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Engineered Extremophiles for Bio-Based Production in Harsh Industrial Environments

Chukwudi Anthony Ugwuanyi

Department of Applied Microbiology Ebonyi State University Nigeria
Email:ugwuanyitony@gmail.com

ABSTRACT

Extremophiles, microorganisms that thrive under extreme environmental conditions, represent a powerful yet underexploited resource for advancing industrial biotechnology. Their unique metabolic and physiological adaptations allow survival in high temperature, pressure, salinity, or acidity, making them ideal candidates for processes that exceed the tolerance limits of conventional microbial hosts. Advances in genetic engineering, synthetic biology, and systems biology have enabled the tailoring of extremophiles into robust chassis organisms for the sustainable production of biofuels, bioplastics, enzymes, and other bioproducts under harsh industrial conditions. This review highlights the classification, significance, and engineering strategies of extremophiles, with a focus on genetic modification approaches such as CRISPR-Cas systems, adaptive evolution, and genome-scale metabolic modeling. Case studies demonstrate successful applications in biofuel production, biodegradable plastic synthesis, and agricultural resilience. Despite promising progress, challenges remain, including difficulties in genetic manipulation, limited understanding of extremophile biology, and the high cost of scale-up. Addressing these barriers through interdisciplinary research and technological innovation could position engineered extremophiles as central players in green chemistry, climate resilience, and the circular bioeconomy.

Keywords: Extremophiles, Industrial Biotechnology, Genetic Engineering, Synthetic Biology and Biofuels and Bioproducts.

INTRODUCTION

Extremophiles thrive in hostile environments and can be exploited to confer resistance to digesters or bioreactors under harsh engineered conditions such as high temperature, high salt and low pH. Industrial processes require such resistance in the face of fluctuating feedstock quality, toxicity by-products, and contamination by invasive species. Genetic engineering and synthetic biology strategies and tools are advancing rapidly, enabling the engineering of extremophile chassis for diverse biofuel, biochemical, and bioproduct generation systems. Polyextremophiles tolerate multiple stresses simultaneously, a necessary trait for more sustainable metabolic engineering approaches. Metabolic pathways that fix carbon dioxide, carbon monoxide, or methane to generate biomass and fuel precursors in extreme environments provide foundation for additional engineering [1]. Extremophiles are microorganisms that inhabit conditions lethal to most life. Their adaptation strategies are the basis of biotechnological applications addressing sustainable development and circular economy. CRISPR-Cas9 gene systems and synthetic biology techniques facilitate strain improvement and prokaryotic research, applicable to numerous biotechnological improvements. Diverse sets of experimental tools enable targeted gene overexpression, silencing or deletion, and genome-wide engineering on industrially relevant bacterial species. Engineered extremophiles enhance resistance to environmental stresses and expedite the repair of damaged biomolecules caused by metal ions or UV light exposure [2]. Rigorous taxonomic scheme and optimal criteria support exploration of diverse extreme environments, increasing the potential to obtain new biotechnologically exploitable microorganisms.

Overview of Extremophiles

Extremophiles are organisms that can thrive and grow under harsh physicochemical conditions existing in natural ecological niches worldwide, which render most life forms incapable of doing so. These microorganisms include bacteria, fungi, and protozoa, which employ a variety of strategies at the molecular, cellular, and physiological levels to survive in extreme ecosystems such as hot springs, glaciers, salt lakes, and alkaline soda lakes. Extremophiles can thrive in conditions unfavorable for common life-forms, such as environments with extreme temperature, pH, light, pressure, and chemical concentrations. They are classified as acidophiles, alkaliphiles, halophiles, psychrophiles, thermophiles, and metalophiles according to the condition they endure [1]. Microbial communities, a key player in the survival capacities of extremophiles, have evolved for billions of years under some of the most severe conditions on Earth, such as volcanic vents, hot springs, acid pools, and salt lakes. While the primary biological role of extremophiles is to maintain their homeostasis and contribute to nutrient, mineral, and biogeochemical cycle balances, their versatility in growth conditions has also found them utility as cell factories in various industrial applications [1, 2].

Definition and Classification

Extremophiles are organisms that thrive under extreme physicochemical conditions, including high or low temperature (hyperthermophiles and psychrophiles), high salinity (halophiles), high pressure (piezophiles), high or low pH (acidophiles and alkaliphiles), and high radiation (radiophiles) [3]. These lifestyles evolved shortly after the appearance of life on Earth and still represent the majority of life's biodiversity [4]. Microbial metabolism sustains the growth and reproduction of extremophiles by accommodating cellular constituents and enzymes to these extremes. During genetic engineering studies they serve as model organisms for the search of mimetic environments capable of supporting contemporary or ancient life. Several biotechnology projects have expressed extremophile genes in mesophilic hosts to improve stress tolerance and physiology.

