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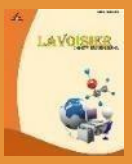

Advances in α-Amylase Technology: Production, Characterization, Engineering, and Functional Applications in Industrial and Biomedical Systems

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Abstract

α-Amylase is a key industrial enzyme widely utilized in food processing, bioenergy production, and various biotechnological applications due to its ability to hydrolyze starch into simpler sugars. This study aims to comprehensively review recent advancements in α-amylase technology, including its production, characterization, mechanisms of action, engineering strategies, inhibition, and applications in industrial, biomedical, and sustainable systems. The research employed a descriptive-analytical literature review method by analyzing 69 relevant scientific publications from reputable databases such as Science Direct, Springer Link, MDPI, and Google Scholar. The findings indicate that microbial-based production, particularly through the utilization of agro-industrial waste, offers a cost-effective and sustainable approach aligned with circular bio economy principles. Enzyme characterization reveals that α-amylase exhibits high adaptability to varying temperature and pH conditions, with performance strongly influenced by physicochemical factors and metal ions. Structural analysis highlights the importance of the GH13 domain in determining catalytic efficiency and substrate specificity. Furthermore, advancements in enzyme engineering, including genetic modification, protein engineering, and immobilization techniques, significantly enhance enzyme stability, reusability, and resistance to extreme conditions. The study emphasizes the importance of enzyme inhibition mechanisms, particularly in medical applications such as diabetes management. Overall, α-amylase technology demonstrates significant potential as a versatile and sustainable biocatalyst. Future developments should focus on optimizing enzyme engineering approaches, exploring novel microbial resources, and integrating advanced biotechnological innovations to enhance efficiency and broaden application potential.

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1. Introduction

Enzymes are biological catalysts that play an essential role in accelerating chemical reactions in both biological systems and modern industrial applications. Beyond merely increasing reaction rates, enzymes contribute to improved selectivity and energy efficiency in complex biochemical processes. In the context of biotechnology, enzymes are not only functional catalysts but also strategic components for enhancing production efficiency while reducing the use of hazardous chemicals. Previous studies have emphasized that enzymes such as α-amylase play a vital role in converting complex substrates into simpler compounds through highly specific catalytic mechanisms (Kuo et al., 2022; Siddikey et al., 2025). In addition, their ability to operate under mild conditions makes them highly advantageous compared to conventional chemical catalysts. Therefore, enzymes have become a fundamental pillar in the development of sustainable and efficient biotechnological industries.

Among the enzymes widely utilized in industrial applications, α-amylase is recognized for its important role in breaking down starch by hydrolyzing glycosidic bonds into simpler sugars, including maltose and glucose. As part of the glycoside hydrolase group, this enzyme demonstrates strong substrate specificity, which supports its use in various carbohydrate-related industrial activities. Furthermore, α-amylase is capable of functioning under different environmental conditions, allowing it to be applied in multiple production settings. Ali et al. (2025) explained that α-amylase has become increasingly important in many industrial fields because of its effectiveness in transforming raw materials into products with higher economic value. Its broad functionality also supports the integration of conventional industrial methods with advances in modern biotechnology.

In the food industry, α-amylase is extensively utilized in bread making, starch processing, and glucose syrup production. The enzymatic degradation of starch allows better control over product texture, leading to improved quality attributes such as softness, volume, and shelf-life stability. Furthermore, enzyme application contributes to enhanced sensory characteristics, including taste and texture. Siddikey et al. (2025) reported that enzymatic processes not only improve production efficiency but also enhance the overall quality of food products. In addition, the use of α-amylase supports process standardization and consistency, which are essential for large-scale food manufacturing. Consequently, α-amylase has become indispensable in supporting innovation in modern food technology.

The increasing industrial demand for enzymes with high stability and efficiency has driven significant advancements in α-amylase production technologies. Current developments focus on improving enzyme yield and optimizing production processes. Microbial-based production has emerged as the most dominant approach due to its ability to produce large quantities of enzymes at relatively low cost. Naik et

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al. (2023) highlighted that microbial systems offer scalability and efficiency, while Sahu et al. (2024) emphasized that fermentation conditions such as pH, temperature, and nutrient composition can be systematically optimized to enhance enzyme production. In addition, advances in bioprocess engineering have enabled more precise control of fermentation systems, further improving productivity and consistency. These advantages make microbial production a highly adaptable solution for industrial needs.

Microorganisms, including bacteria and fungi, are commonly utilized as producers of α-amylase because they possess strong adaptability and flexible metabolic characteristics. Among them, species belonging to the genera *Bacillus* and *Aspergillus* are frequently preferred due to their relatively high enzyme productivity and simple cultivation requirements. Several microbial strains are also able to produce enzymes under challenging conditions, such as elevated temperatures and different pH ranges, making them suitable for industrial processes. Research conducted by Naik et al. (2023) and Sahu et al. (2024) showed that enzyme production can be improved through both submerged and solid-state fermentation methods, particularly when factors such as aeration, nutrient availability, and incubation duration are carefully controlled.

In recent years, sustainability-oriented approaches have been increasingly integrated into enzyme production through the utilization of agro-industrial waste as alternative substrates. This strategy not only adds value to waste materials but also supports circular economy principles in modern industrial systems. Mahfudz et al. (2024) demonstrated substrates such as okara can be effectively used for α-amylase production at lower costs. Similarly, Liaqat et al. (2024) reported the use of agro-industrial waste reduces production costs while minimizing environmental impact, thereby improving overall resource efficiency.

Beyond production, enzyme characterization is a fundamental step in understanding the physicochemical properties and performance of α-amylase under various environmental conditions. Key parameters include optimal temperature, pH, and enzyme stability, which are crucial for determining its suitability for industrial applications. Abedi et al. (2024) revealed that structure-based modifications can significantly enhance thermal stability and catalytic efficiency through conformational changes in the enzyme. Additionally, enzyme engineering techniques such as immobilization have been widely applied to improve stability and reusability. Yandri et al. (2022) reported that immobilized enzymes exhibit enhanced resistance to extreme conditions and offer economic advantages in industrial processes.

Advances in modern biotechnology have further enabled the development of genetically engineered enzymes with improved functional properties. Approaches such as metagenomics and synthetic biology allow the discovery and design of novel enzymes with superior performance characteristics, particularly in terms of stability, specificity, and catalytic efficiency. Wani et al. (2026) explained that these technologies expand the available pool of biocatalysts by exploring uncultured microbial resources, thereby opening new opportunities for the development of highly efficient and adaptable enzymes. Furthermore, the integration

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of computational tools such as protein modeling and molecular simulations has accelerated the rational design of enzymes tailored for specific industrial requirements.

Furthermore, α-amylase has shown growing relevance in several fields, including biomedical and environmental applications. Recent studies have identified this enzyme as a promising biomarker for metabolic analysis (Erta et al., 2025) as well as a potential therapeutic target in diabetes treatment through enzyme inhibition approaches (Marzouk et al., 2025). Its utilization in bioethanol production and waste bioconversion also reflects its contribution to the development of sustainable technologies (Posoongnoen et al., 2025; Sahu et al., 2024). The wide range of these applications indicates that α-amylase functions as a versatile biocatalyst with important roles in different sectors. Therefore, this review is intended to provide a comprehensive discussion of recent developments in α-amylase technology, covering aspects such as production, characterization, engineering, inhibition, and its applications in industrial, biomedical, and sustainability-related systems.

