

FUTURISTIC TRENDS OF EXTREMOPHILIC BACTERIA IN INDUSTRIAL BIOTECHNOLOGY

Abstract

Extreme microorganisms, which thrive in harsh environmental conditions, have gained significant attention in the realm of industrial biotechnology. This chapter presents an overview of emerging trends concerning extreme microorganisms in this domain. It explores various types of extremophiles, such as thermophiles, psychrophiles, acidophiles, alkaliphiles, halophiles, and xerophiles, elucidating their characteristics and potential applications in industrial biotechnology. Moreover, it delves into the diverse applications of extremophiles, including enzyme production, biocatalysis, biofuel production, bioremediation, pharmaceutical, and chemical production, demonstrating how they are poised to revolutionize these sectors. Additionally, it discusses the challenges and opportunities associated with extremophiles, such as cultivation techniques, genetic engineering, and ethical considerations. In conclusion, this chapter underscores the importance of further research and development in the realm of extreme microorganisms, emphasizing their potential to revolutionize industrial biotechnology and shape the future of sustainable and efficient processes..

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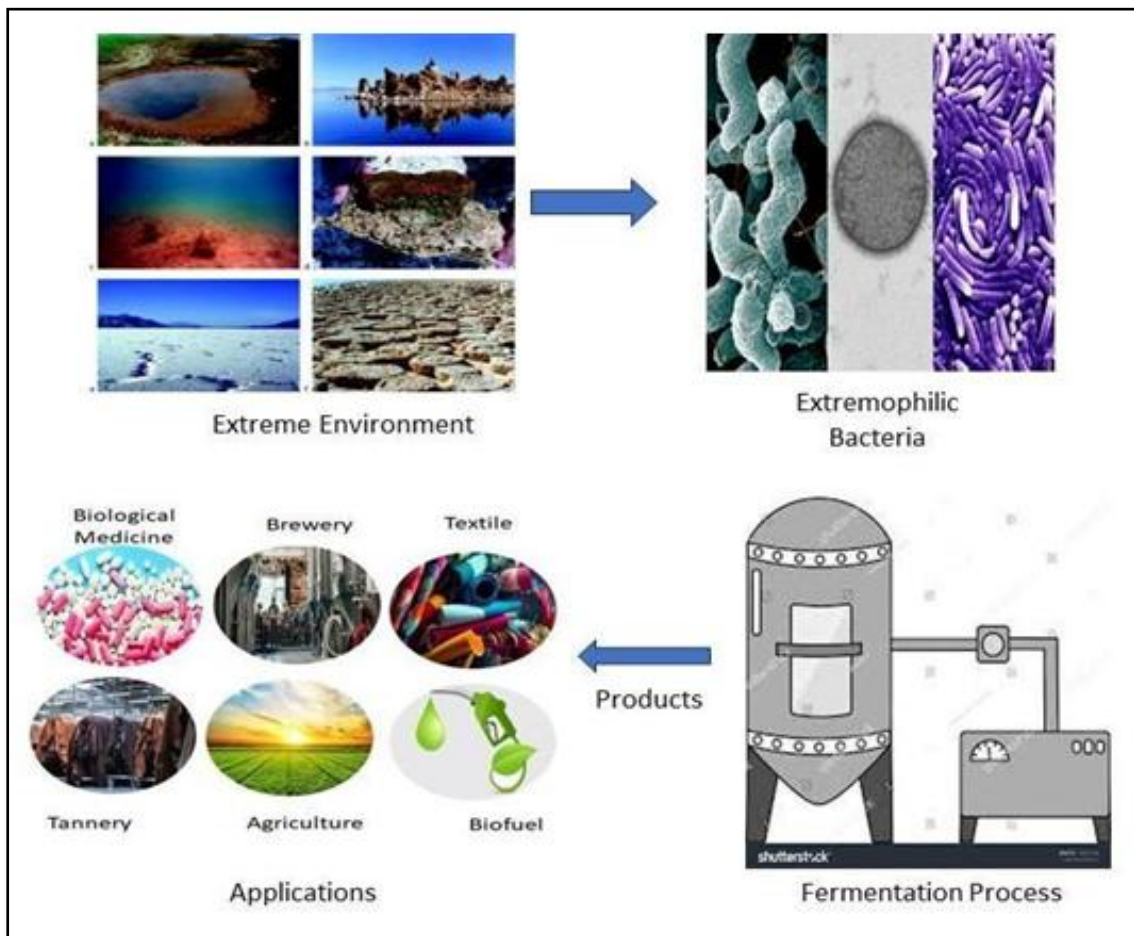
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I. INTRODUCTION

In fascinating world of microbiology certain organisms display an exceptional ability to thrive and flourish in extremes of environment, these extraordinary entities are known as extremophiles, have captivated scientists and researchers alike owing to their remarkable resilience against extreme physical (pH, temperature, pressure and salinity) and chemical (macro and micronutrients required) conditions. These encompass a diverse array of microorganisms, predominantly within the Archaea and Bacteria domains. The study of these extremophiles has not only expanded our understanding of the diversity of life on Earth but has also paved the way for groundbreaking applications in industries. This chapter, focusing on three major subsections, delves into the intriguing world of extremophiles, exploring their diverse adaptations, the enzymes they produce—and their profound significance in industrial processes.

1. Extremophiles: Nature's Resilient Pioneers

Extremophiles represent the pioneers of resilience, pushing the boundaries regarding the conditions in which life can thrive. For instance, hyperthermophiles, a subset of extremophiles, survive in the scorching hydrothermal vents deep within the ocean floor home, where temperatures exceed 80°C (1). In similarly astonishing fashion, psychrophiles have adapted to frigid environments, with optimal growth temperatures as low as -10°C. These remarkable microorganisms can be found in icy polar regions and

high-altitude environments. Likewise, acidophiles flourish in highly acidic surroundings, with pH levels below 3, such as acid mine drainage sites. Alkaliphiles, on the other hand, embrace alkaline environments with pH values exceeding 9, like soda lakes and alkaline soils (2). These organisms survive in harsh conditions as well as produce bioproducts such as extremozymes, pharmaceutical compounds, biofuels etc. These organisms defy conventional wisdom by not only surviving but thriving in such extreme conditions.

Extremozymes: Enzymatic Marvels of Extreme Conditions

One of the most captivating aspects of extremophiles is their ability to produce extremozymes—enzymes that exhibit exceptional stability and activity under extreme conditions. These extremozymes have found their way into a multitude of industrial applications, revolutionizing processes across various sectors. For instance, thermophiles are masters of heat, and their enzymes have revolutionized fields such as biofuel production, where high-temperature reactions can be catalyzed efficiently and sustainably (3). Cold-active enzymes from psychrophiles have transformed the biotechnology landscape, enabling processes that require low temperatures, such as dairy and food industries (4). In the realm of bioremediation, acidophiles and alkaliphiles contribute their extremozymes to cleanse contaminated environments, showcasing their potential in environmental sustainability (5).

- 2. Industrial Applications and Beyond:** The impact of extremophiles and their extremozymes is undeniable across various industries. The biopharmaceutical sector harnesses their unique abilities to synthesize complex molecules under extreme conditions, redefining drug discovery and production (6). In the realm of biorefinery and bioenergy, extremozymes from thermophiles and halophiles are catalyzing reactions that hold the promise of sustainable energy production (7). Furthermore, the detergent industry benefits from the powerful enzymes of alkaliphiles, enhancing the efficacy of cleaning products even in high-pH environments (8).

