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REVIEW

Extremophiles and their enzymatic diversity and biotechnological potential

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Abstract

Extremophiles and their enzymes are explored in this review article, which highlights the peculiar adaptations that these organisms have developed to survive in some of the harshest circumstances on Earth. Microorganisms known as extremophiles challenge our knowledge of habitable environments by living in settings that have extreme characteristics, such as high temperature, salinity, acidity, pressure, alkalinity, radiation, dryness, and many more. The diverse specialized enzymes that these hardy organisms have developed allow them to perform at their best in such harsh circumstances, providing priceless insights into the biochemical and structural adaptations required for survival in hostile environments. An overview of the diversity of extremophiles—which include bacteria, archaea, and even some eukaryotes that are suited to distinct extreme environments is given at the beginning of the article. Following that, it explores the intriguing realm of extremophilic enzymes, emphasizing how exceptionally robust and active they are in harsh environments. The article clarifies the mechanisms behind the remarkable functionality and robustness of extremophile enzymes by thoroughly examining their biochemical characteristics and structural aspects. The review article also examines the various biotechnological uses of extremophile enzymes, including industrial procedures, environmental cleanup, and drug manufacturing.

Keywords Extremophile · rRNA · Biocatalyst · Metagenomics · Thermozymes · Psychrophiles

Introduction

Extremophiles are life forms that flourish in surroundings considered adverse to human existence and reside in environments such as hot springs where temperatures approach the boiling point of water and the deep sea where low temperatures are accompanied by immense water pressure. “Extreme conditions" beyond temperature variations include extreme pH conditions, high pressure, salinity, radiation, and dryness (Fig. 1) [81]. Seasonal temperature changes influence human existence but our adaptation to different temperatures involves avoidance strategies, like building well-insulated houses. This contrasts with extremophiles, which can withstand extreme conditions. Extremophiles have undergone significant adaptations to exist in such extreme conditions, in contrast to humans who employ external measures like insulation instead of modifying somatic cells for temperature challenges. The term "extremophiles" was invented by Mac Elroy in 1974 [73]. (derived from Latin: extremus, Greek: philiā), signifying "extreme-lovers [95].

Most organisms that optimally thrive under such conditions are extremophiles. These organisms not only thrive in extremes but are essentially dependent on them for their existence. In addition to the cellular structural components, enzymes produced by extremophiles help them to adapt effectively under extreme conditions. Enzymes from psychrophiles adapted to cold environments. Extremophiles play a vital role in biotechnology for generative bio-economy. Applications of Biotechnology are already widespread in daily life, such as lactose-free milk production using enzymes like lactose hydrolases. The textile industry employs recombinant enzymes, including cellulases from extremophiles, in processes like stone-washed jeans production. Laundry detergents incorporate enzymes, derived from extremophiles, for efficient stain removal and energy-saving cold-washing processes. Extremophiles, with their unique properties, contribute to drug production, while their cold-adapted enzymes find use in the food and beverage industry for preservation, particularly in milk treatment. Enzymes

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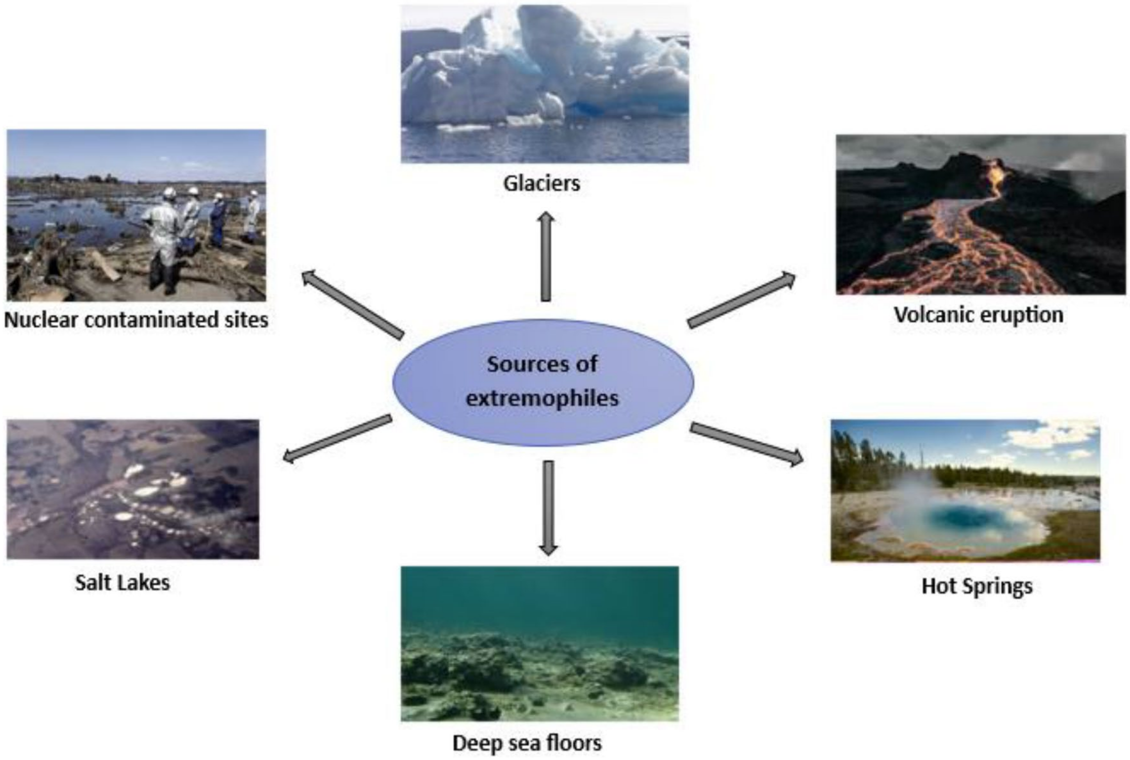

Fig. 1 Sources of extremophiles from different sites

that are functional in cold temperatures and alkaline conditions are extracted from extremophiles, and used in energy-efficient cold-washing processes. Biocatalysts like extremophilic cellulases play a role in color detergents, preventing pilling and preserving textile color.

Overall, extremophiles demonstrate versatility in various industrial applications, advancing biotechnology and contributing to a sustainable bio-economy [95]. These habitats typically exhibit extreme variations in pH, temperature, salinity, desiccation, ionizing radiation, and hydrostatic pressure. Some organisms can tolerate extremes, such as low oxygen levels or contact with metallic substances [48]. The majority of extremophiles are single-celled and fall under the bacteria and archaeal domains [45]. Consider the resilient tardigrade, capable of entering a hibernation state (the 'tun' state) enabling survival across an impressive temperature range from – 272 to 151 °C [106]. It withstands vacuum conditions, ionizing radiation, extreme pressure, and extreme dehydration. Its resilience positions tardigrades as captivating model organisms for space research [104]. Some organisms are crucial for animal nutrition, like certain anaerobes in the mammalian gut and the rumen of ruminants, which exhibit characteristics of extremophiles. There exist such plants that display extremophilic traits, being resistant to high salinity and desiccation.