Types of Extremophiles

Extremophiles are microorganisms that thrive in extreme environmental conditions, such as high/low temperatures, salinity, pressure, vacuum, pH, radiation, and sugar concentrations. They are classified into groups based on these factors [4]. Thermophiles and hyperthermophiles survive at high temperatures, while psychrophiles and psychrotolerants grow at temperatures below 15 °C. Acidophiles and alkaliphiles prefer acidic and alkaline conditions, respectively, whereas halophiles inhabit high-salinity environments. Barophiles, including piezophiles, flourish under elevated pressures, and xerophiles prosper with minimal water availability [4]. Prayer et al. (2020) added that psychrophiles prefer cold, radioresistant organisms tolerate intense radiation and desiccation, and oligotrophs subsist in nutrient-deprived settings. Throughout evolution, the mechanisms enabling extremophiles to withstand multiple stresses have also generated novel genetic diversity. Owing to their ability to survive in harsh industrial environments, extremophiles are difficult to engineer but represent underexploited chassis organisms for bio-based production of valuable chemicals. Engineering approaches include CRISPR-Cas9, synthetic biology parts and devices, and traditional gene modification techniques to enhance stress resistance [1]. Extremophiles have already supported generation of sugars, biofuels, biodegradable plastics, fertilizers, and food additives, with potential for expansion. Pathways of carbon fixation such as the Calvin–Benson–Bassham cycle and the reductive tricarboxylic acid cycle, together with the Biosynthesis of Biomass Pathway (BRITE), provide inferences regarding biomass-associated processes and suggest novel routes for engineering biomass accumulation [1].

Biological Significance

Extremophiles are organisms that thrive in environments characterized by extreme physical parameters. Several types of extremophiles can be distinguished based on the prevailing environmental condition, including thermophiles, psychrophiles, acidophiles, alkaliphiles, barophiles, and halophiles [1, 3]. Because extremophiles possess physiological features that generally distinguish them from mesophiles, they have attracted considerable interest in biotechnological applications under industrially relevant conditions. Extremophiles are able to grow under an array of conditions that are usually detrimental to mesophilic organisms, including high and low temperatures, acid and alkali stress, high osmolarity, and pressure among others. Enzymes and other biomolecules produced by extremophiles and termed extremozymes, display enhanced stability and activity in a variety of extreme physical and chemical conditions, including, but not limited, to high and low temperatures, pH extremes, and high salt concentration, illustrating the potential biotechnological value of extremophiles and their components [1]. The unique attributes of extremozymes have promoted their use in pharmaceutical, food, and fine chemical industries as well as to produce numerous important commodities. With the advance of technologies, such as CRISPR-Cas genome-editing tools and engineered transduction systems, more interest has also been generated in the development of industrially useful extremophiles to serve as synthetic biological chassis to directly manufacture chemicals and fuels in harsh environments [1, 3]. Gaining understanding of how extremophiles survive in such inhospitable conditions can provide important insight for researchers and engineers

to develop engineered organisms for bio-based production. Several carbon-fixation and energy generation pathways have been identified in extremophilic microorganisms, highlighting the outstanding ability of extremophiles to utilize inorganic and organic substrates to produce biomass and energy. These pathways, combined with their evolved ability to survive under unfavorable conditions, make the extremophilic platform an ideal choice for sustainable biofuel and chemical production [1, 3].

Genetic Engineering Techniques

Extremophiles survive in severe environments by elaborating survival mechanisms that may be exploited in industrial processes[1]. Their physiological adaptations constitute a source of biodiversity and improve a variety of bioprocesses, giving rise to the concept of “biorefinery”: the use of renewable biomass in a range of processes to consistently produce fuels, power and chemicals. Among the most commonly used expression hosts are *Escherichia coli* and *Bacillus subtilis*, although natural producers are also explored [2]. Final products are not limited to fuels but include plastics and auxiliary compounds such as flavours, fragrances and additives. Genetic engineering techniques, e.g., CRISPR-Cas9-based gene editing and synthetic biology, promise rapid chassis optimization. The optimal industrial strain is predicted to combine a broad tolerance for genetic modifications with elevated levels of robustness and modular design [2]. Genome-editing techniques have been broadly adopted in model extremophiles to accelerate chassis optimization. The type II CRISPR-Cas9 system from *Streptococcus pyogenes* is the most widely used genome-editing tool and has proven effective in prokaryotes, eukaryotes and archaea. The directed catalysis of specific regions by this system allows multiple unmarked mutations in a single editing round. The resultant strain can survive as four double-strand break events would cause cell death. To enable multiple rounds of directed mutagenesis, the construct developed by Oh et al. incorporates an arabinose-inducible Cas9 system and a guide RNA platform with a simple counter-selection marker. This strategy also establishes a fast, markerless and scarless genome-editing process for up to five loci in *T. saccharophilus* [1].