In this chapter, we embark on a journey through the extremes of life, exploring the remarkable adaptations of extremophiles and the unparalleled capabilities of their extremozymes. As we uncover the intricate mechanisms that enable these microorganisms to thrive in seemingly inhospitable conditions, we delve into their industrial significance, revolutionizing biotechnology, energy production, and environmental restoration. The collaboration between nature's resilient pioneers and human ingenuity opens doors to a future where extremophiles and their extremozymes propel us toward innovative and sustainable solutions for the challenges that lie ahead.

II. EXTREMOPHILIC BACTERIA AND ITS TYPES

Extremophile organisms are living beings that possess the remarkable ability to flourish and propagate in environments characterized by extreme physical conditions such as temperature, pressure, and radiation, as well as challenging chemical parameters like salinity, pH, and redox potential. Most extremophiles belong to the microorganism category, specifically within the Archaea and Bacteria domains. Among these microorganisms, there are polyextremophiles that exhibit the extraordinary capability to withstand and thrive in multiple extreme conditions simultaneously (9).

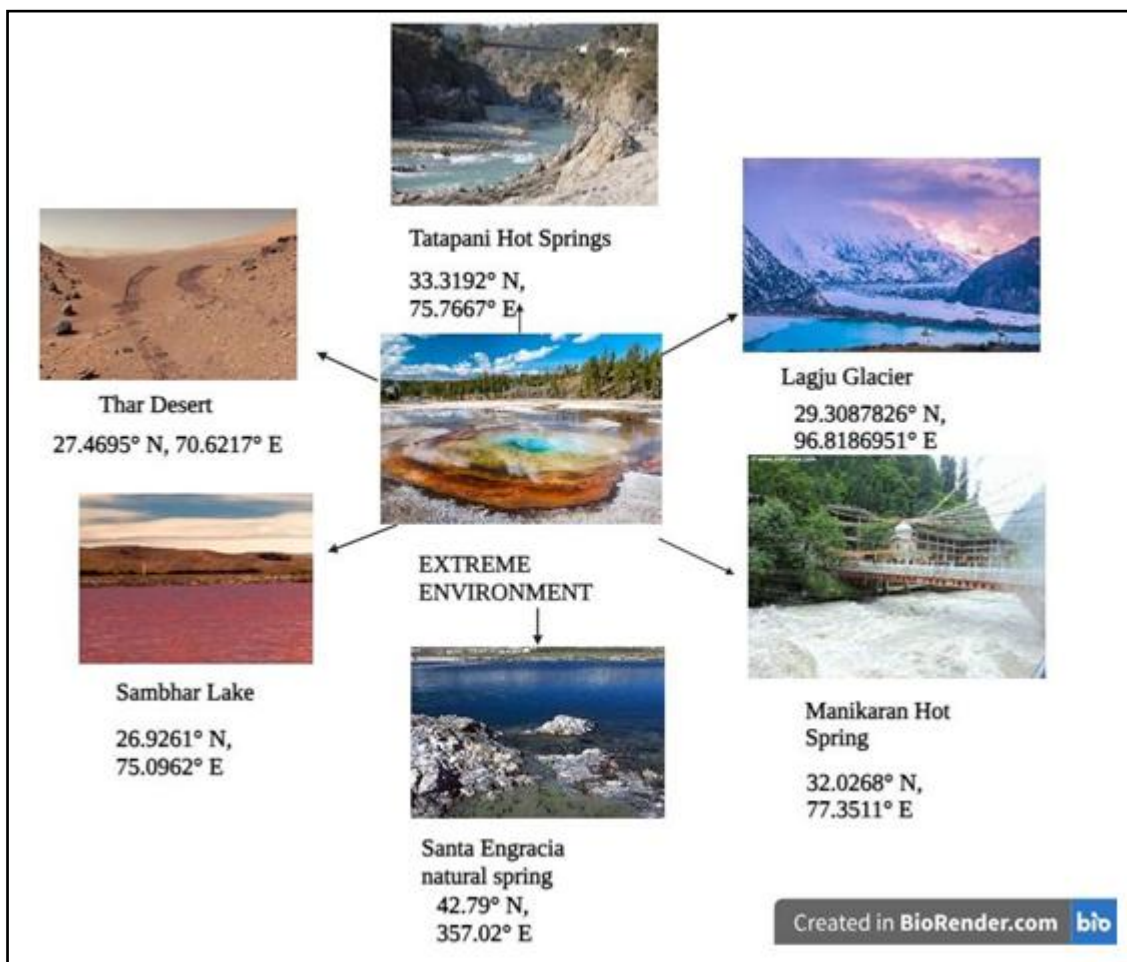


Figure 1: Environmental Source of Extremophiles- A figure represents different environmental conditions with their geographical locations from which different types of extremophiles can be isolated.

1. Overview on the Mechanisms of Extremophiles: It is important to study the mechanism of extremophilic bacteria, as they have diverse mechanisms to survive in harsh conditions, at extremes of environment. For instance, extreme halophilic bacteria forms osmoprotectants such as ectoine and proline etc. These extremophiles can be studied and applied in industrial biotechnology for bioproducts formed by them. It is also necessary to study their mechanisms, that shows how they are surviving at extremes of the environment. 1. It is becoming important to study functioning of these bacteria as research era is shifting towards to isolate extremophiles, what type of potent proteins and enzymes they have, which can be utilized in industrial biotechnology to produce different byproducts. Figure 1, depicts the graph of increasing trends of research towards extremophilic bacteria

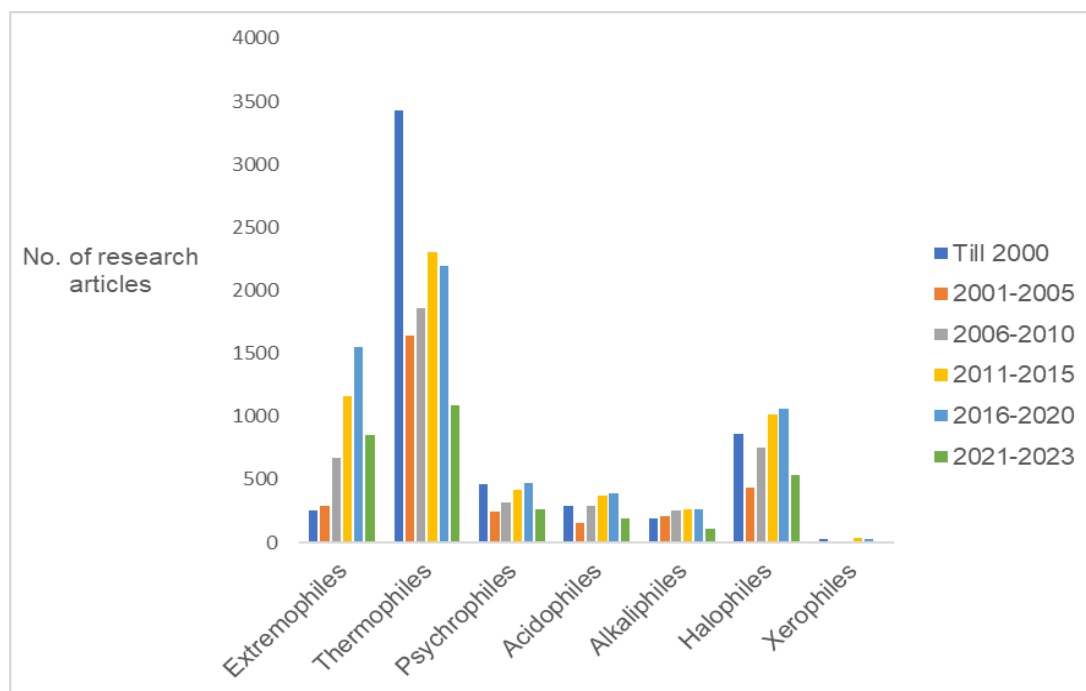


Figure 2: Graph of Increasing Studies on Extremophilic Bacteria vs Different Extremophilic Bacteria

A graph depicting the growth in research focused on extremophilic bacteria in relation to various types of mesophilic bacteria.