Types of extremophiles

There are following types of extremophiles such as Thermophiles, hyperthermophiles, and psychrophiles (Fig. 2) stand out for their resilience to extreme temperature fluctuations [49]. Acidophiles and alkaliphiles exhibit high tolerance to environments with extreme pH levels, while halophiles thrive in high-salinity conditions [49]. Barophiles, or piezophiles, thrive under high pressures and xerophiles can withstand low water activity. In the depths of Earth's oceans, a significant adaptation is observed in psychro-piezophilic microbes. These organisms not only thrive in cold temperatures (below 4 °C) but also withstand the high pressures found at depths exceeding 1000 m (exceeding 10 MPa) [33]. Delving into the depths of Earth's oceans reveals the intriguing adaptation of psychro-piezophilic microbes. These organisms not only withstand cold temperatures (below 4 °C) but also thrive under the high pressures found at depths exceeding 1000 m (exceeding 10 MPa) [34].

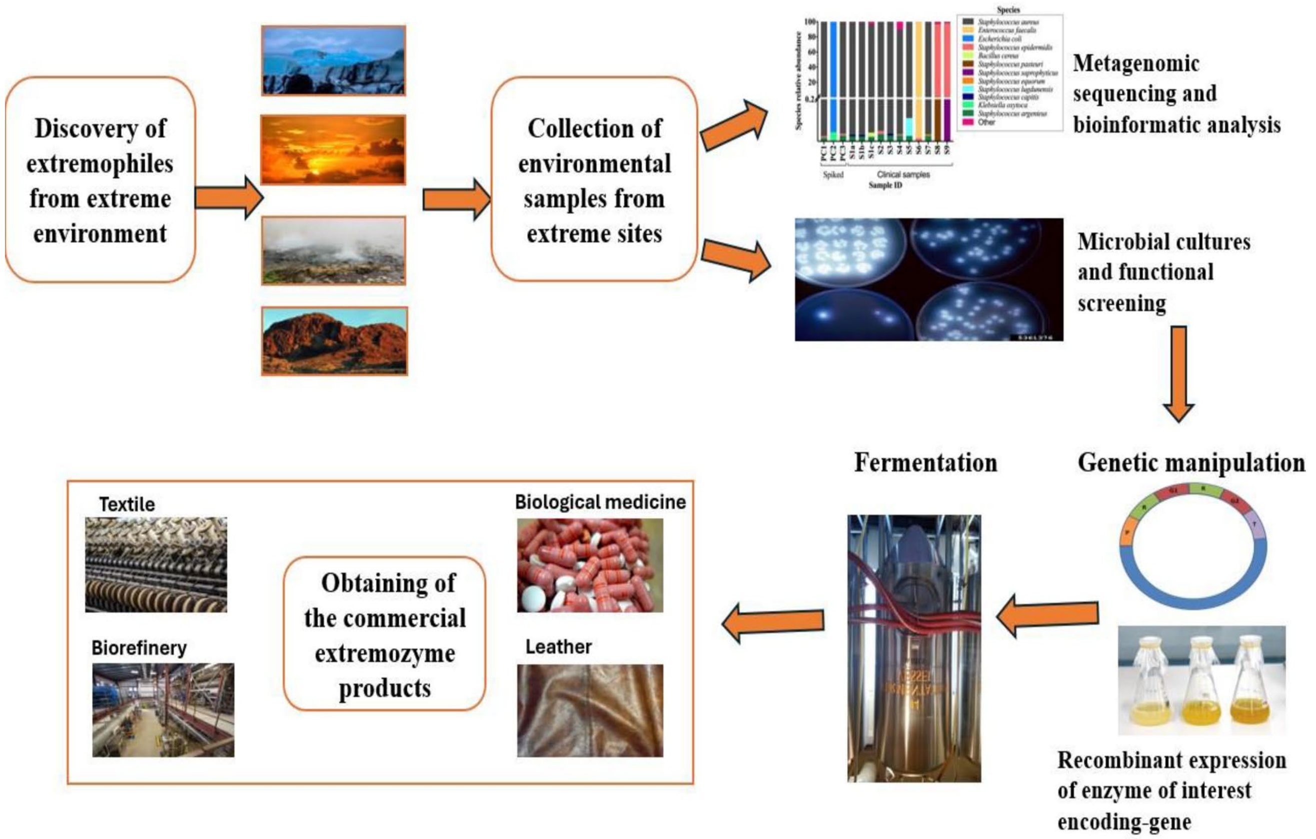

Fig. 2 Schematic view of characterization and classification of extremozymes [109]

Thermophiles

Thermophiles are organisms thriving in high-temperature environmental conditions and exhibiting diverse habitats and characteristics (Table 1). They are categorized into groups: thermophiles, requiring temperatures of 50 °C or higher for optimal growth, and hyperthermophiles, with an optimum growth temperature exceeding 80 °C. Thermophilic extremophiles can survive across abroad temperature range from 0 to 120 °C, and pH values from 0 to 12 making many of them polyextremophiles like thermoalkaliphiles or thermoacidophiles. The isolation of *Thermus aquaticus* in the 1960s marked the discovery of thermophilic microorganisms [49]. Taq DNA polymerase is essential for the PCR was extracted from it.

These organisms are also found in various environments, from domestic settings and industrial processes to natural locals. Hyperthermophiles which thrive near active volcanoes and deep-sea hydrothermal vents tolerate extreme temperatures up to 400 °C [49]. Thermophilic organisms

Table 1 Types of extremophiles modified from [89]

Category	Growth condition	Condition	Isolation ecosystem	Example
Thermopiles	High Temperature	˃ 50–80˚C	Active volcanoes, such as hot springs and deep-sea hydrothermal vents etc	*Thermus aquaticus, Thermoactinomyces vulgaris, T. viridis, T. sacchari and Saccharopolyspora rectivirgula,*
Psychrophile	Low Temperature	< 15˚C	Deep sea, high-elevation regions, polar regions, etc	*Psychromonas ingrahamii, Exiguobacterium sibiricum,*
Halophile	Salinity	[NaCl] ˃0.2M	Dead Sea,Great Salt Lake, Red Sea, and the Eastern Mediterranean	*Halobacterium sp. NRC-1, unicellular β-carotene-rich alga Dunaliellasalina*
Alkaliphile	pH	pH˃9	Rift Valley lakes and soda lakes etc	*Natronococcus occultus, Methanohalophiluszhilinaeae*
Acidophile	PH	pH < 3	Volcanic and geothermal region and Yellowstone	*Picrophilus ashimae, thiobacillus ferrooxidans*

thrive in high temperatures, thanks to the stability of their membrane lipids and a permeability barrier that manages nutrient flow. These organisms exhibit higher G + C content in their DNA, a greater number of charged amino acids on their surfaces, and intramolecular salt bridges, enhancing their high-temperature adaptability [38]. Important hyperthermophiles include *Methanocaldococcus jannaschii* and *Geogemma barossii* (surviving temperatures up to 121 °C). Terrestrial environments with high temperatures have significant thermophile diversity. Metagenomics approaches and 16S rRNA sequencing show the abundance of thermophiles in with *Aquificaceae* and *Thermotogaceae* families classified as hyperthermophiles. The *Sulfolobales*, thermoacidophiles with diverse metabolic activities, include *Pyrococcus furiosus* which is highly studied and utilized in metabolic engineering. The anaerobic thermophiles such as *Caldicellulo disruptor spp*. and *Thermus spp*. contribute to the rich diversity of thermophiles [49].