CRISPR-Cas9 Applications

The CRISPR-Cas9 genome-editing system enables targeted genetic modifications to optimize microorganisms for biotechnological applications. In extremophilic bacteria and archaea, natural hosts of the Class 2 CRISPR-Cas9 system, CRISPR-based tools can probe cellular mechanisms and metabolic potential. A native Type I-F CRISPR-Cas system was characterized in *Zymomonas mobilis*, an ethanologenic bacterium with industrial potential [5]. The endogenous system was repurposed for versatile genome engineering, accomplishing gene knockout, insertion, replacement, in situ tagging, point mutagenesis, and multiplexed gene deletion. This toolkit offers simplicity, convenience, and time savings over traditional methods, eliminating the need for additional selection markers through strong CRISPR-targeting pressure, and lays the foundation for future genome engineering technologies in prokaryotes [6].

Synthetic Biology Approaches

Recent advancements in bioengineering offer promising approaches for the genetic manipulation of extremophilic microbes. Such extremophiles can act as robust chemical factories in systems subject to harsh conditions, facilitating next-generation, bio-based production. Developing synthetic biology tools, genome mining platforms, and closed-loop genetic circuit design could further enable the engineering of extremophiles and accelerate the use of chemical manufacturing platforms based on extremophilic hosts [2]. Extremophiles exhibit superior tolerance and catalytic efficiency in severe environments, making them attractive hosts for bio-based production under harsh conditions. However, manipulating extremophiles remains challenging because existing engineering tools are often incompatible with these organisms. One strategy involves transferring the enzymes of interest from an extremophilic microbe to a mesophilic species with well-established engineering toolkits. For example, industrially relevant enzymes sourced from extremophiles have been expressed in *Escherichia coli*. Complementary approaches aim to engineer the extremophiles themselves [2]. Current genetic engineering approaches include gene deletion, gene disruption, and heterologous gene expression. CRISPR-Cas9 technology has shown particular promise as a powerful genome-editing tool. The development of efficient gene delivery tools is necessary to streamline the genetic tailoring of many extremophiles. Leveraging native CRISPR systems can facilitate the rapid construction of simplified strains, genome-wide knockout libraries, and consume-strain libraries specific to extremophiles. These developments suggest that synthetic biology tools can harness extremophilic hosts for robust bio-based production [2].

Gene Editing for Stress Resistance

The CRISPR-Cas9 genome editing system has been widely used for stress resistance genetic engineering [7]. In particular, models of stress resistance have been used to develop genetic engineering strategies that impart resistance to toxic compounds such as weak acids and other growth inhibitors found in lignocellulosic hydrolysates [8]. Four different gene editing strategies to enhance acid resistance for yeast, *Escherichia coli*, and other bacterial strains were evaluated, including overexpression of proton export genes, suppression of proton import genes, overexpression of acid degradation genes, and overexpression of acid stress response genes. In an

analogous manner, similar approaches could be adapted to enhance tolerance to other important stresses such as high temperature, elevated salinity, and high radiation environments that could be exploited for industrial production. For example, versatile molecular tools that include shuttle vectors and CRISPR-Cas9 systems have been developed for efficient genetic manipulation of the thermophilic bacterium *Parageobacillus thermoglucosidasius*, enabling the engineering strain thermotolerant production. It is imperative to continue to improve knowledge of the genetic basis of stress resistance and develop novel approaches for improving tolerance to industrially relevant conditions, to broaden use of extremophiles as industrial production strains [7, 8].

Metabolic Pathways in Extremophiles

Extremophiles utilize unique metabolic pathways that enable growth under hostile environmental conditions. For instance, pond-dwelling haloarchaea fix carbon via the light-dependent Argonaute-guided hemi-phosphorylative (LAHP) pathway, potentially comprising the earliest photo-driven carbon fixation system. The Calvin-Benson-Bassham (CBB) cycle supports biomass production in salt-adapted thermophilic microbes, while the 3-hydroxypropionate–4-hydroxybutyrate (3-HP-4-HB) and dicarboxylate–4-hydroxybutyrate (DC/4-HB) cycles contribute in acidophilic and acidophilic–thermophilic archaea, respectively. Supplying extremophiles with alternative carbon sources further expands metabolic options for future engineering targets. Models like *Halomonas* TD01 simplify resolution of key pathway enzymes [9]. Systematic comparisons of model capabilities, process requirements, and economic criteria can direct selection of suitable extremophile chassis for specific production scenarios. Cascading operation pathways that exploit multiple strains in sequence reduce the burden on individual organisms, supporting biotechnological operations in increasingly challenging industrial environments [9].