2. Materials and Methods

This study employed a literature review method with a descriptive-analytical approach to comprehensively examine the development of α-amylase technology. A total of 69 relevant scientific publications were analyzed, primarily sourced from reputable international journals. The reviewed literature covered key aspects of α-amylase, including production, characterization, enzyme engineering, and its applications in industrial, biomedical, and sustainable systems. Data sources were obtained from credible scientific databases such as Science Direct, Springer Link, MDPI, and Google Scholar. The use of a literature study provides a strong foundation for identifying research trends and constructing appropriate analytical frameworks (Febrianto et al., 2024; Sugiyono, 2023).

The literature search was conducted systematically using relevant keywords, including “α-amylase production,” “enzyme characterization,” “enzyme engineering,” and “amylase applications.” The selection process was limited to publications from the last ten years to ensure data relevance and novelty. The data collection procedure was carried out through several stages:

- **Identification**: collecting relevant articles from selected scientific databases;
- **Selection**: filtering articles based on relevance, quality, and contribution to the topic;
- **Classification**: grouping selected studies into main themes, namely production, characterization, engineering, and application of α-amylase.

Irrelevant, duplicate, or low-impact studies were excluded to maintain the quality and focus of the review. Data analysis was conducted qualitatively using a descriptive approach through comparison and scientific synthesis. The selected literature was systematically analyzed to identify research patterns, emerging trends, and relationships among findings from different studies. Furthermore, a critical evaluation

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was performed to assess the strengths and limitations of each study, providing a deeper and more balanced understanding of the topic. This approach aligns with qualitative research strategies that emphasize structured analysis and interpretation of data (Nurrisa et al., 2025). The findings were then organized into an integrated scientific narrative to present a comprehensive overview of α-amylase technological advancements and to highlight potential directions for future research.

3. Results and Discussions

3.1 α-Amylase Production

The production of α-amylase represents an important aspect of modern enzyme technology, mainly because of the increasing industrial need for efficient and cost-effective biocatalysts. Generally, this enzyme is produced using microorganisms such as *Bacillus*, *Aspergillus*, and several other bacterial species that are capable of secreting extracellular enzymes in substantial amounts. Ali et al. (2025) stated that these microorganisms are widely chosen due to fast growth, relatively stable genetic characteristics, and adaptability to different production environments. The use of microbial systems also allows production processes to be optimized on an industrial scale through controlled fermentation conditions.

On the other hand, advances in α-amylase production are increasingly associated with the application of sustainability principles, especially through the use of agro-industrial waste as alternative substrates. Naik et al. (2023) highlighted that materials such as bran, tofu residue (*okara*), and starch-processing waste can serve as low-cost and widely available carbon sources. This view is supported by Sahu et al. (2024), who explained that the utilization of these materials not only helps lower production expenses but also assists in reducing environmental waste accumulation. As a result, current developments in α-amylase production are directed not only toward improving efficiency, but also toward supporting circular economy practices and sustainable industrial development.

3.1.1 Microorganism-Based Production

Microorganism-based production of α-amylase is the most dominant method used at the industrial scale. Microorganisms such as *Bacillus subtilis*, *Bacillus licheniformis*, and *Aspergillus niger* are known to have high enzyme production capacity and the ability to adapt to various fermentation conditions. Khattab and Al-Nazzal (2024) explained that bacteria from the genus *Bacillus* are highly advantageous in enzyme production due to their ability to produce thermostable enzymes suitable for industrial applications. Meanwhile, fungi such as *Aspergillus* are more commonly used in solid-state fermentation due to their efficiency in substrate utilization.

In addition to the selection of microorganisms, fermentation techniques also serve as a determining factor in the success of enzyme production. Al-Bedak et al. (2025) demonstrated that optimization of

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fermentation conditions such as temperature, pH, and incubation time can significantly increase enzyme production. Submerged fermentation is generally used for large-scale production, while solid-state fermentation is more advantageous in terms of substrate efficiency and lower energy consumption.

Table 1. Microorganisms and α-Amylase Production Methods

Microorganism	Production Method	Advantages
Bacillus subtilis	Submerged fermentation	High and rapid production
Aspergillus niger	Solid-state fermentation	High substrate efficiency
Bacillus licheniformis	Controlled fermentation	High thermal stability

Based on Table 1, it can be observed that variations in microorganism sources and production methods contribute significantly to the characteristics of the resulting α-amylase. However, to further understand enzyme performance in detail, characterization analysis is required, including parameters such as enzyme activity and stability under various environmental conditions. Therefore, the visualization of these characteristics is presented through experimental results shown in Figure 1.

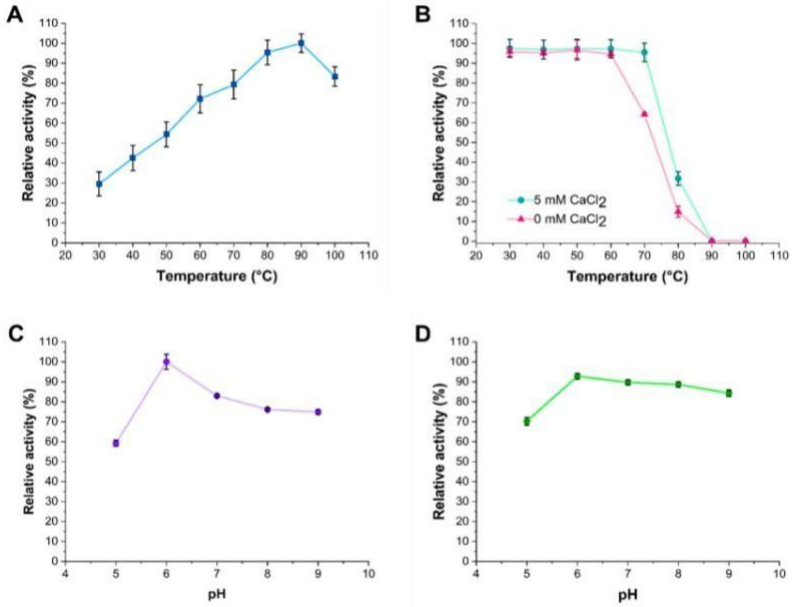

Figure 1. Activity and stability profile of α-amylase under temperature and pH variations (Kholikov etal., 2025)

Figure 1 shows that the relative activity of α-amylase increases with rising temperature until it reaches an optimum condition, before eventually decreasing due to the denaturation of protein structure at high temperatures. This phenomenon reflects the general characteristic of enzymes that have a specific temperature range to maintain their catalytic efficiency. In addition, the presence of Ca^{2+} ions in the reaction system has been proven to enhance the thermal stability of the enzyme, indicating that cofactors play an

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important role in maintaining the structural integrity of the enzyme under extreme conditions (Kholikov et al., 2025).

Furthermore, the enzyme activity profile under varying pH conditions indicates that α-amylase exhibits optimum activity near neutral pH, with relatively high stability within a certain pH range. The decrease in activity under extreme pH conditions suggests conformational changes in the enzyme that affect the active site. These findings confirm that environmental parameters such as temperature and pH are the main determinants in defining enzyme performance, and therefore must be optimally controlled in industrial applications to achieve maximum process efficiency (Kholikov et al., 2025).

The classification of amylase enzymes serves as an important conceptual foundation for understanding the variation in characteristics and mechanisms of action of each enzyme type in both biological and industrial systems. Based on catalytic mechanisms and hydrolysis patterns, amylases are classified into several main groups that exhibit significant differences in enzymatic activity, substrate specificity, and optimal reaction conditions.

