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Protein Biotechnology: The Significance And Applications Of Industrial Polymer-Degrading Enzymes.

Mustafa M. Omar
Department of Forestry, College of Agriculture, Kirkuk University, Kirkuk, IRAQ.
*Corresponding Author: mustafa.mohamed@uokirkuk.edu.iq.

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ABSTRACT

Microorganisms are the primary source of industrial enzymes, with *Bacillus* and *Aspergillus* species being the leading producers. *Bacillus* strains have historically been used to synthesize commercially significant enzymes due to their status as GRAS (Generally Recognized as Safe) and their extensive distribution in the natural environment. Yeast, particularly *Saccharomyces*, may provide many significant enzymes in industry. Primary and secondary screening techniques identify microorganisms that are enabled to produce the target enzyme. Modern genetic engineering techniques have increased yields, such as new recombinant DNA and genetic mutations. The thermophilic microorganisms are used to obtain enzymes with enhanced stability, and they are highly active at temperatures over 80°C. These thermostable enzymes enable several industrial processes that rely on enzyme catalysis to be conducted at higher temperatures, enabling faster response rates. However, most thermophiles have not undergone thorough characterization and are not included in the GRAS list. Global sales of ″Bulk Enzymes ″are approximately US $600 million annually, with 66% attributed to different proteolytic preparations. Isomerases, particularly isomerases, generate around 550 million in yearly sales. Lipases now represent a small portion of overall enzyme sales, but the need for lipases is expected to increase. Massive quantities of degrading enzymes are used in various biotechnological procedures, such as brewing, winemaking, and cheese manufacturing. Enzymatic preparations are used in the brewing, bread-making, and cheese-making sectors. They have made it easier to design other biotechnological methods that generate diverse, commercially significant products to improve the taste, flavour, and appearance characteristics of food items, age meat, clarify juices, and are included in various detergent formulations.

***Keywords*:** Industrial enzyme, Degrading Enzymes, Recombinant DNA, Bulk enzymes, GRAS.

INTRODUCTION

Enzymes are protein molecules that enhance the pace of chemical reactions. Industrial enzymes have been widely used to accelerate industrial processes and produce commodities, therefore earning the title of industrial enzymes. Enzymes have been used since ancient times in many activities, including baking, brewing, and cheese production. These proteins are often produced in restricted quantities and typically undergo thorough purification. Expenses associated with production and advertising are often high, and economic considerations are the main focus [1]; [2]. Recent progress in recombinant DNA technology and improved bioprocesses has facilitated the large-scale manufacture of enzymes in purified and well-described forms. This has enabled the extensive use of enzymes in many industrial sectors, such as chemicals, detergents, textiles, food, animal feed, leather, pulp, and paper. Protein engineering and site-directed evolution have facilitated the development of tailored enzymes with unique capabilities and the capacity to adapt to various process conditions. According to research conducted by Business Communications Co., the global market for industrial enzymes is expected to expand [3]; [4]. The purpose of this subject is to familiarize oneself with protein products referred to as "industrial" or "bulk" enzymes. This group includes amylases, cellulases, lignocellulose-degrading enzymes, pectinases, proteases, and lipases. The majority of these enzymes may be categorized as hydrolytic depolymerases. Industrial enzymes, unlike those used for medicinal or diagnostic purposes, are produced in large quantities, often ranging from thousands to hundreds of thousands of kg per year, and usually undergo little processing. Furthermore, economic variables, particularly manufacturing costs, are very influential in deciding the practical applications of these technologies in most situations. This review focuses on a specific group of essential protein products called "industrial enzymes.

The industrial enzyme sources

Microorganisms are considered the essential and primary source of industrial enzymes. The producer strains often belong to a group of microorganisms categorized as GRAS (Generally Recognized as Safe). *Bacillus* and *Aspergillus* species are the leading producers of these bulk enzymes (Table 1) [5];[6]. *Bacillus* strains have historically been used to synthesize commercially significant enzymes due to their status as GRAS [7] and their extensive distribution in the natural environment.