Carbon Fixation Pathways

Engineering of autotrophic microorganisms extends to heterotrophic hosts to improve carbon fixation efficiency. Calvin cycle enzymes have been introduced into *Escherichia coli*, and synthetic carbon fixation pathways have been designed for formate assimilation through the reductive glycine pathway. Concurrently, omics and genetic engineering advances have elucidated alternative carbon fixation and biomass production pathways executed by extremophiles in harsh environments [10]. Analytical tools including ^{13}C metabolic flux analysis, metabolic modeling, and -omics investigations (genomics, transcriptomics, proteomics, and metabolomics) confirm that autotrophic prokaryotes provide materials and energy necessary for survival in diverse extreme habitats. These processes enable microorganisms to extract essential resources from depleted environments by assimilating basic metals, minerals, water, carbon, and energy [10]. Three major autotrophic carbon fixation pathways, the dicarboxylate/4-hydroxybutyrate cycle, the 3-hydroxypropionate/4-hydroxybutyrate cycle, and the dicarboxylate/ hydroxybutyrate/ hydroxypropionate cycle, operate in extreme environments characterized by optimal temperatures of 70–115°C. Additionally, the Wood–Ljungdahl pathway and the reductive tricarboxylic acid (TCA) cycle, with optimal temperature ranges of 55–85 and 65–95°C respectively, govern carbon fixation in thermophilic and hyperthermophilic bacteria and archaea [10].

Biomass Production Mechanisms

The bio-industrial processes in which the native, multi-extremophilic, radiation-resistant, and metabolically versatile haloarchaeon *Haloferax mediterranei* spreads out in its fullest bloom are those associated with the production of biomass and bioplastics from a variety of substrates [11]. Worldwide, increasing industrialization has led to the adoption of more and more stringent environmental and economic guidelines, which affect the implementation of biotechnological processes of biomass and energy production. These processes must be designed in such a way as to minimize the consumption of natural resources, to optimize the process with respect to energy saving, and to avoid the production of potentially toxic substances during the transformation of renewable sources [11]. A crucial aspect of such applications is the biological response and metabolic adaptation of the microorganisms used; inasmuch as high dilution of the biomass is generally required for initial process optimization, microbial growth in extreme and harsh conditions induces structural changes in the cytoplasmic membrane and physiological changes adapted to the new environmental conditions. Therefore, minerals such as Mg, Ca, K, Mn, Cu, Zn, Fe and Co enter the cytoplasm, as co-factors, activators, and allosteric effectors of enzymes, regulatory elements for biomembrane permeability, inhibitors of auto-oxidation, and agents maintaining chemical stability of the cytoplasm [1, 11].

Applications in Industrial Biotechnology

Extremophiles can be adapted for industrial conditions that standard strains cannot endure, including non-sterile operations [12]. Their metabolic capabilities enable synthesis of bioplastics, biofuels, and other high-value chemicals in challenging environments. Contemporary research focuses on enhancing industrial extant strains, halophiles for salt-tolerant crops, acidophiles for metal extraction and recovery, and thermophiles for biofuel production, through genetic engineering to exploit extremophilic properties [3].

Biofuels Production

The use of extremophilic microorganisms for biofuel production has generated considerable interest, driven by the need for efficient biomass-processing tools and production hosts resistant to harsh process conditions. Examples of promising extremophilic candidates for fuel production at elevated temperature include thermophiles belonging to the genera *Caldicellulosiruptor*, *Pyrococcus*, and *Thermotoga* [1]. Despite significant academic effort to elucidate and engineer their metabolism, production of commodity fuels on a laboratory scale using these organisms has yet to be coupled with successful scale-up to industrially relevant metrics. Production tools from a variety of thermophiles have been applied in metabolic engineering projects to explore the feasibility of generating biofuels from simple and complex biomass at elevated temperatures. Non-model thermophiles are exploited primarily for their ability to metabolize complex carbohydrates, although they are also hosts for fermentation of simple substrates. *Thermoanaerobacter* species have been engineered to generate ethanol and butanol from pentose and hexose substrates. *Thermoanaerobacterium saccharolyticum*, a naturally ethanologenic organism, is well characterized for thermophilic fermentation of pentose and hexose substrates. While this organism has been engineered to produce high titers of ethanol on different fermentation substrates, it exhibits limited growth on polysaccharides such as cellulose and hemicellulose. *Caldicellulosiruptor* species, which can convert a variety of biomass substrates into by-products such as acetate, lactate, hydrogen, and carbon dioxide, have also been investigated for biohydrogen production. *Thermoanaerobacterium aotearoense* is naturally unusually tolerant to ethanol and has been targeted for development of an ethanologenic thermophile. Engineering efforts focused on microbial synthesis of advanced biofuels via introduction of non-native biosynthetic pathways, utilization of synthetic biology tools and genome-scale models, as well as adaptive laboratory evolution and high-throughput screening techniques. Reduction or elimination of by-product formation, expression of prodigious solute transporters, and engineering of rate-limiting carbon conversion steps remain major bottlenecks in efficient production of biofuels and platform chemicals [1, 3]. A major challenge associated with fermentation of biomass is the necessary pretreatment process, which can introduce a variety of contaminants and inhibitors into a substrate saccharification/fermentation pipeline that can significantly reduce microbial fuel output. Additionally, lignocellulosic hydrolyzates generally have low sugar concentrations and their dilution for effective detoxification causes further reduction of fuel output [3]. The ability of extremophiles to grow and produce fuels in inhibitory environments, without the need for extensive detoxification, could therefore be exploited to develop cost-effective biofuel production processes from biomass. Halophilic organisms are excellent candidates for biofuel production in high-salt environments, where contaminants are prevalent in biomass hydrolyzates and additional water for dilution is limited. *Halomonas*, found across a range of salt concentrations (approximately 1.5–15%) and temperatures (around 4–45°C), are naturally endowed with high solvent tolerance, efficient sugar uptake, and accumulation of bioplastic precursors. *Halomonas campaniensis* performs efficient lactate assimilation in high-salt environments, while *Halomonas smyrnensis* is an industrial microbial isolate capable of propionic acid production in high-salt environments. Investigation of various halophiles from different genera (e.g. *Halobacterium*, *Haloferax*, and *Halanaerobium*) and their habitats could identify additional candidates with industrially relevant traits [1, 3].