This data is correct of our knowledge, and collected from pubmed on 14 August 2023

- Protein Stabilization:** Extremophilic bacteria produce proteins that are adapted to withstand harsh conditions such as high temperatures, extreme pH levels, or high salinity. These proteins have unique structural features that enhance their stability and function under extreme conditions (10). For instance, this has been observed in proteins from *P. furiosus*. Enzymes from *P. furiosus* have highly packed hydrophobic cores with many ionic pairs and buried atoms to help diminish unfavorable solvent interactions (4)
- Membrane Adaptations:** The cell membranes of extremophilic bacteria are modified to maintain integrity and functionality in extreme environments. This includes alterations in lipid composition, membrane fluidity, and the presence of specialized transport proteins to regulate ion and nutrient uptake (11). For example, Xerophilic bacteria modify their cell membrane by phospholipid and fatty acid compositions. In *S. enterica*, the lipid A palmitoleoyl acyltransferase gene *ddg*, which replaces lipid A laurate with palmitoleate, is up regulated in response to desiccation, increasing the fluidity of the outer membrane (12).
- Osmoregulation:** Extremophilic bacteria employ osmoregulatory mechanisms to balance internal and external osmotic pressures. They accumulate compatible solutes or osmoprotectants, such as sugars or amino acids, to maintain cellular hydration and

prevent dehydration in environments with low water availability (13).

- **DNA Repair Systems:** Extremophilic bacteria have efficient DNA repair systems that enable them to rectify DNA damage caused by exposure to extreme conditions such as high levels of radiation or oxidative stress (14).
- **Spore Formation:** Some extremophilic bacteria can form spores, which are dormant and highly resistant structures. Spore formation allows bacteria to survive extreme conditions such as desiccation, high temperatures, or high levels of radiation until more favorable conditions arise (15). These mechanisms collectively enable extremophilic bacteria to adapt and thrive in extreme environments that would be detrimental or lethal to most other organisms.

Table1: Types of Extremophilic Bacteria and their Optimum Environment
(Adapted from (15)- The table gives information about categories of extreme bacteria and their favorable environment of growth

Extremophile Microorganism	Favorable Environment for Growth
Hyper Thermophilic	Optimum growth at temperatures above 80 °C
Thermophilic	Grows at temperatures between 60 °C and 85 °C
Psychrophiles	Grows at temperature from 0°C to 15°C
Acidophile	Optimum pH for growth—Below 3
Alkaliphile	Optimum pH for growth—Above 10
Halophile	Requires at least 1M salt for growth
Xerophile	Grows in low water availability, resistant to desiccation

The upcoming section of the chapter deals with the different categories of extremophiles as mentioned in Table 1.

- **Thermophiles:** Thermophiles are organisms that thrive in high-temperature environments, not only enduring these elevated temperatures but often necessitating them for their growth and survival. According to Brock's definition in 1978, a thermophile is an organism capable of thriving at temperatures near or at the upper limit for its taxonomic group. Typically, these organisms grow within a temperature range of 50°C to 121°C, which coincides with the sterilization temperature used in autoclaves. Thermophiles are further categorized into moderate thermophiles, extreme thermophiles, and hyperthermophiles, based on the range of temperatures they can withstand (16).

Thermophiles are known to tolerate extreme temperatures, and certain cellular components like ATP, amino acids, and peptides have shown to be stable even beyond 250°C, suggesting that life's upper temperature limit may surpass current observations. Bacteria classified as extreme thermophiles produce exceptionally stable proteins, which can be easily crystallized for the extraction of robust enzymes, beneficial for structural and functional studies. These hyperthermophilic and

thermophilic proteins require considerable structural integrity to resist unfolding. For example, the bacterium *Thermus thermophilus*, originally isolated from a hot spring's thermal vent in Izu, Japan, is commonly utilized in genetic manipulation research. The DNA gyrase enzyme from this extremophile serves as a model for studying drug resistance mechanisms. DNA gyrase, a type IIA topoisomerase, utilizes ATP hydrolysis to introduce negative supercoiling into closed circular bacterial DNA, making it a significant target for antibacterial drugs, including commonly used fluoroquinolones (3). These benefits arise from the substrate's high solubility, reduced contamination risk in the system, decreased solution viscosity, and improved solvent miscibility, all of which facilitate faster reactions. The biorefinery industry utilizes numerous thermophiles and thermostable enzymes to take advantage of these advantages. The first and second-generation biofuels, paper, and bleaching industries, the crucial ones of which are compiled in Table 2 (15).

Table 2: Types of Extreme thermophiles - Extreme thermophiles, with their optimum temperature, enzymes produced and their applications.

Extreme Thermophile	Optimum Temperature (°C)	Extremozyme Enzyme	Industrial Application	Reference
<i>Thermophilic Anoxybacillus sp. GXS-BL</i>	60	α -amylase	Bioenergy industries, Food, pharmaceuticals, textile, detergent	(17)
<i>Dictyoglomus turgidum Thermostable</i>	80	Thermostable β -glucosidase	Biorefinery, food	(18)
<i>noxybacillus flavithermus Bacillus licheniformis</i>	60	β -galactosidase	Food, bioremediation, biosensor	(19)
<i>Bacillus mojavensis SO-10</i>	70	α -amylase	Biorefinery, food, detergent	(20)
<i>Anoxybacillus thermarum FRM-RBK02</i>	80	α -amylase	Biorefinery, food, detergent	(21)
<i>Janibacter sp. strain R02</i>	80	Thermophilic and halophilic esterase	Detergents, pharmaceutical, leather, food, bioremediation	(22)
<i>Metagenome</i>	115	Thermoactive endoglucanase	Biorefinery	(23)

- **Psychrophiles:** Psychrophilic microorganisms typically thrive within a temperature range of 1-4°C, whereas mesophilic microorganisms prefer temperatures between 30-37°C. In contrast to mesophilic microorganisms, which have a preferred temperature range of 30-37°C, psychrophilic microorganisms can maintain cellular metabolism even at temperatures below 0°C. To adapt to these harsh conditions, psychrophiles have evolved several physiological adaptation mechanisms, including control of membrane fluidity, action of molecular chaperones, and synthesis of antifreeze molecules (2)

These microorganisms can withstand the negative effects imposed by cold environments and are frequently found in such habitats (24).

Cold-adapted enzymes, also known as psychrophilic enzymes, possess specific adaptations in their structural features that provide them with greater flexibility compared to mesophilic and thermophilic enzymes. This flexibility allows for high catalytic activity at low temperatures while maintaining thermostability. These adaptations are the result of long-term selection and are characterized by specific genetic changes. Recent reviews on psychrophilic enzymes have highlighted several important adaptations that contribute to maintaining high flexibility and activity at low temperatures. These adaptations include:

- Decreased core hydrophobicity and increased surface hydrophobicity of cell membrane,
- Changes in amino acid compositions, such as a lower arginine/lysine ratio, an abundance of glycine residues for enhanced conformational mobility, fewer proline residues in loops but more in α -helices, and an increased presence of non-polar residues on the protein surface,
- Weaker protein interactions, including inter-domain and inter-subunit interactions, fewer disulfide bridges, reduced hydrogen bonds and electrostatic interactions, and fewer/weaker metal binding sites.
- Decreased secondary structures and oligomerization, coupled with an increase in the number and size of loops.
- Increased conformational entropy of the unfolded protein state.