In addition, they may be readily cultivated in cost-effective ways. Bacteria belonging to the genus *Bacillus* can produce significant amounts of beneficial enzyme activities, most of which are released directly into the surrounding environment. Several species of *Aspergillus* are used as producer organisms because of their favourable characteristics to *Bacillus* species [8];[9]. Yeast, particularly species of *Saccharomyces*, may provide many enzymes that are significant in industry. Typically, screening techniques identify organisms that can produce the target enzyme. Several wild-type organisms identified using these screening approaches initially generate limited amounts of the desired enzyme [10]. Historically, the enhancement of yields was achieved using techniques of strain improvement, such as mutagenesis, which was then followed by the discovery of microbial strains that exhibited higher production levels. In recent times, the technique of recombinant DNA has been used to enhance production output [11];[12]. Most enzymes of industrial importance are released into the surrounding environment by the microorganisms that produce them. The Exo enzymes make it easier to recover and purify the final product [13]. As mentioned earlier, most bulk enzyme products are crude preparations with several contaminating activities. Every production process must adhere to the standards of good manufacturing practice as they are applied to manufacture enzymes in large quantities. The whole procedure is also subjected to many quality assurance inspections. The technological simplicity of producing bulk enzymes should not be assumed [14].

Table 1: Some critical industrial enzymes, their sources, and their applications [15];[16]

Enzyme	Source	Application
α-Amylase	*B. subtilis* *B. amyloliquefaciens* *B. lichniformans* *Aspergillus oryzae*	Used it to liquefy, reduce viscosity, and aid in the digestion of animal feeds
β-Amylase	*B. polymyxa* *B. circulans*	production of high maltose syrup
Glucoamylase (amyloglucosidase)	*Aspergillus niger* *Rhizopus spp.*	The starch hydrolysis yields dextrose.
Pullulanase	*Bacillus spp.* *Aerobacter aerogenes* *Klebsiella spp.*	Used in the glucose and maltose syrup industries and to process wastes rich in polysaccharides into valuable products from sucrose and glucose
Glucose isomerase	*B. coagulans* *B. stearothermophilus* *Streptomyces spp.* *Arthrobacter*	Production of high-fructose syrup (HFS)
β-Galactosidase (lactase)	*B. coagulans* *Saccharomyces spp.* *Aspergillus spp.* *Streptomyces spp.*	Used in the hydrolysis of milk lactose to avoid lactose intolerance
Invertase (Sucrase)	*Saccharomyces spp.*	Used to soften candies and recover sugar from scrap candy
Cellulase and Hemicellulase	*Trichoderma spp.* *Sporotrichum cellulopilum*	Used to process commercially prepared feed
Pectinase	*Aspergillus niger* *Fusarium spp.*	Used for fruit and vegetable juice clarification (de-pectinization)
Proteases	*Bacillus amyloliquefaciens* *B. subtilis* *Streptomyces spp.* *Aspergillus spp.*	Used in detergent applications as well as in the fermentation, baking, and meat aging processes
Lipase	*Mucor spp.* *Myrioccum spp.*	Used in the dairy industry, as well as in detergent

Thermophilic microorganisms as enzyme producers

The use of thermophilic organisms for obtaining enzymes with enhanced stability. Many thermophiles are extracted from various habitats, including hot springs. The enzymes generated by these organisms are highly active at temperatures over 80°C, a range that would rapidly deactivate identical enzymes produced by mesophiles, which thrive at considerably lower temperatures. These thermostable enzymes would enable several industrial processes that rely on enzyme catalysis to be conducted at higher temperatures. This would be advantageous since it would enable better rates of response. Thermostable enzymes often have better stability and resilience than enzymes from mesophilic species. Most thermophiles have not undergone thorough characterization and, as a result, are not included in the GRAS list [17];[18];[19] [20]. Furthermore,

many of these species pose challenges in cultivation, even when attempted on a limited scale. The limitations of these thermophiles have hindered their widespread use in industry, but there are a few exceptions. Certain species of *Bacillus*, such as thermophilic ones, can thrive at high temperatures. Many enzymes generated by these organisms have garnered significant attention from industry and academia. The recombinant DNA technique enables the creation of thermostable enzymes in (GRAS) organisms [21]; [22] [23].

Sales value of bulk enzymes

Table 2 shows that global sales of bulk enzymes are approximately US $600 million per year. Approximately 66% of these sales are attributed to different proteolytic preparations, generating around 350 million yearly sales. Lipases now represent a small portion of overall enzyme sales, but the need for lipases is expected to increase [24];[25];[13].