Bioremediation

Microbial communities and extremozymes from extremophiles have potential for bioremediation of organic and metal pollutants and radioactive wastes in harsh environments. Isolation of engineered halophilic and other extremophilic strains may offer bioremediation opportunities in wastewater and seawater [1]. Extremophilic microbes are also used to enhance water recycling and reduce operational costs in biofuel production, e.g., thermostable glucosidases to hydrolyze steryl glucosides. The extreme alkaliphile *Bacillus marmarensis* can produce bioproducts in unsterilized wastewater and seawater, improving sustainable production with less water and energy [11]. Resistance to biocides can be harnessed for microbe-based detoxification and bioremediation since many extremophiles are tolerant to biocides and toxic compounds [13].

Bioplastics Synthesis

Extremophiles demonstrate substantial biotechnological promise in sectors encompassing bioplastics, textiles, biofuels, bioremediation, biosensors, and biomining [14]. Bioplastics synthesized by extremophiles primarily belong to polyhydroxyalkanoates (PHA, e.g., poly(3-hydroxybutyrate)), owing to their industrial significance, biocompatibility, and biodegradability [15]. Since petroleum-based plastics underpin an estimated 30% of worldwide oil consumption, sourcing plastic precursors from biological entities offers a sustainable alternative. Engineered extremophiles also hold potential in developing salt-tolerant crops and facilitating selective metal extraction from ores. PHAs represent naturally occurring polyesters produced in response to nutrient imbalances, with over 150 variants identified historically. These polymers accumulate intracellularly as carbon and energy reserves and exhibit distinctive mechanical characteristics, including thermoplasticity, biodegradability, and piezoelectricity. Up to 14,000 tandem PHA repeat units can constitute the polymer. Despite existing industrial synthesis processes,

conventional PHA production fails to satisfy growing market demand. Consequently, engineering extremophiles to augment mineral-rich polymer precursor production may boost application viability. Biosynthesis pathways (e.g., de novo fatty acid synthesis, β -oxidation) underpin PHA monomer assembly, facilitating side chain microstructural design. Engineered approaches leverage cellular metabolic pathways for bioplastics production directly from carbon dioxide, circumventing food substrate inputs [14]. The optimal extremophile chassis would naturally channel carbon flux toward PHA precursors. Halophiles, for instance, utilize the glyoxylate metabolism for poly(β -hydroxybutyrate) (PHB) synthesis; however, the inability to convert acetyl-CoA to pyruvate hampers sugar biomass growth, limiting platform flexibility. Thermophiles generally employ the tricarboxylic acid (TCA) cycle, a comparatively less efficient PHA pathway than glyoxylate metabolism. Consequently, engineered extremophiles constitute a promising source of polymer-building blocks for bioplastics, warranting exploration of alternative safe and robust host chassis [14].

Challenges in Engineering Extremophiles

Environmental conditions in many industries influence growth and productivity, presenting a hedging challenge to biological processes [1]. Industrial platforms employing extremophiles outside their optimal conditions require adaptive genetic engineering approaches to preserve fitness and production. Many industrial processes involve multiple extremes, including acidophilic or alkaliphilic thermohalophiles and thermoacidophiles, necessitating multifactorial engineering strategies [2]. Additional parameters such as temperature, oxygen tolerance, nutrient requirements, cost-effectiveness, growth rate, and maximal cell density further constrain engineering. The formulation of efficient chemical media is critical, as demonstrated by their use in evaluating engineered strains. Metabolic optimization in engineered extremophiles must avoid excessive burden that could limit cellular biomass and productivity. Finally, regulatory policies influence the deployment of genetically modified extremophiles in industrial contexts [1, 2].