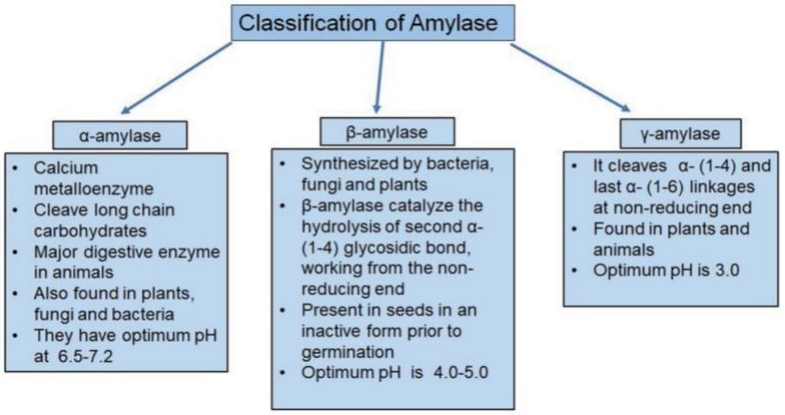

Figure 2. Classification of Amylase Enzymes (Naik et al., 2023; Saranraj & Stella, 2013)

Based on Figure 2, amylase enzymes are commonly divided into α-amylase, β-amylase, and γ-amylase, with each type having different catalytic properties and reaction mechanisms. α-Amylase functions as an endo-acting enzyme that hydrolyzes α-1,4-glycosidic bonds within starch molecules in a random manner, resulting in the formation of shorter-chain oligosaccharides. This enzyme is also classified as a calcium metalloenzyme because calcium ions are important for maintaining its structural integrity and catalytic activity. In terms of distribution, α-amylase can be found in animals, plants, and microorganisms, and generally exhibits optimal activity at a pH range of around 6.5–7.2, which supports efficient enzymatic performance.

In contrast, β-amylase functions as an exo-acting enzyme by hydrolyzing α-1,4-glycosidic bonds from the non-reducing ends of starch chains, gradually producing maltose as the primary product. This enzyme is commonly found in plant tissues, particularly in seeds, and exhibits optimal activity under acidic

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conditions, typically within a pH range of 4–5. Meanwhile, γ-amylase demonstrates a broader activity spectrum, as it is capable of hydrolyzing both α-1,4 and α-1,6-glycosidic bonds, ultimately yielding glucose as the final product. These differences indicate that each type of amylase possesses specific functional roles and application potentials, making a comprehensive understanding of enzyme classification fundamental for designing effective strategies in enzyme production and utilization across various industrial sectors.

Following the production process, the quality and quantity of the enzymes produced are strongly influenced by the complex interaction between the producing microorganisms and the fermentation environment. Kholikov et al. (2025) reported that *Bacillus licheniformis* strains are capable of producing α-amylase with high thermal stability, even under extreme temperature conditions, making them highly promising for large-scale industrial applications. Furthermore, Isabel and Premalatha (2025) revealed that the exploration and isolation of new strains from industrial waste sources can significantly enhance enzyme production potential by utilizing previously underexploited resources.

Moreover, exploratory approaches to microbial diversity provide broad opportunities for discovering enzymes with more adaptive and superior characteristics. Sanjaya et al. (2024) reported that indigenous bacteria isolated from sugar factory waste exhibited competitive α-amylase production compared to commercial strains. This finding highlights that microbial diversity serves as a highly valuable biological reservoir for the development of innovative enzyme bioprocesses, particularly in supporting production efficiency and sustainability in biotechnology-based industries.

3.1.2 Agro-Industrial Waste-Based Production

The agro-industrial waste-based production approach for α-amylase has emerged as a significant innovation in supporting the concepts of biorefinery and bioeconomy. Waste materials such as okara, rice bran, and starch residues contain sufficient nutrients to support the growth of enzyme-producing microorganisms. Mahfudz et al. (2024) demonstrated that okara can be utilized as an effective substrate for α-amylase production by *Bacillus subtilis* at a significantly lower cost compared to conventional substrates. Furthermore, Liaqat et al. (2024) emphasized that the use of agro-industrial waste not only provides economic benefits but also enhances resource utilization efficiency. By employing waste as a substrate, industries can reduce their dependence on expensive raw materials while simultaneously minimizing the environmental impact associated with production processes.

Table 2. Agro-Industrial Waste Substrates for α-Amylase Production

Type of Waste	Microorganism	Advantages
Okara	*Bacillus subtilis*	Low cost, high nutrient content
Rice bran	*Aspergillus* sp.	Abundant availability
Industrial starch waste	*Indigenous bacteria*	Environmentally friendly

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To better understand the effectiveness of microbial-based α-amylase production, it is essential to first examine the characteristics of ideal strains capable of producing enzymes optimally.

Figure 3. Characteristics of Efficient α-Amylase-Producing Strains (Sahu et al., 2024)

This figure illustrates that efficient α-amylase-producing strains generally possess several key characteristics, including ease of maintenance, minimal nutritional requirements, and the ability to produce high enzyme yields with relatively simple downstream processing. Genetic stability and adaptability to environmental conditions are also crucial factors in determining enzyme production performance. These characteristics serve as fundamental criteria in selecting microorganisms for industrial-scale applications.

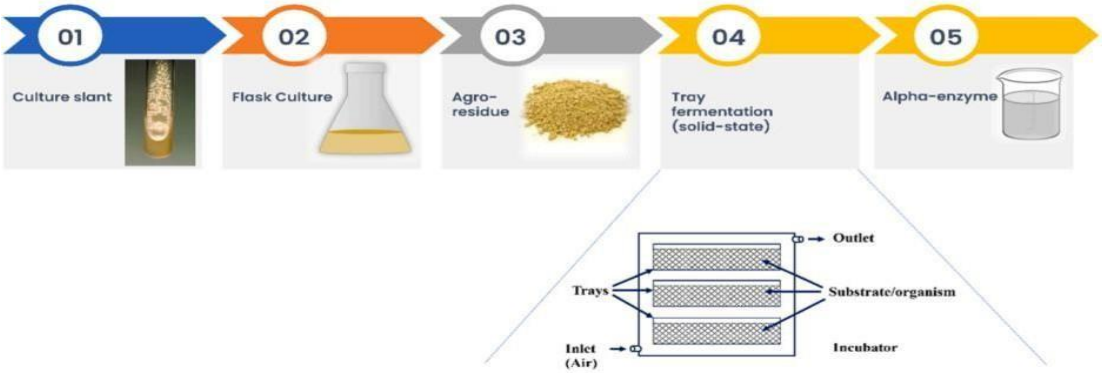

Figure 4. Stages of solid-state fermentation in α-amylase production from agro-industrial substrates (Sahu et al., 2024)

This figure illustrates the systematic stages involved in α-amylase production using the solid-state fermentation method, beginning with the preparation of microbial cultures, followed by inoculation onto agro-industrial waste substrates, and continuing through fermentation and enzyme extraction processes. Each stage plays a critical role in determining the resulting enzyme yield. This method is considered more efficient than submerged fermentation because it allows direct utilization of solid substrates and results in higher enzyme concentrations.

Furthermore, α-amylase production is not only viewed as a biotechnological process but also as an integral component of a sustainable economic system.

