction stage, while glucoamylases and pullulanases are utilized during the saccharification that follows. Pullulanses and glucoamylases are utilized by the food industry to produce glucose syrups, while ß-amylases are employed by the pharmaceutical industry to produce maltose syrups. Amylopullulanases that are thermodynamically stable are utilized in the production of maltose and maltotriose syrups. The ability of these substances to perform both debranching and liquefaction concurrently renders them highly appealing to the food, beverage, and pharmaceutical sectors (Li *et al.,* 2021). Submerged and solid-state fermentation methods that utilize three thermophilic enzymes—-amylase, glucoamylase, and amylopullulanase—successfully saccharified starch (Satyanarayana *et al.,* 2004) without requiring calcium or other enzymes.

Thermophilic Enzymes in the Production of Biofuels

Biofuels are petroleum-like substances that are produced through the process of enzyme-catalyzed fermentations. First-generation biofuels were produced from wheat, sugar beets, and maize. The initial iteration of biofuels encountered obstacles in the form of competition with the worldwide food supply, which ultimately led to a surge in food prices (Bhalla *et al.,* 2013). The increased applicability of second-generation biofuels is due to their utilization of lignocellulose, which is inexpensive, readily available, and frequently harvested from agriculture and forestry. Hemeicellulose, lignin, and cellulose make up lignocellulose. Lyscocellulosic biomass comprises roughly two-thirds cellulose and hemicellulose, which serve as the precursors for the manufacturing of second-generation biofuels. In order for the cellulose/hemicellulose to be accessible to enzymes, pretreatment is required prior to enzymatic hydrolysis of lignocellulose, which has an extremely rigid and compact structure (Alvira *et al.,* 2010). In order to achieve effective pretreatment outcomes, temperatures exceeding 50°C are necessary. This is because elevated temperatures can disrupt the structure of lignocellulose and facilitate the penetration of enzymes through the cell wall of the biomass (Bala and Singh, 2019). The degradation of lignocellulose is most effectively achieved through the combination of cellulase and xylanase due to the specificity of the two enzymes, the near-completion of hydrolysis, and the absence of fermentation inhibitors.

Depolymerization of cellulose is facilitated by cellulases. Endoglucanase (1,4-β-d-glucan glucohydrolase (EC 3.2.1.41)), ß-glucosidase (β-d-glucoside glucohydrolase (EC 3.2.1.21)), and exoglucanase are the three enzymes associated with the term "cellulase" (Lynd *et al.,* 2002). These three enzymes function synergistically to completely hydrolyze cellulose to glucose. Xylanases are required for hemicellulose hydrolysis. Endo-1,4-β-D-xylanases (EC 3.2.1.8), ß-D-xylosidases (E.C 3.2.1.37), ferulic acid esterase (EC 3.1.1.73), acetylxylan esterase (EC 3.1.1.72), α-glucuronidase (EC 3.2.1.139), α-L-arabinofuranosidases (EC 3.2.1.55), and p-coumaric esterase (3.1.1.B10) are all members of the xylanase group of enzymes. The xylan in hemicellulose is hydrolyzed by these enzymes into xylo-oligosaccharides and monosaccharides (Bhardwaj *et al.,* 2019). The degradation of lignocellulose necessitates the combination of multiple enzymes and the application of various strategies, such as the cellulosome, which is a multienzyme complex, and multifunctional megazymes (Singh *et al.,* 2021).

Typically bifunctional, multifunctional megazymes are enzymes with at least two distinct catalytic modules. Xylan, microcrystalline cellulose, untreated grass and rice straw, and microcrystalline cellulose were all

degraded by a hyperthermophilic cellulase/hemicellulase system derived from Caldicellulosiruptor bescii at 75–85°C and pH 5–6. Four multi-domain cellulases comprised the enzyme system, which likely explained its exceptionally high hydrolytic activity. Ca-induced degradation of barley stalks. The efficiency of the cellulase/hemicellulose system derived from the mesophilic fungus *Trichoderma reesei*, which is utilized in commercial enzyme cocktails, was found to be two times lower than that of S. bescii enzyme system (Kanafusa-Shinkai *et al.,* 2013). Comparable to commercially available T, the purified cellulosome derived from Clostridium thermocellum and the thermophilic ß-glucosidase from Thermoanaerobacter brockii converted 91% of the glucan in pre-treated rice stalks. The combination of Reesei cellulase Celluclast 1.5 L and Novozyme-188 yields an economic advantage due to its nearly tenfold reduced enzyme concentration (Waeonukul *et al.,* 2012). Cellulosomes found in *C. Thermocellum* facilitated the complete conversion of glucans from microcrystalline cellulose, in contrast to the commercial enzyme Cellic CTec2 (Novozyme), which achieved a mere 50% conversion (Resch *et al.,* 2013). C. In contrast to calcium, thermocellum exhibited enhanced carbohydrate solubilization of switchgrass when employed as a whole cell catalyst. Novozymes Ctec2 and Htec2 as well as B. bescii were utilized (Holwerda *et al.,* 2019). Fermentations in batches utilizing untreated switchgrass and C. The utilization of clariflavum led to greater solubilization of glucan, xylan, and arabinan (64.7–69.4%) when compared to fermentations utilizing C. as described by Izquierdo *et al.,* (2014). The combination of these studies suggests that thermophilic enzymes have the potential to be utilized in cellulolytic compounds to degrade biomass more efficiently and without requiring pretreatment of the biomass. As a result, operational expenses and time can be reduced.

Thermophilic Enzymes in the Production of Pulp and Paper

Wastepaper and unprocessed lignocellulosic materials (e.g., wood) are utilized as primary materials in the paper industry. Paper production begins with the pulping of wood, which consists of removing lignin from the hemicellulosic fraction while preserving the cellulosic fraction, if possible. The lignocellulose is treated with sodium sulfide, sodium hydroxide, and caustic soda at 150–170°C during the pulping process. This is followed by the mechanical separation of cellulose from lignin and hemicellulose, and bleaching with chemicals containing sodium oxide and chlorine. Conventional pulping methods produce substantial quantities of hazardous byproducts and lack energy efficiency (Gupta *et al.,* 2020). Bio-bleaching and enzymatic bio-pulping effectively address a significant portion of these challenges. Bio-pulping and bio-bleaching processes are efficiently catalyzed by thermophilic and alkaliphilic extremozymes, frequently under milder conditions and without the use of corrosive chemicals, as opposed to conventional chemical pulping.

Xylanases are the principal enzymes utilized in bio-pulping, where they convert lignocellulose and xylan present in the hemicellulose of plant cell walls into xylose monomers. The utilization of thermophilic xylanase eliminates the necessity for pre-cooling prior to the degradation process of lignocellulose, which occurs concurrently with the initial heating phase. By combining xylanases with lignin-degrading enzymes, bio-bleaching of cellulose fiber can be achieved without the use of chlorine, thus minimizing environmental impact. Extensive literature exists regarding thermostable xylanases. Barely thermostable, the majority of commercially available xylanases are derived from fungi (Bhardwaj *et al.,* 2019). A restricted variety of thermophilic xylanases are presently offered in the marketplace. Two thermophilic xylanases are supplied by Verenium Corporation luminase® PB-100 is active between 40 and 70°C, and luminase® PB-200 is active between 60 and 90°C. Enzyme Deinking Technologies offers EnzAid®, which remains active within the temperature range of 50 to 65°C. PRX, a recombinant xylanase that is active between 45 and 65°C, is offered by Sigma Aldrich. Nine fungal and recombinant thermostable xylanases, including recombinant endo-1,4-beta-xylanases from *Thermotoga maritima* and *Geobacillus stearothermophilus*, are available at Megazyme. These enzymes have temperature optima ranging from 50 to 80°C.

Additionally, laccases contribute to the biobleaching of lignin and the manufacturing of paper fibers. As oxidoreductases, laccases convert phenolic compounds to oxygen while utilizing the latter as an electron donor. Lignin, which is composed of phenolic compounds, is an insoluble complex. Enzyme-based delignification systems employ thermostable laccases and peroxidases to separate lignin from cellulose and hemicellulose. Lignin degradation products can also serve as substrates for subsequent reactions (Liu *et al.,* 2020). Espina *et al.,* (2021) report that laccases, which are generated by

thermophiles and hyperthermophiles, exhibit remarkable thermostability. Laccase produced recombinantly by alkalithermophilic Bacillus sp. Swissaustral LLC, based in Athens, Georgia, United States, offers FNT as an enzyme for the research market, which demonstrates maximal activity at 70°C. The cost of producing thermostable laccases on a large scale and the lack of mechanistic research into the enzyme's stability, activity, and substrate interaction prevent their widespread use in lignin degradation.

Thermophilic Enzymes in the Synthesis of Fine Chemicals

Transaminases are pyridoxal-5′-phosphate-dependent enzymes that facilitate the transfer of an amine group from a donor to a receptor, which is typically a ketone or aldehyde. This process yields a new chiral amine and a corresponding ketone or aldehyde. Upon completion of the reaction, the cofactor pyridoxal-5′-phosphate is replenished (Cárdenas-Fernández *et al.,* 2021). Transaminases are utilized in the synthesis of fine compounds and pharmaceuticals, specifically synthetic amino acids, primary amines, and chiral amines (α-, ß-, and ω-amino acids). 40% of pharmaceutical compounds are believed to contain a chiral amine (Cárdenas-Fernández *et al.,* 2021). Synthesis of chiral amines at elevated temperatures is more advantageous due to the increased reaction output and reduced production cost.

At this time, thermostable transaminases are not commercially available. Cloning and expression of numerous thermophilic transaminases from both hyperthermophiles and thermophiles. Two of these stereoselective serine-transaminases generated substantial quantities of hydroxypyruvate (70–77%) when transketolase was present at 65°C and the enzymes were incubated at 50°C for 24 hours. After this period, the enzymes maintained over 80% of their maximal activity (Cárdenas-Fernández *et al.,* 2021). The literature has documented several additional thermostable transaminases that exhibit their highest activity between 65 and 80 degrees Celsius (Atalah *et al.,* 2019). A drug intended for the treatment of type II diabetes, sitagliptin, was effectively synthesized using ω-transaminase, a moderately thermophilic enzyme, resulting in an optically pure compound with a higher yield and reduced waste (Desai, 2011). An additional amino acid besides serine was catalyzed by the serine transaminase produced by the archaeon Sulfolobus solfataricus, which remained stable at 70°C for ten minutes. This property endowed the organism with the capacity to synthesize optically pure drug intermediates (Littlechild, 2015).

Nitrilases facilitate the hydrolysis of nitriles to the corresponding carboxylic acids, resulting in the secondary production of ammonia. Nitrilases find application in the pharmaceutical and precursor synthesis processes. Acidovorax facilis's moderately thermophilic, genetically modified nitrilase produced iminodiacetic acid, a precursor for the production of surfactants and chelating agents, at 40–50°C (Liu *et al.,* 2018). Although several thermophilic nitrilases with maximum activities between 40 and 65 degrees Celsius have been documented in the scientific literature (Shen *et al.,* 2021), no nitrilases are currently available for purchase.

The ability of aminoacylases to convert racemic mixtures of N-acyl amino acids to L- and d-amino acids and amino acid analogues renders them industrially significant. Nitrogen, which is frequently present in pharmaceutical compounds and is derived from amino acids, necessitates optical purity (Littlechild, 2015). A cloned thermophilic l-aminoacylase was isolated from *Thermococcus litoralis*, an archaeon. It is commercially utilized by Chirotech/Dow Pharma and Chirotech/Dr Reddys for the manufacture of l-amino acids and analogs. Its activity ranges from 60 to 90 degrees Celsius (Littlechild, 2015).

Enzymes Psychrophilic

The maximum activity of psychrophilic enzymes occurs between -20°C and-10°C. Psychrophilic enzymes exhibit low-temperature activity through a mechanism that decreases substrate binding affinity (k0.5) and increases catalytic rate (kcat), thereby diminishing the energy required to initiate reactions. This necessitates increased entropy and decreased enthalpy (fewer interactions between substrate and enzyme) and structural stability and flexibility, respectively (Santiago *et al.,* 2016). Adaptations to the structure of psychrophilic proteins that confer enhanced flexibility encompass the following 1) a reduced proportion of lysine to proline residues; 2) an increased number of glycine residues arranged in loops; 3) diminished compactness of the hydrophobic core and heightened hydrophobicity of the surface; 4) diminished strength of protein interactions, including disulfide bridges, electrostatic interactions, and weak metal-binding; and 5) diminished formation of secondary structures (Sarmiento *et al.,* 2015; Mangiagalli *et al.,* 2020). These characteristics maintain the activity of the enzyme at lower temperatures but reduce its stability

above 15°C. Psychrophilic enzymes facilitate biocatalysis at low to moderate temperatures, thereby improving the economics and efficacy of such processes while also reducing their environmental footprint. Laundry detergents and industrial cleaning-in-place containing psychrophilic enzymes.