Psychrophiles

Psychrophiles are organisms adapted to cold environments and are widespread in various habitats including the deep sea, high-elevation regions, polar areas, and sub-glaciers. Psychrophile bacteria demonstrate metabolic activity even in the ice eutectic phase at temperatures as low as – 22 °C. The sub-categories of psychrophiles include 'psychrotroph' and 'psychrotolerant,' based on their growth rates affected by temperature [49]. Cavicchioli argues that such categorization can be misleading. With increasing temperatures, the rate of enzyme-catalyzed reactions typically rises due to the kinetic effect of heat until excessive heat compromises cellular processes. Traditionally, psychrophiles are organisms unable to thrive at temperatures exceeding 20 °C, with optimal growth occurring below 15 °C. Defining optimal conditions for environmental psychrophiles poses challenges, particularly in marine ecosystems, and may not flourish in laboratory conditions leading to misconceptions about their true optimum growth conditions [49]. One key adaptation is the presence of unsaturated fatty acids within their cell membranes, which remain fluid at low temperatures, facilitating the transport of solutes across the membrane. Additionally, Psychrophiles produce cold shock proteins and cryoprotectants, which aid in the synthesis of enzymes adapted to cold conditions. Various factors, including changes in membrane fluidity, decreased levels of transcription and translation, and alterations in ribosome structure, contribute to the adaptation of their cellular machinery at low temperatures. To protect themselves from freezing, psychrophiles accumulate compatible solutes such as glycine betaine, mannitol, and sucrose, or they synthesize antifreeze proteins that inhibit the growth of ice crystals [89].

Halophiles

Halophiles grow in saline environments like the Dead Sea, and Great Salt Lake and also include anoxic brine pools in the Red Sea and Eastern Mediterranean. Oren's definition regarding halophiles is those organisms with maximum growth at NaCl concentrations higher than 0.2 M. Advancements in sequencing and metagenomics approaches have significantly enhanced our understanding of hypersaline environments. They include cold environments, e.g. brine channels in polar ice [49]. Examples such as β-carotene-rich alga *Dunaliella salina*, are widespread halophiles and serve as a model organism for studying photosynthesis. To survive, they employ specific adaptations for osmotic balance, either by synthesizing osmoprotectants (compatible organic solutes) or by balancing internal and external salt concentrations. This involves strategies known as the "low salt-in" (organic solute accumulation) and "high salt-in" (salt accumulation) approaches. Furthermore, halophiles adapt their enzymes with more negatively charged amino acids to facilitate ion exchange and enhance protein solubility [89]. While certain animals, such as the brine shrimp *Artemia*, can withstand high salt concentrations [6].

Acidophiles

Acidophiles are organisms thriving in acidic environments and display diverse characteristics. Environments with pH values below 5 are relatively common on Earth, whereas those with pH values below 3 are less prevalent and are linked to sulfuric acid production. These environments include volcanic and geothermal regions and Yellowstone National Park is the best example [85]. Acidophiles are also present in human-built environments with emerging acidic conditions according to recent studies [59], which have identified diverse acidophilic microbial communities in corroding concrete sewers with pH values of 2–4, influenced by environmental hydrogen sulfide (H2S). These habitats often support a variety of prokaryotic and eukaryotic life, particularly fungi and algae despite their acidity, the first identified acid-tolerant bacterium was *Thiobacillus ferrooxidans* (*Acidithiobacillus ferrooxidans*) in the 1940s, known for catalyzing metal extraction. This chemolithotrophic organism also enhances metal recovery from leachates. Acidophiles include chemolithotrophic species like *Acidithiobacillus ferrooxidans* and *Leptospirillum spp*. have applications in biomining, and efforts are made to engineer them for increased tolerance to fluctuating process conditions [40]. Acidophiles maintain internal pH through mechanisms like membrane impermeability to protons. In archaea, this is facilitated by tetraether lipids in the cell membrane. Adaptations in acidophiles also include reduced membrane pore size, proton efflux mechanisms (antiporters, symporters, and H + ATPases), and the accumulation of buffering amino acids. They may also break down organic acids to release protons and produce chaperones to protect cellular molecules from acid damage [10]. Thermoacidophilic archaea belong to both the Euryarchaeota and Crenarchaeota kingdoms. Significant examples include *Sulfolobus spp*., Thermoplasma, and *Thermogymnomonas* [91].

Alkaliphiles

Microorganisms capable of thriving in alkaline conditions were isolated in the early 1920s [49]. The alkaliphiles thrive at pH at least 9 while their optimal growth occurs around pH 10. The haloalkaliphiles require both alkaline pH and high salinity [49]. Haloalkaliphiles are isolated from extremely alkaline saline environments, such as Rift Valley lakes and soda lakes [49]. Alkaliphiles thriving in high-pH environments maintain the cytoplasmic pH balance and uptake H^+ ions through electrogenic, secondary cation/proton antiprotons to adapt themselves. These microorganisms protect themselves against environmental alkalinity by developing a protective layer outside their cell which is composed of acidic substances like amino acids, teichuronopeptide, and teichuronic acid. Some alkaliphiles produce cytochrome C on their outer membrane to control the movement of protons to enhance the stability of the membrane. Alkaliphile synthesizes phosphoserine aminotransferase which promotes hydrophobic interactions and increases negatively charged amino acids at interfaces to increase resilience in alkaline conditions [30].

Extremozymes and its types

Mesophilic organisms are the source of production of the majority of enzymes despite their benefits these enzymes have restrictions due to their low stability to severe conditions such as high temperatures, pH, and strong ionic strength. However, these organisms can thrive in environments with extreme characteristics—such as temperatures ranging from – 2 to 15 °C or 60–110 °C, high ionic strengths (2–5 M NaCl), and extreme pH levels (< 4, > 9) (Fig. 3). These extremophiles belong to the Archaeal domain, defined through genetic comparisons of 16S rRNA gene sequences, although certain extremophilic bacteria are also identified [16]. Extremophiles yield extremozymes with extreme stability. Due to their stability, they have various applications as biocatalysts and are active under conditions that are considered unsuitable for biological materials [14]. Extremozymes play an advantageous role in enabling eco-friendly processes and producing biodegradable products, hence contributing significantly to the reduction of toxic waste and product footprint [75]. Moreover, a challenge in chemical synthesis lies in the non-tunable physiochemical properties of the desired product, which can be bypassed by using extremozymes.