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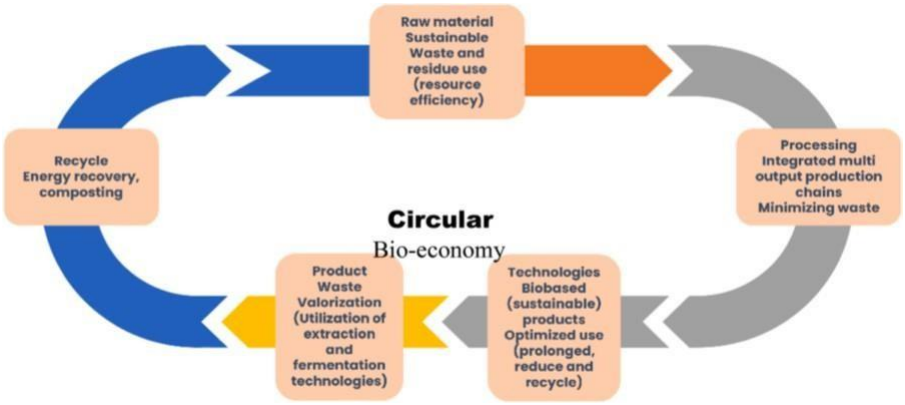

Figure 5. Circular bioeconomy development in enzyme production (Sahu et al., 2024)

This figure depicts the concept of a circular bioeconomy, in which agro-industrial waste is utilized as raw material for enzyme production, subsequently generating various value-added products such as biofuels, food products, and other industrial outputs. The cycle also includes recycling and reuse of residual materials, thereby minimizing waste and enhancing resource efficiency. This approach demonstrates that α-amylase production contributes significantly to supporting sustainability principles. In order to further improve production efficiency, various modern biotechnological approaches have also been developed to optimize enzyme performance.

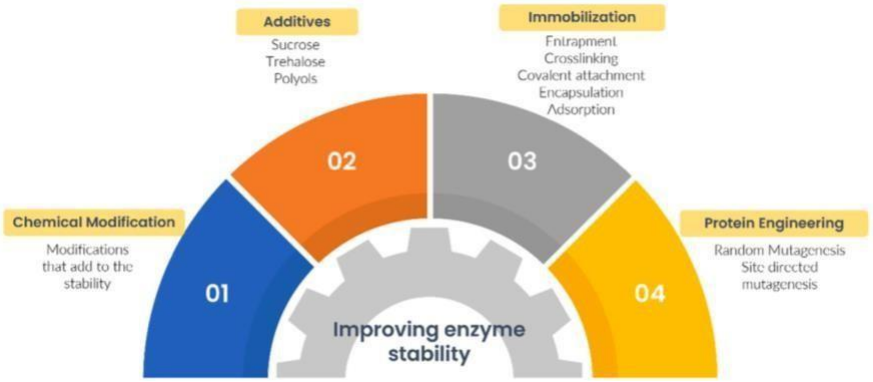

Figure 6. Biotechnological approaches to enhance α-amylase production and stability (Sahu et al., 2024)

This figure presents various strategies for enhancing α-amylase production, including chemical modification, the use of stabilizing additives, immobilization techniques, and protein engineering through mutagenesis. Each approach offers specific advantages in improving enzyme stability, activity, and resistance to extreme conditions. The combination of these strategies enables the optimization of enzyme production at a more efficient industrial scale. The integration between α-amylase production processes and industrial applications can be observed through a comprehensive bioprocess system.

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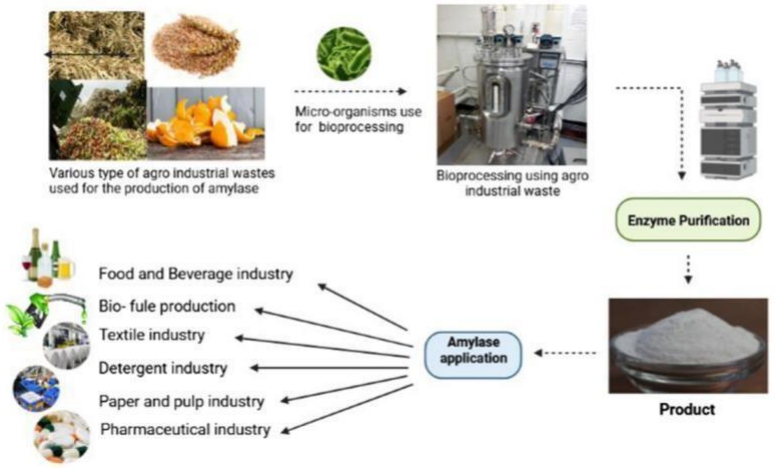

Figure 7. Bioprocess scheme of α-amylase based on agro-industrial waste (Naik et al., 2023)

This figure illustrates the complete workflow of α-amylase production, starting from the utilization of agro-industrial waste as a substrate, followed by fermentation, enzyme purification, and its application across various sectors such as food, biofuel, textile, detergent, and paper industries. This scheme highlights that α-amylase not only plays a role in the production stage but also serves as a key component throughout multiple industrial value chains. Therefore, this integrated bioprocess reflects both the efficiency and flexibility of enzyme utilization across diverse applications.

The implementation of agro-industrial waste as a substrate in α-amylase production cannot be separated from the need for precise process optimization. Each stage, ranging from strain selection and fermentation to biotechnological strategies, as illustrated previously, demonstrates that operational parameters significantly influence enzyme productivity. Solid-state fermentation remains one of the most widely used approaches; however, its effectiveness is highly dependent on factors such as moisture content, substrate particle size, and optimal aeration. Sahu et al. (2024) emphasized that improper control of these parameters can drastically reduce enzyme yield, thereby requiring process engineering approaches to ensure production efficiency is maintained at an industrial scale.

On the other hand, the integration of waste bioconversion concepts, as visualized through circular bioeconomy and industrial bioprocess schemes, indicates a paradigm shift in modern enzyme production. The utilization of waste serves not only as a low-cost alternative substrate but also as part of a sustainable production system capable of generating various value-added products. Posoongnoen et al. (2025) demonstrated that α-amylase produced from waste biomass can be utilized in bioethanol production, thereby creating a more efficient and environmentally friendly production cycle. Consequently, this approach provides not only economic benefits but also strategic significance in supporting the transition toward sustainable biotechnology-based industries.

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3.2 Enzyme Characterisation and Stability

3.2.1 Physical and Chemical Characteristics of α-Amylase

The characterisation of α-amylase is a fundamental step in determining enzyme performance under various environmental conditions. The main parameters examined include optimum temperature, optimum pH, and enzyme activity stability. A study by Albalawi et al. (2025) showed that α-amylase is capable of adapting to high temperatures with good thermal stability, making it highly suitable for applications in heat-based industrial processes.

In addition, external factors such as metal ions and chemical agents also influence enzyme activity. Hossain et al. (2025) found that certain ions can act as either activators or inhibitors, depending on their interaction with the enzyme's active site. This finding highlights that chemical characterization is a crucial aspect in optimizing the application of α-amylase.

Table 3. General Characteristics of α-Amylase

Parameter	Range
Optimum temperature	40–90°C
Optimum pH	5–9
Stability	High under thermophilic conditions

Table 3 shows that α-amylase exhibits high flexibility toward variations in environmental conditions. Enzymes derived from thermophilic microorganisms generally demonstrate greater stability compared to other sources, making them widely utilized in industrial applications (Kholikov et al., 2025).

Table 4. Effect of Ions and Chemical Agents on α-Amylase Activity

Factor	Effect on Activity
Ca^{2+}	Enhances stability
Mg^{2+}	Moderate activator
EDTA	Inhibitor
Heavy metals	Decrease activity

Table 4 shows that interactions with metal ions play a crucial role in determining enzyme activity. The presence of calcium, for example, is essential for maintaining enzyme structural stability.