Laundry detergent production represents the most developed clientele for psychrophilic enzymes. From 2021 to 2026, the global detergent enzymes market is anticipated to expand at a CAGR of 5.8% (Research, 2021). Kumar *et al.,* (2021) state that cold-active laundry detergents decrease energy expenses, diminish carbon emissions, and enhance fabric preservation. Commercially available enzymes include cellulases, proteases, lipases, pectinases, mannanases, and psychrophilic amylases (Table 1). Stains and deposits are degraded by enzymes into soluble compounds that are readily removed with water. Amylases degrade starchy residues caused by foods such as vegetables, cereals, pasta, and potatoes. Lipases hydrolyze lipids in sauces, oil, butter, and fat, whereas proteases hydrolyze proteins in blood, eggs, vegetation, dairy products, and perspiration, among other substances. Mannanases and pectinases exhibit efficacy in degrading stubborn stains originating from gums, fruit products, body moisturizers, and various other personal care items. Cellulases exhibit activity against stains containing oats, including those found in cereals. Cellulases also care for fabrics by decomposing accessible cotton fiber debris, including pills and fuzz, thereby increasing the suppleness of the fabric and decreasing the accumulation of fuzz.

Cellulase enhances the luminance of cotton fabrics through the modification of cellulose fibrils (Al-Ghanayem and Joseph, 2020). The food, beverage, and manufacturing industries also utilize psychrophilic enzymes for cleaning-in-place. Enzymes are utilized in the beverage industry to deblock filters and sanitize apparatus. Molds and biofilms in filters and building surfaces can be degraded by lipases, proteases, amylases, and pullulanases, enabling improved cleansing performance without the need for chemical detergents, surfactants, or organic solvents (Barroca *et al.,* 2017).

Enzymes Phosphoric in the Pharmaceutical Sector

Commercial applications of psychrophilic enzymes can be found in the pharmaceutical sector. Kirk and Christensen (2002) reported on a cold-active lipase isolated from *Candida antarctica* that exhibited activity against carboxylic acids, primary alcohols with long to medium chains, and secondary and tertiary alcohols. This property suggests that the lipase may find utility in the organic synthesis of flavor, cosmetic, and pharmaceutical compounds.

Candida antarctica lipase B (CALB) finds utility in various fields, such as the resolution of alcohols and amines and the modification of polysaccharides; it is also employed in the synthesis of calcium antagonists, aroma esters, and cosmetics (Huston, 2008). Novozym 435, an immobilized form of CALB, finds extensive commercial application and has been utilized in amidation reactions, esterification reactions (e.g., to produce fine compounds and optically pure products) (Ortiz *et al.,* 2019). It has been demonstrated that additional psychrophilic enzymes are capable of esterifying aroma esters at 10–20°C (Mhetras *et al.,* 2021).

The versatility of therapeutic applications for psychrophilic proteases can be attributed to their superior ability to catalyze biological processes at lower temperatures while maintaining favorable safety profiles in comparison to mesophilic proteases. Topical applications benefit from the efficacy of psychrophilic proteases; the enzymes' increased flexibility enables them to function effectively in low-water environments, such as those found in the vicinity of membrane proteins and within the lipid layer of mucus.

Additionally, psychrophilic proteases exhibit a localized effect; when externally administered at room temperature, they demonstrate high activity; however, as they approach equilibrium with the balmy in vivo environment, their activity diminishes (Fornbacke and Clarsund, 2013). In a wound healing model, psychrophilic proteases derived from Antarctic krill, specifically acidic endo- and exopeptidases, exhibited superior efficacy in debriding necrotic ulcers compared to a saline control.

Without causing significant systemic adverse effects, they enhanced wound healing, decreased the degree of necrotic tissue, and improved tissue granulation (Mekkes *et al.,* 1998).

Enzymes Phosphoric in Molecular Biology

Affymetrix, ArcticZymes, New England Biolabs, nucleases, uracil-DNA N-glycosylases, proteases, and

DNA ligases—all of which have been cold-adapted—are commercially available as molecular biology instruments (Barroca *et al.,* 2017; Mangiagalli *et al.,* 2020; Li *et al.,* 2020). Due to the fact that psychrophilic enzymes become inactive at temperatures above 20°C and are susceptible to inactivation via moderate heat treatment, they are highly desirable for use in molecular biology. This eliminates the necessity for laborious chemical or column-based purification procedures, while moderate thermal treatment maintains the integrity of double-stranded DNA and RNA in preparation for further procedures.

During the cloning process, alkaline phosphatase dephosphorylates the 5′ end of linear DNA in order to inhibit re-circularization. Commercially available Antarctic phosphatase was initially derived from an Antarctic bacterium and subsequently enhanced through the process of directed evolution (New England Biolabs, based in Ipswich, MA, United States) (Sarmiento *et al.,* 2015). ArcticZymes (Tromsø, Norway) offers an alkaline phosphatase that has been cold-adapted from arctic crustaceans. Single-stranded DNA, including probes, is unaffected by double-stranded nuclease digestion. DNA is extracted from RNA preparations utilizing double-stranded nuclease (Mangiagalli *et al.,* 2020; Li *et al.,* 2020). Available from Affymetrix and ArcticZymes, heat-labile nucleases become inactive after 30 minutes of exposure to 65–70°C. Uracil is effectively eliminated from uracil-containing DNA by uracil-DNA N-glycosylase. They are utilized in site-directed mutagenesis single nucleotide polymorphism genotyping, PCR, and reverse-transcriptase PCR. A range of commercially available cold-active uracil-DNA N-glycosylases exhibit their highest activity at temperatures between 20 and 25°C and deactivate at temperatures between 40 and 50°C (Sarmiento *et al.,* 2015; Mangiagalli *et al.,* 2020; Li *et al.,* 2020).

Protein contamination in DNA and RNA preparations is eliminated by proteases. The advantageous characteristic of psychrophilic proteases is that their heat sensitivity permits inactivation at temperatures that do not disrupt the structure of RNA and DNA. A marine-derived cold-stable proteinase is manufactured by ArcticZymes (Mangiagalli *et al.,* 2020; Li *et al.,* 2020). By catalyzing the formation of 3′–5′ phosphodiester bonds between deoxynucleotides, DNA ligase binds together DNA fragments that have abrupt or staggered ends. Staggered DNA ends anneal more efficiently at temperatures ranging from 3 to 10 degrees Celsius. Presently accessible ligases derived from T4 and T7 bacteriophages exhibit their highest efficiency at 37°C and decelerate significantly below that temperature, necessitating ligation periods in excess of 12 hours. In order to accomplish this, a psychrophilic ligase will be advantageous, as it will facilitate ligation effectively at the low temperature necessary for annealing. There is a scarcity of literature regarding psychrophilic DNA ligases, and there is currently no commercially available product. The psychrophilic DNA ligase Vib-Lig was cloned from *Aliivibrio salmonicida*, an obligate psychrophile. According to Berg *et al.,* (2019), its activity peaked at 20°C and remained active within the temperature range of 10–35°C. Genolette *et al.,* (2000) cloned a psychrophilic DNA ligase isolated from the Antarctic Sea water bacterium Pseudoalteromonas haloplankis; the enzyme retained over 50% of its maximal efficiency at 4°C and exhibited maximum catalytic efficiency at 18°C.

Psychrophilic Enzymes in the Industry of Food and Beverage

For centuries, enzymes have been utilized as food constituents, additives, and processing aides. In addition to their traditional applications in fermentation, dairy production, and bread and pastry production, enzymes are also utilized in the recent development of nutraceuticals and functional foods. In recent years, low-temperature processes have increasingly supplanted high-temperature processes. Barroca *et al.,* (2017) found that low-temperature procedures have a reduced cost, diminished microbial and fungal contamination and spoilage, an extended expiration life, and a smaller environmental footprint.

After infancy, nearly 70% of the global population cannot metabolize lactose. B-galactosidases are capable of hydrolyzing lactose into galactose and glucose, thereby facilitating the digestion of dairy products by eliminating lactose. One advantage of psychrophilic ß-galactosidases is their ability to hydrolyze lactose in dairy products efficiently in a bulk process prior to pasteurization and packaging at storage temperature (4–8°C) (Mangiagalli *et al.,* 2020; Mangiagalli and Lotti, 2021). Additionally, ß-galactosidases with psychrophilic properties can be utilized to value whey, a byproduct of cheese production. Syrups rich in glucose and galactose that result from the hydrolysis of whey with ß-galactosidase have the potential to be utilized as sweeteners (Kumari *et al.,* 2021). ß-galactosidases

possess transglycosylation activity as well. During the hydrolysis of lactose, tris and tetrasaccharides are formed; these saccharides not only serve as low-calorie sweeteners but also promote the growth of Bifidobacteria in the intestine, making them potential prebiotics (Benešová *et al.,* 2005). Additionally, oligosaccharide synthesis by psychrophilic ß-galactosidases derived from the marine bacteria Alteromonas sp. has been documented. ANT48 is a species of Marinomonas. *Bacillus pseudoalteromonas* sp. BSi20414." Arthrobacter sp. 22b. 32bc (Lotti and Manganagalli, 2021). The majority of documented psychrophilic ß-galactosidases are found in the Arctic and Antarctic regions, and their activity spans a wide temperature range of 4–50°C (Kumari *et al.,* 2021). ß-galactosidase is produced by the archaeon Halorubrum lacusprofundi and is active between -5 and 60 degrees Celsius (Karan *et al.,* 2013). A psychrophilic ß-galactosidase is employed in the initial phase of the low-calorie sweetener tagatose's production (Van de Voorde *et al.,* 2014). During the maturation and production of cheese, the dairy industry also employs cold-adapted proteases, lipases, and phospholipases (Barroca *et al.,* 2017; Mangiagalli *et al.,* 2020; Li *et al.,* 2020).

Enzymes utilized in baking applications reduce the production of polyacrylamide and enhance product quality while minimizing the need for chemical additives. Although the majority of currently utilized enzymes are thermotolerant and mesophilic, producing dough at moderate temperatures provides a substantial cost advantage. Xylanases are indispensable to the bread-making process, as they convert hemicellulose to soluble carbohydrates, thereby producing bread that is both elastic and tender. Reports regarding psychrophilic xylanases are scarce. Three Flavobacterium sp. psychrophilic xylanases were isolated. In comparison to commercially available mesophilic xylanases, MSY-2, Pseudoalteromonase haloplanktis, and an unidentified bacterial isolate enhanced the flexibility and structure of dough and crumb (Dornez *et al.,* 2011).

Fish descaling, skin removal, degreasing, and oil extraction have all been accomplished with the aid of psychrophilic proteases, lipases, and chitinases (Barroca *et al.,* 2017). Psychrophilic proteases are utilized to enhance the flavor and tenderize meat. An psychrophilic protease originating from Pseudoalteromonas species. He *et al.,* (2004) found that at 0°C, SM9913 liberated more flavor amino acids from the surface of marine fish, shrimp, and pork than a mesophilic protease.

Pectin is degraded by pectinases, an enzymatic class consisting of pectin esterases, pectate lyases, and polygalactoronases. Pectin is a fruit and plant-derived fiber. Pectinases are employed in the processing of fruits and vegetables to enhance clarification, augment extraction efficiency, and decrease the viscosity of fruit juice. Commercially accessible psychrophilic pectinases are exceedingly scarce. Lallzyme®, an aseptic niger polygalacturonase, pectin lyase, and pectin esterase, is utilized for the clarification of liquids and wine at room temperature (Sarmiento *et al.,* 2015). It is active between 5 and 20°C.

Acidophilic Enzymes

The activity of acidophilic enzymes is at its peak at pH levels below 3. An in-depth examination of the structural modifications required to maintain stability at low pH is still lacking. However, studies on acid-stable amylases have documented a greater abundance of acidic amino acids (e.g., glutamate) and a reduced number of basic amino acids on the protein surfaces (Parashar and Satyanarayana, 2018). Between 4 and 5 are the acidic isoelectric points of acidophilic amylases. The protonation of the majority of surface amino acids occurs at pH values below 3, which leads to a reduction in their negative charge and subsequent protein stabilization. An increase in pH facilitates the deprotonation of a greater number of amino acids, which induces unfolding as a result of the repulsion between the surplus negative groups. Particularly utilized in bioremediation, microbial fuel cells, and the production of sugar syrups from starch, acid stable enzymes have an extensive range of applications.