Table 2: Estimated value of annual worldwide sales of major enzymes

Enzymes type	Market value (US$ million)
Proteases	330
Carbohydrases	150
Isomerases	50
Lipase	20

Industrial applications of bulk enzymes

Massive quantities of enzymes are used in several biotechnological procedures. Several ancient technologies, like brewing, winemaking, and cheese manufacture, may be traced back to the earliest periods of human history. During these occurrences, humans inadvertently used microbes to get the necessary enzymes for converting beginning substances into end products, such as ethanol or cheese [26];[27]. Enhanced comprehension of the molecular mechanisms behind these transformations has enabled the subsequent use of purified enzymes for targeted industrial objectives. These enzyme preparations are now used to enhance or substitute for the natural enzymatic complement. Enzymatic preparations are used in the brewing, bread-making, and cheese-making sectors. These enzyme preparations have also made it easier to design other biotechnological methods that generate diverse commercially significant products, improve the taste, flavor, and appearance characteristics of several food items, and tenderize the meat and clarify liquids. They are also included in various detergent formulations [28];[29].

Carbohydrate degrading enzymes

Polysaccharide-degrading enzymes are an essential category of bulk enzymes highly significant in industrial applications. This category includes enzymes like amylases, pectinases, cellulases, glucose isomerase, invertase, and lactase, all of which have substantial market opportunities [30]. Table 3 is a compilation of industrially significant carbohydrates.

The group of these enzymes that analyze starch into smaller components is known as amylolytic enzymes, also called amylases. This category belongs to α-amylase, β-amylase, gluco-amylase, pullulanase, and iso-amylase. Starch analysis by enzymatic degradation produces glucose, maltose, and other sugars [31]. Maize, wheat, rice, and tubers of potatoes are rich sources of starch; these are mainly used from waste from food industry factories as low-cost sources for producing high syrups of glucose, fructose, or maltose. These syrups have a significant significance in the food and confectionery sectors. Moreover, they may be manufactured more competitively than sucrose, derived from conventional sources like sugar beet or sugar cane [32];[33].

Table 3: Some industrially important carbohydrates [34].

Monosaccharides	Disaccharides	Polysaccharides
Galactose	Maltose	Cellulose
Arabinose	Cellobiose	Hemicellulose
Glucose	Sucrose	Starch
Fructose	Lactose	Glycogen

Amylase

The α-amylase activity in a wide range of organisms in nature, including microorganisms, is an enzyme that acts internally, catalyzing the random analysis of αl→ 4 glycosidic bonds inside the starch substrate. These enzymes cannot analyze αl→6 glycosidic connections seen at the junctions of amylopectin chains [35]. Except is produced by the bacteria *Thermoactinomyces vulgaris*, which can analyze αl→6 and αl→4. All α-amylases that have been studied so far are classified as metalloproteins. Furthermore, these enzymes can catalyze the breaking of internal αl→4 in glycogen and several other oligosaccharides [36].

The α-amylase is a high heat resistance used for industrial applications are usually produced from *Bacillus* species bacteria, specifically *B. amyloliquefaciens* and *B. licheniformis*. The enzyme activity's temperature and pH are 90°C and 6.0, respectively. The molecular weight is 55,200 Da and contains 483 amino acids [37]; [38]. This enzyme is also produced by fungi, especially *Aspergillus* species, such as *A. oryzae* and *A. niger* [39].

Glucoamylase and amyloglucosidase are extracellularly produced by several fungi species, particularly *Aspergillus* and

Rhizopus. These enzymes hydrolysis αl→4 glycosidic linkages and also αl→ 6 glycosidic linkages branch in amylopectin at a slower pace. It converts starch into a liquid to produce glucose syrup, a process called starch saccharification [40]. While amyloglucosidase is heat-sensitive, high pH is unstable at temperatures over 60 °C. These enzymes are used in industrial applications to produce glucose and high-fructose syrup directly in food and manufacture crystalline glucose [41].