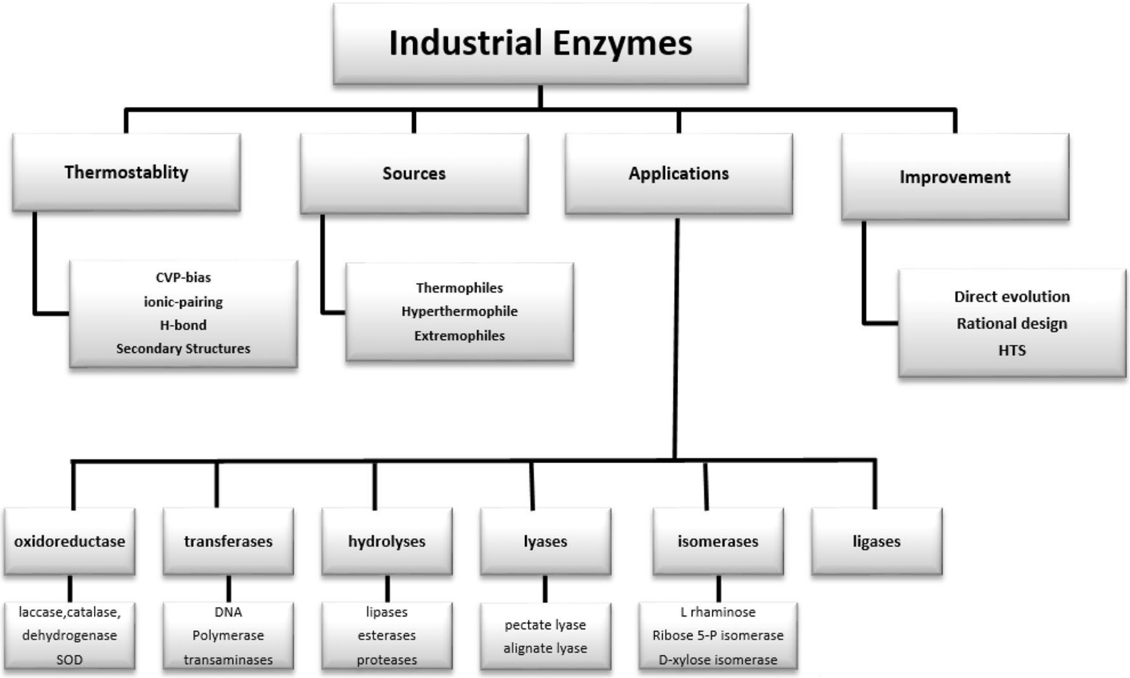

Fig. 3 Industrial applications of the extremozymes

The enzymatic characteristics like enantioselectivity, chemo selectivity, region selectivity, stereoselectivity, and chloroselectivity, have made biocatalysis extremely capable for the production of structure-controlled materials [32, 35]. Although extremozymes yield is lower as compared to mesophilic enzymes, their inherent characteristics like reliability, constancy, cost-effectiveness, and high clarity throughout production and extraction play major roles in utilizing these enzymes for industrial purposes [19, 33]. Esterases / lipases, glycosidases, aldolases, nitrilases /amidases, phosphatases, and racemases are the classes of extremozymes. Psychrophilic enzyme's importance in improving yields of heat-sensitive products is also found, halophilic enzymes, which work in high salt concentration, provide a role for biocatalysis in low-water environments, while thermophilic enzymes are extremely challenging various proteases, detergents, and chaotropic agents. Their resistance often extends to the effects of organic solvents [96].

Thermozymes

Thermozymes are resistant to irretrievable deactivation even at high temperatures, approximately 55 °C. This characteristic makes them new catalysts with major research concerns, especially in biologically tough environments (Table 2). Thermozymes consist of various enzymes like cyclodextrin glucanotransferases, β-Glucosidase, chitinases, prolidases, cellulases, and keratinases [51, 70]. From all extremozymes,

thermophilic enzymes have prevalent viable use, primarily due to their natural stability and best activity at high temperatures [41]. This stability does not come from any specific single trait but rather arises from a combination of specific structural and dynamic qualities. The three-dimensional structure of these enzymes has also been explored, uncovering this fact [27]. The factors responsible for their stability include unique surface charges, core hydrophobicity, exposed thermo-labile amino acids, and improved elasticity due to shorter loops. These attributes are majorly responsible for their stability [108]. The factors influencing thermozyme stability are increased hydrophobicity and compactness, more ionic bonds, reduced length and flexibility of loops, stronger N- and C-termini interactions, and reduced reliance on heat-sensitive amino acids [103]. These are examples of thermozymes such as Proteases, lipases, glucoamylase [57], glucosidases, amylases [54], pollulanases, cellulase, xylanases, esterase, DNA polymerases, and dehydrogenases [7]. Thermozymes have various applications such as detergents, food and feed breaking, fermentation, chitin modifications, health foodstuffs, paper bleaching, stereo-specific reactions (such as transesterification), oxidation reactions, genetic modification, desulfurization of waste gas, and diagnostic reactions [22, 52, 77].

Psychrophilic enzymes

Psychrophiles, organisms living in cold environments, synthesize enzymes to remain permanently active at near-zero temperatures. Psychrozymes have greater structural flexibility compared to their homologous proteins. This elasticity enables these proteins to undertake conformational changes, improving their efficiency in carrying out reactions at lower temperatures. At colder temperatures, enzyme activity, biochemical reaction rates, cellular membrane fluidity, and rates of molecular diffusion decrease. Meanwhile, the solubility of gases and the viscosity of water increases. Moreover,

Table 2 Thermozymes and its characteristics

Enzymes types	Organisms	Expression of enzyme	Temperature	Optimum pH	Applications	References
Xylanase	*Dictyoglomus thermophilum*	Recombinant	75 °C	6.5	Role in research and diagnostic purposes	[42]
	Thermotoga neapolitana	Recombinant	102 °C	5.5–6.0		[15]
	Nonomuraea flexuosa	Recombinant	80 °C	8.0	Food and biofuel production	
Nitrilase	*Pyrococcus abyssi*	Recombinant	80 °C	7.4	Not thermostable so no commercial application is Currently available	[72]
	Bacillus pallidus	native	65 °C	7.6		[64]
	Pyrococcus sp. M24D13	native	90 °C	7.0		[29]
Lipase	*Bacillus sp. HT19*	Recombinant	70 °C	9.0	Role in transesterification, pulp treatment, food Processing, food industry	[8]
	Stenotrophomonas maltophilia Psi1	Recombinant	70 °C	8.0		[46]
	Pyrococcus furiosus	Recombinant	80 °C	7.0		
GDH	*Pyrococcus furiosus*	Native	85 °C	N/A	Has application in research and diagnostic purposes, cheese development	[29]
	Thermococcus litoralis	Native	95 °C	8.0		[8]
	Bacillus sp.	Native	65 °C	8.0		[15]
Laccase	*Aquifex aeolicus*	Recombinant	75 °C	7.0	Has applications in textile and gum industry, micro biocides production, in phenolic compounds	[64]
	Bacillus sp. PC-3	Native	60 °C	7.0		[46]
	Thermobacullum terrenum	Recombinant	92 °C	5.5		[15]

hydrogen bonds are strengthened, leading to protein denaturation [24, 98].

High activity at lesser temperatures and reduced stability are the inherent characteristics of psychrophilic enzymes that offer numerous advantages across different applications. These enzymes are already employed in the industrial enzyme markets consisting of food, feed, and technical sectors. Furthermore, they are expanded in pharmaceutical and fine chemical synthesis areas [11]. Some of the commercial success of these enzymes consists of cold-adapted lipase role in various pharmaceutical, cosmetics, and flavor compounds for organic synthesis [55]. Xylanases for improving bread quality [11], various hydrolases in detergents [90], and other enzymes (like alkaline phosphate, nuclease, and uracil-DNA N-glycosylase) have applications in molecular biology [11].

