DOI: https://doi.org/10.54393/fbt.v5i4.199

FUTURISTIC BIOTECHNOLOGY
https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Vol 05 Issue 04, (Oct-Dec, 2025)

Review Article

OPEN ACCESS

Extremophilic α-Amylases: Structural Adaptations, Discovery Strategies, and Industrial Applications (2020-2025)

Ibrar Ul Haq[1*], Hassan Saeed[1], Hooria Wasim[2], Muhammad Ahsan[1], Ansar Khan[3] and Abdillahi Ismail Mohamed[2]

[1]Center of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan
[2]Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan
[3]Department of Biotechnology, University of Malakand, Malakand, Pakistan

ARTICLE INFO

Keywords:
Extremophiles, Enzyme Stability, Metagenomics, Protein Engineering, Industrial Biotechnology, Starch Hydrolysis

How to Cite:
Haq, I. U., Saeed, H., Waseem, H., Ahsan, M., Khan, A., & Mohamed, A. I. (2025). Extremophilic α-Amylases: Structural Adaptations, Discovery Strategies, and Industrial Applications (2020-2025): Extremophilic α-Amylases: Structural Adaptations and Discovery Strategies. Futuristic Biotechnology, 5(4), 09-17. https://doi.org/10.54393/fbt.v5i4.199

***Corresponding Author:**
Ibrar Ul Haq
Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan
ibrarmeraj@gmail.com

Received Date: 5th October, 2025
Revised Date: 19th November, 2025
Acceptance Date: 24th November, 2025
Published Date: 31st December, 2025

ABSTRACT

The use of the enzyme α-amylases is a large-scale industrial enzyme used in the manufacture of food and beverages, textiles, detergents, paper, pharmaceuticals, and biofuels. Conventional microbial α-amylases, primarily Bacillus and Aspergillus-based ones, have been in use for many years, but their effectiveness is often limited by the harsh conditions of industrial processes. Extremophilic enzymes such as thermophiles, halophiles, acidophiles, alkaliphiles, and psychrophiles are an attractive alternative to resilient α-amylases with exceptional thermostability, pH tolerance, salt resistance, and, in some cases, cold activity. This review sums up recent developments (2020-2025) in the discovery, biochemical characterization, as well as industrial application of extremophilic α-amylases. New culture-independent technologies, such as metagenomics, high-throughput functional screening, and machine learning-guided enzyme mining, are highlighted because they help to increase the number of genes in α-amylases of previously unculturable microorganisms. The discussion is centered on structural and mechanistic understanding concerning enzyme stability with reference to comparison to conventional counterparts. Although considerable advances have been made, there are still several gaps in the exploration of unexplored habitats, structural explanation of identified new enzymes, and cost-effectiveness of industrial applications. A combination of extremophilic scaffolds with protein engineering, synthetic biology, and sustainable fermentation has great potential for the realization of tailored α-amylases to serve advanced bioprocesses. The advances make extremophilic α-amylases an important source of industrial biotechnology innovation.

INTRODUCTION

It is a metalloenzyme (EC 3.2.1.1) that catalyzes the hydrolysis of internal 1, 4 glycosidic bonds in starch, glycogen, and related polysaccharides in the presence of calcium to produce maltose, glucose, and limit dextrins [1]. Thanks to using starch in plants as the main source of carbohydrates, as well as in many industries as one of the main raw materials, 2-amylases are the crucial biocatalysts in starch processing. Today, they form some of the most manufactured commercial enzymes, with a presence of about 25-30% of the world's enzyme market, just being surpassed by proteases [2]. The worldwide market of α-amylase is expected to grow at a compound annual growth rate (CAGR) of 5.9 percent between the year 2023 and 2033. In 2023, the market is estimated to be valued at over USD 1,840.8 million, and this is expected to reach approximately USD 2,692.5 million in 2033 [3]. They have extensive applications in the food and beverage sector, brewing, baking, making sweeteners, in the textile industry to desize fabrics, in the detergent industry to remove stains made by starch, in the pulp and paper industry to reduce their viscosity, the pharmaceutical industry and as a source of biofuels among other things, indicating their widespread

DOI: https://doi.org/10.54393/fbt.v5i4.199

relevance in economics [4]. The major use of α-amylase was demonstrated (Figure 1).

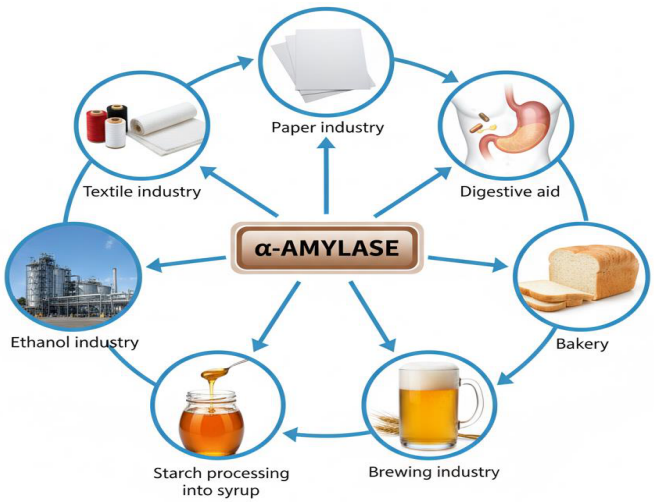

Figure 1: Major Industrial Sectors Utilizing α-Amylase

Its applications are important in the food and beverage sector (bakery, brewing, starch processing into syrups), the textile and paper industry, and in the production of ethanol and as a digestive aid in pharmaceuticals and nutraceuticals. In the past, Bacillus species (e.g., B. licheniformis, B. amyloliquefaciens) and filamentous Aspergillus species have been used as the major microbiological sources of commercial alpha-amylase production because of their high secretion capacity, established fermentation techniques, and regulatory approvals [5]. The enzymes are often found to show poor functioning in high-pressure industries characterized by high temperatures, harsh pH, or heavy salt. This can lead to denaturation of enzymes, loss of activity, and increased production costs due to the need to have stabilizers or process adjustments [6]. The paper gives a comparative overview of traditional microbial α-amylases and extremophilic α-amylases, their optimal temperature, pH, halotolerance, and applications. Stability is better at high temperature, strong pH, and high salinity than conventional Bacillus and Aspergillus enzymes, and extremophilic α-amylases are therefore better adapted to harsh industrial environments (Figure 2).