Enzymes Acidophilic to Starch Processing

Three stages are required to produce oligosaccharide syrups from starch gelatinization of a starch purée at 90–100°C, liquefaction with α-amylase, and digestion by glucoamylases. The native starch solution has a pH ranging from 3 to 5. By eliminating the requirement to neutralize the starch slurry prior to liquefaction, acidophilic amylases facilitate the production of oligosaccharides from unprocessed starch in a time and cost-effective manner. Numerous reports have been devoted to acidophilic amylases and glucoamylases. Acidothermophilic bacteria (e.g., Bacillus, Alicyclobacillus) and archaea (Thermoplasma, Sulfolobus, Picrophilus) generate the majority of these substances, which exhibit their highest activity levels at

pH levels ranging from 3 to 6 (Bertoldo *et al.,* 2004; Sharma *et al.,* 2016; Parashar and Satyanarayana, 2018). However, the availability of commercially available acidophilic amylases is restricted, primarily because acidophilic microorganisms obscure the secretion of extracellular amylases. Inadequate expression and the development of inclusion bodies have rendered large-scale heterologous expression of acidophilic amylases ineffective as well (Parashar and Satyanarayana, 2018). Two acidophilic amylases are manufactured by Genencor International (Palo, Alto, CA, United States) Spezyme Xtra, which is active at pH 5.5–6.0, and Stargen™ 001, which is active at pH 4.0–4.5. Active at a pH of 4.5, Valley Research (South Bend, Indiana, United States) manufactures Ultra-Thin. Several amylases are manufactured by Novozymes (Franklinton, NC, United States) Termamyl® SC DS, which is active at pH 5, Liquozyme® SC, which is active at pH 5.7–6.0, Liquozyme® Supra 2.2X, which is active at pH 5.5, and LpHera®, which is active at pH 4.5. Additionally, Spirizyme ®, a blend of glucoamylases active at pH 3.5–5.5, is manufactured by Novozymes.

Acidophiles in Bioremediation

Acid mine drainage (AMD) results from the exposure of sulfide minerals in the Earth caused by mining or significant construction activities. When exposed to oxygen and water, the majority of sulfide minerals oxidize to sulfuric acid, which then enters the groundwater and surface. Significant contributors to water pollution and heavy metal dispersion in the environment are AMD. The pH range of AMD is 2 to 8, and it is composed primarily of metals and sulfides. Traditional approaches to managing AMD entailed alkalinizing the acidic effluent to a pH level that exceeded the optimal range for iron-oxidizing bacteria, or alternatively, utilizing pulverized limestone to treat the effluent and impede the acid production rate (Skousen *et al.,* 2019). These approaches exhibit inefficiency, entail substantial operational expenses, and produce considerable volumes of solid sediment that necessitate proper disposal.

Acidophilic microbes, primarily Acidiphilum, Acidithiobacillus, Acidisphaera, and Leptospirillum, inhabit AMD (Li *et al.,* 2020). In addition to their acidophilic nature, these microorganisms are resistant to toxic metals including cadmium, chromium, nickel, and arsenic and are capable of oxidizing and reducing iron and sulfur. Bioreactors containing acidophilic iron-oxidizing bacteria (e.g., Leptospirillum ferroxidans) and sulfate-reducing bacteria (e.g., *Acidithiobacillus ferroxidans, A. ferrivorans*) are utilized in lieu of individual enzymes in AMD bioremediation strategies. The extracellular oxidoreductases secreted by these microorganisms exhibit stability at pH levels considerably lower than their cytoplasmic pH (approximately pH 5) (Sharma *et al.,* 2012). Sulfate reduction and metal precipitation are facilitated by the fermentation process and the passage of AMD through the bioreactors (Villegas-Plazas *et al.,* 2019). In order to generate alkalinity, sulfate reduction transforms sulfate into sulfide. Sulfide then combines with dissolved metals to produce intractable metal sulfides. Drainage devoid of metals and neutralized exits the bioreactor. Numerous studies have documented bioreactors designed to treat AMD that have achieved heavy metal removal rates exceeding 90% (Johnson and Hallberg, 2005; Chen *et al.,* 2016; Rodrigues *et al.,* 2019; Sun *et al.,* 2020).

Microbial Fuel Cell Acidophiles

Microbial fuel cells (MFCs) are an auspicious technological advancement that facilitates the simultaneous production of bioelectricity and effluent bioremediation. Two compartments—anode and cathode—are separated by a proton-selective membrane to form an MFC. Electrochemically active microorganisms oxidize organic and/or inorganic waste material in the anodic compartment, thereby liberating carbon dioxide, protons, and electrons. Electrons are transferred to the cathode through an external electrical circuit, in which oxygen typically serves as the acceptor of electrons. Oxygen reduction has a sluggish kinetics that is accompanied by substantial overpotentials. Application of acidophilic bacteria that oxidize iron, such as A. The kinetics of oxygen reduction at acid pH was significantly enhanced by the presence of ferroxidans in the cathode compartment (Izadi *et al.,* 2019). Nevertheless, *A. Ferroxidans* have the ability to oxidize iron at a neutral pH; however, at pH 2, oxygen reduction and energy gain are more pronounced (Ferguson and Ingledew, 2008). An MFC comprised of Ferroplasma sp. and Acidithiobacillus sp. mixed cultures. Leptospirillum sp. as well. The electrical power output of cathode biocatalysts increased by a factor of four (Stom *et al.,* 2021). Leiva *et al.,* (2018) reported that arsenic and iron were effectively extracted from AMD by means of a single chamber air cathode MFC employing surface sorption and bioprecipitation of microorganisms derived from acidic iron/arsenic-rich surface detritus.

Additionally, the existence of acidophilic microorganisms in the anodic compartment is beneficial. According to Jadhav and Ghangrekar (2009), an increase in the pH difference between the anode and cathode compartments of an MFC results in a reduction in internal resistance, which subsequently enhances power output.

According to Sharma *et al.,* (2016), the power output of a single chamber microbial fuel cell utilizing anaerobic anodic biocatalysts was found to be significantly higher at anodic pH 6, as opposed to anodic pH 7 or 8. Sadly, the limited capacity for large-scale implementation of MFCs is a result of their low power outputs and the expense of scaling up.

Hydrophilic Alkali Enzymes

The activity of alkaliphilic enzymes is at its peak at pH values exceeding 9. In contrast to neutrophilic counterparts, their isoelectric points are more alkaline due to the greater abundance of arginine and histidine residues and the reduced abundance of glutamate residues. It is hypothesized that the formation of ionic interactions between arginine residues and aspartate residues is crucial for the stability of the enzyme under alkaline conditions (Fujinami and Fujisawa, 2010). As laundry detergent additives, alkaliphilic enzymes were initially implemented on a significant scale. Alkaline detergents, which are frequently encountered in washing machines and dishwashers, do not cause denaturation of alkaline lipases, proteases, amylases, and cellulases. Furthermore, these enzymes exhibit resistance to chelating agents and long-term storage, including those containing bleaching agents (Fujinami and Fujisawa, 2010).

In leather processing, alkaliphilic proteases have also been implemented. In addition to collagen, additional globular and fibrous proteins, including elastin, albumin, globulin, and keratin, are found in leather. Hair adhered to the epidermis is eliminated throughout the leather processing, along with non-collagenous substances like fat and flesh. By employing substantial quantities of lime, acid, sodium sulfide, detergents, pigments, and solvents, conventional leather processing generates hazardous byproducts (Wanyonyi and Mulaa, 2020). Leather processing is accomplished enzymatically, which reduces costs, environmental impact, and processing time. Protease-enzymed soaking of hides and skins in alkaline solutions effectively eliminates hair and non-collagen proteins and initiates fiber opening of the hide and skin. The alkaline pH significantly diminishes microbial contamination, a significant issue associated with traditional immersion methods. In lieu of lime and sodium sulfide, alkaline protease was utilized to entirely dehair fish skin and hides (Wanyonyi *et al.,* 2016). Tanneries utilize alkaline lipases to eliminate inherent fat from hides and skins. By doing so, they enhance the color and visual appeal of the final product, as well as facilitate the production of watertight leather (Hasan *et al.,* 2006).

Haemophilic Proteases

The maximum activity of halophilic enzymes occurs at sodium concentrations that exceed 1.5 M. Na+ is predominantly derived from sodium chloride, although sodium carbonate and bicarbonate may also be present. Additionally, numerous halophilic enzymes can withstand molar concentrations of potassium. Enzyme surface modifications that respond to elevated salinity levels consist of a profusion of aspartate and glutamate residues and an acidic isoelectric point (Oren, 2013).

The exceptional water-binding properties of acidic amino acids maintain the solubility of the protein in dehydrating environments induced by salinity. Numerous halophilic enzymes are tolerant of organic solvents due to the fact that, similar to elevated salinity, organic solvents diminish water activity. An additional adaptive mechanism consists of a reduced number of hydrophobic amino acid residues exposed to the solvent and a diminished protein core. Protein flexibility is enhanced at high salt concentrations due to weak hydrophobic interactions; an ionic environment prevents a weak hydrophobic core from becoming overly rigid (Brininger *et al.,* 2018).

Biotechnology, bioremediation, and biopharmaceutics hold significant promise for the use of halophilic enzymes, which maintain their stability and solubility under low-water conditions and can be scaled up in processes utilizing organic solvents and brine without the need for stringent sterile conditions.

In contrast to other extremozymes, however, the production and deployment of halophilic enzymes on a significant scale remain underdeveloped. The reason for this is that numerous halophilic microorganisms, specifically haloarchaea, are susceptible to disintegration at low salinity levels. Furthermore, the cultivation of

halophilic microorganisms on a large scale can result in the deterioration and corrosion of stainless steel apparatus (Haque *et al.,* 2020). As numerous halophilic proteins misfold and aggregate at low salinity, heterologous expression of halophilic proteins on a large scale may also be problematic (Martínez-Espinosa, 2020).

The incorporation of halophilic amylases and proteases into detergent formulations has been documented (Patel and Saraf, 2015; Mokashe *et al.,* 2018). Since granular detergents contain NaCl as an ingredient, salt stability of enzymes is essential.

Biocatalysis utilizes halophilic extremozymes immobilized or free, in non-conventional and organic solvents. Halophilic cellulases, xylanases, and laccases find utility in the manufacturing of biofuels, particularly in procedures involving the pretreatment of lignocellulose with ionic liquids or organic solvents (Mesbah and Wiegel, 2017; Amoozegar *et al.,* 2019; Kasirajan and Maupin-Furlow, 2021; Motamedi *et al.,* 2021). Lipases free and immobilized from Haloarcula sp. Biodiesel yields exceeding 85% were achieved by G41 and *Bacillus lichenformis* when using soybean oil and myrtus oil, respectively (Qiu *et al.,* 2021). Halophilic esterases and lipases are utilized in the synthesis of polyunsaturated and short chain fatty acids, which find application in nutraceuticals and dietary supplements (Salihu and Alam, 2015).

The aroma ester methyl acetate was produced in both free and immobilized form in n-hexane using a lipase derived from the halothermophile Rhodothermus marinus (Memarpoor-Yazdi *et al.,* 2017). Halophilic lipases can be utilized to generate lipophilic antioxidants through the modulation of natural polyphenols due to their chemoselectivity and regioselectivity (Delgado-García *et al.,* 2018).

Incorporating halophilic extremozymes into reverse micelles enables their use in the development of biosynthetic processes in non-aqueous media. P-nitrophenylphosphate phosphatase (pNPPase) and malate dehydrogenase (hMDH), both of which are halophilic enzymes found in archaea, were effectively encapsulated in reverse micelles composed of cetyltrimethylammonium bromide in cyclohexane (Marhuenda-Egea and Bonete, 2002). Archaeal enzymes, in particular, are among the halophilic enzymes that lose activity and unfold at low salt concentrations. Singh and Singh (2017) state that the preservation of the catalytic activity of halophilic enzymes within reverse micelles eliminates a significant barrier to their implementation on a large scale.

Enzymes that are Barophilic and Radiophilic

Radiophiles are capable of thriving and proliferating in environments characterized by elevated levels of oxidative stress and radiation, such as gamma, ultraviolet, and X-ray radiation (Sysoev *et al.,* 2021). Isolation of radiophilic enzymes has not been documented; however, radiophilic microorganisms have been employed in decontaminating radioactive waste environments and bioprecipitating uranium from diluted nuclear waste (Brim *et al.,* 2003; Appukuttan *et al.,* 2006 as whole cells). Radiophile-produced extremeolytes have possible applications.

Extensive DNA damage can be repaired by radiophiles, and their extremolytes have potential applications in cancer prevention and treatment. When administered topically, mycosporine-like amino acids derived from the red alga *Porphyra rosengurttii* prevented the formation of sunburn cells and other structural changes in the epidermis of hairless mice (De La Coba *et al.,* 2009). (Choi *et al.,* 2014) Deinoxanthin, which was generated by the radiophile Deinococcus radiodurans, induced apoptosis in three human cancer cell lines, indicating its potential as an anticancer agent. Due to its ability to repair DNA damage induced by ionizing radiation, bacteriophages Halobacterium and Rubrobacter produce bacteriopherin, which can prevent cutaneous cancer (Raddadi *et al.,* 2015).

Barophiles are high-pressure-tolerant microorganisms that flourish between 70 and 80 MPa. Barophiles from the depths of the ocean have been isolated. While literature on barophiles is limited, high-pressure sterilization of food items has utilized proteins produced by barophiles (Arora and Panosyan, 2019).

pH Extremophile proteases can be acidophiles (active at low pH), alkaliphiles (active at high pH), or neutrophilic (active at neutral pH).

Acidophilic proteases These enzymes function best in acidic environments, with optimal pH values below 5. They are found in extremophiles that live in environments like volcanic hot springs or acid mine drainage.

Table.1 Summary of the main strategies to improve the culturability and isolation of extremophilic microorganisms from extreme environments.