Table 3: Types of Extreme psychrophiles- Extreme psychrophile with their optimum temperature, enzymes produced and their applications

Extreme Psychrophiles	Optimum Temperature °C	Extremozyme	Industrial Application	Reference
<i>Pseudomonas sp. MM15</i>	4°C to 42°C.	Endoglucanase (Cel5M)	Cellulose degradation	(25)
<i>Arthrobacter sp. 20B</i>	30–37°C	galactosidase	Food processing	(26)
<i>Planomicrobium sp. 547</i>	20-21°C	Protease	detergent and pharmaceutical	(27)
<i>Cryptococcus diffluens</i>	32 and 40°C	D-galacturonate reductase	Food, pharmaceutical	(28)
<i>Bacillus sp. N2a (BNC)</i>	40°C	Catalase	food industry	(29)

- **Acidophiles:** Microorganisms adapted to acidic environments, known as *acidophilic microorganisms*, possess a remarkable ability to survive under extremely low pH conditions (less than pH 3). They achieve pH homeostasis by effectively controlling proton permeation. For instance, microorganisms belonging to genera such as *Thermoplasma*, *Ferroplasma*, and *Sulfolobus* are capable of regulating proton permeation even under these extremely low pH conditions. This is achieved through the presence of a highly impermeable cell membrane, primarily composed of tetraether lipids. These lipids possess a diverse range of polar head groups and a bulky isoprenoid core, contributing to the membrane's impermeability and enabling these microorganisms to thrive in acidic environments (2).

Microorganisms that thrive in acidic environments have developed several survival mechanisms. One crucial adaptation lies in their cell membranes, which effectively block the passage of protons. In the Archaea domain, this impermeability is attributed to unique characteristics such as tetraether lipids, distinctive lipid head-group structures, a substantial isoprenoid core, and the ability of ether linkages to resist acid hydrolysis. These features distinguish them from organisms in the Bacteria and Eukarya domains (30). *Acidophiles* also employ strategies such as reducing membrane channel pore size, observed in *Acidithiobacillus ferrooxidans*, and maintaining a net positive charge inside the cell to counteract the high concentration of H⁺ ions in their acidic surroundings. Some *acidophilic* species, like *Bacillus* and *Thermoplasma*, utilize active proton pumping for intracellular pH regulation. While an identifiable acid response signature in *acidophile* genomes remains elusive, it is interesting to note that *acidophile* genomes tend to be smaller than those of neutrophiles, though the reasons and potential evolutionary advantages of this difference are yet to be fully understood. These adaptations provide valuable insights into the unique biology of *acidophilic microorganisms* and their ability to thrive in acidic environments (31). Tab 4 lists the type of acidophilic bacteria, and their industrial application.

Table 4: Types of Extreme Acidophile-Types of extreme acidophilic bacteria with their optimum pH, enzymes produced and their industrial applications.

Extreme Acidophiles	Optimum pH	Industrial Application	Reference
<i>Acidianus brierley</i>	1.5–2.0	Ferric iron-reducing	(34)
<i>Acidianus copahuensis</i>	2.5–3.0	Ferric iron-reducing	(32)
<i>Acidianus manzaensis</i>	1.2–1.5	Ferric iron-reducing	(33)
<i>Acidianus sulfidivorans</i>	0.8–1.4	Ferric iron-reducing	(34)
<i>Acidothiobacillus ferrooxidans</i> BY-3	<1.8	Bioleaching	(36)

<i>At. Ferrooxidans</i>	1.5–4.5	Bioleaching	(37)
<i>Acidithiobacillus ferrivorans</i>	2.5	Bioprecipitation	(38)
<i>Acidocella aromatica</i>	2.5	Bioreduction	(39)

- Alkaliphiles:** Alkaliphiles are organisms that thrive in alkaline environments, typically with a pH exceeding 9, and they tend to exhibit optimal growth even at a pH of as high as 10. These extremophiles can either be obligate or facultative depending on their capacity to tolerate alkaline conditions. Facultative alkaliphiles are capable of growing within a pH range of 7.0 to 9.5, while obligate alkaliphiles, like *Bacillus krulwichiae*, demonstrate their best growth between pH 10.0 and 12.0. Alkaliphiles often coexist with neutrophilic microorganisms under mildly alkaline pH conditions, thriving in specific extreme environments. Alkaliphile bacteria have molecular defenses against high pH that include symporter (proteins that simultaneously transport two molecules across a membrane in the same direction) and antiporter (membrane protein that transports two molecules at the same time in the opposite direction) system activation. An electrochemical gradient of Na⁺ and H⁺ is produced by electrogenic antiporters, and the symporter system allows the uptake of Na⁺ and other solutes into the cells. These systems enable the influx of protons and solutes inside the cell due to the alteration in the distribution of ions (e.g., Na⁺), maintaining the hydro saline homeostasis and thermodynamic stability of the cell. The transporters are controlled, probably by signaling from a transmembrane pH sensor (35). Table 5 consists of extreme alkaliphiles, their pH at which they function and their potential use.

Table 5: Extreme alkaliphiles- Industrial application of extreme alkaliphiles with their optimum pH is listed.

Source	Optimum pH	Stable pH range	Optimum temperature (°C)	Reference
<i>Aneurinibacillus thermoaerophilus</i> lipase expressed in <i>E. coli</i> (LipAT)	10	7-10	45	(40)
<i>Microbulbifer</i> sp. YNDZ01	10	7-10	70	(41)
<i>Burkholderia gladioli</i> BRM58833	9	8-10	50	(5)
<i>Serratia</i> sp. W3	9	5-10	55	(42)
<i>Burkholderia gladioli</i> BRM58833	9	8-10	50	(5)

<i>Bacillus mycoides</i> strain CV18	9	7-10	30	(43)
<i>Anoxybacillus flavithermus</i> HBB-134 9 6-11 50 Bakir and Metin, 2016	9	6-11	50	1
<i>Bacillus smithii</i> BTMS11	8	7-10	50	(44)
<i>Pseudomonas</i> sp.	8.2	7-9	45	(8)

- Halophiles:** Extreme halophiles are a specific group of microorganisms that require high salt for their growth. They are categorized based on their optimal NaCl concentration requirement for growth. Depending on their salt concentration requirements these have been further categorized as slight halophiles (0.2 M), moderate halophiles (0.5–2.5 M), borderline extreme halophiles (>2.5–4.0 M), and extreme halophiles (>4.0–5.9 M). Halophilic microorganisms possess varied environmental habitats for their survival including salars, saline lakes, oceans, polar ice, and coastal areas. On the other hand, halotolerants are microorganisms capable of growing both in the presence and absence of NaCl. Among halotolerants, the ones thriving in the presence of >2.5 M NaCl are considered extremely halotolerant (35).