Comparative Chart

Feature	Conventional α-Amylase	Extremophilic α-Amylase
Optimal Temperature	Typically mesophilic, ranging from 37∘C (human) to 70°C (bacterial).	Often highly thermophilic, with optimal temperatures above 70°C and some functional up to 100°C or more.
Optimal pH	Generally neutral to slightly acidic, typically pH 6.0 to 7.5.	Highly variable, with optimal activity in a broad range, from acidic (pH 4.0) to alkaline (pH 10.5).
Halotolerance	Low tolerance; activity is often inhibited by high salt concentrations.	High tolerance; many are active and stable in high salt environments, tolerating several molar concentrations of NaCl.
Sensitivity to Solvents	Generally low tolerance; can be denatured by organic solvents.	High tolerance; often stable and functional in the presence of organic solvents.

Figure 2: Comparison of Conventional and Extremophilic Microbial α-Amylases

Microorganisms known as extremophiles, which flourish in conditions previously deemed inhospitable to life, present a significant and largely unexplored source of resilient biocatalysts [7]. Organisms such as thermophiles found in geothermal springs, compost heaps, and hydrothermal vents generate enzymes that maintain stability at elevated temperatures. Halophiles, which inhabit salt flats and saline lakes, produce enzymes that can withstand high salt concentrations [8]. Acidophiles, sourced from acidic mines or hot pools, along with alkaliphiles from soda lakes, demonstrate stability across varying pH levels [9]. Meanwhile, psychrophiles from polar and alpine regions develop cold-active enzymes that function effectively at low temperatures [10]. α-Amylases derived from these organisms frequently exhibit a range of beneficial characteristics, including thermostability, halotolerance, and pH stability. These traits can contribute to minimizing contamination risks, decreasing energy consumption, and enhancing process efficiency in industrial applications [11]. The representative global habitats of microorganisms that thrive in extreme conditions, including thermophilic, halophilic, acidophilic/alkaliphilic, and psychrophilic environments, all of which are capable of producing α-amylases, were illustrated. Thermophiles (red) are associated with geothermal springs and hydrothermal zones; halophiles (blue) inhabit saline lakes and salt flats; acidophiles/alkaliphiles (orange) occur in acidic mines and soda lakes; psychrophiles (light blue) are found in polar and alpine environments. Callouts indicate representative locations (Figure 3).

DOI: https://doi.org/10.54393/fbt.v5i4.199

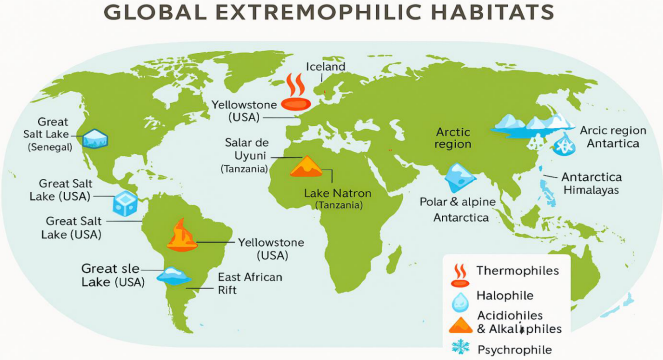

Figure 3: Global Distribution of Representative Extremophilic Habitats

The culture-independent methodologies of the recent past, especially metagenomics, amplicon sequencing, and high-throughput functional screening, have given us a wide knowledge of microbial diversity beyond the small number of culturable species [12, 13]. These approaches have been used to identify a large number of new α-amylase genes whose properties may be improved using protein structure modeling, homology-based mining, and machine learning techniques [14]. However, few comprehensive reviews have been conducted to capture such improvements. The present review provides a revised synthesis of microbial α-amylases in extreme environments with their structural and biochemical adaptations, their discovery methods, and their use in the food processing sector, textile sector, detergents, paper production, pharmaceutical sectors, as well as biofuel production. Traditional alpha-amylases, predominantly found with Bacillus and Aspergillus strains, control the market but usually exhibit low stability in high temperatures, extreme pH, or high salt content. These shortcomings add to production expenses and limit them in severe industrial processes. Thermophiles, halophiles, acidophiles, alkaliphiles, and psychrophiles are examples of extremophilic microorganisms that provide enzymes with impressive thermostability, halotolerance, and pH adaptability that are more suited to the needs of industry. New technologies (within the last five years, 2020-2025), including metagenomics, functional screening, structural biology, and machine learning controlled enzyme mining, have increased the finding of novel α-amylase genes by previously unculturable microbes. Synthetic biology and protein engineering are also making the optimization of extremophilic scaffolds for particular uses feasible. Nevertheless, there are still significant knowledge gaps in terms of studying unexplored habitats, resolution structures, enhancing heterologous expression, and creating cost-efficient scale-up techniques. This review aims to fill this gap between environmental enzyme diversity and commercial application by combining ecological, biochemical, and technological views on the research in extremophilic 2-surrendered Aylase, presenting both the present and possible future developments of this research. The left track shows culture-dependent screening (isolation, fermentation, and activity assays), while the right track shows culture-independent approaches (DNA extraction, metagenomic sequencing, bioinformatic mining, and functional screening). Both approaches converge at the characterization of new α-amylase candidates for industrial application (Figure 4).