Strategy	Approach	Microbial target	Previous uses	Advantages	Disadvant-ages	References
Culture media and incubation settings for microbial growth	Standard and alternative culture media, modifications of composition and incubation conditions	Generalist and specialized microorganisms	Environmental and clinical microbes. Commonly used for cultivation of microbes from extreme environments	-Easy formulation and manipulation -Established methods and commercial options -Low cost -The equipment is not necessarily complex	Cultivation bias of isolating more members of Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria	Yaşar Yıldız S, Radchenkova N, 2024
	Enriched culture	Generalist and specialized microorganisms	Environmental and clinical microbes. Commonly used for Archaea	-Enrichment of wanted groups by manipulating the conditions (e.g., adding inhibitors or growth factors)	-Long incubation period	Bae *et al.,* (2005); Könneke *et al.,* (2005); Klein *et al.,* (2022)
				-Separation of specific species from a mixed community	-In some cases, lack of pure cultures	
	Co-culture	Autotrophic and syntrophic microbes	Environmental and clinical microbes	-Microbial interactions	-May require optimization for some requirements	Plugge and Stams (2002); Stewart *et al.,* (2012); Sánchez-Andrea *et al.,* (2018); Pillot *et al.,* (2020)
				-Growth of microbes that depend on specific (sometimes unknown) compounds generated by other microorganisms	-Lack of pure cultures	
				-Low cost		
	Culturomics	Generalist and selective microorganisms	Environmental microbes and human gut microbiota	-High-throughput isolation	-Labor-intensive. Large numbers of samples and data to process	Lagier *et al.,* (2012); Khelaifia *et al.,* (2018); Yasir *et al.,* (2019); Sood *et al.,* (2021)
				-Multiple and simultaneous microbial growth under different conditions	-Significant cost	

Diffusion-based devices and *in-situ* cultivation	Cultivation chambers	Generalist microbes	Microorganisms from soil, marine environments, activated sludge	-*In situ* cultivation -Small, low-cost device	Competition between cells may occur in the chamber, leading to selectivity	Kaeberlein *et al.,* (2002); Bollmann and Lewis (2007)
	Isolation chips	Generalist microbes	Environmental microbes	-High-throughput cultivation -*In-situ* cultivation -Easy observation of colonies under a microscope	Difficulty of loading cells into the chip wells	Nichols *et al.,* (2010); Berdy *et al.,* (2017)
	Multiwell microbial culture chip	Generalist microbes	Environmental microbes	-High cultivation efficiency - High-throughput screening for phenotypes and microbial products -Rapid changes in environment and analysis under a variety of culture conditions	Difficult to pick and recover microcolonies from the wells	Ingham *et al.,* (2007)
	Hollow-fiber membrane-based chamber	Generalist and specialized microorganisms	Environmental microbes	-Simple handling -Rapid molecular exchange	-Oversize device -Culture of microbes from surface water layer	Aoi *et al.,* (2009); Fu *et al.,* (2017)
	Paper-based analytical device	Generalist microbes	Environmental, human and clinical microbes	- Multifunctional, used for biological and chemical purposes -Low cost -Efficient for clinical diagnostics	Selective growth of well-known cultured microbes	Kim and Yeo (2016); Noiphung and Laiwattanapaisal (2019); Levy *et al.,* (2020)
Targeted cell-sorting and cultivation	Reverse genomics	Targeted microbial groups	Human oral microbiome	Isolation and cultivation of targeted microbes	-Significant cost -Use of different robust equipment -Required technical abilities	Cross *et al.,* (2019); Ibrahim *et al.,* (2022)

	FACS	Targeted microbial groups	Environmental and clinical microbes	Isolation and cultivation of targeted microbes	-Not compatible for anaerobe growth -In some cases, it is not possible to culture	Alvarez-Barrientos *et al.,* (2000); Zengler *et al.,* (2002); Espina *et al.,* (2022)
	Live-FISH	Targeted microbial groups	Marine microbes	-Isolation and cultivation of targeted microbes -No sophisticated probe design is required	-Advances skills in fluorescence due to variations in fluorescent signals can decrease the detectability of some microorganisms	Batani *et al.,* (2019)
	Micromanipulators and laser manipulation system	Generalist and selective microorganisms	Marine microbes, human cells, microorganisms from deep-sea brine pools and hydrothermal systems	-Selection of cells of interest from a mixed microbial community -Pure colonies obtained by sorting cells with microscope	-Required technical abilities -Laser manipulation	Huber *et al.,* (1995); Huber *et al.,* (2000); Antunes *et al.,* (2008a,b)

Figure.1 Representation of Enzyme Immobilization

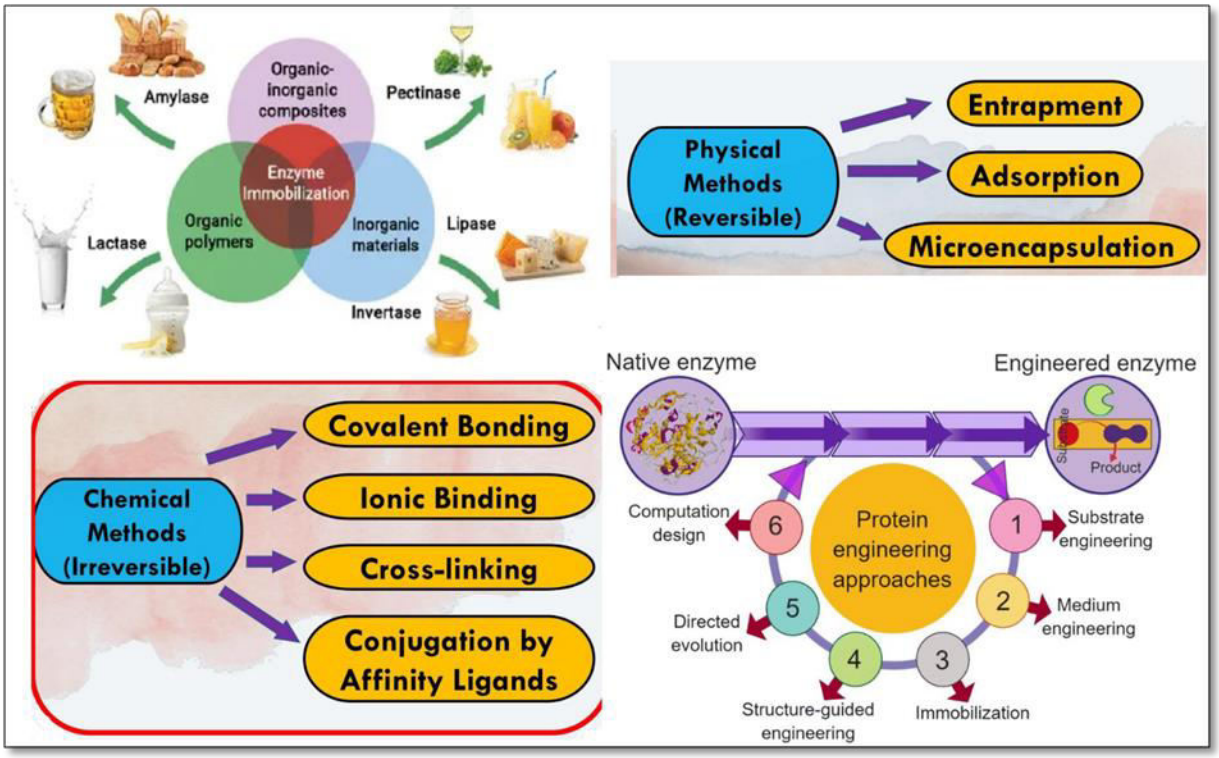


Figure.2 Extremozyme and its types with specific characteristic Copyright @ Noha M. Mesbah, 2020