Halophilic enzymes

Halophilic enzymes exhibit higher activity at sodium concentrations exceeding 1.5 M, mainly derived from sodium chloride, but sodium carbonate/bicarbonate can also be a sources. Moreover, these enzymes also withstand high potassium concentration. Acidic isoelectric point and an abundance of aspartate and glutamate residues on the enzyme surface make them adaptive to high salt concentrations [79]. These enzymes are normally very unstable in environments with low salt concentrations. The first enzyme to be purified was malate dehydrogenase. Higher salt concentration affects protein conformational stability. So, these enzymes have developed a specific mechanism to tolerate such conditions [63]. The activity of the enzyme is also dependent on salt nature. For example, few enzymes show higher activity in the presence of KCl than NaCl.3-hydroxy-3-methylglutaryl-coenzyme activities enhanced with higher KCl concentration and decrease with higher NaCl concentration [92]. The genes were cloned and expressed in Haloferax volcanii to generate recombinant proteins. These proteins will have enhanced functional properties such as tolerance to NaCl, temperature, pH, and stability in the presence of chemical additives. This helps in improving our comprehension of the characteristics of halophilic protease. In a recent study, a gene responsible for encoding the extracellular protease Hly176B from the haloarchaeon Haloarchaeobius sp. FL176 was cloned and expressed in E. coli. The expressed enzyme exhibited good tolerance to certain metal ions, surfactants, and organic solvents and exerts its optimal enzyme activities at 40 °C, pH 8.0, and 0.5 M NaCl [108]. Halophilic enzymes have various applications in biotechnology, bioremediation, and biopharmaceuticals due to their stability in low-water conditions and can be scaled up in processes with organic solvents and brine. However, these cannot be utilized at a large scale behind other extremozymes due to mechanical fragility and lysis in low salinity Under low salinity halophilic proteins tend to misfold and aggregate [43]. So, large-scale heterologous expression of halophilic proteins can also be challenging [68]. Proteases and amylases adapted to elevated saline concentrations have been included in detergent composition [69]. Xylanases, cellulases, and laccases can be utilized in biofuels production and lignocellulosic pretreatment involving organic solvents or ionic liquids [69, 71]. Short-chain and polyunsaturated fatty acids are major components in nutraceuticals and dietary supplements are synthesized by utilizing halophilic lipases and esterases [88]. (Some of the halophilic enzymes are discussed in Table 3):

Acidophilic enzymes

These enzymes have the most favorable activity at pH < 5. At pH below 3, protonation of surface amino acids occurs,

Table 3 Halophilic enzymes and their applications

Enzymes types	Organisms	Temperature	pH	Application	[39]
α-amylase	*Klebsiella pneumoniae (CC ICC no. 10018)*	45 °C	6.5	has application in the processing of food and biosynthesis	[39]
α-amylase	*Klebsiella pneumoniae (CC ICC no. 10018*	50 °C	7.0	has application in the processing of food and biosynthesis	[39]
Chitinase A	*Vibrio carchariae*	37 °C	5.5	used in the pharma and food industries	[39]
cyclodextrin glycosyltransferase	*Bacillus circulans STB01*	50 °C	6.5	has a role in the pharmaceutical industry	[39]
Lipase	*Haloferax lucentensis GUBF2*	70 °C	6	has a role in food industry, pharmaceutical industry, cosmetic and biofuel production	[39]
Lipase	*Haloferax mediterranei*	60 °C	7	has a role in food, textile, and pharmaceutical industry,	[3]
Esterase	*Halobacterium sp. NRC-1*	80 °C	6	has applications in bioremediation, detergent industry, and biocatalysis	[21]

creating less negative charge and stable proteins. At higher pH, other amino acids will be deprotonated, causing unfolding due to the repulsion of excess negative groups [82]. Acidophiles generally share other extremophilic environment traits such as thermophilicity, halophilicity, or heavy-metal resistance. Enzymes stable in acidic conditions have various applications in starch, baking, fruit juice processing, animal feed, and pharmaceutical industries, with several already being commercialized. Acidophiles play a role in the bioleaching of metals from low-grade ores [97]. Amylases are one of the vital enzyme groups, widely utilized in converting starch into sugar syrups, and they play an essential role in both the bread and textile industries. An Acidophilic *Stenotrophomonas maltophilia* was found to be functional in the biodegradation of polycyclic aromatic hydrocarbons [26].

Alkaliphilic enzymes

Alkaliphilic enzymes have the best activity at a pH level below 9. Because of the abundance of arginine and histidine residues and lower glutamate residues, they have more alkaline isoelectric points [36]. Alkaline lipases, proteases, amylases, and cellulases exhibit stability in the alkaline detergents usually used in laundry and dishwashers [105]. *Bacillus sp.* produces alkaline cellulase which is reported as the first alkaliphilic enzyme. Alkaliphilic proteases have also been useful in the processing of leather. Moreover, alkaliphilic xylanase has a role in the bleaching method of Kraft and soda pulps devoid of adjusting the pH, which enhances economic viability [65].

Applications of extremozymes

Biotechnology is the field of biology in which we use microorganisms for the benefit of mankind. Scientists have discovered some microorganisms that can survive under harsh conditions. Some of the microorganisms develop the metabolic system that enables them to deal with harsh environments. Such types of microorganisms are typically characterized as extremophiles and the protein or enzyme components produced by them are referred to as extremozymes. Their ability to thrive in harsh environments makes them very useful in industrial applications [23]. Enzymes released by extremophilic microorganisms have many industrial applications as compared to those from mesophilic organisms. Extremozymes possess several properties such as they are thermostable, able to live in salty environments, and also can live in extreme cold environments. They also play a main role in the development of the economy as well as the reason for great advances in the field of research [31]. These applications result in a wide range of resistant molecules that undergo several industrial applications. These molecules are heat resistant, salt resistant, alkali resistant, acid resistant, etc. [31]. Lignocellulolytic extremozymes, a diverse group of extracellular proteins that include ligninolytic and cellulolytic enzymes, are crucial for the development of biofuels that could replace fossil fuels. These enzymes aid in breaking down lignin, making the production of biofuels feasible. Thermostable lignin peroxidase is used to remove dyes, lignin by-products, and pollutants from the environment. In the textile industry, thermostable laccases are utilized for bleaching processes. Over recent decades, the biological conversion of biomass into biofuel has become more economically viable and environmentally friendly compared to traditional chemical or thermal methods, leading to a growing focus on enzymatic solutions despite the current reliance on these older technologies [94].

Industrial and biotechnological applications of extremozymes:

Different types of extremozymes play a role in a wide range of industrial applications. Enzymes from thermophilic microorganisms, Psychrophiles, alkalophiles, and Acidophiles show a wide variety of applications (Fig. 2). Thermophiles have been studied by scientists for the last decades. Many enzymes such as chitinases, cellulase, amylases, peptases, xylanases, pectinases, and lipases are characterized as thermozymes. Extremozymes not only possess electrostatic interactions but also physical properties to be active at high temperatures [31]. Protease hydrolyzes protein into amino acids and peptides. Many of the thermostable enzymes are of crucial importance in many industrial processes but proteases are sometimes.

not suitable to be used at high temperatures because they undergo autolysis such as self-digestion of proteases. The major thermostable protease that is currently in use is thermolysin which plays an important role in the production of dipeptides phenyl methyl esters that are also used as precursors in the production of aspartame. Some proteases also play an important role in the cleaning of DNA before amplification in the PCR [18]. Most of the other applications of proteases are their use in the making of detergents. Some of the other thermozymes play a role in the backward reaction involved in the breakdown of oligosaccharides. Lipases and esterase enzymes play a crucial role in several biotechnological applications such as textile processing, waste treatment, and as additives in detergents [94].