DISCOVERY OF NOVEL α-AMYLASES

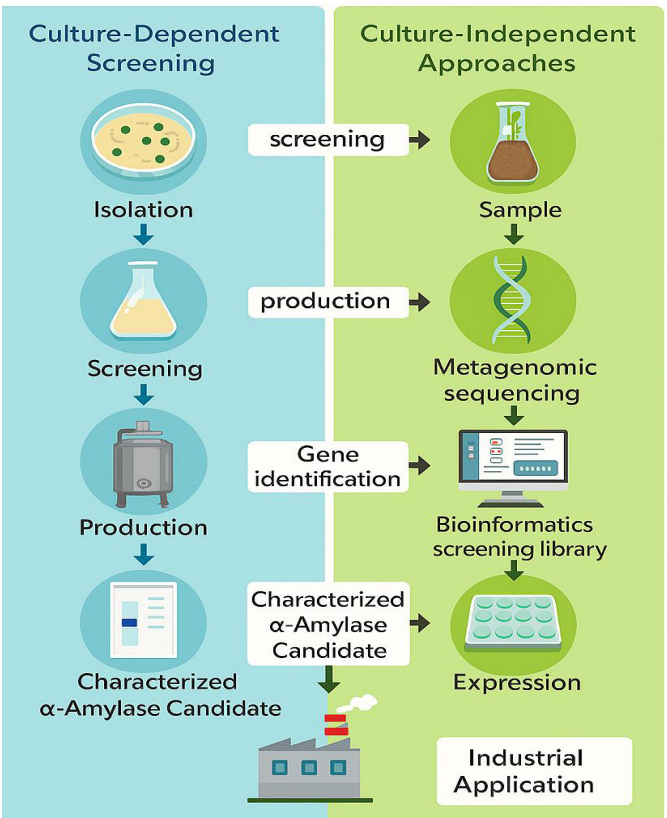

Figure 4: Workflow for Discovery of Novel α-Amylases

Classification

α-Amylases (EC 3.2.1.1) are found in the glycoside hydrolase (GH) family and are mostly defined by their action on the polysaccharide chains [15]. Two main types of amylolytic enzymes are possible. Endo-amylases break internal α-1,4 bonds of starch and other related polysaccharides, producing oligosaccharides of variable length. Exo-amylases, such as α-amylase (EC 3.2.1.2) and glucoamylase (EC 3.2.1.3), sequentially cleave α-1,4 or α-1,6 bonds at the non-reducing terminus of the polypeptide to yield maltose or glucose [16]. The most outstanding endo-hydrolase in such categories is α-amylase, with its high catalytic ability and extensive substrate selectivity. It finds widespread application in the food industry, detergent industry, and biofuel industry because of its flexibility and cost-effectiveness [17]. A schematic categorization of the

DOI: https://doi.org/10.54393/fbt.v5i4.199

amylolytic enzymes into endo-type and exo-type is distinguishing between endo-type enzymes, which cut internal α-1,4 bonds, and exo-type enzymes, which cleave one or two terminals of the chains.

Mechanism of Action

The hydrolysis of starch is catalyzed by alpha-amylase using the retaining, double-displacement reaction that involves a covalent glucosyl-enzyme intermediate. A conserved catalytic triad of a nucleophilic aspartate, an acid/base glutamate, and a stabilizing aspartate residue at the catalytic domain facilitates this reaction [18, 19]. Active-site water molecules coordinate and play a key role in the glycosylation phase and determine the reaction rate [20]. Cofactors such as bound Ca 2 ions have an effect on domain interfaces, but neighbouring Cl ions alter acid-base arrangements and further enhance the activity of the catalyst, particularly in chloride-dependent subfamilies. The combination of these dynamics, along with the help of metal/anion-induced modifications, can be considered the significant determinant of the activity, stability, and commercial performance of alpha-amylase. The catalytic action of 2010 kb of alpha-amylase with its preserved active-site residues and necessary metal/anion cofactors. Left: internal 14-alpha-1,4 bond is cleaved by endo-amylases (e.g., 2-amylase, EC 3.2.1.1) into oligosaccharides. Right: exo-amylases(e.g., α-amylase, EC 3.2.1.2; glucoamylase, EC 3.2.1.3; pullulanase, EC 3.2.1.41) remove glucose/maltose units from chain termini. Examples indicate common sources and representative industrial applications(Figure 5).

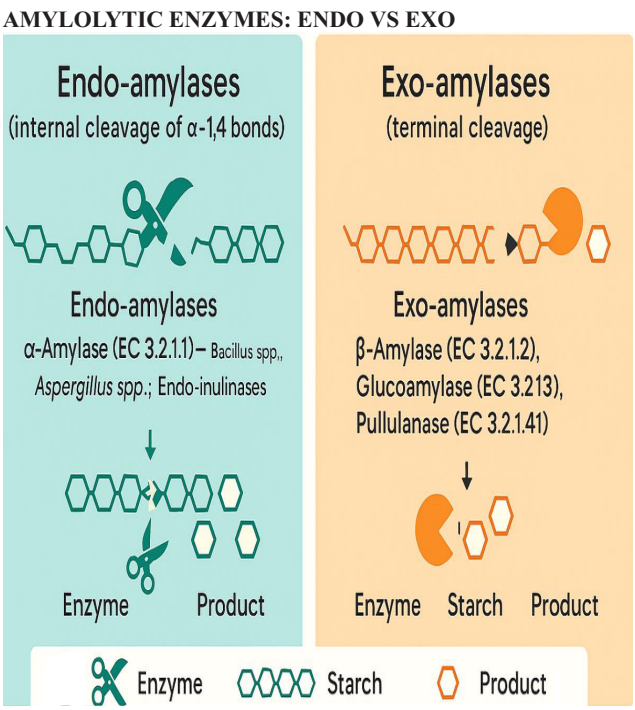

Figure 5: Classification of Amylolytic Enzymes into Endo and Exo-Acting Types

The mechanism employed by the enzyme is a retaining double-displacement reaction that includes an Asp nucleophile, an acid /base Glu, and a stabilizing Asp. The structural stability of the ion is supplied by calcium ions (Ca 2 +), whereas the allosteric modulators are chloride ions (Cl -). Schematically, the two-step formation of the covalent glucosyl enzyme intermediate and hydrolysis to give maltose/glucose is depicted.