Beta-amylase. Unlike α-amylase, β-amylases are extracellular enzymes analysis αl→ 4 glycosidic bonds starting from the non-reducing end. This reaction results in the formation of maltose units in the β form, as seen in Figure 1. Similar to α-amylases, β-amylases cannot analyze αl→6 bonds by hydrolysis. Beta-amylase cannot convert amylopectin into maltose because of branch points. Conversely, it can be analyzed the amylose because it does not contain any branch. Typically, when β-amylase is used to analyze most starches, it produces a mixture of maltose and oligosaccharides [42]. β-amylase activity is found in several higher plants. For instance, the enzyme in barley may convert starch into maltose, which occurs during the malting process. β-amylases have also been derived from several bacterial sources, particularly *Bacillus circulans* [35]. Alpha-l→6 bonds are enzymes that catalyze the hydrolysis of αl→6 bond bonds of amylopectin. Debranching enzymes are essential for the thorough analysis of starch since both α- and β-amylases are unable to analyze the αl→6 bond found in amylopectin. Several additional αl→6 glycosidases have superior efficacy and velocity in the hydrolysis of branch-point linkages compared to amyloglucosidase. Out of these enzymes, pullulanase and isoamylase are the most essential [43]. These enzymes often enhance the saccharification process, as seen in Figure 2.

Pullulan is a polysaccharide that contains 1500 glucose units. The main structure comprises three glucose molecules connected by two αl→4 links. The connection between each maltotriosyl unit and the next unit is made by an αl→6, as seen in Figure 3. Pullulanase and isoamylase can analyze αl→6 bonds and, therefore, analysis of pullulan, while isoamylase cannot. Pullulanase was first discovered in the early 1960s inside a particular strain of *Aerobacter*. Several types of bacteria, such as *Bacillus* and *Streptococcus*, produce it. Isoamylase, produced by several microbial species, was first isolated from yeast. *Pseudomonas amyloderamosa*, a mutant strain, is known for its high production of extracellular isoamylase, which is extensively used in many sectors [44]; [45].

Many pullulanases with distinct functions have recently been identified in different thermophilic species. Producer microorganisms include a wide array of *Clostridia* species and several strains of *Thermoanaerobium*. Most exhibit remarkable heat stability and retain their activity for long periods at temperatures above 90°C. A distinguishing feature of some novel pullulanase enzymes, commonly referred to as amylopullulanases, is their ability to hydrolyze αl→6 glycosidic bonds in particular carbohydrates, such as pullulan and α1→4 linkages. Conventional pullulanase cannot analyze the α1→4 bound in pullulan or amylose [46].

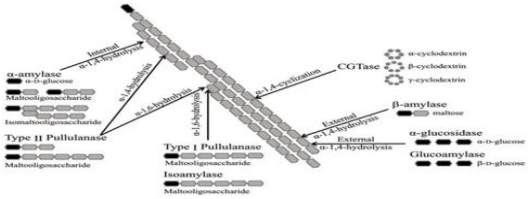

Figure 1: Starch hydrolysis by α-amylase and β-amylase [47]

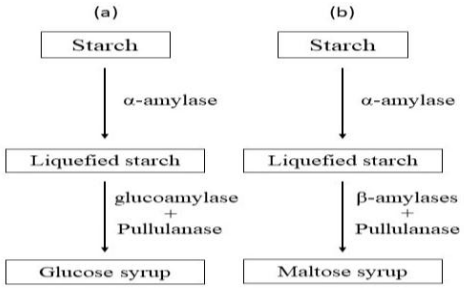

Figure 2: Illustrates the hydrolytic analysis of starch, which produces valuable end products that are significant in several industries. For the sake of clarity, process factors such as pH and temperature modifications, as well as the inclusion of stabilizers, have been excluded [48]

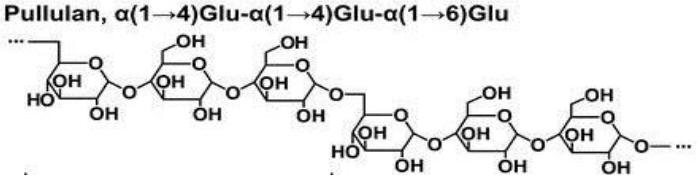

Figure 3: Structure of Pullulan [45]

Glucose isomerase

The enzymatic hydrolysis of easily accessible and low-cost starch enables the cost-effective generation of substantial amounts of glucose syrup. While it is possible to consume glucose syrup directly, many are converted to high-fructose syrups because fructose has a sweetness level about twice as high as glucose (Table 4). As a result, it is more appealing for commercial applications as a sweetening agent in the ice cream and soft drink industries [49]. Glucose may undergo chemical or enzymatic isomerization to be transformed into fructose (Figure 4). The use of alkali is essential in chemical conversion; nevertheless, this approach is limited due to poor yields and unwanted side reactions. Glucose isomerase, an enzyme, facilitates the conversion process at average temperatures and pH levels close to neutral, resulting in a syrup that contains 42% fructose. Recent advancements in process refinement now enable the manufacturing of syrups with elevated levels of fructose [50].