Halophiles have evolved two primary adaptation mechanisms to prevent the diffusion of NaCl into their cells and thrive in hypersaline environments. The first mechanism involves the accumulation of inorganic ions, particularly KCl, to balance the osmotic pressure. This strategy is primarily employed by aerobic and extremely halophilic archaea, as well as some anaerobic halophilic bacteria. On the other hand, most halophilic bacteria and eukarya utilize a different approach by accumulating water-soluble organic compounds of low molecular weight known as compatible solutes or osmolytes. These compatible solutes serve to maintain a low intracellular salt concentration and act as stabilizers for biological structures, enabling the cells to adapt not only to high salt concentrations but also to conditions such as heat, desiccation, cold, and even freezing. Remarkably, this allows halophiles to thrive in environments with pH levels as high as 10 and temperatures exceeding 50°C. Some halophiles synthesize osmolytes such as ectoine, which are small organic molecules and maintain the integrity of cells. These organic molecules do not intervene in enzymatic activity. Some halophiles scavenge osmoprotectants or compatible solutes from other organisms by using transporter systems as they do not produce their own compatible solutes. For example, *Halobacterium salinarum* has been known to show chemotactic activities toward osmoprotectants (15).

Table 6: Types of halophile- Extreme halophiles, with their optimum temperature, enzymes produced and their applications.

Strain	Salt tolerance (M)	Enzymes	Potencial Use	Reference
<i>Haloferax sp.</i>	3.4	α -amylase	surfactants and detergents.	45
<i>Halomonas nitroreducens 11ST</i>	0.5–3.4	poly(3-hydroxybutyrate) PHB	bioplastics	46
<i>Shewanella sp. Asc-3</i>	0–0.5	fibrinolytic enzyme	treat and prevent CVDs.	47
<i>Exiguobacterium sp. SH31</i>	0.4	not yet known	capable of reducing arsenate and chromium and explored for bioremediation applications.	35
<i>Microbacterium sp.</i>	0–1.2	carbohydrate-active enzyme (CAZyme)	polysaccharides degradation.	48

- **Xerophiles** (least evaluated): Xerotolerant extremophile organisms have the remarkable ability to survive in arid environments where the water activity level is less than 0.75_{a_w} (low water activity) (35).

Xerotolerant bacteria have different adaptations such as:

- **Behavioral Adaptations:** In dry environments, xerotolerant bacteria employ two key strategies to survive. First, they utilize spore formation, which enables them to transition into a non-replicative but viable state. Second, they engage in the formation of exopolysaccharides (EPS) and biofilms. These mechanisms assist in maintaining a hygroscopic nature, effectively shielding the cells from desiccation. Furthermore, the hydrophilic properties of EPS play a crucial role in facilitating rapid water absorption rates (12).
- **Physiological Adaptations:** The bacterial cellular membrane, serving as the primary interface between the intracellular environment and the external surroundings, undergoes substantial alterations in response to changes in external water availability. Bacteria subjected to severe fluctuations in water conditions often exhibit adaptations in their membrane's phospholipid and fatty acid compositions. These adaptations include heightened ratios of saturated to unsaturated fatty acids, an increase in trans- to cis-mono-unsaturated fatty acids,

and an elevated conversion of monoenoic fatty acids to cyclopropane fatty acids.

First: Cell Membrane Adaptations: Accumulation of Compatible Solutes

Under desiccating conditions, cells employ different strategies to cope with the challenge of water loss. They accumulate small molecules and salts within the cell to restore osmotic equilibrium, allowing proteins to function even at low water activity (aw) levels, facilitating adaptation to xeric stress. Extreme halophiles follow a "salting-in" approach, importing inorganic ions (referred to as osmolytes) to match extracellular osmotic pressure. In contrast, xerotolerant bacteria employ a two-step "salting-out" process. Initially, they swiftly gather charged solutes like potassium and glutamate in response to osmotic stress. Subsequently, these charged solutes are replaced by neutral organic solutes, which are better suited for organic reactions. These organic solutes maintain long-term osmotic balance and act as stabilizers by preventing the unfolding and denaturation of proteins and other large molecules (12). The study of xerophilic bacteria till now is limited to their isolation and to study their growth characteristics which is given in Table 7, Types of xerophilic bacteria, growth characteristics and their source. **Table 7: Extreme xerophilic bacteria-** Table describes about different strains of xerophiles and their growth characteristics along with their source

Extremophilic	Organisms	Growth characteristics	Geographical location/source
Xerophile	<i>Arthrospira platensis</i> , <i>Bacillus halophilus</i> , <i>Chromohalobacter canadiensis</i> , <i>C. marismortui</i> , <i>C. israeliensis</i> , <i>Dunaliella</i> spp., <i>Halospirulina rapelicola</i> , <i>Halomonas campisalis</i> , <i>H. elongata</i> , <i>H. salina</i> , <i>H. halophila</i> , <i>Methanococcus doii</i> , <i>Natrococcus occultus</i> , <i>Oreniasalinaria</i> , <i>Salinibacter ruber</i> , <i>Zygocharoccharomyces rouxii</i>	Organisms able to grow at low water activity, and resistant to high desiccation	Atacama Desert in Chile, Brackish Sea water, Great Salt Lake, Rock surfaces, Soda lakes (Egypt, Kenya, Tanzania), Salterns (Spain, Mexico), Subterranean halite

III. APPLICATIONS OF EXTREMOPHILES IN INDUSTRIAL BIOTECHNOLOGY

The chapter so far clearly suggests that extremophiles and extremozymes exhibit remarkable abilities to facilitate catalytic reactions across a diverse spectrum of conditions, spanning extreme environments, as well as heightened catalytic efficiency. These intriguing attributes of extremophiles and extremozymes hold substantial potential for various practical applications. They play a significant role in fields such as bioremediation, agriculture, polymer production, bioenergy generation, and diverse industrial sectors (49). which are compiled in Figure 2. Certain most widely used and crucial applications have been described in detail in the upcoming sections:

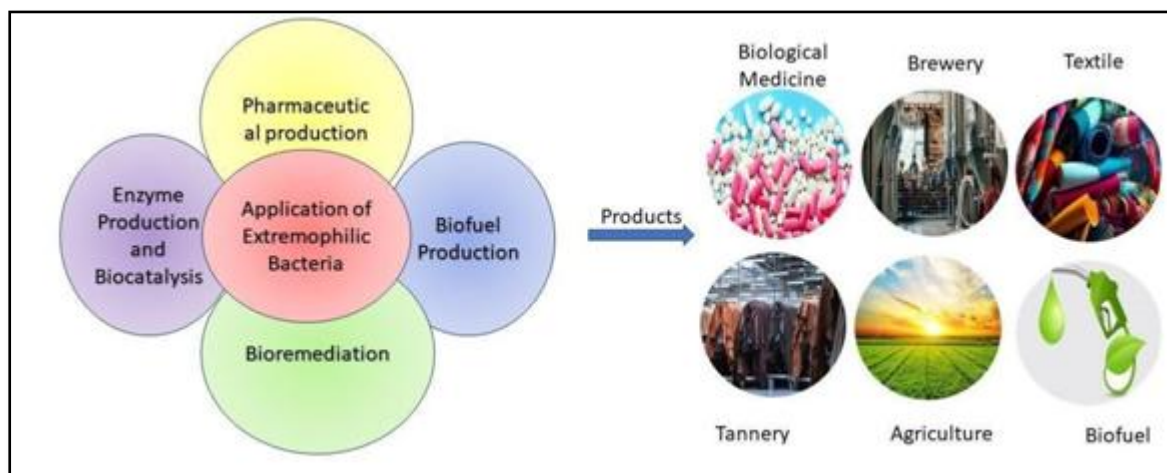


Figure 3: Application of Extremophiles -A diagram describes application of extremophilic bacteria in different industries such as production of bioactive compounds, biofuels, extremozymes and cleaning of environment at harsh conditions.