The Structural and Functional Features

The alpha-amylases are usually monomeric proteins with a molecular weight ranging between 45 and 60 kDa [21]. They exhibit three domain folds: Domain A (the β/α barrel catalytic domain), Domain B (a loop area needed in binding Ca 2), and Domain C (a β-sheet domain commonly involved in binding raw starch). This modular design enables the α-amylases to interact with a large number of substrates and maintain their functions in different working conditions [22]. The variations of loop-regions, surface residues, and metal-binding sites between extremophilic and α-amylases are considered to be the causes of their higher stability at high temperature levels, salinity, or extreme pH levels [23]. Extremophilic Micro-organisms as Novel Suppliers of 2-Amylases [24]. The α-amylases that are secreted by these microorganisms are physically and functionally adapted to survive high temperatures, high PH, high salinity, or low temperatures. These features make them attractive replacements for conventional microbial α-amylases in the industry [25, 26]. Findings: The species of microbes and their location and optimum temperature and pH. (Table 1).

Table 1: Representative Extremophilic Microorganisms Producing α-Amylases and Their Key Biochemical Properties

Microorganism (Species)	Environment/ Habitat	Optimal Temp (°C)	Optimal pH	Unique Traits/ Notes
Geobacillus stearothermophilus	Hot springs (thermophile)	70-80	5.5-6.5	High thermostability, rapid starch liquefaction
Anoxybacillus flavithermus	Compost/ thermal soils	65-75	6.0-7.0	Calcium-independent α-amylase (REF)
Haloferax mediterranei	Salt flats (halophile)	40-50	6.5-7.5	Active at 3–4 M NaCl
Halomonas meridiana	Saline lakes (halophile)	35-45	7.0-8.0	Broad salt tolerance
Alicyclobacillus acidocaldarius	Acidic hot springs (acidophile)	55-65	3.0-4.0	pH stability at low pH
Bacillus alcalophilus	Soda lakes (alkaliphile)	40-50	9.0-10.5	High alkaline stability
Pseudoalteromonas haloplanktis	Polar sea (psychrophile)	0-15	6.0-7.0	Cold-active α-amylase
Colwellia psychrerythraea	Arctic sediments (psychrophile)	0-10	6.0-7.0	Catalysis at low temperature

DOI: https://doi.org/10.54393/fbt.v5i4.199

This table summarizes major extremophilic microbial species reported between 2020 and 2025, highlighting their native habitats, optimal temperature and pH for α-amylase activity, and unique adaptive traits such as thermostability, halotolerance, acid/alkali stability, and cold activity.

Thermophilic Organisms

Thermophilic archaea and bacteria thrive in geothermal springs, compost heaps and hydrothermal vents [27]. Their amylases have 2, 3-alpha2 stabilities and activities at high temperatures above 70° C and they are capable of rapid liquefaction of starch as well as reduced viscosity without the use of additional stabilizers [28, 29]. Some examples include Geobacillus stearothermophilus, Anoxybacillus flavithermus, and some archaeal species of thermal springs [30]. Hermophilic microorganisms of geothermal environments generate α-amylases that are stable and active at high temperatures, allowing efficient liquefaction of starch under industrial conditions.

Halophiles

Halophiles live in the salt lakes, solar salterns and saline soils, producing α-amylases that remain active in high salt levels [31]. Halotolerant enzymes reduce the risk of contamination and stabilise industrial reactions within a brine or high-ionic strength environment. Other notable producers include Haloferax spp., Halomonas meridiana and Natrialba magadii [32-34].

Acidophiles and Alkaliphiles

Alpha-amylases that are produced by acidophilic and alkaliphilic bacteria in the acidic mines or soda lakes have the ability to remain active at extreme levels of pH widening the scope of their application in food processing, detergents and in producing biofuels [35]. They include Alicyclobacillus acidocaldarius (acidophilic) and Bacillus alcalophilus(alkaliphilic)[36].

Psychrophilic Organisms

The cold-active alpha-amylases synthesized by psychrophilic polar region and alpine microorganisms enable low-temperature catalysis thus reducing energy costs in processes like cold-wash detergents as well as the fermentation of chilled food [37, 38]. Such species as Pseudoalteromonas haloplanktis and Colwellia psychrerythraea[39].

Other Potential Market Niches.

Other than well-studied thermophilic, halophilic, acidophilic/alkaliphilic, and psychrophilic environments, deserts, deep-sea hydrothermal vents, and cave microbiomes are ecosystems with novel α-amylases of unknown properties. Metagenomic studies of such environments have demonstrated numerous families of amylase genes. The continued research can reveal the existence of enzymes with unique substrate specificities or stability properties that can be used in new industrial applications.

Biochemical and Functional Properties of Extremophilic α-Amylases

Extremophilic 2-amylases are biochemically and structurally distinct to enable their stability and functioning in harsh conditions. These properties make them unlike the conventional microbial α-amylases which make them suitable to specific industrial use [28].