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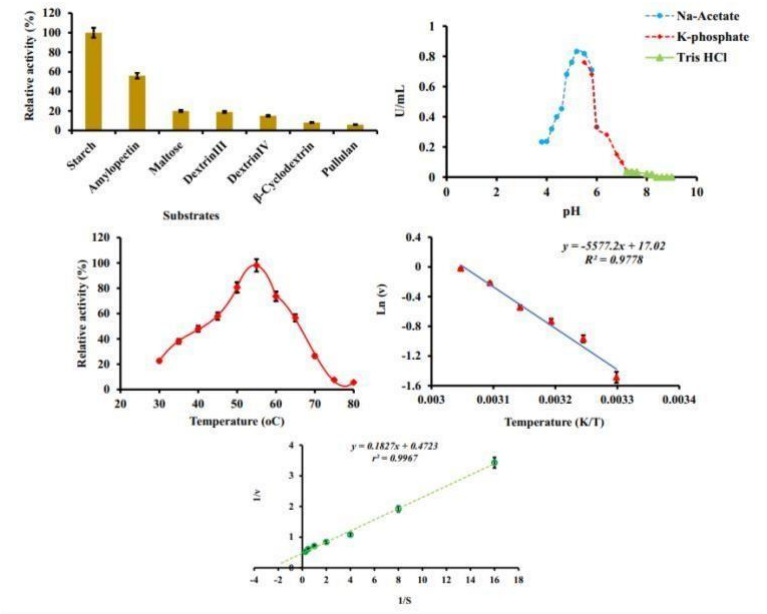

Figure 8. Graph of pH effect on α-amylase activity (Albalawi et al., 2025)

Based on Figure 8, α-amylase activity is significantly influenced by pH and temperature. In section (b), the enzyme exhibits optimum activity at a specific pH, while in section (c), enzyme activity increases up to the optimum temperature before declining due to protein denaturation.

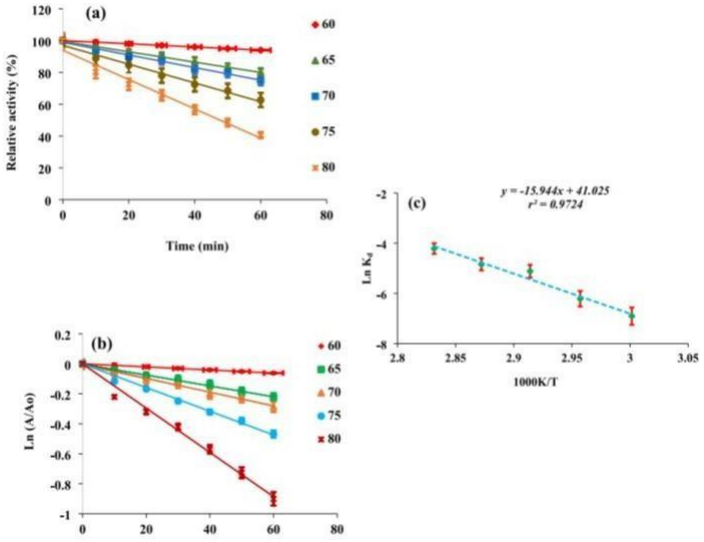

Figure 9. Graph of the effect of pH on enzyme activity (Alhazmi & Alshehri, 2025)

Thermal stability is an important parameter in industrial enzyme applications. Based on the study by Albalawi et al. (2025), α-amylase exhibits a gradual thermal denaturation pattern within the temperature range of 60–80°C, indicating significant changes in protein structure. Figure 9 shows that increasing temperature leads to a decrease in enzyme activity due to irreversible denaturation processes. In addition, kinetic analysis indicates that enzyme inactivation follows a pseudo-first-order model, describing the rate

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of enzyme degradation during heating. The Arrhenius curve in Figure 9 also demonstrates the relationship between temperature and the activation energy of enzyme inactivation, which serves as an important indicator in determining enzyme stability under extreme conditions.

Overall, the characterization findings suggest that α-amylase possesses considerable flexibility under different environmental conditions, including both physical and chemical factors. Its capability to remain active across broad temperature and pH ranges indicates its strong potential for application in several industrial fields, particularly the food, bioethanol, and textile industries. Moreover, the influence of external components such as metal ions on enzyme activity demonstrates the importance of controlling reaction conditions in order to achieve optimal enzymatic performance

Furthermore, the combination of physical, chemical, and thermal stability properties provides a broader understanding of α-amylase behavior within biocatalytic systems. The enzyme's resistance to elevated temperatures and its capacity to preserve structural activity under varying conditions are important advantages for large-scale industrial utilization. Therefore, understanding these characteristics is essential not only for enzyme characterization but also for supporting the development of more effective, adaptable, and sustainable enzyme technologies in the future.

3.2.2 Structural and Enzyme Activity Analysis

Structural analysis of enzymes is an important approach to understanding the relationship between molecular conformation and catalytic activity. The secondary and tertiary structures of α-amylase determine the enzyme's ability to bind substrates as well as the efficiency of starch hydrolysis reactions. Abedi et al. (2024) stated that structure-based modifications can significantly enhance both thermal stability and catalytic efficiency of the enzyme.

In addition, multi-analytical approaches enable the identification of specific domains within the enzyme that are responsible for catalytic activity. Zinck et al. (2026) demonstrated that variations in the GH13 domain structure influence substrate specificity and enzyme efficiency.

Table 5. Relationship Between Structure and Function of α-Amylase

Enzyme Structure	Main Function
Secondary structure	Maintains structural stability
Tertiary structure	Determines the active site
GH13 domain	Primary catalytic activity
Glycosylation	Enhances stability

Table 5 shows that each level of enzyme structure contributes specifically to the activity and stability of α-amylase. Furthermore, advanced characterization also includes analysis of molecular structure

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and evolutionary relationships within the α-amylase family. This approach is essential for understanding how structural variations and conservation of catalytic residues influence enzyme activity and substrate specificity. To clarify this relationship, phylogenetic-based visualization is conducted to illustrate the relationships among enzymes within the GH13 subfamily.

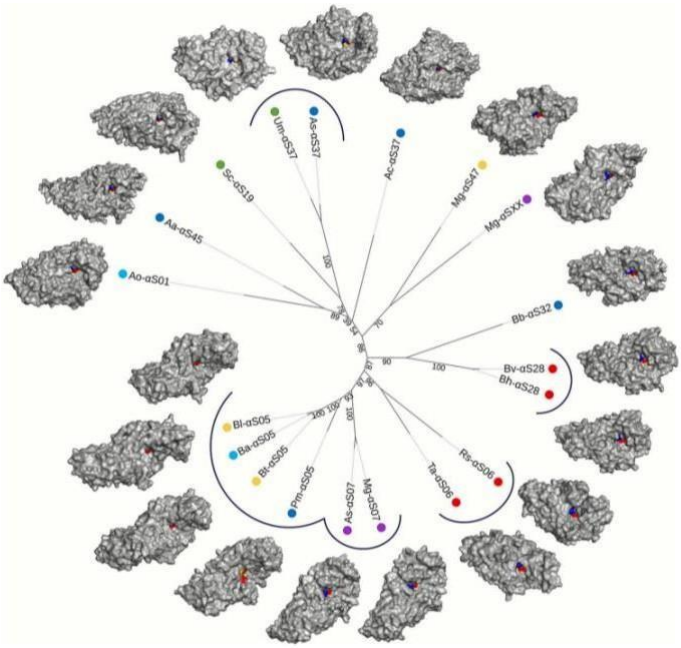

Figure 10. Domain diagram and phylogenetic tree of GH13 enzymes (Zinck et al., 2026)

Figure 10 presents a radial representation of the phylogenetic tree of the GH13 α-amylase enzyme family, constructed based on similarities in core protein sequences. This visualization shows the clustering of enzymes into several distinct subfamilies, reflecting structural and functional variations among members of the GH13 family. Each branch of the phylogenetic tree represents evolutionary relationships between enzymes, while clustering based on PCA analysis highlights differences in molecular characteristics such as molecular weight and domain structure distribution.