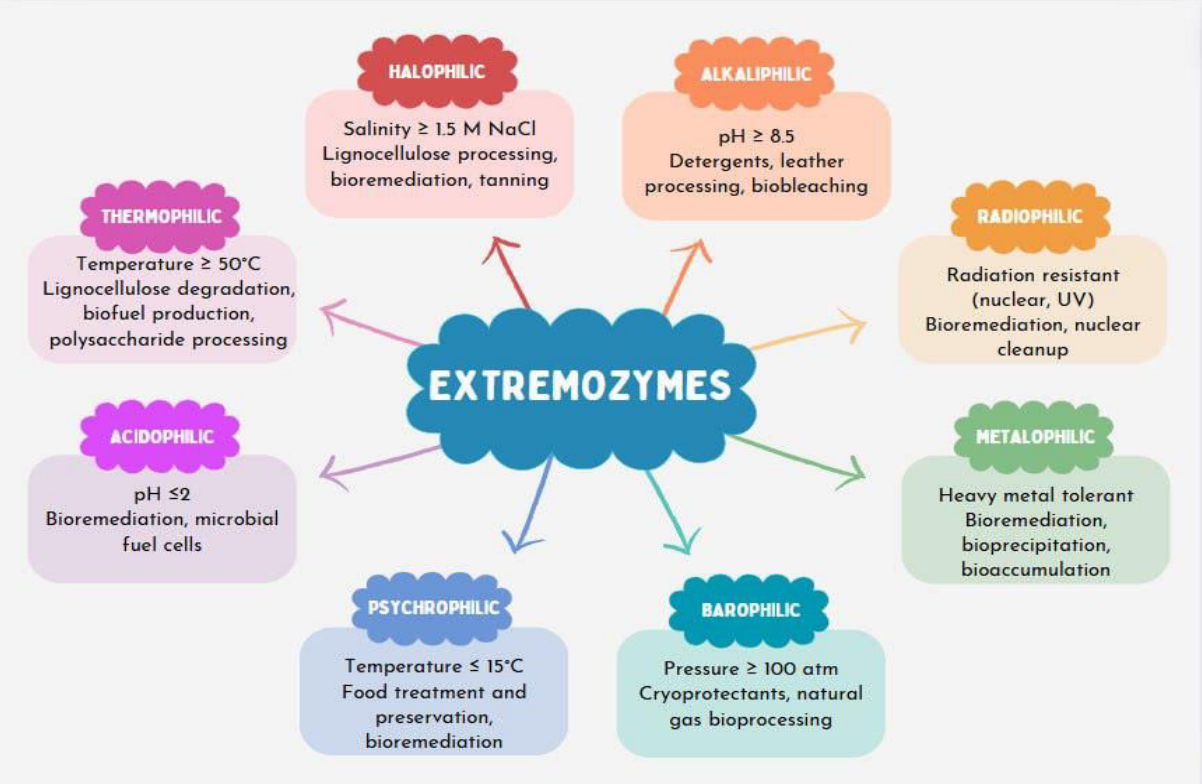


Figure.3 Application of Extremophiles Copyright @ Furhan, J. 2020

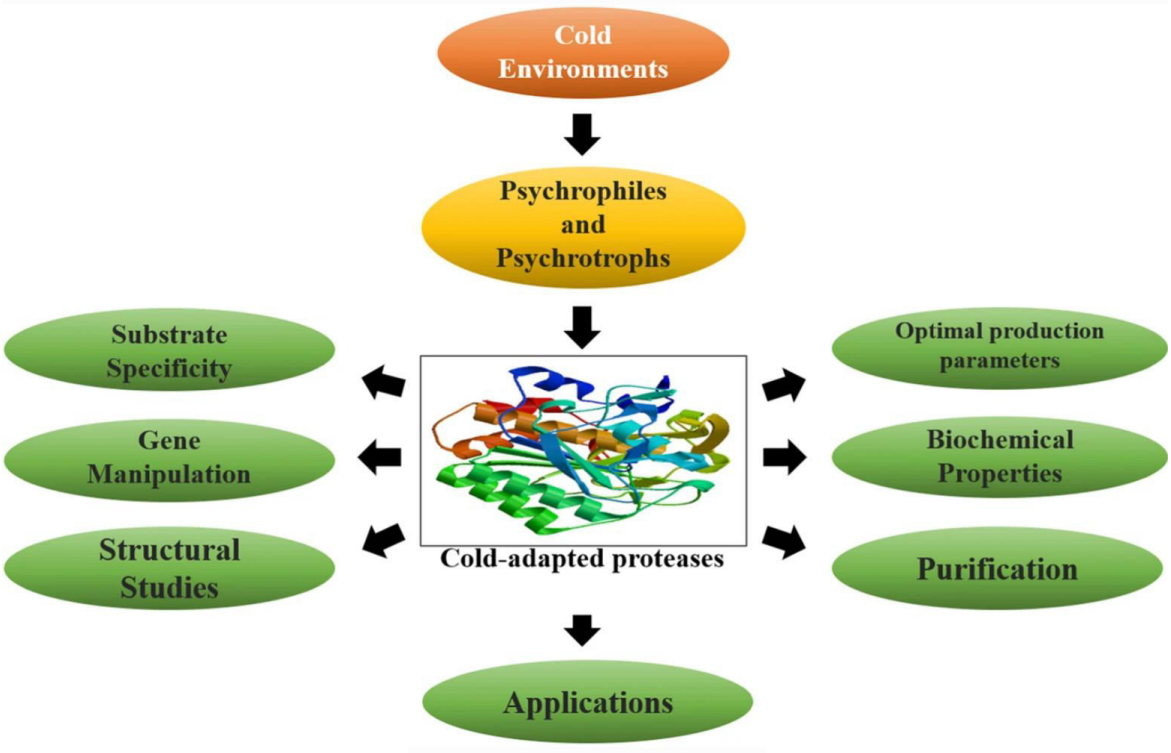


Figure.4 Leather Enzymatic Process

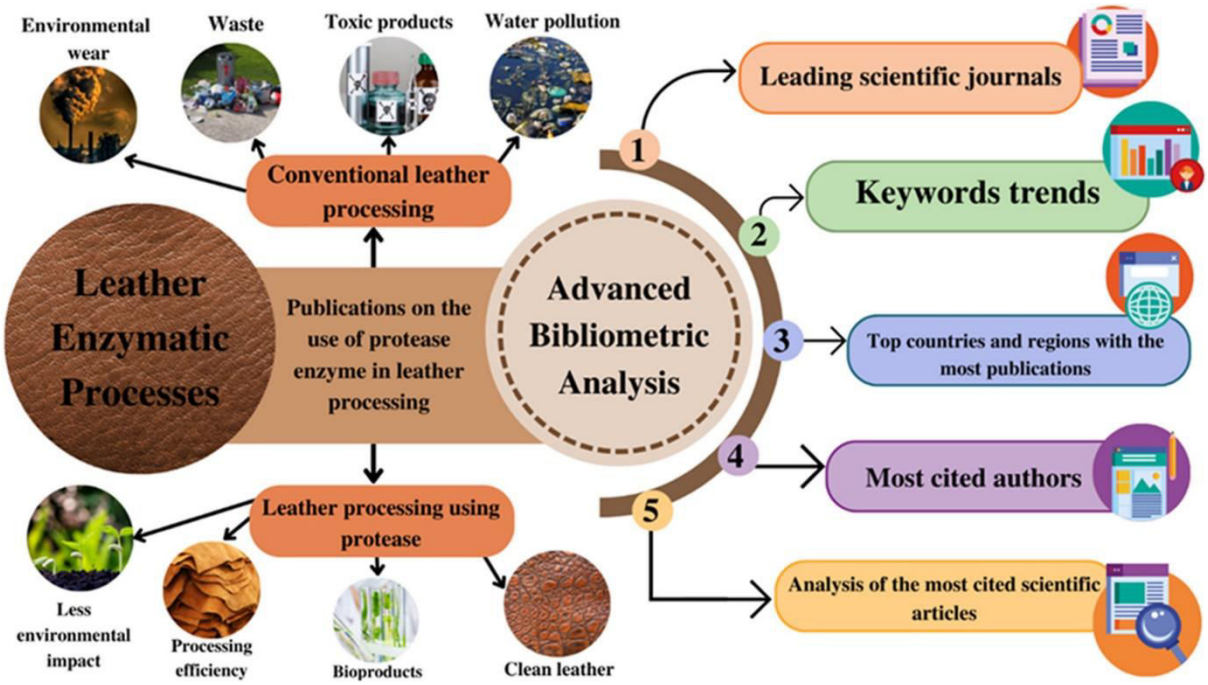
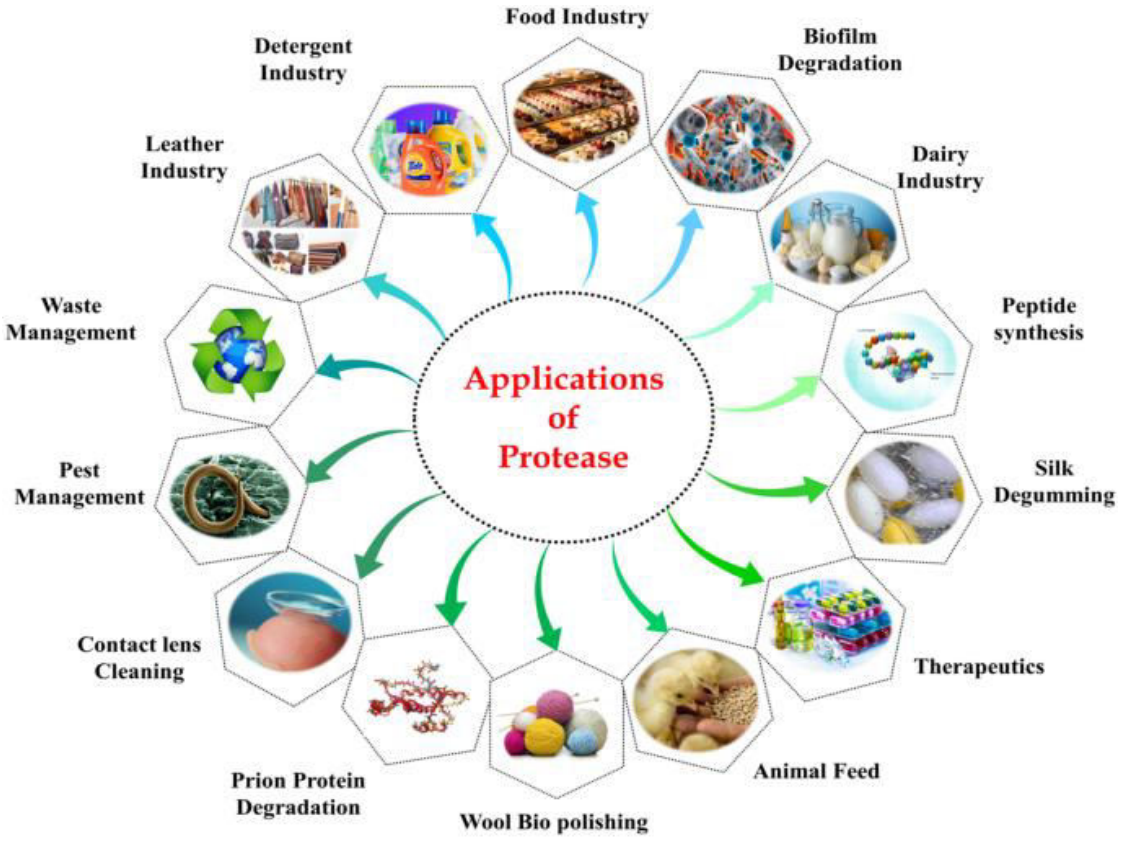


Figure.5 Application of Microbial Protease



Applications of Extremophile Proteases in Leather Processing

Conventional leather processing relies heavily on harsh chemicals that raise environmental concerns. Extremophile proteases offer a more eco-friendly alternative for several key steps in leather processing

Dehairing

Traditional dehairing methods use lime and sometimes sulfides, which are harmful chemicals.

Extremophile proteases, particularly those from alkaliphiles, can effectively remove hair from hides at high pH levels. This eliminates the need for harsh chemicals and reduces the environmental impact of the process.

Bating

Bating is a process that removes non-collagenous proteins, dirt, and other impurities from the hide.

Conventional bating utilizes enzymes like trypsin and pancreatin, which require specific temperature and pH ranges.

Extremophile proteases with broader temperature and pH tolerance can offer more flexibility and potentially reduce processing times.

Soaking and Rehydration

Soaking helps rehydrate dried hides and prepares them for further processing.

Some extremophiles produce enzymes with lipase activity, which can break down fats and oils present in the skin.
This can improve the efficiency of the soaking process and enhance the quality of the final leather.

Advantages of using Extremophile Proteases

Environmentally friendly They eliminate or significantly reduce the use of harsh chemicals.
Efficiency Some extremophiles demonstrate faster enzymatic activity compared to traditional methods.
Specificity Certain extremophile proteases can target specific proteins, leading to cleaner and more consistent leather quality.
Process flexibility Their wider temperature and pH tolerance allows for more adaptable processing conditions.

Challenges and Future Directions

Cost-effectiveness Currently, the production cost of extremophile proteases is higher than traditional enzymes.
Optimization Further research is needed to optimize the performance of these enzymes for specific leather processing applications.
Large-scale production Scaling up the production of extremophile proteases is crucial for broader industrial use.

Overall, extremophile proteases hold significant promise for revolutionizing leather processing towards a more sustainable and efficient future. As research and production costs decline, we can expect these innovative enzymes to become a mainstay in the leather industry.

Advantages and Challenges of Extremophile Proteases

Extremophile proteases offer exciting possibilities for various industrial applications due to their unique properties. However, there are both advantages and challenges associated with their use.

Advantages

Broader Activity Range They can function in extreme temperatures (thermophilic, psychrophilic), pH levels (acidophilic, alkaliphilic), pressure (piezophilic), and salt concentrations (halophilic) beyond the capabilities of conventional enzymes.
Environmentally Friendly Their use can reduce reliance on harsh chemicals in industrial processes like leather tanning and detergent production.
High Efficiency Some extremophile proteases exhibit faster reaction rates than traditional enzymes.
Specificity Certain enzymes can target specific proteins, leading to more precise and consistent results.
Novel Applications Their unique properties open doors for innovative applications in bioremediation, medicine, and food processing.

Challenges

Cost-Effectiveness Currently, the production cost of

extremophile proteases is higher compared to traditional enzymes.

Process Optimization Further research is needed to tailor their performance for specific industrial applications.

Large-Scale Production Scaling up production is crucial for wider industrial adoption.

Enzyme Stability Some extremophile proteases might require specific storage and handling conditions due to their adaptation to extreme environments.

Limited Availability Discovery and isolation of new extremophile enzymes with desired properties is an ongoing process.

Overall, extremophile proteases offer significant advantages in terms of their broad activity range and potential for environmentally friendly processes. However, overcoming challenges related to production cost, optimization, and stability is crucial for their widespread adoption in various industries.

Future Perspectives of Extremophile Proteases

Extremophile proteases are a rapidly evolving field with immense potential for various applications. Here's a glimpse into the future possibilities

1. Advanced Enzyme Engineering

Techniques like directed evolution and protein engineering can be employed to create novel extremophile proteases with even more desirable properties.

This could involve enhancing their activity, stability under broader conditions, or targeting specific substrates for precise applications.

2. Cost-Effective Production

Optimizing production methods and utilizing alternative hosts for enzyme expression can significantly reduce the cost of extremophile proteases.

This will make them more competitive with traditional enzymes, paving the way for wider industrial adoption.

3. Metagenomic Exploration

Advancements in metagenomics can help discover novel extremophile enzymes with unique properties from diverse environmental samples.

This vast untapped resource holds the potential for uncovering enzymes with functionalities yet to be explored.

4. Integration with Biocatalysis

Extremophile proteases can be integrated with other enzymes and biomolecules to create complex biocatalytic systems.

This could lead to the development of efficient and eco-friendly processes for various industries, such as bioremediation, biofuel production, and biopolymer synthesis.

5. Personalized Medicine

Extremophile proteases with specific activity under human body conditions might be useful in developing personalized medicine approaches.

Tailored enzymes could potentially target specific disease-causing proteins within the body for therapeutic purposes.

Challenges and Opportunities

- Addressing intellectual property rights associated with extremophile discovery and enzyme engineering is crucial.
- Developing efficient delivery and storage methods for extremophile proteases might be necessary for certain applications.
- Public awareness and education about the benefits of extremophile enzymes can promote their use in sustainable industrial practices.
- Overall, the future of extremophile proteases is bright. As research advances and production costs decline, we can expect these versatile enzymes to play a crucial role in creating a more sustainable and bio-based future across various industrial sectors.

They reduce the environmental impact by providing a greener alternative to the abrasive chemicals that have traditionally been used in leather processing. Effectiveness Their operation across a wide range of conditions enables greater adaptability and, potentially, quicker processing times. Particular extremophile proteases are capable of selectively targeting particular proteins, thereby enhancing the cleanliness and uniformity of leather. Despite the presence of obstacles such as cost-effectiveness and large-scale production, the outlook for the future appears optimistic. Cost-reduction strategies and developments in enzyme engineering have the potential to increase the competitiveness of extremophile proteases. Further investigation has the capacity to unveil enzymes that are not only more efficient but also more adaptable for particular stages of leather processing. In general, extremophile proteases provide a potent instrument for revolutionizing the leather processing sector towards greater environmental consciousness and sustainability. Their implementation establishes a pathway towards a future in which the

manufacturing of premium leather requires only minimal ecological disruption, thereby satisfying the increasing market demand for sustainable methods.