Table 4: This study examines the comparative sweetness of several widely found sugars of significant value in industrial applications. To facilitate comparisons, sucrose has been designated with a sweetness rating of 100% [51].

Sugar	Sweetness (%)
Sucrose	100
Fructose	130
Glucose	70
Maltose	40

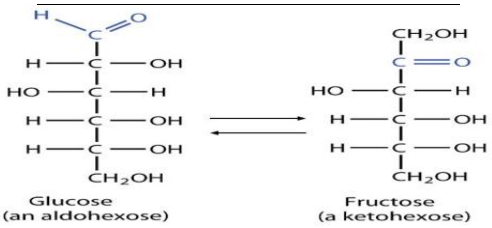

Figure 4: The process of isomerization converts D-glucose, which is an aldohexose, into fructose, which is a ketohexose

Microbial species-derived glucose isomerase has been used commercially to manufacture high-fructose syrups. The enzyme may be produced from several bacterial species, including *Aerobacter*, *Bacillus*, and *Streptomyces albus*. The two bacterial species are *Lactobacillus brevis* and *Actinoplanes missouriensis*. Glucose isomerase derived from plants is also used to convert glucose into fructose [52]. Most microorganisms produce glucose isomerase, an intracellular enzyme. Intracellular enzymes must be broken to separate it, and then using the appropriate technique for purification, glucose isomerase is more difficult to produce and separate commercially than extracellular enzymes [53].

Industrial importance of starch conversion

The final products from starch analysis are used in various culinary items and beverages. Some substances are used only as food sweeteners, while others are added to provide a specific texture to the food product. Starch analysis enzymes are used to produce juices and bread [50].

Yeast cells lack the enzyme capacity to analyze starch and other complex carbohydrates found in grains. Instead, they only use glucose, monosaccharides, and disaccharides as energy sources. Before the fermentation process, grain germination is encouraged. The sprouting process activates endogenous enzymes that may analyze stored starch, cellulose, and other structural polysaccharides, which may analyze stored starch into glucose and other carbohydrates. Yeast cells can ferment these sugars via a process known as malting [53].

Instead of getting more amylolytic enzymes from seeds like people usually do, amylolytic enzymes from bacteria not found in plants can now be used instead or in addition to seeds. The concentration of β-amylase in grains used for bread-making stays consistent, but the amount of α-amylase is small before germination. Adding fungal α-amylase to the flour enhances the starch analysis, making the dough more manageable and facilitating yeast fermentation. As a result, this encourages fermentation, leading to a larger loaf size and improving the bread's texture [5].

Additional sugar-degrading enzymes

Several more sugar-degrading enzymes have been used, notably in industrial settings, although with some limitations. Lactase, also known as β-galactosidase (Figure 5), catalyzes the hydrolytic cleavage of lactose, the sugar in milk. This process results in the production of glucose and galactose. The process of analysis of lactose by hydrolysis is significant for several reasons. Before absorption from the small intestine, lactose in consumed milk must be digested into monosaccharides [54]. While babies often have significant levels of intestinal lactase activity, several adult human cultures lack this enzyme almost entirely. Adults of northern European descent continue to manufacture substantial amounts of intestinal lactase throughout their lifespan. Adults with little or no lactase activity are lactose intolerant and cannot analyze lactose in their diet. In these cases, the consumed lactose leads to gastrointestinal disorders and stimulates the occurrence of diarrhea. This phenomenon dramatically limits the use of milk as a source of nutrients for several adult populations. The milk lactose is first subjected to enzymatic hydrolysis to overcome this challenge. Immobilized lactase preparations derived from species of *Aspergillus* and

Saccharomyces have been effectively used in this process. In addition, lactase-containing tablets have been developed for lactose-intolerant patients to consume orally before consuming milk or milk products [55].