Thermal Stability

High temperature thermophilic α-amylases have increased optimum temperatures (typically over 70°C) and high temperature half-lives, which reduce the viscosity of starch slurries, speed up processing, and reduce the risk of contamination [40]. Improved thermostability is often due to increased hydrogen bonding, additional salt bridges, reduced hydrophobic cores of reduced size, and greater loop region rigidity [41].

pH Stability

The acidophilic and alkaliphilic 1/ 2 -amylases can be catalytically active in extreme pH and at the same time they can be used at pH 2-4 and also at pH 9-11 [42] The large pH stability would ensure that the processes of adjusting the pH would be significantly reduced in the industrial processes, which would lead to higher efficiency and lower costs [43]. Some of the structural adaptations include in the modification of surface charge distribution and the strengthening of Ion-pair networks [44].

Salt Tolerance

Halophilic alpha-amylases are active at high ionic strength (i.e. 3M NaCl) and thus, at that concentration, other enzymes would precipitate or denature. This feature works to the advantage of brine-based processes and improves the stability of enzymes to proteolysis [45]. To guarantee solubility, halophilic enzymes tend to have an increased concentration of acidic amino acids on their surfaces and reduce their hydrophobicity [46].

Cold Activity

Psychrophilic -amylases make efficient use of low temperature (0-15°C) to catalyze hydrolysis of starch which may be used in cold-wash laundry product and chilled food fermentation with low energy consumption [47]. Their high catalytic turnover and low thermostability are evidence of active-site loops that are flexible, as well as a small number of stabilizing interactions.

Substrate Specificity and Raw Starch Affinity

The substrate specificity of α-amylases extremophiles has a broad range of substrate specificity in comparison to traditional enzymes, which have a low affinity to raw starch. The features can reduce the number of pretreatment steps and increase yields of the starch-based processes [48]. This affinity is increased by aromatic residue-binding domains or surface loops. Industrial uses of extremophilic α-Amylases Applications The enzyme is useful in industries

DOI: https://doi.org/10.54393/fbt.v5i4.199

to substitute traditional enzymes in diverse functionalities. They have the advantage of being naturally stable in high temperature, extreme pH or high salinity conditions to promote shorter processing times, less risk of contamination, and less need to use stabilizing additives [49]. Alpha-amylases are thermophilic enzymes involved in the liquefaction of starch used in brewing, baking, as well as preparation of sweeteners like high fructose syrups and maltodextrins [50]. They have a high tolerance to high temperatures and can be directly incorporated into hot mash or dough processes and as a result, reduce cooling processes and increase efficiency [51].

Paper and Textile Industries

In the case of textiles, 3-amylases are used in the removal of starch-based finishes in fabrics. Enzymes that are thermostable promote short desizing times and water saving. In pulp and paper industry, alpha-amylases reduce the viscosity of the pulp, hence increasing drain and sheet forming [52].

Surfactants

Psychoactive 0-amylases are cold-active enzymes, which can be used at low washing temperatures to save energy during domestic and commercial laundry. They still remain active in highly salty and alkaline detergents formulations, outperforming conventional enzymes [53, 54].

Biofuels and Biorefineries

By eliminating the additional cooling stages and providing the possibility of saccharification and fermentation in one step, thermophilic α-amylases promote the effectiveness of the starch-to-ethanol transformation. This saves on energy use and enhances yield on production of bioethanol [55].

Pharmaceuticals and Special Applications.

Extremophilic 6-amylases have been studied in terms of their possible uses as digestive enzymes, diagnostic enzymes and in controlled-release formulations in which they must endure atypical conditions [56].

Identifying Research Gaps and Future Directions

Nevertheless, in spite of the major achievements of the isolation and characterization of a-amylases of extremophiles, there are some critical gaps in knowledge. Limited Exploration of Ecosystem Research has mainly focused on readily available geothermal springs, saline lakes and soda lakes. There are many untapped niches, such as deep-sea sediments, desert-dwelling crusts, cave microbiomes and extreme oligotrophic environments, that are not well characterized. The use of higher sampling and global cooperation can reveal new enzyme families with unanticipated properties. Clues to Structure and Mechanism despite many new α-amylase genes having been discovered, few have high-resolution 3D structures. To explore the atomic basis of thermostability, halotolerance, and pH stability through crystallography, cryo-EM, and computational modeling is necessary to engineer the rational enzyme. Several metagenomic studies have been performed based on functional metagenomics and high-throughput screening in which sequence-based mining is commonly applied, whereas functional screening has been constrained by throughput and expression issues. The use of novel microfluidic and cell free systems of expression can facilitate a greater number of active enzymes that are acquired directly out of environmental DNA, protein engineering and directed evolution. A combination of extremophilic scaffolds with site-directed mutagenesis, domain swapping, and directed evolution can be used to create tailored α-amylases that can be used in specific industrial settings. The inclusion of machine learning models in stability or substrate prediction could reduce the experimental effort (REF) that is needed. Regulation, scale up and sustainability. Regulatory approval, cost of production and impact to the environment are subject to commercial acceptance of a product. The future directions vital are optimization of the fermentation processes involving extremophilic enzymes, research on GRAS (generally regarded as safe) status and use of renewable substances during fermentation.

CONCLUSION

Because they are stable in harsh environments including high temperatures, salinity, and fluctuating pH, extremophilic α-amylases have a great deal of promise for industrial use. Enzyme discovery is now a systematic, predictive approach rather than a random screening method because to developments in metagenomics, bioinformatics, and machine learning. Continued integration of protein engineering, synthetic biology, and sustainable processing is anticipated to produce highly effective, personalized enzymes, despite obstacles in structural characterization, large-scale production, and regulatory approval. To fully realize the industrial and environmental benefits of extremophilic α-amylases, interdisciplinary collaboration will be essential.