1. Enzyme Production and Biocatalysts: The need of an hour is to work with extremophiles as mesophilic enzymes are tailored for standard conditions while extremozymes are isolated from extremophilic bacteria so they can survive in extreme scenarios, making them crucial players in industries requiring robust performance amidst adversities (50). They can be used in different industries such as to permit them in biorefinery, agriculture, the chemical industry, bioremediation, biomedicine, and environment pollution control.

These microorganisms hold significant promise for the large-scale synthesis of enzymes. The present industrial enzyme market, valued at approximately US\$ 7,100 million, is witnessing a consistent annual growth rate of 8%. Projections indicate that by 2024, enzymes derived from microorganisms will establish dominance in the market. The substantial industrial demand for microbial enzymes can be attributed to their ready availability and cost-effectiveness in production (35).

Table 8: Comparison between normal enzymes and extremozymes - This table deals with the difference between enzymes produced by mesophiles and extremophiles.

Characteristic	Mesophilic Enzymes	Extremozymes
Temperature Sensitivity	Optimal at 20-50°C	Can function at high temperatures (50-121°C)
pH Range	Optimal pH around 6-8	Can tolerate extreme pH values (acidophiles (<3) or alkaliphiles (pH <9))
Stability	Relatively stable under normal conditions	More stable under extreme conditions

Applications	Common in various industries and research	Used in extreme condition-dependent processes (e.g., bioremediation, biofuels)
Enzyme Engineering	Subject to enzyme engineering for optimization	Engineered for enhanced performance or adaptation
Industrial Importance	Enzymes used in traditional processes like food, pharmaceuticals, textiles, etc.	Essential for processes requiring extreme conditions, e.g., high-temperature food processing, biofuel production from extreme environments, and bioremediation in extreme pH environments.

The utilization of extremophilic microorganisms within industrial processes has experienced significant growth over the past two decades. More than 3,000 enzymes have been successfully isolated and identified from extremophiles, with a considerable proportion finding application in biotechnological and industrial contexts. The extremozymes play a pivotal role as they are highly stable and there is low risk of contamination by mesophilic organisms while their cultivation as they are grown in harsh conditions as comparable to them. The polymer degrading enzymes are important as it is difficult to hydrolyse polymers, for instance cellulose, starch, and polyethylene glycol etc. It is important to hydrolyse them as they are abundant on earth which becomes pollutants for humans. These enzymes are classified as polymer-degrading agents, including amylases, proteases, cellulases, and xylanases. Moreover, ongoing research indicates that extremozymes hold promise as innovative catalysts for diverse industrial applications. Notably, certain extremophiles have transitioned into commercial availability, as outlined in Table 9.

Table 9: Commercially available extremozymes- This table deals with commercially available enzyme, their brand and year of commercialisation

Brand	Enzyme	Company	Application	Reference
Optimax®	Glucoamylase and pullulanase	Genencor	Starch saccharification	(51)
IndiAge® NeutraFlex	Cellulase	Genencor	Textile and garment processing	(51)
Lipoclean®, Lipex®, Lipolase® Ultra, Kannase, Liquanase®, Polarzyme®	Lipases	(Novozyme)	Detergent	(52)
Novoshape® Pectinase 62L Lallzyme®	Pectinases	Novozymes Biocatalysts Lallemand	Food and beverages	(51)

Luminase® PB-100 and PB-200	Xylanases	Verenium	Pulp and paper	(53)
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- 2. Biofuel Production:** Extremophiles and their enzymes are well-positioned to play pivotal roles in a variety of bioprocessing applications, including the conversion of non-food biomass into biofuels. These exceptional microorganisms thrive in extreme conditions, encompassing high temperatures, acidity, alkalinity, or salinity, and have showcased their ability not only to produce biofuels but also unique value-added products. The utilization of extremophilic microorganisms in biofuel and value-added product production processes can deliver cost-effective benefits owing to several factors: (i) a decreased risk of unwanted microbial contamination, (ii) heightened reaction rates, (iii) improved product yields, and (iv) reduced environmental risks (54).

Extremophiles and their enzymes are strategically positioned to play pivotal roles across various bioprocessing applications, notably in the conversion of non-food biomass into biofuels. These remarkable microorganisms thrive in extreme conditions, spanning high temperatures, acidity, alkalinity, or salinity, and have demonstrated their capacity not just for biofuel production but also for crafting distinctive value-added products. Leveraging extremophilic microorganisms in the processes of biofuel and value-added product production holds the promise of cost-effectiveness, thanks to several advantages: (i) mitigated risks of unwanted microbial contamination, (ii) accelerated reaction kinetics, (iii) enhanced product yields, and (iv) minimized environmental hazards (55).

Extremophiles' contributions to biofuel production have become tangible and are growing fast. The development of thermophilic whole cell biocatalysts of industrial relevance is one of the most significant recent contributions. Developments in genetic systems and strain production will continue to push new innovations to achieve a sustainable supply of biofuels.

- 3. Bioremediation:** the accumulation of hazardous contaminants in the environment poses a significant threat to both the ecosystem and human health, resulting from various anthropogenic and natural factors. Mitigating the contamination levels has become a formidable challenge, necessitating the exploration of effective strategies. Among these approaches, bioremediation has emerged as a promising, cost-effective, and sustainable solution. Extremophile-based bioremediation has garnered considerable attention. These unique microorganisms, adapted to thrive in extreme environments, offer potential for efficient and targeted remediation of contaminants. Harnessing the capabilities of extremophiles presents a promising avenue for addressing the environmental impacts of hazardous contaminants. These microorganisms possess unique enzymatic features and physiological properties that render them highly promising for diverse biotechnological applications. Notably, these applications encompass the bioremediation of hazardous pollutants present in water and sediments, along with the production of essential biomolecules for medical and industrial purposes. Among these microorganisms, metalophiles and acidophiles, which have adapted to thrive in environments with high concentrations of heavy metals, are particularly valuable in processes such as bioremediation and biomining (56). Their remarkable capabilities offer considerable potential for addressing environmental challenges and advancing various industries. On

the contrary, radiophiles have proven to be well-suited for the management of environments contaminated with nuclear waste. In the agricultural context, extremophilic desert bacteria that can endure low water activity conditions present potential advantages in water management for drought-stressed plants. These diverse applications showcase the adaptability and untapped potential of extremophilic microorganisms in tackling various environmental and industrial issues (57).

Halophilic microorganisms have demonstrated their usefulness in bioremediation processes owing to their unique structural and functional properties such as their cell wall, capsule, S-layer proteins, extracellular polymer substances (EPS), and siderophores which enable them to effectively biosorb metals even under extreme environmental stress (58).

There are different types of pollutants which are degraded by extremophilic bacteria are listed in table 10.