Environmental Stressors

Environmental stressors encompassing physical and chemical conditions such as temperature, pH, oxygen availability, salinity, pressure, and toxic agents pose major challenges to the engineered deployment of biological systems in harsh settings. In their native settings, extremophiles frequently encounter diverse extremes and have evolved mechanisms to support growth in various habitats and across a broad range of stresses. While many extremophiles tolerate singular water-activity limiting stresses, some withstand simultaneous extremes in both water activity and temperature. Even when strains tolerate a particular environmental stress individually, growth can be severely compromised when it is combined with additional stressors [3]. Provided adequate nutrients, temperature is the primary variable affecting growth rates. Temperatures exceeding the maximum tolerated generally cause irreversible denaturation of enzymes, nucleic acids, membranes, and cytoskeletal elements. Irreversible damage to most other intracellular constituents occurs either concomitantly or within minutes of the disruption of any one of these key systems. Conversely, physical disruption from low temperatures depresses growth rates until the minimum tolerated temperature is reached, at which point ice crystal formation causes irreversible membrane leakage and cellular rupture [3].

Metabolic Burden

Recombinant strains are indispensable for exploring the mechanisms of extremophiles, expanding molecular tools for these organisms and facilitating their diverse industrial applications. Beyond fermentation engineering where overexpressed enzymes demand cofactor regeneration that often shifts metabolic fluxes heterologous protein production imposes a substantial metabolic burden on host cells [16]. During induction, host cells allocate excessive resources to synthesizing heterologous proteins, as well as for transcription, translation and post-translational modifications. This heavy demand often reduces energy supply for growth and maintenance, resulting in decreased viability, elongation of doubling time, reduction in biomass production, decreased growth rate and unintended variations in production capacity and yield that complicate further applications [16]. An additional challenge is the accumulation of toxic by-products such as acetate and ammonium, which further impede growth and protein production. Halophiles can mitigate metabolic burden owing to their ability to thrive in environments with low nutrient availability and high concentrations of toxic substances [17]. In contrast to non-halophile industrial microorganisms such as *Escherichia coli*, *Halomonas* strains have the potential to directly degrade lignin-derived compounds, including ferulic acid, vanillic acid and 4-hydroxybenzoic acid [2].

Regulatory Considerations

A global regulatory framework specific to extremophiles is currently lacking, although many countries monitor genetically modified extremophiles on a case-by-case basis. Certain extremophiles, such as thermophiles, are likely to be regulated as pathogenic or risk-grouped organisms because of their increased growth temperature and metabolic rate relative to mesophilic organisms [3]. Handling genetically modified halophilic archaea for industrial-scale production raises concerns related to biosecurity and biosafety. In China, genetically modified self-cloning organisms and exotic organisms are regulated by the “Measures on the Administration of Safety of

Biotechnology Research and Development" (June 1996) and the "Regulations on Administration of Human Genetic Resources" (June 1998). The handling of biohazardous materials requires approval under the "Regulations on Biosafety Management of Pathogenic Microbial Laboratories" (Ministry of Health, 2004). *Ralstonia eutropha* H16 (1 year continuous culture at 75 °C on hydrogen and carbon dioxide), *Hydrogenimonas* EPR 70 and their genetically modified derivatives are classified as pathogenic or risk-grouped organisms because of their increased growth temperature and specificity for hydrogen and sulfur oxidation, whereas halophiles are not accredited as pathogenic or risk-grouped organisms [3]. Tailoring or controlling the gene expression of engineered extremophiles in harsh conditions requires a thorough understanding of global protein–DNA interactions in the cell. Most of the global DNA-binding proteins in archaea make physical contact with the nucleic acid by recognizing the major groove [3].

Case Studies of Engineered Extremophiles

Thermophiles serve as crucial candidates for biofuels production and bioremediation in harsh industrial settings; they also enable the facile secretion of heterologous proteins without the need for cell lysis [1]. The discovery, characterization, and construction of halophilic hosts and genetic tools have facilitated the production of ectoine and polyhydroxyalkanoates in halophiles [1]. The construction and wide application of halophilic protocell models, based on biological components, further support genetic engineering in these organisms. Gas vesicles have been used to vectorially display heterologous proteins in halophiles. Addressing the future challenges concerning the supply of low-cost raw materials, enhancement of genetic toolkits, and understanding of the polar photic zone environment will be essential to develop well-engineered halophilic cells, paving the way for sustainable industrial biotechnology [1]. Acidophilic, acid-tolerant, and aciduric extremophiles have been exploited in engineered systems designed to selectively bind and recover copper, rare earth metals, and gold at pH values below 4 and temperatures exceeding 70 °C. *Sulfolobus islandicus* is intrinsically acid- and thermotolerant, thrives at high temperatures (>70 °C), and has been engineered to produce industrial cellulose and metal-binding peptides without a metabolic burden associated with plasmid maintenance, enabling precise and tunable protein expression. Investigations into the pH limits of other microbial metabolisms will assist the development of industrial processes under conditions that limit water activity and reduce contamination risks associated with higher pH settings. Bioleaching has become a foundational method for extracting metals such as copper, gold, and cobalt from deep ore bodies unsuitable for traditional mining. Future scenarios predict frequent ore accessibility challenges, extensive low-grade ore stockpiling, and increasingly stringent environmental regulations. To alleviate processing strain, attention has shifted toward microbes that function at significantly reduced pH values below [1]. Hydrogeochemistry dictates that potential microbe-inhabited environments span a pH range from +1.5 to -1.5. For example, the neoarchaeon ARMAN thrives in extremely acidic conditions in bioreactors [1].