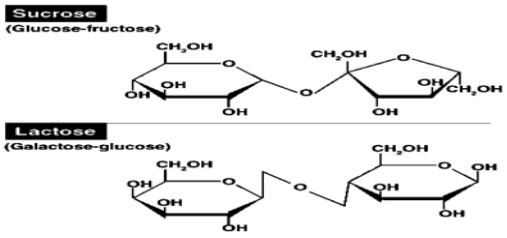

Figure 5: Structure of the disaccharides, (a)

sucrose and (b) lactose

The cheese-making business generates substantial amounts of a by-product known as whey, which poses disposal challenges. Unsurprisingly, whey has a high lactose concentration. The hydrolysis of lactose, which produces glucose and galactose, can make this by-product a valuable food or feed supplement [56]. Sucrase, also known as invertase, is used in industrial applications to facilitate the hydrolysis of sucrose (Figure 5), forming glucose and fructose. This enzyme is often used in the confectionery industry to create the dense filling in many soft-centered candies. Additionally, it has been used to produce ham, synthetic honey, and marzipan. Fungi, such as *Aspergillus* or certain yeast species, are often used for the large-scale production of invertase in industrial settings [57].

Lignocellulose-degrading enzymes

Plant biomass has the most significant amount of naturally occurring glucose reserves on Earth. Biomass is often described as any organic matter that undergoes growth and may be used as an energy source. The majority of plant biomass is composed of three main polymeric substances: cellulose (40%), hemicellulose (33%), and lignin (23%). The continuous regeneration of plant biomass via photosynthesis guarantees the unlimited availability of this material. An estimated yearly synthesis of around 4×1010 tons of cellulose by higher plants has been reported. This implies that more than 70 kg of cellulose is produced per individual daily. Enzymes with the ability to analyze cellulose are highly sought-after in the industrial sector [58].

The cellulose substrate. Cellulose is a polysaccharide made up of glucose subunits that are connected by β1-4 glycosidic links. It is a linear and unbranched molecule. Cellulose molecules exhibit significant variation in length, ranging from as little as 3000-4000 glucose residues to as much as 8000-12000 glucose molecules. Each glucose molecule consists of up to 20,000 units. Most cellulose molecules are composed of subunits that are rotated at a 180° angle about each other. As a result, the disaccharide cellobiose (Figure 6) possesses neighboring residues.

The present ongoing structure. Cellulose is a compound comprised of glucose units linked together by 14 glycosidic bonds, different from the links found in amylose. Individual cellulose molecules, composed of alternating glucose molecules, form elongated structures typically organized into bundles or fibrils of multiple parallel cellulose molecules. This arrangement is due to the β form of the connection, spatial configuration, and conformational shape. The stability of these structures is maintained by an intricate network of hydrogen bonds that occurs both inside and between the cellulose molecules. Hydrogen bonds between glucose residues increase the molecule's overall rigidity. Cellulose fibrils consist of crystalline sections with a highly structured structure and amorphous, less organized portions [59].

Hemicelluloses and lignin are two components found in plant cell walls. Hemicelluloses are typically polysaccharides with relatively lower molecular weights. The main types of hemicellulose found in cell walls include xylans, mannans, and glucans. Xylan is the main kind of hemicellulose, composed of a chain of D-xylose units connected in a β1-4 linkage [60].

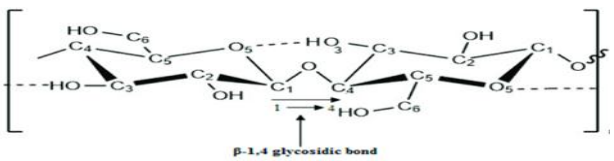

Figure 6: Diagram depicting the arrangement of a segment of the cellulose main chain

Lignin is an intricate aromatic polymer that is present mainly in high-end plants. Hemicellulose is primarily located inside the cell walls of plant cells. The combination creates a solid structure similar to cement, with the cellulose fibrils arranged in an organized manner inside it. Hemicellulose and lignin enhance the cell wall's overall structural integrity. More than 20% of the composition of tree trunks is made up of lignin, a substance found in the woody part of the trunk. The molecule in issue is a polymer composed chiefly of three alcohol molecules: sinapyl, coniferyl, coumaryl, and alcohol (as shown in Figure 7).

The cross-linking of these alcohols seems to be random, resulting in the formation of lignin molecules that are widely scattered. Angiosperm lignins typically comprise equal proportions of sinapyl and coniferyl alcohol monomers. Conversely, lignins in gymnosperms comprise coniferyl units, whereas lignins in grasses comprise all three alcohols [61].

Cellulases. Agricultural and residential trash also includes significant amounts of cellulose initially obtained from plant material. Wood and wood pulp have a cellulose content of more than 40%, whereas straw and bagasse have a cellulose content ranging from 30% to 50%. Newspapers may contain as much as 80% cellulose. Cotton consists mainly of cellulose. Glucose is produced when cellulose is fully hydrolyzed. Developing a highly efficient and cost-effective method to convert cellulolytic material into glucose would have significant industrial implications. Cellulases are enzymes that can analyze cellulose by hydrolysis [62].