- 4. Pesticide Degrading Microbes as Extremophiles: Pesticides are chemical substances designed to deter, eliminate, or hinder insects and pests during the agricultural process.** While they offer practical benefits, pesticides carry the risk of contaminating soil, water, vegetation, and other flora. These chemical agents also pose a threat to human health, as they can harm the neurological system, digestive system, and endocrine system (59). The extensive and widespread use of pesticides has led to significant soil contamination and degradation. Extremophilic microorganisms have demonstrated the ability to effectively break down various pesticide components that find their way into the environment. For instance, the organophosphorus insecticide monocrotophos was successfully eliminated from wastewater through bioremediation involving *Arthrobacter atrocyaneus*, *Bacillus megaterium*, and *Pseudomonas mendocina*. Additionally, a psychrotolerant microorganism, *Sphingobacterium sp. C1* isolated from Shimla, Himachal Pradesh, India, exhibited the capacity to degrade the organophosphorus pesticide chlorpyrifos at different temperatures, making it a promising candidate for chlorpyrifos remediation in cold environments (60).
- 5. Removal of Hydrocarbons:** Crude oil extraction and transportation processes can occasionally lead to oil spills, which, in turn, contaminate extreme environments. In these challenging settings, conventional microorganisms typically employed in bioremediation exhibit limited metabolic capabilities, resulting in suboptimal degradation. They struggle to function at their best or even survive in such extreme environments due to their inability to withstand the harsh physical and chemical conditions of these habitats. Therefore, it is advisable to explore the use of extremophilic microbes for bioremediation in extreme environments contaminated with crude oil, as extremophiles possess the potential to adapt to and thrive in adverse environmental conditions (61).
- 6. Removal of Heavy Metals:** Extremophiles can be used in those industries, which use heavy metals to produce their products such as leather making industries (tannery), paper manufacturing industry, and lead- acid battery manufacturing factory. Bioleaching and alkylation and reduction are two types of techniques followed by extremophilic microorganism to bioremediation heavy metal contamination site.
 - **Bioleaching:** Both pyrometallurgy and hydrometallurgy are favorable for efficient and

rapid treatment of metal-bearing sludge. These processes use highly acidic environments and are done at elevated temperatures. However, these methods have various environmental issues. Some produce toxic pollutants such as dioxins, highly acidic water, and furans. Therefore, extremophile microorganisms are appropriate to extract the various valuable metals from e-waste through an economical and ecofriendly approach (62). Extremophilic microorganisms and archaeobacteria are majorly employed for the biomining and recovery of minerals from the ores via the process of biooxidation and bioleaching. These bioprocesses offer multiple advantages such as anaerobic metabolism, redox pathways, intercellular communication, biofilm formation (63), and resistance to heat, acid and metals of (thermo) acidophiles consortia (e.g., *Leptospirillum spp.*, *Acidithiobacillus spp.*, *Sulfobacillus spp.*, *Ferropasma spp.*, *Acidiplasma spp.*) (64). Insoluble metal sulfides are converted into soluble metal sulfates by iron- and sulfur-oxidizing extremophiles, thereby facilitating the recovery of economically relevant metals (e.g., Cu, Fe, Au, Ni, and Zn) (65).

7. Alkylation and Reduction: *Herminiimonas arsenicoxydans* ULPAs1 can oxidize As^{+3} to As^{+5} and is known for its ability to mobilize heavy metals through alkylation and reduction reactions (56).

- Metal immobilization involves the conversion of harmful heavy metals, such as hexavalent chromium, into a non-toxic form like trivalent chromium. Bioaccumulation, on the other hand, refers to the energy-dependent uptake of heavy metals and radionuclides by extremophiles, followed by their physiological utilization or storage. Extremophiles possess tolerance to high metal concentrations, enabling them to convert these metals from toxic to non-toxic forms. The metals imported by these extremophiles can be securely bound to different proteins and peptide ligands (66).
- **Biotransformation:** Extremophiles convert heavy metals and radionuclides into non-toxic or recoverable forms through biotransformation. Biotransformation is of two types – (1) redox conversion of inorganic forms and (2) interchange between organic and inorganic forms via methylation–demethylation reaction. Extremophiles can obtain energy through the oxidation of heavy metals like Fe, Sulfur (S), manganese (Mn), and arsenic (As) or can utilize arsenic (As), selenium (Se), chromium (Cr), and uranium (U) in anaerobic respiration as terminal electron acceptors via dissimilatory reduction. Certain microbes also utilize metals as a part of their metal resistance pathway. Such as aerobic and anaerobic reduction of Cr (VI) to Cr (III); reduction of Se (VI) to elemental selenium; reduction of U(VI) to U(IV); and reduction of Hg (II) to elemental (56).

8. Removal of Radionuclides: A radionuclide refers to an unstable atom containing surplus nuclear energy, often generated through processes such as fission, neutron stimulation, cyclotrons, and generators. These radionuclides find application in various industries and mining operations, playing a role in inspecting welds, detecting leaks, assessing corrosion rates, erosion, and other factors. However, the presence of radionuclide contamination is linked to severe health risks, including cancer development and DNA damage (67). Certain microorganisms, known as radiophilic or radiotolerant organisms, exhibit the ability to thrive in environments with high levels of radiation, and survive in high levels

of ionizing radiation for example gamma rays and uv rays. Leveraging this capability, radiophilic microorganisms can be employed to address radionuclide-related issues using techniques like biosorption, biomineralization, bioprecipitation, biostimulation, and biotransformation (67). Among these extremophiles, *Deinococcus radiodurans* stands out as one of the most resilient microorganisms discovered so far in terms of remediating radionuclide pollution. This remarkable organism, due to its exceptional radiotolerance, has demonstrated effectiveness in mitigating uranium contamination by efficiently removing uranyl ions from the environment (56). This category of extremophiles is important as it is surviving in radiation and can help clean those environments which consist of radio- waste. Table 10 consists of the type of extremophile, and the pollutant that they degrade.

Table 10: Pollutants degraded by type of extremophilic bacteria - This table deals with type of contaminant, type of extremophilic microorganism

Type of Contaminant	Type of Extremophile	Microorganism	References
Hydrocarbon	Halophile	<i>Acinetobacter Marinobacter</i> <i>Pseudomonas</i> <i>Ochrobactrum</i> <i>Enterobacter cloacae</i> , <i>Stenotrophomonas maltophila</i> <i>Cycloclasticus</i> <i>are Achromobacter</i> <i>Rhodococcus Micrococcus</i> <i>Arthrobacter</i> <i>Rhodanobacter</i>	(68)
	Thermophile	<i>Bacillus</i> sp. strain JF8 <i>Bacillus thermoleovorans</i> <i>Bacillus subtilis</i> BUM <i>A. fulgidus</i> 24	(69) (70) (71) (72)
	Psychrophile	<i>Rhodococcus</i> , <i>Sphingomonas</i> or <i>Pseudomonas</i>	(73)
	Acidophile	<i>Acidocella</i> 26 <i>Acidiphilium</i>	(74)
Heavy metals	Thermophile	<i>Anoxybacillus</i> sp. 28	(75)
	Metal Tolerant	<i>Pseudomonas</i> strain As-11 29	(76)
Radio nuclide	Radiophile	<i>Deinococcus radiodurans</i> <i>Geobacter</i>	(77)
		<i>Bacillus Micrococcus</i>	(78)

Pesticide		<i>Arthrobacter atrocyaneus Bacillus megaterium Pseudomonas mendocina</i>	(79)
	Psychrophile	<i>Sphingobacterium sp. 35</i>	(80)

9. Pharmaceutical and chemical Production : Extremophilic microorganisms exhibit significant potential for generating a diverse array of biologically active compounds relevant to biomedical applications, which are in high demand in various industries. Sahli et al. (2017) highlighted the extraction of bioactive agents from extremophilic plants, *Juncus maritimus* and *Limonium virgatum*, demonstrating attributes such as antiradical, antimicrobial, antiviral, and cytotoxic properties (49). However, capitalizing on the medical potential of extremophiles presents challenges, notably the intricate process of isolating and refining extremolytes (81). Even when suitable extremolytes are successfully isolated and their applications identified, the transformation of these compounds into specific drugs is a protracted and laborious endeavor. Furthermore, the current regulatory framework imposes constraints on the expeditious development of these potential drugs. This primarily results from the complexities involved in conducting comprehensive functional analyses of specific molecules derived from extremophiles (81). Table 11 provides a list of bioactive compounds produced by different bacteria.