Thermophilic Bacteria in Biofuel Production

Thermophilic bacteria are a focus for biofuel production because they can survive in a temperature range of 40 °C to 72 °C and produce thermostable enzymes [18]. Genes from *Geobacillus thermoglucosidasius* have been engineered to work alongside native metabolism for ethanol production, and further metabolic modifications are underway to enhance carbon and electron ion efficiency [19]. Thermophilic bacteria isolated from environmental waste have been genomically characterized for multiple thermostable extracellular enzyme production. These bacteria activate genes encoding enzymes such as cellulase, xylanase, pectinase, amylase, and protease, which are advantageous for biotechnological applications. *Thermoanaerobacter thermohydrosulfuricus* sustains maximal growth at an optimal temperature of 70 °C and produces a variety of extracellular enzyme-coding genes across these categories. Continuous ethanol fermentation of dilute acid-treated corn stover hydrolysate has been conducted with the thermophilic bacterium *Thermoanaerobacter* BG1L1, which also demonstrates high ethanol tolerance. Several studies have examined how temperature influences ethanol tolerance and modeling simulations of *Thermoanaerobacter* species. Growth rate affects ethanol production by *Thermoanaerobacter ethanolicus* when cultured on glucose or xylose, with variable ethanol yields reported from steady-state continuous cultures on these substrates. Enzymatic activities and fermentation yields related to ethanol production have been investigated in *Clostridium thermocellum* and *Thermoanaerobium brockii*. The glucose fermentation pathway of *Thermoanaerobium brockii* has been elucidated, and both *Thermoanaerobacter brockii* and *Thermoanaerobacter yonseiensis* are capable of converting amino acids into branched-chain alcohols. Recent advances include the use of novel extremely thermophilic bacteria for single-step ethanol production directly from lignocellulose. Additionally, metabolic engineering efforts targeting *Geobacillus thermoglucosidasius* aim to achieve high-yield ethanol synthesis. Bioprocessing agricultural waste into ethanol also utilizes extremophiles, while thermophiles more broadly serve the production of various fuels and chemicals [18, 19].

Halophiles for Salt-Tolerant Crops

Salinization of arable land is one of the major causes for crop destruction worldwide [20]. Though the majority of the commercial crops are sensitive to saline environments, several wild plants that grow naturally in salty soils can tolerate salinity, such as halophytes and halotolerant plants. Halophiles are completely dependent on saline environments and cannot survive in salt concentrations below a critical range. Halotolerant plants and bacteria, in particular, can grow in the absence of salt as well as at high salt concentrations [20]. Bacteria isolated from the coastal Patenga area are halotolerant, as they can tolerate up to 20% sodium chloride in their growth medium. Such bacteria are exposed to various environmental stresses, including salinity, pH, heavy metals, and presence of other halotolerant species. Therefore, seawater (salt water) alone cannot completely inhibit the growth of halotolerant bacteria, and they survive in that environment for a longer period of time. The isolation of salt-tolerant microorganisms is important for their many industrial applications, such as the production of polyhydroxyalkanoates (PHAs), bioplastics, enzymes, and biodegradable and renewable biopolymers [20].

Acidophiles in Metal Recovery

Many metals originate from sulfide minerals, which are refractory minerals and difficult to process through flotation methods. Additionally, high sulfur content can damage smelters. Therefore, bioleaching is widely used for mineral pre-processing or metal extraction. Similar to bioleaching of ores such as copper, uranium, and nickel, acidophiles play an important role in metal recovery from electronic waste, wastewater treatment, and bioremediation. *Ta. Genus* organisms (formerly classified as *Acidithiobacillus*) are widely studied for acidophilic bioleaching. *Acidithiobacillus ferrooxidans* is a gram-negative, chemolithoautotrophic acidophile known to be effective in metal bioleaching. Bioreaction occurs at the contact interface of *A. ferrooxidans* and minerals. For example, copper and iron are accumulated on the cell surface. *A. ferrooxidans* attaches not only to mineral surfaces, but also to inert substrates and the EPS matrix [21]. Iron- and sulfur-oxidation mutants indicate that both iron- and sulfur-oxidation pathways and the initial attachment stage are essential for cell adhesion during chalcopyrite bioleaching; however, effects of energy sources are different for various minerals and the relationship between energy metabolism and biofilm formation remains to be unveiled. A high rate of copper and iron recovery from chalcopyrite under extreme thermoacidophilic conditions is promising for improving the treatment of refractory materials and is attributed to the enhanced kinetics of oxidative mechanisms of chalcopyrite bioleaching. Additionally, a variety of studies have examined the population dynamics of bioleaching microbiology, including quantitative monitoring techniques and the effects of temperature on the community composition [22].