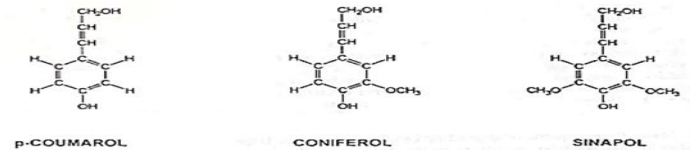

Figure 7: The molecular structure of the alcohol compounds present in lignin [64]

A single enzyme does not hydrolyze cellulose hydrolyzed by a group of enzyme activities. The degradation of cellulose-based material in the natural environment is a slow process influenced by several causes. Only a few numbers of microbes possess cellulase systems that can comprehensively and systematically analyze cellulose into glucose molecules. The organized crystalline arrangement of individual cellulose molecules inside cellulose fibers makes it resistant to enzymatic degradation. However, parts of cellulose that lack a defined structure, known as amorphous regions, are analyzed faster. Furthermore, the inherent strong bond between cellulose and hemicellulose, lignin, and sometimes pectin poses an additional obstacle for cellulases to access their desired substrates effectively. Cellulolytic materials may undergo several pretreatments to enhance the accessibility of cellulose molecules for enzymatic degradation. Using such treatments on a large scale would make manufacturing glucose from cellulose not cost-effective. The process of hydrolysis requires costly pretreatments, which are not economically favorable. Additionally, the high expenses related to the manufacture of cellulolytic enzymes further reduce its economic viability [63].

Cellulolytic enzymes are produced by several microorganisms, particularly fungi (Table 5). Certain bacterial species also possess the ability to degrade cellulose. Several cellulases synthesized by bacteria seem attached to the cell wall and cannot be analyzed using natural lignocellulose materials. Many fungi that can analyze cellulose produce significant amounts of cellulases outside their cells, effectively analyzing the cellulose substrate. Thus, it is unsurprising that fungal cellulases have garnered the highest interest. Some types of fungi, particularly *Trichoderma* species like *T. koningii*, *T. reesei*, and *T. viride*, with *Penicillium funiculosum*, which can synthesize cellulase enzymes. Cellulases are produced in small quantities by most wild-type producer strains, and the enzymes generally exhibit disappointingly specific activities. While mutational approaches have yielded hyperproducing strains, their specific activity remains low. Such product inhibition has focused attention on the study of genes encoding cellulases. Such investigations, coupled with ongoing biochemical research, may facilitate the production of more efficient cellulase-digesting systems. The industrial use of cellulose hydrolysis. Cellulases and related enzymes have a large potential for industrial use. The glucose released from the substrate lysis by cellulase may be utilized in processed foods for human and animal feed. They can use in other processes of fermentation that provide helpful end products such as organic acids, amino acids, methane, single-cell protein, methanol, butanol, and ethanol. Cellulases may directly enhance the digestibility of meals that contain a significant amount of fiber and improve food taste, texture, and other characteristics. Although there are many potential uses for cellulases, they have only been used in a limited number of industrial processes thus far. This results from technological challenges and economic considerations [65].

Although there are technological and economic obstacles, some cellulolytic formulations have been used to a limited extent in industrial settings, primarily as digestive aids. These preparations have been administered to economically essential compounds derived from plants. Molecular organisms, used to improve the process of extracting different biological substances, have also enabled the utilization of cellulases, particularly the cellulose-binding domains of cellulases, for many inventive applications. Several cellulases possess distinct cellulose-binding domains located next to the catalytic regions. Sequences of nucleotides that encode cellulose-binding domains have been artificially created and combined with genes that encode other proteins. The resulting recombinant product maintains the original protein's biological functionality while exhibiting a strong affinity for cellulose due to adding a newly acquired cellulose binding domain. This may selectively purify the genetically engineered product using a cellulose column. Alternatively, it may fixate the protein onto cellulose columns [66]. This is an overview of cellulose hydrolysis. The enzymatic hydrolysis of this polymer is not only complex but remains a challenge due to the different types and numbers of enzymes needed for comprehensive degradation.