Table 11: Bioactive Compounds Produced by Extremophile- Type of extremophile, bioactive compounds produced by them and their application.

Extremophile	Category of extremophile	Bioactive compound	Application	Reference
<i>Halomonas smyrnensis</i>	Halophile	Microbial levan production	organic fertilizer	(82)
<i>Pseudoalteromonas haloplanktis</i>	Psychrotrophic	Sterols/steroids	biopharmaceutical production	(83)
<i>Psychrobacter namhaensis</i>	Marine psychrophile	Probiotics	as feed diet additive	(84)
<i>Leptospirillum ferriphilum</i>	Acidophiles	Glycolic acid	bioactive compound	(49)
<i>Pseudomonas aeruginosa</i>	—	Glycolipids	Bioremediation	(85)
<i>Bacillus subtilis</i>	—	Lipopeptides	Biomedical application	(85)
<i>Rhodococcus, Mycobacterium</i>	—	Trehalolipid	Bioremediation	(85)
<i>Arthrobacter</i>	—	Trehalolipid	Biocontrol agent	(85)

IV. CHALLENGES AND OPPORTUNITIES

Cultivating extremophilic bacteria, which thrive in extreme conditions like high temperature, high salinity, or extreme pH, poses significant challenges. These microorganisms demand specialized environmental mimicry, often necessitating the replication of harsh conditions not typical in standard laboratory settings. Finding and collecting extremophiles from their often remote and harsh natural habitats can be logistically demanding, while maintaining sterile conditions to avoid contamination is crucial. The development of growth media that accurately mirrors the nutrient and chemical compositions of extreme environments, such as high-temperature or high-pressure conditions, requires meticulous formulation. Additionally, factors like slow growth rates, the need for specialized equipment to maintain extreme conditions, and ethical considerations around sample collection further complicate the cultivation and study of extremophilic bacteria. Nevertheless, these challenges are met with enthusiasm because extremophiles offer valuable insights into the limits of life on Earth and possess biotechnological potential.

- 1. Obstacles at Large Scale Production:** The constrained expansion rate of these organisms, along with the modest output of essential compounds, and the specialized machinery essential for their cultivation, have imposed restrictions on the volume of these organisms produced (81). Scaling up the manufacturing of extremozymes presents challenges as well. For example, when employing halophilic bacteria to synthesize Polyhydroxylalkanoate (PHA) under severe conditions, the demand for a high-salinity environment leads to recurrent and costly equipment maintenance (3).
- 2. Designing Suitable Growth Media:** Overcoming these obstacles can involve innovative strategies like employing bioreactors tailored to endure the unique environmental extremities required by these organisms. Another approach is the insertion of genes related to extremozymes into mesophilic hosts, enabling them to function in more manageable conditions. Bridging various disciplines is essential to uncover the underlying mechanisms of these biological systems and to characterize them for enhanced comprehension and biotechnological applications.
- 3. Scarce Biocatalytic Resources:** Despite the inherent benefits of extremozymes, the pool of available extremophilic biocatalytic tools remains limited. Working with extremophiles or extremozymes necessitates adapting or creating novel methodologies, assays, and techniques that function under non-standard conditions. Many conventional tools from microbiology and biochemistry aren't applicable to extremophilic research due to their inability to withstand extreme conditions. For instance, standard agar-based plating is impractical for hyperthermophiles due to high temperatures causing agar to melt and water to evaporate. Technical disparities persist between laboratory enzyme production and the eventual commercial product, posing a challenge for novel biocatalyst development. Addressing these scientific hurdles is crucial to fully exploit the potential of extremozymes (4).
- 4. Technical Limitations:** Challenges arise not only during the discovery of novel biocatalysts but also when optimizing these biocatalysts for industrial use. Utilizing extremophiles directly as producers of extremozymes is ideal but presents complexities. Operating bioreactors under extreme conditions, such as high or low pH, high temperatures, or high salt concentrations, can shorten the lifespan of sensors and seals in these systems. In some cases, extremophiles do not thrive optimally in bioreactors,

attributed to issues like the accumulation of toxic compounds resulting from Maillard reactions at high temperatures. This is exemplified in the cultivation of *Aeropyrum pernix* (86).

V. BIOSAFETY AND BIOSECURITY CONCERNS

The apprehensions related to black biology are centered on its potential implications for pathogens, particularly those with a historical association or potential for use in biological weapons (BW) and bioterrorism activities. The intention behind introducing genetic modifications into pathogens encompasses a wide array of objectives. These spans from extending the longevity of preserved pathogens in controlled environments to conferring resistance against antibiotics or vaccines. Additionally, there is the aim to enable pathogens to evade natural immune responses, such as resisting fever or avoiding macrophage endocytosis, as well as increasing the lethality rate among infected hosts, including humans, livestock, and crops.

To illustrate, Daly's work delves into how extremophiles and their genes, bearing specific traits, could be harnessed for the development of genetically enhanced biological weapons. In a specific scenario, thermophiles—organisms adapted to extreme heat—could provide attributes for creating more resilient and heat-resistant BW. This could enable the BW to endure explosive dispersal from a missile or withstand elevated temperatures within human hosts during infection (87).

VI. CONCLUSION

The chapter deals with different types of extremophilic bacteria and their robust mechanism that is surviving in those conditions in which life cannot survive. They can stabilize their proteins, enzymes, cell membrane at extremes of physical and chemical conditions. They are different types of extremophiles such as thermophiles, psychrophiles, acidophiles, alkaliphiles, and halophiles. Extremophiles have already demonstrated their utility on an industrial scale. Their robust enzymes, known as extremozymes, have revolutionized processes across diverse sectors, including biofuels, pharmaceuticals, and waste management. The inherent stability of these enzymes allows for enhanced activity under both extreme and moderate conditions, contributing to more efficient and sustainable industrial processes.

Despite their immense potential, there are challenges associated with utilizing extremophiles in industrial biotechnology. Scaling up their cultivation poses obstacles due to the need for specialized equipment and stringent control of extreme conditions. Furthermore, the transition from laboratory-scale success to large-scale industrial application requires overcoming technical barriers and refining the production processes. Addressing biosafety and biosecurity concerns is paramount in harnessing the power of extremophilic bacteria. The unique mechanisms that allow extremophiles to survive extreme conditions must be thoroughly understood to prevent unintended consequences. Mismanagement or accidental release of extremophiles with potentially harmful traits could have far-reaching consequences.

Moreover, the potential misuse of extremophiles in bioterrorism or biological warfare presents a significant challenge. Genetic modifications could be introduced to pathogens,

enhancing their virulence, resilience, and evasion capabilities. This highlights the need for strict regulations, robust containment protocols, and international collaboration to ensure the responsible handling of extremophilic organisms.

In conclusion, the integration of extremophilic bacteria into industrial biotechnology offers a promising path toward innovation and progress. Capitalizing on their unique adaptations and enzymatic capabilities can lead to more sustainable and efficient industrial processes. However, a balanced approach is crucial, considering both their benefits and potential risks, to ensure that extremophiles are harnessed safely and ethically for the betterment of society.

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