There are over 3,000 identified enzymes, with approximately 65% finding applications in industries such as detergent, textile, pulp, paper, and starch, while 25% are utilized in the food processing sector. Amylase, for instance, is being integrated into biochemical processes that operate at elevated temperatures, offering a cost-effective alternative to high-cost reactants. Furthermore, extremozymes also exhibit reactions at high rates, possess the capability to break down or eliminate xenobiotics (foreign chemical compounds to a specific biological system), and can modulate the hyperaccumulation of substances like heavy metals, pollutants, and radionuclides [31]. Peizophilic enzymes show application in industries as well as in the production of food products. These enzymes show high effectiveness in detergent industries. Acidophilic enzymes play role in the bioleaching of low-grade ores. They are also used in food processing industries; fruit juice making, starch as well as baking industries. They also have significant applications in bioremediation as well as in bioaccumulation [97]. Many of the microorganisms can survive under the presence of high salt that are known as halophiles. These halophiles are characterized into three types depending on the optimal salt concentration such as extreme, moderate, and slightly halophilic enzymes. These enzymes play a role in biodiesel production, polyunsaturated fatty acids, and food. Their application in industries as well as in biotechnology is not restricted to their optimal ability to survive under high salt but also that they can tolerate elevated temperatures as well as they can survive in organic solvents. Halophilic enzymes play a crucial role in various stability and solubility mechanisms, particularly in the presence of elevated concentrations of sodium chloride and potassium chloride. These enzymes also exhibit interactions with organic solvents and contribute to the three-dimensional structure of the enzyme. The significance of halophilic enzymes lies in their activities, especially in creating optimal culture conditions for enzymatic activity, aligning with their specific salt requirements to avoid enzyme inhibition [31]. Extremozymes also used in the degradation of lignin, cellulose, and hemicelluloses that are used in the production of biofuel.

Research related to extremozymes increasing day by day because of their wide range of applications. Some of the enzymes that are produced by Psychrophiles can form catalytic action at low temperatures due to their structural flexibility. The application of extremozymes in white, red, and blue biotechnology is of great importance for the benefit of mankind (Table 4). White or industrial biotechnology addresses environmental and economic challenges linked to rising energy demands and the escalating costs of products made from petroleum. It allows enzymes to undergo the conversion of renewable resources like waste and byproducts into valuable substances such as fine chemicals, biopolymers, biomaterials, and biofuel. Grey or environmental biotechnology utilizes biocatalysts for the remediation of polluted sites, while red or medical/pharmaceutical biotechnology allows microorganisms for pharmaceutical production. Currently, a majority of commercially available enzymes are sourced from bacteria or fungi, with only a limited number of origins from archaea. These archaea-derived enzymes are predominantly produced by mesophiles that are mostly masked by harsh conditions present in many of the processes that are performed in industries [86].

Table 4 Applications of extremozymes (Modified from [31])

Types	Conditions for growth	Source	Enzymes	Applications
Acidophile	The optimum PH for growth is at or below 3–4	Volcanic spring acid mine range, USA	Amylase, glucoamylase protease	Processing of starch, production of single cell protein from shellfish waste. In livestock feed to improve digestibility
Alkaliphile	Optimum PH for growth is above 10	Soda Lakes, Utah USA	Proteases cellulase	Detergents, feeds, and foods The production of wine and beer through fermentation, the baking of bread, and the processing of fruit juice
Halophiles	Organisms require approximately 1 M salt for their growth	Salt lakes	Protease Dehydrogenases	Peptide synthesis As a biocatalyst in organic media as well as asymmetric chemical synthesis
Hypolith	Organisms line in rocks inside cold deserts	Desert and the islands of Cornwallis and Devon	NI	NI
Piezophile	Organisms require hydrostatic pressure of 40 MPa or higher for their growth	Deep ocean, Antarctic ice	Piezophilic cellulase, DNA polymerase	Production of antibiotics Processing of food products
Psychrophiles	An organism that thrives best at temperatures around or below 10 °C and has a maximum growth temperature of 20 °C	Antarctic ice, ice, snow, and arctic ocean	Protease Amylase Cellulase Dehydrogenases	In food and detergent production Bakery products and detergents Textiles, detergents and feed Biosensors

Thermostable DNA polymerases find a large number of applications in various molecular biology techniques such as DNA amplification, sequencing, and labeling. The use of archaeal DNA polymerases is particularly advantageous in polymerase chain reaction (PCR) processes due to their high fidelity, which helps minimize errors in amplification in the resulting PCR products. On the other hand, heat-resistant DNA ligases play a crucial role in the formation of primer in sequence and function as key enzymes in ligase detection reaction (LDR) or ligase chain reaction (LCR) processes. Their catalytic activity is especially valuable for nick-joining reactions at elevated temperatures, typically ranging from 90 to 100 degrees Celsius. This high-temperature capability is essential for ensuring efficient and specific DNA strand joining, particularly in applications that require stability and accuracy under extreme thermal conditions [20].

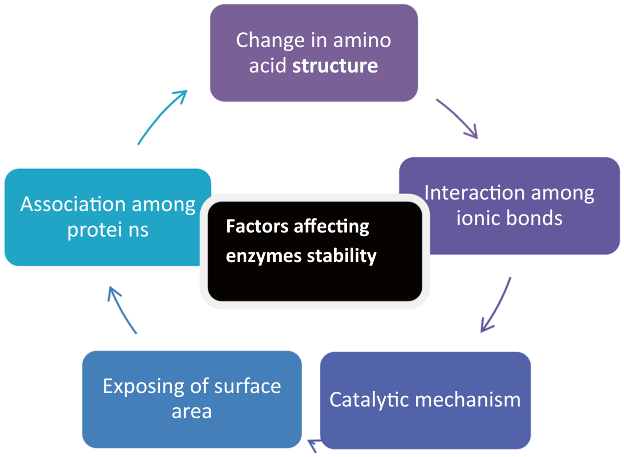

Fig. 4 Factors affecting the stability of extremozymes

Applications of extremozymes in biorefineries

Bioethanol, often considered a leading alternative or supplement to gasoline, is widely used in China, where many gas stations incorporate it as an additive. This type of biofuel, known as "second-generation bioethanol," is produced from lignocellulosic biomass through a four-step process: initial biomass pretreatment, enzymatic hydrolysis, fermentation, and finally, distillation. Utilizing lignocellulosic materials for bioethanol production is environmentally friendly and supports sustainable development. However, this second-generation technology still faces challenges, particularly regarding high production costs, and requires further advancements to become more cost-effective. Extremophiles, with their innate resilience to the harsh conditions present in bioethanol production, offer significant advantages over common terrestrial microorganisms. Specifically, thermophilic microorganisms and their enzymes show considerable promise in efficiently converting lignocellulose into bioethanol [108].

Factors affecting the stability of extremozymes

The stability of extremozymes in harsh environments is influenced by a variety of factors (Fig. 4). Here are some key factors that affect extremozymes' stability.

- Change in structure of amino acid

Amino acids that are prone to instability, such as cysteine, asparagines, and aspartic acid, can experience rapid covalent changes under harsh conditions like high temperature, pressure, and pH, resulting in protein denaturation. By positioning these sensitive amino acids within the protein's hydrophobic core, the overall stability of the protein can be enhanced [28].

- Interaction among ionic bonds

Proteins found in extremophiles frequently feature ion pairs that are crucial for their stabilization, particularly in proteins from hyperthermophiles where the hydrophobic effect is less pronounced. Examination of the crystal structures of these extremophilic proteins has shown extensive networks of ionic interactions. These ionic interactions are effective over longer distances compared to hydrophobic interactions [28].

- Association among proteins

Under extreme conditions, Oligomeric proteins can denature, initially by the separation of their subunits, which is then followed by the irreversible denaturation of each monomer. Extremozymes, enzymes from extremophiles, often possess more intricate Oligomeric configurations compared to similar enzymes from mesophilic organisms, which may enhance their stability [28].