Author Contributions

Gandharv Singh Rawat: Investigation, formal analysis, writing—original draft. Satish Mohabe: Validation, methodology, writing—reviewing. Jitendra Malviya:—Formal analysis, writing—review and editing. Vikah Jadhav: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Akal, A. L., Karan, R., Hohl, A., Alam, I., Vogler, M., Grötzinger, S. W., *et al.,* (2018). A Polyextremophilic Alcohol Dehydrogenase from the Atlantis II Deep Red Sea Brine Pool. FEBS Open Bio 9 (2), 194–205. https://doi.org/10.1002/2211-5463.12557

Al-Ghanayem, A. A., and Joseph, B. (2020). Current Prospective in Using Cold-Active Enzymes as Eco-Friendly Detergent Additive. Appl. Microbiol. Biotechnol. 104 (7), 2871–2882. https://doi.org/10.1007/s00253-020-10429-x

Alvira, P., Tomás-Pejó, E., Ballesteros, M., and Negro, M. J. (2010). Pretreatment Technologies for an Efficient Bioethanol Production Process Based on Enzymatic Hydrolysis A Review. Bioresour. Techn. 101, 4851–4861. https://doi.org/10.1016/j.biortech.2009.11.093

Amoozegar, M. A., Safarpour, A., Noghabi, K. A., Bakhtiary, T., and Ventosa, A. (2019). Halophiles and Their Vast Potential in Biofuel Production. Front. Microbiol. 10, 1895. https://doi.org/10.3389/fmicb.2019.01895

Appukuttan, D., Rao, A. S., and Apte, S. K. (2006). Engineering of Deinococcus Radiodurans R1 for Bioprecipitation of Uranium from Dilute Nuclear Waste. Appl. Environ. Microbiol. 72 (12), 7873–7878. https://doi.org/10.1128/aem.01362-06

Arora, N. K., and Panosyan, H. (2019). Extremophiles Applications and Roles in Environmental Sustainability. Environ. Sustainability 2 (3), 217–218. https://doi.org/10.1007/s42398-019-00082-0

Atalah, J., Cáceres-Moreno, P., Espina, G., and Blamey, J. M. (2019). Thermophiles and the Applications of Their Enzymes as New Biocatalysts. Bioresour. Techn. 280, 478–488. https://doi.org/10.1016/j.biortech.2019.02.008

Bala, A., and Singh, B. (2019). Cellulolytic and Xylanolytic Enzymes of Thermophiles for the Production of Renewable Biofuels. Renew. Energ. 136, 1231–1244. https://doi.org/10.1016/j.renene.2018.09.100

Barroca, M., Santos, G., Gerday, C., and Collins, T. (2017). "Biotechnological Aspects of Cold-Active Enzymes," in Psychrophiles From Biodiversity to Biotechnology. Editor R. Margesin (Cham Springer International Publishing), 461–475. https://doi.org/10.1007/978-3-319-57057-0_19

Beaufils, C.; Man, H.-M.; de Poulpiquet, A.; Mazurenko, I.; Lojou, E. From Enzyme Stability to Enzymatic Bioelectrode Stabilization Processes. *Catalysts* 2021, *11*, 497. https//doi.org/10.3390/catal11040497

Beckham, G. T., Resch, M. G., Donohoe, B. S., Baker, J. O., Decker, S. R., Bayer, E. A., *et al.,* (2013). Fungal Cellulases and Complexed Cellulosomal Enzymes Exhibit Synergistic Mechanisms in Cellulose Deconstruction. Energy Environ. Sci. 6 (6), 1858. https://doi.org/10.1039/c3ee00019b

Benešová, E., Marková, M., and Králová, B. (2005). α-Glucosidase and β-Glucosidase from Psychrotrophic Strain Arthrobacter Sp. C2-2. Czech J. Food Sci. 23, 116–120. https://doi.org/10.17221/3380-CJFS

Berenguer-Murcia, Á., Ortiz, C., Ferreira, M. L., Barbosa, O., Dos Santos, J. C. S., Rodrigues, R. C., *et al.,* (2019). Novozym 435 the "perfect" Lipase Immobilized Biocatalyst? Catal. Sci. Technol. 9 (10), 2380–2420. https://doi.org/10.1039/c9cy00415g

Berg, K., Leiros, I., and Williamson, A. (2019). Temperature Adaptation of DNA Ligases from Psychrophilic Organisms. Extremophiles 23 (3), 305–317. https://doi.org/10.1007/s00792-019-01082-y

Bertoldo, C., Dock, C., and Antranikian, G. (2004). Thermoacidophilic Microorganisms and Their Novel Biocatalysts. Eng. Life Sci. 4 (6), 521–532. https://doi.org/10.1002/elsc.200402155

Bhalla, A., Bansal, N., Kumar, S., Bischoff, K. M., and Sani, R. K. (2013). Improved Lignocellulose Conversion to Biofuels with Thermophilic Bacteria and Thermostable Enzymes. Bioresour. Techn. 128, 751–759. https://doi.org/10.1016/j.biortech.2012.10.145

Bhardwaj, N., Kumar, B., and Verma, P. (2019). A Detailed Overview of Xylanases an Emerging Biomolecule for Current and Future Prospective. Bioresour. Bioproc. 6 (1), 40. https://doi.org/10.1186/s40643-019-0276-2

Bharmoria P, Tietze AA, Mondal D, Kang TS, Kumar A, Freire M G. (2024) Do Ionic Liquids Exhibit the Required Characteristics to Dissolve, Extract, Stabilize, and Purify Proteins? Past-Present-Future Assessment. Chem Rev.;124(6)3037-3084. https://doi.org/10.1021/acs.chemrev.3c00551. Epub

Brim, H., Venkateswaran, A., Kostandarithes, H. M., Fredrickson, J. K., and Daly, M. J. (2003). Engineering Deinococcus Geothermalis for Bioremediation of High-Temperature Radioactive Waste Environments. Appl. Environ. Microbiol. 69 (8), 4575–4582. https://doi.org/10.1128/aem.69.8.4575-4582.2003

Brininger, C., Spradlin, S., Cobani, L., and Evilia, C. (2018). The More Adaptive to Change, the More Likely You Are to Survive Protein Adaptation in Extremophiles. Semin. Cel Develop. Biol. 84, 158–169. https://doi.org/10.1016/j.semcdb.2017.12.016

Cárdenas-Fernández, M., Sinclair, O., and Ward, J. M. (2021). Novel Transaminases from Thermophiles from Discovery to Application. Microb. Biotechnol. 15 (1), 305–317. https://doi.org/10.1111/1751-7915.13940

Chen, L.-X., Huang, L.-N., Méndez-García, C., Kuang, J.-L., Hua, Z.-S., Liu, J., *et al.,* (2016). Microbial Communities, Processes and Functions in Acid Mine Drainage Ecosystems. Curr. Opin. Biotechnol. 38, 150–158. https://doi.org/10.1016/j.copbio.2016.01.013

Chien, A., Edgar, D. B., and Trela, J. M. (1976). Deoxyribonucleic Acid Polymerase from the Extreme Thermophile *Thermus aquaticus*. J. Bacteriol. 127 (3), 1550–1557. https://doi.org/10.1128/jb.127.3.1550-1557.1976

Choi, Y. J., Hur, J. M., Lim, S., Jo, M., Kim, D. H., and Choi, J. I. (2014). Induction of Apoptosis by Deinoxanthin in Human Cancer Cells. Anticancer Res. 34 (4), 1829–1835.

de la Fuente, M., Lombardero, L., Gómez-González, A., Solari, C., Angulo-Barturen, I., Acera, A.,...& Barreda-Gómez, G. (2021). Enzyme therapy current challenges and future perspectives. *International Journal of Molecular Sciences*, *22*(17), 9181.

Delgado-García, M., Rodríguez, J. A., Mateos-Díaz, J. C., Aguilar, C. N., Rodríguez-Herrera, R., and Camacho-Ruíz, R. M. (2018). in Enzymes in Food Technology Improvements and Innovations. Editor M. Kuddus (Singapore Springer Singapore), 243–262.

Desai, A. A. (2011). Sitagliptin Manufacture a Compelling Tale of green Chemistry, Process Intensification, and Industrial Asymmetric Catalysis. Angew. Chem. Int. Ed. 50 (9), 1974–1976. https://doi.org/10.1002/anie.201007051

Distaso, M. A. (2019). *Metagenomics approaches to discover new industrially-relevant enzymes*. Bangor University (United Kingdom).

Dornez, E., Verjans, P., Arnaut, F., Delcour, J. A., and Courtin, C. M. (2011). Use of Psychrophilic Xylanases Provides Insight into the Xylanase Functionality in Bread Making. J. Agric. Food Chem. 59 (17), 9553–9562. https://doi.org/10.1021/jf201752g

Elend, C., Schmeisser, C., Hoebenreich, H., Steele, H. L., and Streit, W. R. (2007). Isolation and Characterization of a Metagenome-Derived and Cold-Active Lipase with High Stereospecificity for (R)-ibuprofen Esters. J. Biotechnol. 130 (4), 370–377. https://doi.org/10.1016/j.jbiotec.2007.05.015

Eppinger, J., Karan, R., Mathew, S., Muhammad, R., Bautista, D. B., Vogler, M., *et al.,* (2020). Understanding High-Salt and Cold Adaptation of a Polyextremophilic Enzyme. Microorganisms 8 (10), 1594.

https://doi.org/10.3390/microorganisms8101594

Espina, G., Atalah, J., and Blamey, J. M. (2021). Extremophilic Oxidoreductases for the Industry Five Successful Examples with Promising Projections. Front. Bioeng. Biotechnol. 9 (654), 710035. https://doi.org/10.3389/fbioe.2021.710035

Ferguson, S. J., and Ingledew, W. J. (2008). Energetic Problems Faced by Micro-organisms Growing or Surviving on Parsimonious Energy Sources and at Acidic pH I. Acidithiobacillus Ferrooxidans as a Paradigm. Biochim. Biophys. Acta (Bba) - Bioenerg. 1777 (12), 1471–1479. https://doi.org/10.1016/j.bbabio.2008.08.012

Fornbacke, M., and Clarsund, M. (2013). Cold-adapted Proteases as an Emerging Class of Therapeutics. Infect. Dis. Ther. 2 (1), 15–26. https://doi.org/10.1007/s40121-013-0002-x

Fujinami, S., and Fujisawa, M. (2010). Industrial Applications of Alkaliphiles and Their Enzymes-Ppast, Present and Future. Environ. Technol. 31 (8-9), 845–856. https://doi.org/10.1080/09593331003762807

Furhan, J. Adaptation, production, and biotechnological potential of cold-adapted proteases from psychrophiles and psychrotrophs recent overview. J Genet Eng Biotechnol 18, 36 (2020). https://doi.org/10.1186/s43141-020-00053-7

Gallo, G., Puopolo, R., Carbonaro, M., Maresca, E., and Fiorentino, G. (2021). Extremophiles, a Nifty Tool to Face Environmental Pollution From Exploitation of Metabolism to Genome Engineering. Int. J. Environ. Res. Public Health 18 (10), 5228. https://doi.org/10.3390/ijerph18105228

Genolette, S. W., Karan, R., Strillinger, E., Bader, S., Frank, A., Al Rowaihi, I. S., *et al.,* (2000). Identification and Experimental Characterization of an Extremophilic Brine Pool Alcohol Dehydrogenase from Single Amplified Genomes. ACS Chem. Biol. 13 (1), 161–170. https://doi.org/10.1021/acschembio.7b00792

Grötzinger, S. W., Vogler, M., Karan, R., Renn, D., Vancea, A., Vielberg, M. T., *et al.,* (2020). Crystal Structure and Active Site Engineering of a Halophilic γ-Carbonic Anhydrase. Front. Microbiol. 11, 742. https://doi.org/10.3389/fmicb.2020.00742

Gupta, G. K., Kapoor, R. K., and Shukla, P. (2020). "Advanced Techniques for Enzymatic and Chemical Bleaching for Pulp and Paper Industries," in Microbial Enzymes and Biotechniques Interdisciplinary Perspectives. Editor P. Shukla (Singapore Springer Singapore), 43–56. https://doi.org/10.1007/978-981-15-6895-4_3

Haque, R. U., Paradisi, F., and Allers, T. (2020). Haloferax Volcanii for Biotechnology Applications Challenges, Current State and Perspectives. Appl. Microbiol. Biotechnol. 104, 1371–1382. https://doi.org/10.1007/s00253-019-10314-2

Hasan, F., Shah, A. A., and Hameed, A. (2006). Industrial Applications of Microbial Lipases. Enzyme Microb. Techn. 39 (2), 235–251. https://doi.org/10.1016/j.enzmictec.2005.10.016

He, H., Chen, X., Li, J., Zhang, Y., and Gao, P. (2004). Taste Improvement of Refrigerated Meat Treated with Cold-Adapted Protease. Food Chem. 84 (2), 307–311. https://doi.org/10.1016/s0308-8146(03)00242-5

Holwerda, E. K., Worthen, R. S., Kothari, N., Lasky, R. C., Davison, B. H., Fu, C., *et al.,* (2019). Multiple Levers for Overcoming the Recalcitrance of Lignocellulosic Biomass. Biotechnol. Biofuels 12 (1), 15. https://doi.org/10.1186/s13068-019-1353-7

Homaei A A, Sariri R, Vianello F, Stevanato R. (2013) Enzyme immobilization an update. 29;6(4)185-205. https://doi.org/10.1007/s12154-013-0102-9. PMID 24432134; PMCID PMC3787205.