Future Perspectives

Extremophiles possess unique physiological adaptations to harsh conditions. Their applications in industrial biotechnology are becoming increasingly attractive; genetic engineering has expanded beyond model organisms such as *Escherichia coli* and *Saccharomyces cerevisiae*. Microbes capable of surviving extreme industrial or municipal bioreactor stresses are in demand. This review surveys genetic engineering techniques for extremophiles, offering an overview of carbon fixation pathways suitable for engineering and identifying outstanding challenges. When fully developed, engineered extremophiles as well as their enzymes, biomass, and biochemicals—may contribute to bio-based manufacturing activities currently depending heavily on fossil fuels, often within corrosive or otherwise aggressive settings [21, 22].

Emerging Technologies

New microbial engineering techniques have recently emerged to cultivate non-model, recalcitrant strains such as extremophiles that demonstrate potential for bio-based chemical production. Genomic databases have increased, driving research on non-model organisms, and genetic editing techniques such as CRISPR-Cas9, synthetic biology, and homologous recombination have been applied to investigate fundamental biological properties and growth at scale [1]. Techniques including metabolic engineering, adaptive laboratory evolution, and epigenetic modification have enhanced intracellular fluxes and improved resistance to environmental stresses, increasing chemical production through strain development. Extremophilic microorganisms thriving in habitats with extreme physical or chemical conditions belong to physiological groups such as thermophiles, hyperthermophiles, psychrophiles, piezophiles, acidophiles, alkaliphiles, and halophiles. They inhabit environments characterized by elevated temperatures, lowered temperatures, high pressures, acidic pH, alkaline pH, and high salinity, respectively. Adaptations to these stresses occur across levels of genome, transcriptome, proteome, and membrane physiology and biochemistry; these biological states facilitate energy harvesting, carbon fixation, and biomass production at extremes, offering opportunities for exploration in biological sciences and applications in intensive bio-based production with low environmental impact [1].

Sustainability Considerations

In modern industrial societies, energy and material consumption is increasing, and the resulting waste streams are causing serious sustainability concerns. Bio-based production processes using extremophiles offer a sustainable

alternative to the fossil fuel-based economy. Biological material conversion using extremophiles generally occurs under ambient conditions, and the resultant bioproducts are typically biodegradable and biocompatible [1]. Bio-based manufacturing using extremophiles represents a sustainable model. As extremophiles operate under conditions that suppress microbial contamination, there are no sterilization requirements or only limited sterilization requirements. Any contaminating microbes can be inhibited using the appropriate environmental stressor (e.g., extreme pH, high temperature) without the addition of expensive sterilization equipment. Because industrial fermentations are not sterilized, and culture media can therefore be constructed from bulk commodities, the fermentation cost can be reduced by approximately half. As relative percentages, economic analyses of bioethanol production showed that raw materials constitute more than 80% of production costs, whereas maintenance and energy contributed less than 10% each. In anaerobic fermentations, the production of CO₂ is avoided; instead, H₂ is generated, providing additional advantages from an environmental and sustainability perspective [1, 22]. A variety of engineered extremophiles have already demonstrated potential for deployment in industrial biotechnology, but there are many challenges that remain. As an example, an engineered haloarchaeon (*Halobacterium*) was shown to produce α -amylase, but the high salt concentration required over many weeks of cultivation proved to be a major barrier. *Sulfolobus* structures can exhibit significant heterogeneity during scale-up, and it is currently difficult to engineer both transcriptional and translational regulation in these cells. Engineered halophiles have been shown to accumulate polyhydroxybutyrates from acetyl-CoA but achieve low yields of this value-added commodity bioproduct, limiting their commercial appeal. Extremophiles in bio-based production remain a very active research topic, and it is likely that these challenges will ultimately be addressed. However, it is clear that there is considerable scope for improving extremophile-based production applications [1, 22].

CONCLUSION

Engineered extremophiles stand at the frontier of industrial biotechnology, offering solutions where conventional microbial systems fail. Their natural ability to withstand high salinity, acidity, pressure, or temperature enables industrial processes to operate under extreme conditions, reducing contamination risks and improving efficiency. Advances in CRISPR-based editing, synthetic biology, and systems-level modeling have made it increasingly possible to design extremophile strains for the targeted production of biofuels, biodegradable plastics, pharmaceuticals, and stress-tolerant crops. However, realizing their full potential requires overcoming key challenges, including genetic intractability, limited commercial scalability, and regulatory hurdles. The integration of computational tools, omics technologies, and collaborative research can accelerate progress in this field. Looking ahead, engineered extremophiles could play a pivotal role in sustainable energy, food security, and environmental conservation supporting a resilient bioeconomy aligned with climate change mitigation and circular economy principles.

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