Authors Contribution

Conceptualization: IUH
Methodology: IUH, HW, MA, AK
Formal analysis: IUH
Writing review and editing: IUH, HS, HW, AK, AIM

All authors have read and agreed to the published version of the manuscript.

DOI: https://doi.org/10.54393/fbt.v5i4.199

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Feller G, Bonneau M, Da Lage JL. Amyrel, A Novel Glucose-Forming α-Amylase from Drosophila with 4-α-Glucanotransferase Activity by Disproportionation and Hydrolysis of Maltooligosaccharides. Glycobiology. 2021 Sep; 31(9): 1134-44. doi: 10.1093/glycob/cwab036.

[2] Prongjit D, Lekakarn H, Bunterngsook B, Aiewviriyasakul K, Sritusnee W, Champreda V. Functional Characterization of Recombinant Raw Starch Degrading A-Amylase from Roseateles Terrae HL11 and Its Application on Cassava Pulp Saccharification. Catalysts. 2022 Jun; 12(6): 647. doi: 10.3390/catal12060647

[3] Verified Market Reports. Alpha-Amylase Market Size, Share, Industry Analysis and Forecast. 2025. Available from: https://www.verifiedmarketreports.com/product/alpha-amylase-market.

[4] Rehman A, Saeed A, Asad W, Khan I, Hayat A, Rehman MU *et al.* Eco-friendly Textile Desizing with Indigenously Produced Amylase from Bacillus Cereus AS2. Scientific Reports. 2023 Jul; 13(1): 11991. doi: 10.1038/s41598-023-38956-3.

[5] Nguyen VM, Ndao A, Peterson EC, Blais JF, Adjallé K. Bacillus Species: Evolving Roles in Bio-Based Detergents. Processes. 2025 Jun; 13(6): 1885. doi: 10.3390/pr13061885.

[6] Tadesse M, Liu Y. Recent Advances in Enzyme Immobilization: The Role of Artificial Intelligence, Novel Nanomaterials, and Dynamic Carrier Systems. Catalysts. 2025 Jun; 15(6): 571. doi: 10.3390/catal15060571.

[7] Marzban G, Tesei D. The Extremophiles: Adaptation Mechanisms and Biotechnological Applications. Biology. 2025 Apr; 14(4): 412. doi: 10.3390/biology14040412.

[8] Rekadwad BN, Li WJ, Gonzalez JM, Punchappady Devasya R, Ananthapadmanabha Bhagwath A, Urana R *et al.* Extremophiles: The Species That Evolve and Survive Under Hostile Conditions. 3 Biotech. 2023 Sep; 13(9): 316. doi: 10.1007/s13205-023-03733-6.

[9] Sepe F, Costanzo E, Ionata E, Marcolongo L. Biotechnological Potential of Extremophiles: Environmental Solutions, Challenges, and Advancements. Biology. 2025 Jul; 14(7): 847. doi: 10.3390/biology14070847.

[10] Kuddus M, Roohi, Bano N, Sheik GB, Joseph B, Hamid B *et al.* Cold-active microbial enzymes and their biotechnological applications. Microbial Biotechnology. 2024 Apr; 17(4): e14467. doi: 10.1111/1751-7915.14467.

[11] Kholikov A, Vokhidov K, Murtozoyev A, Tóth ZS, Nagy GN, Vértessy BG *et al.* Characterization of a Thermostable α-Amylase from Bacillus licheniformis 104. K for Industrial Applications. Microorganisms. 2025 Jul; 13(8): 1757. doi: 10.3390/microorganisms13081757.

[12] Nam NN, Do HD, Loan Trinh KT, Lee NY. Metagenomics: An Effective Approach for Exploring Microbial Diversity and Functions. Foods. 2023 May; 12(11): 2140. doi: 10.3390/foods12112140.

[13] Yiping SH, Nan LI, Zongqiang WA. Recent Advances in the Culture-Independent Discovery of Natural Products Using Metagenomic Approaches. Chinese Journal of Natural Medicines. 2024 Feb; 22(2): 100-11. doi: 10.1016/S1875-5364(24)60585-6.

[14] Van der Flier F, Estell D, Pricelius S, Dankmeyer L, Van Stigt Thans S, Mulder H *et al.* Enzyme Structure Correlates with Variant Effect Predictability. Computational and Structural Biotechnology Journal. 2024 Dec; 23: 3489-97. doi: 10.1016/j.csbj.2024.09.007.

[15] Gómez-Villegas P, Vigara J, Romero L, Gotor C, Raposo S, Gonçalves B, Léon R. Biochemical Characterization of the Amylase Activity from the New Haloarchaeal Strain Haloarcula sp. Hs Isolated in the Odiel Marshlands. Biology. 2021 Apr; 10(4): 337. doi: 10.3390/biology10040337.

[16] Lahiri D, Nag M, Banerjee R, Mukherjee D, Garai S, Sarkar T *et al.* Amylases: biofilm inducer or biofilm inhibitor? Frontiers in Cellular and Infection Microbiology. 2021 Apr; 11: 660048. doi: 10.3389/fcimb.2021.660048.

[17] Abedi E, Kaveh S, Hashemi SM. Structure-Based Modification of α-Amylase by Conventional and Emerging Technologies: Comparative Study on the Secondary Structure, Activity, Thermal Stability and Amylolysis Efficiency. Food Chemistry. 2024 Mar; 437: 137903. doi: 10.1016/j.foodchem.2023.137903.

[18] Huma T, Mustafa G, Sethi A, Maryam A, Ali M. Computational Approach to Study Role of Active Site Amino Acids of Alpha-Amylases in Thermostability of Pyrococcus fu-riosus and Bacillus sp. Biochemistry and Molecular Biology Journal. 2021.