In addition, Figure 10 also illustrates the locations of key catalytic residues, consisting of one glutamate and two aspartates, as well as arginine residues that play a role in stabilizing the enzyme's active structure. The presence of these residues within the three-dimensional structure confirms that the catalytic activity of α-amylase is strongly influenced by its spatial configuration and the conservation of its molecular structure. This phylogenetic and structural analysis provides important insights into enzyme mechanisms and serves as a foundation for protein engineering aimed at improving catalytic efficiency and specificity.

3.2.3 Enzyme Stability and Immobilization Techniques

Enzyme stability is a key factor in determining the success of α-amylase applications across various industrial sectors, particularly in processes involving high temperatures, extreme pH variations, and

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complex operational conditions. Unstable enzymes tend to undergo denaturation, a structural change in proteins that results in the permanent loss of catalytic activity. Enhancing enzyme stability has therefore become a major focus in the development of modern biocatalysts. Various studies indicate that enzyme stability is influenced by intrinsic factors such as molecular structure and active residue interactions, as well as extrinsic factors including reaction environment and the presence of inhibitors (Erta et al., 2025; Gerlits et al., 2026; Freitas, 2026). Furthermore, the application of enzymes in the food and biotechnology industries requires assurance of safety and efficiency for sustainable use (EFSA FEZ Panel et al., 2025).

With the advancement of enzyme technology, various approaches have been developed to improve enzyme stability and performance, one of which is immobilization techniques. This method enables enzymes to be bound to solid matrices, reducing the likelihood of denaturation and allowing for repeated use. This approach is also aligned with the concept of sustainable biocatalysis, which emphasizes efficiency and reduced operational costs (Devi et al., 2026; Dkhar et al., 2025). In addition, the development of support materials such as polymers and cyclodextrin-based compounds has contributed to improving the effectiveness of enzyme immobilization (Benkovics et al., 2019). In this context, innovations in chemical engineering and compound synthesis also play a significant role in developing more stable and efficient immobilization systems (Carvalho et al., 2021; Cao et al., 2025).

Table 6. Comparison of Free Enzymes and Immobilized Enzymes

Parameter	Free Enzyme	Immobilized Enzyme
Stability	Low	High
Reusability	Cannot be reused	Can be used repeatedly
Operational cost	Higher	More efficient
Initial activity	High	Slightly decreased

Table 6 shows that although free enzymes have higher initial activity, immobilized enzymes offer significant advantages in terms of stability and efficiency of use. The ability to be reused makes immobilized enzymes more economical in the long term, especially in large-scale industrial processes. In addition, immobilization also plays a role in maintaining the conformational structure of enzymes so that they remain active under extreme conditions, thereby increasing the overall enzyme lifespan (Baktir et al., 2005; Albertelli et al., 2026).

Table 7. Immobilization Techniques and Their Characteristics

Immobilization Method	Principle	Advantages
Adsorption	Physical interaction on surface	Simple and inexpensive
Covalent	Chemical bonding with matrix	High stability
Entrapment	Trapped within gel/matrix	Protects enzyme from environment
Cross-linking	Bonds between enzymes	Does not require a matrix

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Table 7 shows that various immobilization techniques have their own characteristics and advantages that can be adjusted according to application needs. Covalent and cross-linking methods, for example, provide higher stability due to strong bonds between molecules, while adsorption methods are simpler but less stable. The selection of an appropriate immobilization method depends on the type of enzyme, operational conditions, and the intended industrial application. In some cases, combinations of methods are also used to improve enzyme efficiency and resistance to extreme conditions (El Ashry et al., 2013; Buev et al., 2026).

In addition, immobilization techniques have also been proven to enhance enzyme resistance to extreme temperature and pH, as well as reduce the effects of inhibitors that may interfere with enzyme activity. In this context, the interaction between enzymes and inhibitor molecules also becomes an important concern, as it can affect overall catalytic efficiency (Basso et al., 2025). Therefore, understanding the mechanism of enzyme interaction with its surrounding environment is key in developing optimal immobilization systems.

In conclusion, immobilization techniques represent a strategic solution for improving the stability and efficiency of α-amylase in various industrial applications. The integration of biochemical approaches, material engineering, and modern enzyme technology enables the development of more adaptive, efficient, and sustainable biocatalyst systems. Thus, the utilization of immobilized enzymes is expected to continue to grow alongside increasing industrial demand for environmentally friendly and economical processes (Graf, 2026).

3.3 Mechanism of Action of α-Amylase

The identification mechanism of orphan enzymes is carried out through a multi-stage manual curation process that combines intensive literature search with protein sequence matching using the BLAST algorithm (Shearer et al., 2014). This process aims to link enzyme activities that have been characterized in the laboratory with genomic data through searches in databases such as BRENDA and MetaCyc. More broadly, enzymes and biocatalysis play a crucial role in facilitating efficient and specific chemical reactions across various sectors (Kuo et al., 2022). In the development of sustainable biocatalysis, the “Solid State Biocatalysis” mechanism was introduced to process substrates with low water solubility, in which both substrates and products remain in suspension during the enzymatic reaction (Křen & Bojarová, 2020). On the other hand, innovations in enzyme purification continue to be developed, such as the implementation of universal purification protocols for fibrinolytic enzymes from Bacilli (Xin et al., 2019), although modern

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biocatalysis applications also include efficient one-pot cascade synthesis to produce complex structures without requiring intermediate purification steps.

In the fields of health and functional food, the mechanism of digestive enzyme inhibition has become a major focus. Phenolic acids, such as p-hydroxybenzoic acid and ferulic acid, act by inhibiting α-amylase activity through non-covalent interactions, including hydrogen bonding and hydrophobic interactions at the enzyme binding pocket (Shi et al., 2025). These interactions cause changes in the microenvironment of tryptophan and tyrosine residues in the enzyme, which are characterized by a red shift in the fluorescence spectrum. A similar mechanism is found in grape seed polyphenols that perform competitive or mixed-mode inhibition of pancreatic lipase by binding stably to catalytic residues (Kamah et al., 2026). Molecular docking simulations confirm that phytochemicals such as beta-sitosterol and stigmasterol occupy the active site of intestinal enzymes, thereby physically blocking polysaccharide breakdown (Shanak et al., 2025). A deeper understanding of molecular interactions can be strengthened by atomic-level structural insights, as demonstrated by X-ray and neutron crystallography studies that reveal the catalytic mechanisms of complex enzymes such as RNase H at room temperature (Gerlits et al., 2026).

The mechanism of enzymatic regulation is also influenced by plant structural components such as lignin. Lignin acts as an inhibitor of functional enzymes by forming hydrogen bonds and electrostatic interactions that cause steric hindrance in enzymes such as tyrosinase and α-glucosidase (Li et al., 2025). In the food industry sector, enzyme activity in metabolic pathways can be induced by the presence of soluble saccharides. In the bacterium *Lactococcus lactis*, sugar metabolism alters the NADH/NAD+ ratio, which induces the activity of key enzymes in the β-oxidation pathway for the synthesis of methyl ketones that contribute to the aroma of dairy products (Li & Ma, 2018). In addition, modification of enzymatic hydrolysis conditions has been shown to significantly affect biochemical properties and antioxidant capacity in food products, such as in sardine fish hydrolysates (Sandoval-Gallardo et al., 2020). Enzymatic activity is also innovatively utilized to induce copigmentation in pomegranate juice concentrate to maintain color stability during storage (Türkyılmaz et al., 2025).