- Surface area exposed by solvents

Extremozymes often have unique properties at the solvent-exposed surface area that contribute to increased stability. For example, halophilic enzymes may have a highly negative surface charge that enhances solubility and flexibility at high salt concentrations [28].

- Catalytic mechanism

Some of the scientists suggest that catalytic processes are typically similar between mesophilic enzymes and their extremophilic equivalents. For example, an analysis comparing recombinant β-glucosidases from the hyperthermophile *P. furiosus* and the mesophilic Agrobacterium *faecalis* revealed comparable features in aspects like substrate specificity, pH dependency, kinetic isotope effects, and linear free energy relationships [28].

Novel developments in extremozymes identification using multi-omics methods

Omics' is the term that encompasses meta-genomics, meta-transcriptomics, meta-proteomics, and metabolomics, and has been crucial in the recent discovery of novel, more active enzymes for biorefinery. Furthermore, as highlighted by [4], bioinformatics and algorithms are crucial in the design of in-situ type mutagenesis and genetic shuffling strategies aimed at enhancing protein stability for prospective commercial bio-refinery applications. These methods are highly beneficial for the production of tolerant extremozymes in biotechnology. The majority of microscopic microbes found in various places are difficult to culture, and the target enzyme cannot be obtained using the available procedures from the extensive pool of natural enzyme resources. As a consequence, "omics" technologies have given scientists a potent technique for finding novel enzymes in nature [52]. In the last twenty years Our understanding has advanced significantly throughout the years about extremophile survival mechanisms in harsh environments because to whole genome sequencing technologies. It encourages comprehension and application of the transport mechanism, enzymatic mechanism, metabolic pathways, and substrate biotransformation of extremophiles. Understanding the mechanism of robust enzymes as well as details regarding the extreme enzymes' three-dimensional structure are made possible by genomics research. The lignin breakdown routes have been elucidated, and a minimum of five lignin metabolic processes were postulated by the annotation and analysis of *Comamonas SP 35* genomic data combined with GC–MS metabolic studies [109]. It has several uses in terms of genetically engineering chassis cells to generate highly valuable lignin. The identification of the synthetase system of naturally occurring PHAs and the metabolic mechanism of poisonous aromatic compounds were made possible by a bioinformatics examination of *Pseudomonas sp.* MPC6, a psychrotolerant extremophile, over its whole genome. It was believed that *Pseudomonas sp*. MPC6 would be a suitable choice to utilize as a biopolymer plant [78]. A combination of evolutionary-driven methods (e.g., instructed evolution, artificial biology) with computational and structure-based analysis has greatly improved the identification of novel extreme enzymes with high industrial application potential in recent years [1, 47].

Sequence-based techniques like Meta-transcriptomics, meta-proteomics, and metagenomics have significantly boosted the finding of novel illogical techniques due to the emergence of a substantial public genetic information database [22, 74]. After extensive analysis, the genomes of the *Anaerobranca*,*Pyrococcus*, *Thermoplasma*,*Thermotoga*, and *Thermus* genera produce enzymes appropriate for commercial usage in biorefineries. With more than 120 thermophilic microorganisms, their genome data is accessible in public databases [25]. Furthermore, “omics" technology has the potential to overcome the research bottleneck concerning bacteria that are not culturable and discover new, highly active enzymes and metabolic routes that can be used in biorefineries, the central processing facilities for sustainable biofuel generation. study of an organism's entire ribonucleic acid transcript content using transcriptomics methods, encompassing both coding and non-coding RNAs, within a cell. These techniques can provide information on gene functions and genome-wide information, which may reveal molecular mechanisms associated to specific biological processes. In the post-genomic era, RNA sequencing technology has become one of the major areas of concern and has expanded researchers' understanding of RNA-based gene regulation. As stated by [50, 67], A deeper comprehension of the regulatory and functional networks of microorganisms adapting to their living conditions can be gained through transcriptome analysis of extremophiles, which can provide light on the frequent shifts in the expression of genes in severe environments. According to a multi-omics analysis*, Thermus filiformis* uses various strategies for thermal adaptation, such as oxidative stress caused by high temperatures, it suppresses the cycle of tricarboxylic acid and glycolysis genes; the pentose phosphate route, also known as the glycolysis pathway, is the main mechanism by which the breakdown of glucose is accomplished and accumulation of antioxidant enzymes related to scavenging free radicals and oxaloacetic acid [66]. During the biomass pretreatment process, it was discovered that over 14 different types of supplementary oxidoreductases and glycosyl hydrolase enzymes genes—12 of which came from intestinal microorganisms—may have a role in the breakdown of the lignin elements and associated redox networks, according to transcriptome analysis of termites' digestive systems. These results offered novel perspectives on biorefinery and implied that termites had a distinct digestive system [39]. The field of transcriptional manipulation has been demonstrated in numerous research to be an effective technique for enhancing recombinant microorganisms. Higher enzyme activity will result from it, and wild-type strains' pH, ion demand, and product selectivity will all change [44]. Modified α-amylase has led to improved industrial applications, as shown by the halophilic *thermotolerant Bacillus strains cu-48* [12].

Recognition of extremophiles' remarkable resistance to harsh climatic conditions has drawn increasing interest from proteome analyses of these organisms. Proteome technology has made it possible to learn enough about extremophile survival mechanisms and has encouraged the application of extremophiles in the biofuels area [13]. Using lignin as a substrate, a proteome analysis of *Bacillus ligniniphilus L1* showed that over 30 different types of upregulated enzymes, including ferredoxin, cytochrome oxidase, oxidoreductase, and peroxiredoxin, are responsible for the breakdown of lignin. Additionally, a massive number of environmental response factors were discovered, such as the DNA integrity scanning protein repressor LexA. the transcriptional regulator that effectively controls lignin's use as a substrate, the primary glycolytic gene regulator, and the HPr-like protein that catabolite represses [109]. *Oleispira antarctica RB-8*, an obligate hydrocarbon-degrading psychrophile, has been discovered to express an n-alkane oxidative route that includes a fatty acid desaturase, a fatty acid ligase, two alcoholic dehydrogenases, two aldehyde dehydrating enzymes, and two alkane monooxygenases, according toa shotgun proteomics study using LC–MS/MS. Proteomics is more beneficial for their commercial application since it can better comprehend the features of the enzyme than the current approaches. Proteomics and gene-recombinant protein modification, for instance, are used to enhance the properties of enzymes, including solvent tolerance, pH, specificity, heat stability, and greater activity [5, 100]. Utilizing the data from transcriptome, proteomics, and genome technologies, it is possible to find novel targets and apply metabolic engineering to produce strains for the biorefinery sector. Thus, multi-omics technologies can be used to study metabolic regulators and pathways in detail to enhance strain performance and productivity [17, 37].