[19] Samanta S. Structural and Catalytical Features of Different Amylases and their Potential Applications. Jordan Journal of Biological Sciences. 2022 Jun; 15(2). doi: 10.54319/jjbs/150220.

DOI: https://doi.org/10.54393/fbt.v5i4.199

[20] Ramakrishnan K, Johnson RL, Winter SD, Worthy HL, Thomas C, Humer DC *et al.* Glycosylation Increases Active Site Rigidity Leading to Improved Enzyme Stability and Turnover. The Federation of European Biochemical Societies Journal. 2023 Aug; 290(15): 3812-27. doi: 10.1111/febs.16783.

[21] Tagomori BY, Dos Santos FC, Barbosa-Tessmann IP. Recombinant Expression, Purification, and Characterization of an α-Amylase from Massilia Timonae. 3 Biotech. 2021 Jan; 11(1): 13. doi: 10.1007/s13205-020-02505-w.

[22] Sidar A, Albuquerque ED, Voshol GP, Ram AF, Vijgenboom E, Punt PJ. Carbohydrate Binding Modules: Diversity of Domain Architecture in Amylases and Cellulases from Filamentous Microorganisms. Frontiers in Bioengineering and Biotechnology. 2020 Jul; 8: 871. doi: 10.3389/fbioe.2020.00871.

[23] Ahmad A, Rahamtullah, Mishra R. Structural and Functional Adaptation in Extremophilic Microbial α-Amylases. Biophysical Reviews. 2022 Apr; 14(2): 499-515. doi: 10.1007/s12551-022-00931-z.

[24] Amils R, Ellis-Evans C, Hinghofer-Szalkay H. Life in extreme environments. New York, NY, USA: Springer. 2007 Sep. doi: 10.1007/978-1-4020-6285-8.

[25] Jaiswal N and Jaiswal P. Thermostable α-Amylases and Laccases: Paving the Way for Sustainable Industrial Applications. Processes. 2024 Jun; 12(7): 1341. doi: 10.3390/pr12071341.

[26] Khan MF. Recent Advances in Microbial Enzyme Applications for Sustainable Textile Processing and Waste Management. Science. 2025 Apr; 7(2): 46. doi: 10.3390/sci7020046.

[27] Burkhardt C, Baruth L, Meyer-Heydecke N, Klippel B, Margaryan A, Paloyan A *et al.* Mining Thermophiles for Biotechnologically Relevant Enzymes: Evaluating the Potential of European and Caucasian Hot Springs. Extremophiles. 2024 Apr; 28(1): 5. doi: 10.1007/s00792-023-01321-3.

[28] Kim JA, Kim MJ, Yim JH, Kim IC, Rhee JS, Han SJ. Isolation and Characterization of Low-Temperature and High-Salinity Amylase from Halomonas sp. KS41843. Fermentation. 2025 Aug; 11(8): 465. doi: 10.3390/fermentation11080465.

[29] Yandri Y, Tiarsa ER, Suhartati T, Satria H, Irawan B, Hadi S. The Stability Improvement of α-Amylase Enzyme from Aspergillus fumigatus by Immobilization on a Bentonite Matrix. Biochemistry Research International. 2022; 2022(1): 3797629. doi: 10.1155/2022/3797629.

[30] Valenzuela B, Solís-Cornejo F, Araya R, Zamorano P. Isolation of Thermophilic Bacteria from Extreme Environments in Northern Chile. Microorganisms. 2024 Feb; 12(3): 473. doi: 10.3390/microorganisms12030473.

[31] Dutta B and Bandopadhyay R. Biotechnological Potentials of Halophilic Microorganisms and Their Impact on Mankind. Beni-Suef University Journal of Basic and Applied Sciences. 2022 May; 11(1): 75. doi: 10.1186/s43088-022-00252-w.

[32] Khan MA, Sahile AA, Jan R, Asaf S, Hamayun M, Imran M *et al.* Halotolerant Bacteria Mitigate the Effects of Salinity Stress on Soybean Growth by Regulating Secondary Metabolites and Molecular Responses. BioMed Central Plant Biology. 2021 Apr; 21(1): 176. doi: 10.1186/s12870-021-02937-3.

[33] Mainka T, Weirathmüller D, Herwig C, Pflügl S. Potential Applications of Halophilic Microorganisms for Biological Treatment of Industrial Process Brines Contaminated with Aromatics. Journal of Industrial Microbiology and Biotechnology. 2021 Apr; 48(1-2): kuab015. doi: 10.1093/jimb/kuab015.

[34] Neagu S, Stancu MM. Novel Halotolerant Bacteria from Saline Environments: Isolation and Biomolecule Production. BioTech. 2025 Jun; 14(2): 49. doi: 10.3390/biotech14020049.

[35] Zhu D, Adebisi WA, Ahmad F, Sethupathy S, Danso B, Sun J. Recent Development of Extremophilic Bacteria and Their Application in Biorefinery. Frontiers in Bioengineering and Biotechnology. 2020 Jun; 8: 483. doi: 10.3389/fbioe.2020.00483.

[36] González E, Vera F, Scott F, Guerrero C, Bolívar JM, Aroca G *et al.* Acidophilic Heterotrophs: Basic Aspects and Technological Applications. Frontiers in Microbiology. 2024 May; 15: 1374800. doi: 10.3389/fmicb.2024.1374800.

[37] Kumari M, Padhi S, Sharma S, Phukon LC, Singh SP, Rai AK. Biotechnological Potential of Psychrophilic Microorganisms as the Source of Cold-Active Enzymes in Food Processing Applications. 3 Biotech. 2021 Nov; 11(11): 479. doi: 10.1007/s13205-021-03008-y.