Innovations in enhancing enzyme stability largely involve immobilization mechanisms. Lipase immobilized on multi-walled carbon nanotubes (MWNT) through covalent binding shows a significant increase in thermal stability, although there is a slight change in the secondary structure of the enzyme (Verma et al., 2013). This immobilization approach is also highly aligned with the principles of green chemistry, which are increasingly being integrated as sustainable experiments in laboratory education (Linkwitz & Eilks, 2021). For energy applications, the mechanism of photocatalytic water splitting using sensitized dyes mimics natural photosynthesis; sunlight excites electrons to the conduction band of

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semiconductors to reduce protons into hydrogen gas (Watanabe, 2017). Finally, the utilization of specific enzymes such as dextranase (B7DEX) demonstrates a mechanism for hydrolyzing α-1,6 glycosidic bonds in dental plaque triggered by divalent metal cations (Ca2+ and Mg2+), making it a potential agent for the prevention of dental caries (Baktir et al., 2005).

3.4 Engineering and Modification of α-Amylase

Enzyme engineering is an important strategy in improving the performance of α-amylase for various industrial applications, especially under extreme process conditions such as high temperatures, extreme pH, and the presence of chemical inhibitors. This approach involves genetic mutation techniques, protein structure modification, and the integration of modern biocatalysis technologies that enable the development of enzymes with more stable and efficient characteristics. As biotechnology advances, α-amylase engineering is not only focused on increasing catalytic activity, but also on sustainability and efficiency in industrial processes (Devi et al., 2026; Křen & Bojarová, 2020).

In addition, structure- and computation-based approaches enable the identification of key residues in the enzyme active site as well as the prediction of mutation impacts on catalytic activity. Technologies such as molecular docking and protein engineering provide opportunities to design enzymes with higher substrate specificity and resistance to inhibitors (Li et al., 2026; Kamah et al., 2026). Thus, enzyme engineering becomes a fundamental basis in the development of next-generation biocatalysts.

Table 8. α-Amylase Engineering Methods and Their Impacts

Engineering Method	Principle	Impact on Enzyme
Genetic mutation	Changes in DNA sequence	Increases activity & stability
Protein engineering	Modification of protein structure	Increased substrate specificity
Immobilization	Binding to a matrix	Increased stability & reuse
Chemical modification	Addition of functional groups	Resistance to inhibitors
Domain engineering	Modification of GH13 domain	Increased catalytic efficiency

This table shows that each engineering method has a different approach but complements one another in improving α-amylase performance. Genetic mutation and protein engineering, for example, focus on the molecular level to optimize enzyme structure, while immobilization and chemical modification place greater emphasis on enhancing stability under operational conditions.

Table 9. Applications of Engineered α-Amylase

Application Field	Role of Modified α-Amylase
Food industry	Improvement of texture quality and fermentation
Bioenergy	Hydrolysis of biomass into simple sugars

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Pharmaceutical	Enzymatic target for diabetes therapy
Biosensor	Specific detection of enzyme activity
Chemical industry	Catalyst in the synthesis of complex compounds

This table demonstrates that α-amylase engineering not only enhances enzyme performance but also broadens its potential applications in various modern industrial sectors. The advancement of α-amylase through engineering approaches has contributed significantly to improving industrial process efficiency. In the bioenergy sector, for instance, the use of modified α-amylase has been reported to improve the hydrolysis of lignocellulosic biomass into fermentable sugars, thereby supporting more efficient bioethanol production (Islam et al., 2025). The findings suggest enzyme engineering contributes not only to increased enzymatic activity but also to the advancement of sustainable energy development.

On the other hand, modern biocatalysis-based approaches enable the use of α-amylase in more complex systems, including in combination with other enzymes or active compounds. Studies show that interactions between enzymes and small molecules such as inhibitors or complex substrates can be utilized to control enzyme activity specifically (Li et al., 2018; Li et al., 2025). Even lignin and other phenolic compounds can act as natural inhibitors that affect α-amylase performance, making it important to consider this aspect in enzyme design (Li et al., 2025).

In addition, the application of immobilization methods together with structural engineering offers further benefits related to enzyme stability and repeated usage. Immobilized enzymes generally show greater resistance to extreme environmental conditions and can utilized in sustainable systems, including biosensors and industrial bioreactors (Jung et al., 2026; Linkwitz & Eilks, 2022). These characteristics highlight the flexibility of α-amylase as a biocatalyst for a wide variety of applications. Within the food and healthcare sectors, α-amylase engineering also contributes to the development of functional products and enzyme-based therapeutic approaches. For example, inhibition of α-amylase activity has become an important strategy in the development of antidiabetic compounds aimed at regulating blood glucose levels (Quazi et al., 2022; Kamah et al., 2026). In addition, engineered α-amylase is applied to improve the quality of food products, including bread and fermented beverages (Reichenberger et al., 2025).

Furthermore, the exploration of enzyme structure and domains such as GH13 opens opportunities for more specific and targeted engineering. Variations within this domain enable the development of enzymes with more efficient raw starch hydrolysis capabilities, which is highly relevant in the food and bioenergy industries (Radzlin et al., 2024; Kohli et al., 2020). This approach is also supported by advances in molecular structure analysis and protein visualization techniques (Moon et al., 2026). In addition, the integration of synthetic chemistry and biotechnology further expands the possibilities of enzyme

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modification. Various approaches in the synthesis of bioactive compounds and molecular modification demonstrate that enzymes can be engineered to function in more complex chemical systems (Honcharov et al., 2025; Mei et al., 2015; Pytel et al., 2026). This shows that α-amylase engineering is not limited to the field of biology but also involves interdisciplinary sciences.

Finally, the utilization of biological resources such as plant- and microorganism-derived enzymes is also a focus in the development of sustainable α-amylase. Enzymes derived from natural sources have great potential as environmentally friendly biocatalysts that can be applied in various industrial applications (Dkhar et al., 2025; Haidar et al., 2024). Thus, the engineering and modification of α-amylase not only improve enzyme performance but support the development of greener and more sustainable technologies.

3.5 α-Amylase Inhibitors in Various Fields

Amylase enzymes play a crucial role in carbohydrate metabolism in humans, animals, and insects. Specifically, α-amylase functions to catalyze the hydrolysis (breakdown) of α-1,4-glycosidic bonds in starch molecules. This breakdown process produces smaller carbohydrate fragments in the form of oligosaccharides, which can then be further degraded into monosaccharides such as glucose. Due to its central role in releasing sugars from more complex carbohydrates, controlling how actively this enzyme works has become a major focus across various fields, ranging from medical treatment to agricultural crop protection. (Arbetelli, L. A. et al., 2026)

The primary mechanism through inhibitors reduce amylase activity involves the direct suppression of the enzyme's catalytic function. In general, amylase inhibitors act by binding specifically to the active or catalytic site of the enzyme. This interaction commonly occurs through hydrogen bonding and π-interaction with important amino acid residues, including ALA 106, ASP 197, and TRP 59. By occupying the active site, the inhibitor behaves similarly to the natural substrate, thereby preventing starch molecules from interacting with the enzyme and slowing down the hydrolysis process (Quazi et al., 2022).