Hübscher, U., Georlette, D., Jónsson, Z. O., Van Petegem, F., Chessa, J.-P., Van Beeumen, J., *et al.,* (2000). A DNA Ligase from the Psychrophile Pseudoalteromonas Haloplanktis Gives Insights into the Adaptation of Proteins to Low Temperatures. Eur. J. Biochem. 267, 3502–3512. https://doi.org/10.1046/j.1432-1327.2000.01377.x

Huston, A. L. (2008). "Biotechnological Aspects of Cold-Adapted Enzymes," in Psychrophiles From Biodiversity to Biotechnology. Editors R. Margesin, F. Schinner, J.-C. Marx, and C. Gerday (Berlin, Heidelberg Springer Berlin Heidelberg), 347–363. https://doi.org/10.1007/978-3-540-74335-4_20

Izadi, P., Fontmorin, J-M., Fernández, L. F. L., Cheng, S., Head, I., and Yu, E. H. (2019). High Performing Gas Diffusion Biocathode for Microbial Fuel Cells Using Acidophilic Iron Oxidizing Bacteria. Front. Energ. Res. 7, 93. https://doi.org/10.3389/fenrg.2019.00093

Izquierdo, J. A., Pattathil, S., Guseva, A., Hahn, M. G., and Lynd, L. R. (2014). Comparative Analysis of the Ability of Clostridium Clariflavum Strains and Clostridium Thermocellumto Utilize Hemicellulose and Unpretreated Plant Material. Biotechnol. Biofuels 7 (1), 136. https://doi.org/10.1186/s13068-014-0136-4

Jadhav, G. S., and Ghangrekar, M. M. (2009). Performance of Microbial Fuel Cell Subjected to Variation in pH, Temperature, External Load and Substrate Concentration. Bioresour. Techn. 100 (2), 717–723. https://doi.org/10.1016/j.biortech.2008.07.041

Jin, Z., Li, X., Ji, H., Zhai, Y., and Bai, Y., (2021). Characterizing a Thermostable Amylopullulanase from Caldisericum Exile with Wide pH Adaptation and Broad Substrate Specificity. Food Biosci. 41, 100952. https://doi.org/10.1016/j.fbio.2021.100952

Jitendra Malviya. 2021. Potential of Protease from Bacillus species for Biomedical and Industrial Applications.Int.J.Curr.Microbiol.App.Sci. 10(5) 560-574.

Johnson, D. B., and Hallberg, K. B. (2005). Acid Mine Drainage Remediation Options a Review. Sci. Total Environ. 338 (1), 3–14. https://doi.org/10.1016/j.scitotenv.2004.09.002

Jorquera, M. A., Graether, S. P., and Maruyama, F. (2019). Editorial Bioprospecting and Biotechnology of Extremophiles. Front. Bioeng. Biotechnol. 7, 204. https://doi.org/10.3389/fbioe.2019.00204

Kanafusa-Shinkai, S., Wakayama, J. i., Tsukamoto, K., Hayashi, N., Miyazaki, Y., Ohmori, H., *et al.,* (2013). Degradation of Microcrystalline Cellulose and Non-pretreated Plant Biomass by a Cell-free Extracellular Cellulase/hemicellulase System from the Extreme Thermophilic Bacterium Caldicellulosiruptor Bescii. J. Biosci. Bioeng. 115 (1), 64–70. https://doi.org/10.1016/j.jbiosc.2012.07.019

Karan, R., Capes, M. D., Dassarma, P., and Dassarma, S. (2013). Cloning, Overexpression, Purification, and Characterization of a Polyextremophilic β-galactosidase from the Antarctic Haloarchaeon Halorubrum Lacusprofundi. BMC Biotechnol. 13, 3. https://doi.org/10.1186/1472-6750-13-3

Karshikoff, A., Nilsson, L., and Ladenstein, R. (2015). Rigidity versus Flexibility the Dilemma of Understanding Protein thermal Stability. Febs J. 282 (20), 3899–3917. https://doi.org/10.1111/febs.13343

Kasirajan, L., and Maupin-Furlow, J. A. (2021). Halophilic Archaea and Their Potential to Generate Renewable Fuels and Chemicals. Biotechnol. Bioeng. 118 (3), 1066–1090. https://doi.org/10.1002/bit.27639

Katsimpouras, C., and Stephanopoulos, G. (2021). Enzymes in Biotechnology Critical Platform Technologies for Bioprocess Development. Curr. Opin. Biotechnol. 69, 91–102. https://doi.org/10.1016/j.copbio.2020.12.003

Kim, T. D., Le, L. T. H. L., Yoo, W., Jeon, S., Lee, C., Kim, K. K., Lee, J. H., (2020). Biodiesel and flavor compound production using a novel promiscuous cold-adapted SGNH-type lipase (*Ha*SGNH1) from the psychrophilic bacterium *Halocynthiibacter arcticus*. *Biotechnology for biofuels*, *13*, 55. https://doi.org/10.1186/s13068-020-01696-x

Kirk, O., and Christensen, M. W. (2002). Lipases from *Candida antarctica* Unique Biocatalysts from a Unique Origin. Org. Process. Res. Dev. 6 (4), 446–451. https://doi.org/10.1021/op0200165

Knapic, L. (2012). *Computational methods for rational screening and engineering of enzyme properties* (Doctoral dissertation, Università degli studi di Trieste).

Kumar, A., Mukhia, S., and Kumar, R. (2021). Industrial Applications of Cold-Adapted Enzymes Challenges, Innovations and Future Perspective. 3 Biotech. 11 (10), 426. https://doi.org/10.1007/s13205-021-02929-y

Landeta, C., Boyd, D., and Beckwith, J. (2018). Disulfide Bond Formation in Prokaryotes. Nat. Microbiol. 3 (3), 270–280. https://doi.org/10.1038/s41564-017-0106-2

Leiva, E., Leiva-Aravena, E., Rodríguez, C., Serrano, J., and Vargas, I. (2018). Arsenic Removal Mediated by Acidic pH Neutralization and Iron Precipitation in Microbial Fuel Cells. Sci. Total Environ. 645, 471–481. https://doi.org/10.1016/j.scitotenv.2018.06.378

Li, X., Han, H., Ling, Z., Khan, A., Virk, A. K., Kulshrestha, S., (2019). Improvements of Thermophilic Enzymes From Genetic Modifications to Applications. Bioresour. Techn. 279, 350–361. https://doi.org/10.1016/j.biortech.2019.01.087

Li, Z., Liu, W., Gu, Z., Li, C., Hong, Y., and Cheng, L. (2015). The Effect of Starch Concentration on the Gelatinization and Liquefaction of Corn Starch. Food Hydrocolloids 48, 189–196. https://doi.org/10.1016/j.foodhyd.2015.02.030

Littlechild, J. A. (2015). Enzymes from Extreme Environments and Their Industrial Applications. Front. Bioeng. Biotechnol. 3, 161. https://doi.org/10.3389/fbioe.2015.00161

Liu, T., Wang, Y., Luo, X., Li, J., Reed, S. A., Xiao, H., *et al.,* (2016). Enhancing Protein Stability with Extended Disulfide Bonds. Proc. Natl. Acad. Sci. U.S.A. 113 (21), 5910–5915. https://doi.org/10.1073/pnas.1605363113

Liu, Y., Luo, G., Ngo, H. H., Guo, W., and Zhang, S. (2020). Advances in Thermostable Laccase and its Current Application in Lignin-First Biorefinery A Review. Bioresour. Techn. 298, 122511. https://doi.org/10.1016/j.biortech.2019.122511

Liu, Z., Ning, C., Yuan, M., Yang, S., Wei, X., Xiao, M., *et al.,* (2020). High-level Expression of a Thermophilic and Acidophilic β-mannanase from Aspergillus Kawachii IFO 4308 with Significant Potential in Mannooligosaccharide Preparation. Bioresour. Techn. 295, 122257. https://doi.org/10.1016/j.biortech.2019.122257

Lopez-Lopez, O., Cerdan, M. E., and Siso, M. I. (2014). New Extremophilic Lipases and Esterases from Metagenomics. Curr. Protein Pept. Sci. 15 (5), 445–455. https://doi.org/10.2174/1389203715666140228153801

Lozano, P., Bernal, J. M., Nieto, S., Gomez, C., Garcia-Verdugo, E., and Luis, S. V. (2015). Active Biopolymers in green Non-conventional media a Sustainable Tool for Developing Clean Chemical Processes. Chem. Commun. 51, 17361–17374. https://doi.org/10.1039/c5cc07600e

Lynd, L. R., Weimer, P. J., Van Zyl, W. H., and Pretorius, I. S. (2002). Microbial Cellulose Utilization Fundamentals and Biotechnology. Microbiol. Mol. Biol. Rev. 66, 506–577. https://doi.org/10.1128/mmbr.66.3.506-577.2002

Mallick, P., Boutz, D. R., Eisenberg, D., and Yeates, T. O. (2002). Genomic Evidence that the Intracellular Proteins of Archaeal Microbes Contain Disulfide Bonds. Proc. Natl. Acad. Sci. U.S.A. 99 (15), 9679–9684. https://doi.org/10.1073/pnas.142310499

Mangiagalli and Lotti, M. (2021). Cold-Active β-Galactosidases Insight into Cold Adaptation Mechanisms and Biotechnological Exploitation. Mar. Drugs 19 (1), 43. https://doi.org/10.3390/md19010043

Mangiagalli M., Brocca, S., Orlando, M., and Lotti, M. (2020). The "cold Revolution". Present and Future Applications of Cold-Active Enzymes and Ice-Binding Proteins. New Biotechnol. 55, 5–11. https://doi.org/10.1016/j.nbt.2019.09.003

Marhuenda-Egea, F. C., and Bonete, M. J. (2002). Extreme Halophilic Enzymes in Organic Solvents. Curr. Opin. Biotechnol. 13 (4), 385–389. https://doi.org/10.1016/s0958-1669(02)00338-5

Martínez-Espinosa, R. M. (2020). Heterologous and Homologous Expression of Proteins from Haloarchaea Denitrification as Case of Study. Int. J. Mol. Sci. 21 (1), 82. https://doi.org/10.3390/ijms21010082

Mathur, E. J., Lundberg, K. S., Shoemaker, D. D., Adams, M. W. W., Short, J. M. and Sorge, J. A., (1991). High-fidelity Amplification Using a Thermostable DNA Polymerase Isolated from Pyrococcus Furiosus. Gene 108 (1), 1–6. https://doi.org/10.1016/0378-1119(91)90480-y

Mekkes, J. R., Le Poole, I. C., Das, P. K., Bos, J. D., and Westerhof, W. (1998). Efficient Debridement of Necrotic Wounds Using Proteolytic Enzymes Derived from Antarctic Krill a Double-Blind, Placebo-Controlled Study in a Standardized Animal Wound Model. Wound Repair Regen. 6, 50–57. https://doi.org/10.1046/j.1524-475x.1998.60108.x

Memarpoor-Yazdi, M., Karbalaei-Heidari, H. R., and Khajeh, K. (2017). Production of the Renewable Extremophile Lipase Valuable Biocatalyst with Potential Usage in Food Industry. Food Bioproducts Process. 102, 153–166. https://doi.org/10.1016/j.fbp.2016.12.015

Mesbah, N. M. (2019). Covalent Immobilization of a Halophilic, Alkalithermostable Lipase LipR2 on Florisil Nanoparticles for Production of Alkyl Levulinates. Arch. Biochem. Biophys. 667, 22–29. https://doi.org/10.1016/j.abb.2019.04.004

Mesbah, N. M., and Wiegel, J. (2017). A Halophilic, Alkalithermostable, Ionic Liquid-Tolerant Cellulase and its Application in In Situ Saccharification of Rice Straw. Bioenerg. Res. 10 (2), 583–591. https://doi.org/10.1007/s12155-017-9825-8

Mhetras, N., Mapare, V., and Gokhale, D. (2021). Cold Active Lipases Biocatalytic Tools for Greener Technology. Appl. Biochem. Biotechnol. 193 (7), 2245–2266. https://doi.org/10.1007/s12010-021-03516-w

Mokashe, N., Chaudhari, B., and Patil, U. (2018). Operative Utility of Salt-Stable Proteases of Halophilic and Halotolerant Bacteria in the Biotechnology Sector. Int. J. Biol. Macromolecules 117, 493–522. https://doi.org/10.1016/j.ijbiomac.2018.05.217

Motamedi, 2021. "The Principles of Bioprocess Technology," in Bioprocess Technology Kinetics and Reactors. Editor A. Moser (New York, NY Springer New York), 13–65. https://doi.org/10.1007/978-1-4613-8748-0_2

Movahedi, A. A., Motamedi, E., Sadeghian Motahar, S. F., Maleki, M., Kavousi, K., Ariaeenejad, S., Moosavi- *et al.,* (2021). Upgrading the Enzymatic Hydrolysis of Lignocellulosic Biomass by Immobilization of Metagenome-Derived Novel Halotolerant Cellulase on the Carboxymethyl Cellulose-Based Hydrogel. Cellulose 28 (6), 3485–3503. https://doi.org/10.1007/s10570-021-03727-8

Mutlu-Ingok, A., Kahveci, D., Karbancioglu-Guler, F., and Ozcelik, B. (2022). "Applications of Extremozymes in the Food Industry," in Microbial Extremozymes. Editor M. Kuddus (London, Cambridge, San diego, Oxford Academic Press), 197–206. Available at https//www.sciencedirect.com/science/article/pii/B9780128229453000129. https://doi.org/10.1016/b978-0-12-822945-3.00012-9

Oren, A. (2013). Life at High Salt Concentrations, Intracellular KCl Concentrations, and Acidic Proteomes. Front. Microbiol. 4, 315. https://doi.org/10.3389/fmicb.2013.00315

Parashar, D., and Satyanarayana, T. (2018). An Insight into Ameliorating Production, Catalytic Efficiency, Thermostability and Starch Saccharification of Acid-Stable α-Amylases from Acidophiles. Front. Bioeng. Biotechnol. 6, 125. https://doi.org/10.3389/fbioe.2018.00125

Patel, S., and Saraf, M. (2015). "Perspectives and Application of Halophilic Enzymes," in Halophiles Biodiversity and Sustainable Exploitation. Editors D. K. Maheshwari, and M. Saraf (Cham Springer International Publishing), 403–419. https://doi.org/10.1007/978-3-319-14595-2_15

Petri, R., & Schmidt-Dannert, C. (2004). Dealing with complexity evolutionary engineering and genome shuffling. *Current opinion in biotechnology*, *15*(4), 298-304.