[38] Liu Y, Zhang N, Ma J, Zhou Y, Wei Q, Tian C *et al.* Advances in Cold-Adapted Enzymes Derived from Microorganisms. Frontiers in Microbiology. 2023 Apr; 14: 1152847. doi: 103389/fmicb.2023.1152847.

[39] Casillo A, D'Angelo C, Parrilli E, Tutino ML, Corsaro MM. Membrane and Extracellular Matrix Glycopolymers of Colwellia Psychrerythraea 34H: Structural Changes at Different Growth Temperatures. Frontiers in Microbiology. 2022 Feb; 13: 820714. doi: 10.3389/fmicb.2022.820714.

[40] Ullah I, Khan MS, Khan SS, Ahmad W, Zheng L, Shah SU *et al.* Identification and Characterization of

DOI: https://doi.org/10.54393/fbt.v5i4.199

Thermophilic Amylase Producing Bacterial Isolates from the Brick Kiln Soil. Saudi Journal of Biological Sciences. 2021 Jan; 28(1): 970-9. doi: 10.1016/j.sjbs.2020.11.017.

[41] Arfah RA, Sarlan S, Karim A, Anita A, Ahmad A, Taba P et al. Systematic Review on Isolation, Purification, Characterization, and Industrial Applications of Thermophilic Microbial α-Amylases. Karbala International Journal of Modern Science. 2024; 10(3): 6. doi: 10.33640/2405-609X.3367.

[42] Gupta N, Beliya E, Paul JS, Tiwari S, Kunjam S, Jadhav SK. Molecular Strategies to Enhance Stability and Catalysis of Extremophile-Derived α-Amylase Using Computational Biology. Extremophiles. 2021 May; 25(3): 221-33. doi: 10.1007/s00792-021-01223-2.

[43] Zhang H, Zhang F, Wu F, Guo L, Xu X. Purification and Characterization of Endogenous α-Amylase from Glutinous Rice Flour. Foods. 2025 May; 14(10): 1679. doi: 10.3390/foods14101679.

[44] Tincu CE, Bouhadiba B, Atanase LI, Stan CS, Popa M, Ochiuz L. An Accessible Method to Improve the Stability and Reusability of Porcine Pancreatic α-Amylase Via Immobilization in Gellan-Based Hydrogel Particles Obtained by Ionic Cross-Linking with Mg2+ Ions. Molecules. 2023 Jun; 28(12): 4695. doi: 10.3390/molecules28124695.

[45] Atif F, Maqsood N, Ali W, Ali W, Irfan M. Extremophiles and Their Enzymatic Diversity and Biotechnological Potential. Systems Microbiology and Biomanufacturing. 2024 Jul; 4(3): 833-49. doi: 10.1007/s43393-024-00275-7.

[46] Ortega-Quintanilla G and Millet O. On the Molecular Basis of the Hypersaline Adaptation of Halophilic Proteins. Journal of Molecular Biology. 2025 Sep: 169439. doi: 10.1016/j.jmb.2025.169439.

[47] Tenchov R and Zhou QA. Extremophiles: Unlocking Bioactive Compounds and Biotechnological Innovation from Life at the Edge. 2025. doi: 10.26434/chemrxiv-2025-t7lqf.

[48] Baroroh U, Yusuf M, Rachman SD, Ishmayana S, Syamsunarno MR, Levita J *et al.* The Importance of Surface-Binding Site towards Starch-Adsorptivity Level in α-Amylase: A Review on Structural Point of View. Enzyme Research. 2017; 2017(1): 4086845. doi: 10.1155/2017/4086845.

[49] Kumar A, Dhiman S, Krishan B, Samtiya M, Kumari A, Pathak N *et al*. Microbial Enzymes and Major Applications in the Food Industry: A Concise Review. Food Production, Processing and Nutrition. 2024 Oct; 6(1): 85. doi: 10.1186/s43014-024-00261-5.

[50] Far BE, Ahmadi Y, Khosroshahi AY, Dilmaghani A. Microbial alpha-Amylase Production: Progress, Challenges and Perspectives. Advanced Pharmaceutical Bulletin. 2020 May; 10(3): 350. doi: 10.34172/apb.2020.043.

[51] Rashwan AK, Younis HA, Abdelshafy AM, Osman AI, Eletmany MR, Hafouda MA *et al*. Plant Starch Extraction, Modification, and Green Applications: A Review. Environmental Chemistry Letters. 2024 Oct; 22(5): 2483-530. doi: 10.1007/s10311-024-01753-z.

[52] Aydin D. Enzymes in Textile Industry: Towards to Sustainable Textile Processes. Trends in Text, Engineering, Fashion, and Technology [Internet]. 2023 Jul; 8(5). doi: 10.31031/TTEFT.2023.08.000697.

[53] Hamid B, Bashir Z, Yatoo AM, Mohiddin F, Majeed N, Bansal M *et al*. Cold-Active Enzymes and Their Potential Industrial Applications—A Review. Molecules. 2022 Sep; 27(18): 5885. doi: 10.3390/molecules27185885.

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

[55] Vučurović V, Katanski A, Vučurović D, Bajić B, Dodić S. Simultaneous Saccharification and Fermentation of Wheat Starch for Bioethanol Production. Fermentation. 2025 Feb; 11(2): 80. doi: 10.3390/fermentation11020080.

[56] Liu F, Zheng X, Liao W, Ren X, Ma C, Zhang G *et al.* Heterologous Expression and Biochemical Characterization of a New α-Amylase from Nocardiopsis aegyptia HDN19-252 of Antarctic Animal Origin. Marine Drugs. 2025 Apr; 23(4): 159. doi: 10.3390/md23040159.