In the medical field, especially in diabetes mellitus management, amylase inhibitors are utilized to help regulate blood glucose levels. The inhibition of amylase activity can decrease the rate at which glucose is released into the bloodstream, thus reducing rapid increases in blood sugar following the consumption of carbohydrate-rich foods. Acarbose is one example of a clinically used inhibitor that functions by delaying carbohydrate digestion and sugar absorption in the intestine. Furthermore, researchers continue to develop new synthetic compounds, including 1,2,4-triazole derivatives, which are designed to inhibit α-amylase activity more effectively (Naik et al., 2023).

Interestingly, amylase inhibitors are also naturally produced by plants as a defense system against pest attacks. Herbivorous insects rely heavily on α-amylase enzymes in their digestive systems to break

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down starchy seeds they consume. As a defense mechanism, plants produce inhibitory molecules (both protein and non-protein) that specifically target insect amylase enzymes, thereby reducing the extent of crop damage. These natural plant-derived inhibitors work very specifically by mimicking starch substrates and binding to catalytic residues of insect amylase through hydrogen bonding, effectively preventing the insects from digesting their food. (Basso, M. F. et al., 2025)

A deeper understanding of the mechanism of amylase inhibitors has opened significant opportunities for future innovation, both in pharmacology and biotechnology. In the pharmaceutical field, since synthetic inhibitors may cause adverse side effects when used long-term, researchers are exploring the effectiveness of polyherbal teas (mixtures of natural plant-based ingredients) that may exhibit more body-friendly amylase inhibitory activity. In agriculture, genes encoding amylase inhibitors from certain plants are now being explored for overexpression in transgenic food crops, aiming to enhance plant resistance to insect pests in a natural and efficient manner. (Marzaok, M. A. et al., 2025)

3.6 Benefits and Applications of α-Amylase in Various Fields

In the continuously evolving ecosystem of modern bioindustry, enzymes have transformed from merely biological catalysts into key pillars that provide sustainable benefits for the creation of efficient and high-quality food systems. Microbial enzymes, particularly those produced through solid-state and submerged fermentation techniques, offer fundamental benefits in the form of significant cost efficiency in global industrial-scale production. In the bakery industry sector, the specific application of amylase and protease enzymes provides tangible benefits in modifying dough rheology, which not only improves fermentation quality but also effectively extends product shelf life by inhibiting starch retrogradation or staling (Chowdhury et al., 2024). Furthermore, the precise integration of enzyme biotechnology offers benefits in modifying food macromolecular components to enhance nutritional profiles as well as sensory characteristics that are more adaptive to modern consumer preferences (Devi et al., 2026).

The exploration of these innovations also brings extensive benefits to techniques for extracting bioactive components from natural materials in order to maximize their functional potential for human health. The use of a combination of pretreatment in the form of steam explosion optimized with a mixture design strategy has been proven to provide benefits in increasing the antioxidant activity of crude polysaccharides isolated from edible fungi (Zheng et al., 2024). On the other hand, the utilization of plant-based biocatalysts offers benefits in the form of high biocompatibility and exceptional operational stability, particularly in the development of biosensor devices to detect specific analytes such as pesticide residues in real time (Dkhar et al., 2025). These advantages emphasize that the exploration of plant-based natural resources holds unlimited potential benefits when managed with appropriate green technology approaches.

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Bioprocess efficiency at the industrial scale is highly dependent on a deep understanding of enzyme structural stability and molecular conformation, where the implementation of new technologies such as ultrasonication and pulsed electric fields provides significant benefits in enhancing amylolytic activity through the induction of secondary structure changes in α-amylase (Abedi et al., 2024).

In characterizing this performance, the use of a multi-analytical approach on the GH13 α-amylase profile offers benefits in accurately mapping molecular degradation patterns in potato starch, which are visualized through Principal Component Analysis (PCA) (Zinck et al., 2026). To provide benefits in the form of operational resilience under extreme industrial conditions, immobilization techniques Cross-Linked Enzyme Aggregates (CLEA) serve as a solution to enhance thermal stability and ensure enzyme reusability (Alemdaroğlu et al., 2026). Enzyme immobilization on polystyrene media provides significant practical benefits in simplifying analytical testing procedures in the laboratory (Jung et al., 2026).

The utilization of enzymes also provides crucial benefits in the bioenergy sector through the optimization of more efficient and economical lignocellulosic biomass deconstruction. The addition of amylase in the hydrolysis process of sorghum combined with ionic liquid treatment demonstrates a synergy that delivers maximum benefits in converting carbohydrates into fermentable sugars for biofuel production (Islam et al., 2026). In the field of advanced materials, the development of Pickering emulsions stabilized by chitosan nanoparticles provides benefits as enhancing interfacial stability as well as regulating the digestion kinetics of active compounds in biological systems (Geng et al., 2024). In addition, innovations in active packaging based on ZnO integrated with plant polyphenols provide dual benefits, namely as environmentally friendly (biodegradable) materials while also functioning as antioxidant protective agents that maintain food product quality during distribution and storage (Song et al., 2023).

In the medical field, the application of enzyme technology offers revolutionary benefits for the development of non-invasive diagnostics and more precise therapeutic interventions. Salivary amylase activity (sAA) has identified to provide benefits as a potential metabolic biomarker for monitoring glucose homeostasis status and plays a role in regulating appetite mechanisms through interactions with gut hormones (Erta et al., 2025). As a preventive effort against the increasing incidence of degenerative diseases, the discovery of multi-target peptides from barley (Qingke) offers benefits in simultaneously inhibiting α-amylase and α-glucosidase enzyme activities, which is highly important for the clinical management of type 2 diabetes (Li et al., 2026). Similar health benefits have also been observed in in-vitro studies of the traditional formula Brahmi Nei, which has been proven effective in suppressing blood glucose spikes through the mechanism of inhibiting carbohydrate-digesting enzyme activity (Shalini et al., 2025).

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The future of the global bioindustry will continue to grow rapidly by leveraging the benefits of exploring microbial diversity through metagenomic technologies supported by artificial intelligence (AI) and machine learning. These advanced technologies provide extraordinary benefits in discovering next-generation biocatalysts from environmental DNA without reliance on conventional laboratory culture methods (Wani et al., 2026). Other functional benefits found in use of biosurfactants from *Pseudomonas syringae*, which exhibit high antimicrobial efficacy with low cytotoxicity toward human cells (Haidar et al., 2024). These efforts strengthened by the optimization of superior strains such as *Talaromyces islandicus* as the use of precision analytical methods such as HPTLC-nanoGIT, which provide benefits in accelerating enzyme inhibitor screening (Al-Bedak et al., 2025; Müller et al., 2026). This entire series of innovations confirms that the development of bioprocesses provides fundamental benefits for the creation of a smarter, environmentally friendly, and sustainable future industry for global well-being (Ali et al., 2025).

5. Conclusions

This review discusses development of α-amylase technology, include its production, characterization, mechanisms, engineering, inhibition, and applications in industrial, biomedical, and sustainable fields. The findings show that α-amylase is a versatile biocatalyst with high adaptability and broad industrial potential. Microbial production using agro-industrial waste has also been shown to support efficient and sustainable processes. In addition, enzyme performance is influenced by factors such as temperature, pH, metal ions, and structural characteristics within the GH13 domain that affect catalytic activity and stability.

Advancements in enzyme engineering, such as genetic modification, protein engineering, and immobilization methods, have improved the stability, reusability, and resistance of α-amylase under extreme conditions, thereby increasing its industrial potential. In addition, studies on inhibition mechanisms provide important benefits for medical, agricultural, and food-related applications. These developments indicate α-amylase technology plays an important role in modern biotechnology. Future research is expected to focus on improving engineering strategies, exploring new microbial sources, and integrating advanced technologies to support efficiency, sustainability, and broader application.

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