Extremophiles' use of synthetic biology for biorefinery

Over the last 20 years, synthetic biology has grown to become a significant field of multidisciplinary study that combines metabolic and genetic manipulation to modify genetic circuits to manufacture required molecules utilizing *E. Coli* and *S. cerevisiae*. Since then, *E. coli* and S. *cerevisiae have* been used in the majority of synthetic biology research [2]. But to create strains that are significant for the industry, scientists have recently started using a variety of extremophiles, such as *Deinococcus* species, *Geobacillus* species, *Halomonas* species, *Pyrococcus* species, *Thermococcus* species, *Thermus* species, etc. Through the use of synthetic genetic circuits, synthetic biology techniques can be useful in engineering organisms to thrive in altered or necessary environments and to generate a variety of bioactive compounds with significant industrial and pharmaceutical applications [58]. The production of artemisinic acid, a precursor to artemisinin, in *S. cerevisiae* is one of the advances in synthetic biology that increases production and reduces the costs of artemisinin [80]. The production of branched-chain higher alcohols from renewable resources via a synthetic non-fermentative genetic circuit in *E. coli* is one important way that synthetic biology has advanced the field of biofuel and bioenergy [9]. Despite defined advancements in lignin valorization, several bottleneck situations have emerged, which can be attributed to lignin's heterogeneous traits and high recalcitrance [62]. The three-step process of high-value bioconversion of lignin entails depolymerization, degradation of aromatics, and synthesis of the desired final product. Thus, system-level discovery of processes and pathways using combined (meta)genomics, (meta) proteomics, (meta)transcriptomics, (meta) secretomes, and metabolomics techniques have enabled the development of lignin conversion synthetic genetic circuits. Furthermore, strains of ligninolytic bacteria that have been metabolically modified serve as suitable hosts for highly valuable lignin fermentation. Furthermore, strains that can grow effectively when there are large concentrations of aromatic chemicals provoked by depolymerization of lignin —which frequently hinders the process of growth of microorganisms—can be developed through metabolic engineering and standard adaption approaches. Lin and colleagues have developed a strain of *Pseudomonas putida* (A514) that can produce PHA using insoluble kraft lignin as the sole carbon source. This strain behaves using peroxidase-based depolymerization, the breakdown of aromatic residues, and the rechanneling of β-oxidation products [60]. Researchers have been drawn to employ the thermoacidophilic Sulfolobus species as one of the most promising sites for metabolic manipulation and artificial biology due to its metabolic diversity and stability [93]. It is possible to modify regulatory systems and reroute metabolic processes to increase manufacturing in *Sulfolobus acidocaldarius* and *Sulfolobus islandicus* through entire genome sequencing, marker-free in-frame deletion mutants, and homologous expression of protein through ectopic integration of foreign genes [104]. With the help of a synthetic genetic circuit that includes superoxide dismutase and heat shock proteinHB8 and MB4 of *Thermoanaerobacter tengcongensis*, and *Thermus* thermophiles, respectively, the *S. cerevisiaeINVSc1*strain thrives at 42°C and yields significant more ethanol in comparison to the wild type [99]. It is noteworthy that alcohol dehydrogenases that are extremophiles are highly efficient catalysts for the generation of butanol in systems without cells [53]. According to [107], alkali lignin can be chemically depolymerized into vanillin and syringate using metabolically engineered *E. coli strains*, which can subsequently be bioconverted into cis, cis-muconic acid, and pyrogallol, respectively. Making Use of hydrothermally depolymerized lignin aromatics as a feedstock, to produce cis and cis-muconic acid [56], metabolically modified *P. putida KT2440 MA-9*, which is subsequently polymerized into nylon after being hydrogenated to create adipic acid. It has been demonstrated that removing vanillin dehydrogenase from commercially significant strains increases the amount of vanillin that can be produced from lignocellulose biomass [61]. As an alternative, the thermoregulated-genetic structure can be employed, which involves the two vital enzymes expressed heterologously in *E. coli*, like as enoyl-CoA hydratase/aldolase (Ech) and feruloylCoA synthetase (Fcs) of the thermophilic *actinomycete Amycolatopsis* thermoflava N1165. With the use of ferulic acid as a source, this system enables E. coli to create vanillyl alcohol at 30◦C. Vanillin is subsequently produced at 50◦C by the enzymatic actions of Fcs and Ech from vanillyl alcohol [76].

Research needs and future perspective

Due to their distinct metabolic routes and protein frameworks that enable them to mineralize and decolorize the dye under particular ecological settings, microbes are now used more frequently in the dye degradation process [31]. Comprehensive research on the enzymes, genes, and metabolic pathways underlying extremophile decolorization is currently required for wastewater treatment applications. It should be investigated whether it is possible to locate, isolate, clone, and transfer the genes encoding the degrading enzymes to find putative super degrading microbes from extremophiles. Since bacterial biomass offers sources of both carbon and nitrogen, numerous investigations have shown that it is a suitable biosorbent material for textile dye bioremediation [69, 83]. As a result, hybrid adsorbent systems have low running costs and great dye removal efficiency. The processes underlying the connection between colors and the biomass of living and dead cells are intricate, nevertheless. Furthermore, the effects of final biomass disposal and dye effluents following absorption remain unchecked and untreated.

With the removal of hemicellulose, cellulose, and lignin, compost is thought to be a great substrate supply for the fermentation process that produces polyenzymes [83, 87]. As a result, bacteria that produce multiple enzymes could be suitable candidates for different waste degradation processes when they are polypolluted. It's interesting to keep in mind that most bacterial strains that exhibit robust stress adaptation have been studied as potential multiple enzyme producers in recent studies [83, 84]. As a consequence, additional useful study on the ecology, taxonomy, and molecular characteristics of these unique bacteria has to be done to comprehend the relationship between their high severe tolerance and their ability to produce enzymes during the toxicant degradation process. In the presence or absence of oxygen, dyes can undergo biological deterioration. While the first step of azo-linkage cleavage primarily involves the breakdown of azo dyes in anaerobic conditions, bacteria may almost solely destroy the aromatic amines generated in the second step in an aerobic environment. As a result, combining the anaerobic and aerobic phases of a treatment process can increase process efficiency and speed [101]. Extremolytes are additional significant compounds that need to be taken into account. These are tiny organic compounds that build up inside cells and are either produced by extremophilic bacteria or ingested by them. Through the formation and stabilization of protective water layers, they are essential in preserving the macromolecules and cell structures of extremophiles [102]. Furthermore, additional research should be done on the compositional and functional alterations of the biosurfactants released by extremophiles during dye degradation. Extremophiles should be viewed as a wonderful alternative to many of the present conventional approaches because they have an extensive number of superior applications and potential applications. It should be pursued as a separate and independent step to continue developing novel isolation techniques to study fastidious or unculturable bacteria. These techniques include designing the nutritional components of culture media, equipment, and growth conditions based on features, adaptation techniques, and metabolic pathways. Nevertheless, because of the alteration in their intricate metabolic pathways under harsh conditions, research on specific physiological characteristics and molecular characteristics of these polyenzyme-producing extremophiles is more difficult. It is important to confirm the long-term impacts of utilizing extremophilic bacteria and their byproducts with every initial dye effluent intake.

Conclusion

Extremophiles, organisms thriving in extreme conditions, and their enzymes, known as extremozymes, are pivotal in industrial biotechnology, revolutionizing processes across multiple sectors. From biofuel production to pharmaceuticals and food manufacturing, extremozymes enhance efficiency, quality, and sustainability. They also play a crucial role in environmental cleanup through bioremediation efforts, breaking down pollutants and toxins. In agriculture, extremozymes boost soil fertility and plant growth, reducing reliance on chemical inputs. Beyond traditional industries, extremozymes contribute to nanotechnology advancements, enabling the creation of customizable nanomaterials.

Looking forward, extremophiles offer potential solutions for space exploration bioremediation. Overall, extremophiles and their enzymes are essential agents driving innovation, sustainability, and exploration across various fields.

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