Piroozmand, F., Barati, F., Hosseini, F., Vafaee, R., Sabouri, Z., Arab, S. S., Shadfar, N., (2024). In-silico approaches to investigate enzyme immobilization a comprehensive systematic review. *Physical Chemistry Chemical Physics*.

Puri, M., Singh, N., Mathur, A. S., Gupta, R. P., Barrow, C. J. and Tuli, D. K., (2021). Enzyme Systems of Thermophilic Anaerobic Bacteria for Lignocellulosic Biomass Conversion. Int. J. Biol. Macromolecules 168, 572–590. https://doi.org/10.1016/j.ijbiomac.2020.12.004

Qiu, J., Han, R., and Wang, C. (2021). Microbial Halophilic Lipases A Review. J. Basic Microbiol. 61 (7), 594–602. https://doi.org/10.1002/jobm.202100107

Raddadi, N., Cherif, A., Daffonchio, D., Neifar, M., and Fava, F. (2015). Biotechnological Applications of Extremophiles, Extremozymes and Extremolytes. Appl. Microbiol. Biotechnol. 99 (19), 7907–7913. https://doi.org/10.1007/s00253-015-6874-9

Renganathan, P., Dumorné, K., Córdova, D. C., and Astorga-Eló, M., (2017). Extremozymes A Potential Source for Industrial Applications. J. Microbiol. Biotechnol. 27 (4), 649–659. https://doi.org/10.4014/jmb.1611.11006

Resch B (2013). BCC Research Report. Global Markets for Enymes in Industrial Applications. USA BCC Research LLC. Bio030L.

Research E M (2021). Global Detergent Grade Enzymes Market Report and Forecast 2021-2026. USA Expert Market Research.

Reshmy, R., Madhavan, A., Arun, K. B., Binod, P., Sirohi, R., Tarafdar, A., *et al.,* (2021). Design of Novel Enzyme Biocatalysts for Industrial Bioprocess Harnessing the Power of Protein Engineering, High Throughput Screening and Synthetic Biology. Bioresour. Techn. 325, 124617. https://doi.org/10.1016/j.biortech.2020.124617

Rodrigues, C., Núñez-Gómez, D., Silveira, D. D., Lapolli, F. R., and Lobo-Recio, M. A. (2019). Chitin as a Substrate for the Biostimulation of Sulfate-Reducing

Bacteria in the Treatment of Mine-Impacted Water (MIW). J. Hazard. Mater. 375, 330–338. https://doi.org/10.1016/j.jhazmat.2019.02.086

Salihu, A., and Alam, M. Z. (2015). Solvent Tolerant Lipases A Review. Process Biochem. 50 (1), 86–96. https://doi.org/10.1016/j.procbio.2014.10.019

Santiago, M., Ramírez-Sarmiento, C. A., Zamora, R. A., and Parra, L. P. (2016). Discovery, Molecular Mechanisms, and Industrial Applications of Cold-Active Enzymes. Front. Microbiol. 7, 1408. https://doi.org/10.3389/fmicb.2016.01408

Sarmiento, F., Peralta, R., and Blamey, J. M. (2015). Cold and Hot Extremozymes Industrial Relevance and Current Trends. Front. Bioeng. Biotechnol. 3, 148. https://doi.org/10.3389/fbioe.2015.00148

Satyanarayana, T., Noorwez, S. M., Kumar, S., Rao, J. L. U. M., Ezhilvannan, M., and Kaur, P. (2004). Development of an Ideal Starch Saccharification Process Using Amylolytic Enzymes from Thermophiles. Biochem. Soc. Trans. 32 (2), 276–278. https://doi.org/10.1042/bst0320276

Schröder, C., Burkhardt, C., and Antranikian, G. (2020). What We Learn from Extremophiles. ChemTexts 6 (1), 8. https://doi.org/10.1007/s40828-020-0103-6

Sharma, A., Kawarabayasi, Y., and Satyanarayana, T. (2012). Acidophilic Bacteria and Archaea Acid Stable Biocatalysts and Their Potential Applications. Extremophiles 16 (1), 1–19. https://doi.org/10.1007/s00792-011-0402-3

Sharma, A., Parashar, D., and Satyanarayana, T. (2016). "Acidophilic Microbes Biology and Applications," in Biotechnology of Extremophiles Advances and Challenges. Editor P. H. Rampelotto (Cham Springer International Publishing), 215–241. https://doi.org/10.1007/978-3-319-13521-2_7

Shen, J.-D., Cai, X., Liu, Z.-Q., and Zheng, Y.-G. (2021). Nitrilase a Promising Biocatalyst in Industrial Applications for green Chemistry. Crit. Rev. Biotechnol. 41 (1), 72–93. https://doi.org/10.1080/07388551.2020.1827367

Singh, A., and Singh, A. K. (2017). Haloarchaea worth Exploring for Their Biotechnological Potential. Biotechnol. Lett. 39 (12), 1793–1800. https://doi.org/10.1007/s10529-017-2434-y

Singh, S. P., Kumari, M., Padhi, S., Sharma, S., Phukon, L. C., and Rai, A. K. (2021). Biotechnological Potential of Psychrophilic Microorganisms as the Source of Cold-Active Enzymes in Food Processing Applications. 3 Biotech. 11 (11), 479. https://doi.org/10.1007/s13205-021-03008-y

Skousen, J. G., Ziemkiewicz, P. F., and Mcdonald, L. M. (2019). Acid Mine Drainage Formation, Control and Treatment Approaches and Strategies. Extractive Industries Soc. 6 (1), 241–249. https://doi.org/10.1016/j.exis.2018.09.008

Stom, D. I., Zhdanova, G. O., Kalashnikova, O. B., Bulaev, A. G., Kashevskii, A. V., Kupchinsky, A. B., *et al.,* (2021). Acidophilic Microorganisms Leptospirillum sp., Acidithiobacillus sp., Ferroplasma Sp. As a Cathodic Bioagents in a MFC. Geomicrobiology J. 38 (4), 340–346. https://doi.org/10.1080/01490451.2020.1856980

Sun, Z. Paul, C., Hanefeld, U., Hollmann, F., Qu, G., Yuan, B., (2024). Enzyme engineering for biocatalysis. *Molecular Catalysis*, *555*, 113874.

Sysoev, M., Grötzinger, S. W., Renn, D., Eppinger, J., Rueping, M., and Karan, R. (2021). Bioprospecting of Novel Extremozymes from Prokaryotes—The Advent of Culture-independent Methods. Front. Microbiol. 12, 196. https://doi.org/10.3389/fmicb.2021.630013

Tang, F., Chen, D., Yu, B., Luo, Y., Zheng, P., Mao, X., *et al.,* (2017). Improving the Thermostability of Trichoderma Reesei Xylanase 2 by Introducing Disulfide Bonds. Electron. J. Biotechnol. 26, 52–59. https://doi.org/10.1016/j.ejbt.2017.01.001

Tatta, M., Mewis, K., and Hallam, S. J. (2011). The Art and Design of Functional Metagenomic Screens. Curr. Opin. Biotechnol. 22 (3), 465–472. https://doi.org/10.1016/j.copbio.2011.02.010

Van de Voorde, I., Goiris, K., Syryn, E., Van Den Bussche, C., and Aerts, G. (2014). Evaluation of the Cold-Active Pseudoalteromonas Haloplanktis β-galactosidase Enzyme for Lactose Hydrolysis in Whey Permeate as Primary Step of D-Tagatose Production. Process Biochem. 49 (12), 2134–2140. https://doi.org/10.1016/j.procbio.2014.09.010

Vester, J. K., Glaring, M. A., and Stougaard, P. (2015). Improved Cultivation and Metagenomics as New Tools for Bioprospecting in Cold Environments. Extremophiles 19, 17–29. https://doi.org/10.1007/s00792-014-0704-3

Vieille, C., and Zeikus, G. J. (2001). Hyperthermophilic Enzymes Sources, Uses, and Molecular Mechanisms for Thermostability. Microbiol. Mol. Biol. Rev. 65, 1–43. https://doi.org/10.1128/mmbr.65.1.1-43.2001

Villarino, L., Chordia, S., Alonso-Cotchico, L., Reddem, E., Zhou, Z., Thunnissen, A. M. W.,...& Roelfes, G. (2020). Cofactor binding dynamics influence the catalytic activity and selectivity of an artificial metalloenzyme. *ACS catalysis*, *10*(20), 11783-11790.

Villegas-Plazas, M., Sanabria, J., and Junca, H. (2019). A Composite Taxonomical and Functional Framework of Microbiomes under Acid Mine Drainage Bioremediation Systems. J. Environ. Manage. 251, 109581. https://doi.org/10.1016/j.jenvman.2019.109581

Waeonukul, R., Kosugi, A., Tachaapaikoon, C., Pason, P., Ratanakhanokchai, K., Prawitwong, P., *et al.,* (2012). Efficient Saccharification of Ammonia Soaked rice Straw by Combination of Clostridium Thermocellum Cellulosome and Thermoanaerobacter Brockii β-glucosidase. Bioresour. Techn. 107, 352–357. https://doi.org/10.1016/j.biortech.2011.12.126

Wang, P., Li, L., Liu, Z., Zhang, M., Meng, D., Liu, X., *et al.,* (2020). Insights into the Metabolism and Evolution of the Genus Acidiphilium, a Typical Acidophile in Acid Mine Drainage. mSystems 5 (6), e00867–00820. https://doi.org/10.1128/mSystems.00867-20

Wang, X., Li, S., Yang, X., Yang, S., and Zhu, M., (2012). Technology Prospecting on Enzymes Application, Marketing and Engineering. Comput. Struct. Biotechnol. J. 2 (3), e201209017. https://doi.org/10.5936/csbj.201209017

Wanyonyi, W. C., and Mulaa, F. J. (2020). in Alkaliphiles in Biotechnology. Editors G. Mamo, and B. Mattiasson (Cham Springer International Publishing), 195–220.

Wanyonyi, W. C., Onyari, J. M., Shiundu, P. M., and Mulaa, F. J. (2016). New Eco-Friendly and Clean Method of Processing Hides and Fish Skins into Leather Using Alkaline Protease Enzyme. Patent, (KE/P/2015/2231).

Wiltschi, B., Cernava, T., Dennig, A., Casas, M. G., Geier, M., Gruber, S.,...& Wriessnegger, T. (2020). Enzymes revolutionize the bioproduction of value-added compounds From enzyme discovery to special applications. *Biotechnology advances*, *40*, 107520.

Xue, Y.-P., Liu, Z.-Q., Lu, M.-M., Zhang, X.-H., Cheng, F., Xu, J.-M., *et al.,* (2018). Significant Improvement of the Nitrilase Activity by Semi-rational Protein Engineering and its Application in the Production of Iminodiacetic Acid. Int. J. Biol. Macromolecules 116, 563–571. https://doi.org/10.1016/j.ijbiomac.2018.05.045

Yaşar Yıldız S, Radchenkova N. Exploring Extremophiles from Bulgaria Biodiversity, Biopolymer Synthesis, Functional Properties, Applications. Polymers. 2024; 16(1)69. https://doi.org/10.3390/polym16010069

Zhang, L., Sun, R., Li, Y., Lin, N., Ou, C., Wang, X., *et al.,* (2020). Removal of Heavy Metals Using a Novel Sulfidogenic AMD Treatment System with Sulfur Reduction Configuration, Performance, Critical Parameters and Economic Analysis. Environ. Int. 136, 105457. https://doi.org/10.1016/j.envint.2019.105457

Zhu, D., Adebisi, W. A., Ahmad, F., Sethupathy, S., Danso, B., & Sun, J. (2020). Recent development of extremophilic bacteria and their application in biorefinery. *Frontiers in Bioengineering and Biotechnology*, *8*, 483.

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