Table 5: Selected microbial sources of cellulases [65]

Microorganism

Fungi		Actinomyces	Bacteria
Acremonium cellulolytic	*Chrysosporium*	*Actinomyces sp.*	*Clostridium thermocellum*
Aspergillus aculeatus	*Schizophyllum commune*	*Thermoactinomyces sp.*	*Ruminococcus albus*
Aspergillus fumigatus	*Sclertium rolfsii*	*Thermomonospora curvata*	
Aspergillus niger	*Sporotrichum cellulophilum*		
Fusarium solani	*Talaromyces emersonii*		
Irpex lacteus	*Thielavia terrestris*		
Penicillium funiculosum	*Trichoderma koningii*		
Phanerochaete	*Trichoderma reesei*		

Pectin and pectic enzymes

It is a complex carbohydrate that is a structural component in higher plants. Pectin is usually found inside the cellular structure, namely in the cell wall and middle lamella. Enzymes that can analyze pectin may be obtained from both plants and microorganisms. The lysis of pectin is crucial for developing plant cells and fruit maturation. Fungi primarily generate pectic enzymes, which are extensively used in many industrial processes. Recombinant DNA technology has made identifying, replicating, and analyzing genes that encode various enzymes easier. It has also allowed for the production of these enzymes in other organisms [67].

The pectic substrate is the specific material that serves as a source of pectin, a complex carbohydrate found in plant cell walls. The protopectin or galacturonic acid is the predominant compound in pectic preparations, making up 60–80% of the total molecules. The sugars galactose, arabinose, rhamnose, and xylose are often included in pectic preparations. Rhamnogalacturonans are the primary components of pectic compounds. The primary structure comprises α-D-galacturonate units connected by αl→ 4 bound. The main chain is branched by side chains of varying lengths, often composed of Galacturonic, Galatians, Arabinans, or Arabinogalactans (Figure 8) [68].

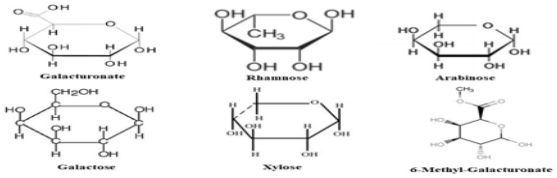

Figure 8: The structural formulas of the most prevalent monomers discovered in pectin

Pectin and enzymes that break down pectin are essential in industry because they can make thick, gel-like solutions when heated with sugar in an acidic environment. Pectin is usually extracted from the peel of citrus fruits or the pulp of sugar beets. Pectin is advantageous because it can serve as a gelling agent, emulsifier, or thickening agent in many food items. Utilizing the pectin on top of the substrate makes the production of jams and jellies with a lower sugar content achievable. These enzymes are especially preferred for producing fruit juice by mechanically pressing the corresponding fruits. Commercial pectic enzyme formulations often lead to a significant increase in juice production. Fungal pectinase preparations for the enzymatic destruction of pectin are often employed to optimize juice extraction from grapes and apples. Using exogenous pectinolytic enzymes enhances juice extraction from soft fruits, including raspberries, strawberries, and blackberries [69]. Commercial pectinase preparations often clarify "sparkling" fruit juice preparations, including apple and pear juice. Recently squeezed, most of these juices include elevated amounts of soluble pectins, responsible for the distinct thickness and cloudiness often seen in these products. Haze is often a result of decreased solubility of the juice's constituents, which is associated with pectins. Commercial pectinase preparations partially degrade the pectins, leading to a substantial decrease in the product's viscosity. Moreover, the partial breakdown of the pectin causes the haze particles to become unstable, clumping together and settling out of the solution. The residue may be readily removed using centrifugation or filtering, producing a sparkling and clear juice. The use of pectic enzymes decreases the thickness of the solution, enabling the production of highly concentrated juice extracts [70]. Pectinase is an enzyme that softens fruits and vegetables through maceration, transforming fruit or vegetable tissue into a mixture. This process is associated with fruit softening during ripening and produces fruit nectars, pulp-containing drinks, and baby food products. Pectic enzymes also extract citrus oils and colors from orange and lemon peels. Pectinase preparations are typically made from fungi like *Aspergillus* or *Penicillium*. Following stabilizers, preservatives, and other chemicals, precipitation concentrates and partially purifies the enzymes. Commercial pectinase preparations typically include various pectinolytic and additional enzymes [71];[72].

Proteolytic enzymes

Proteolytic enzymes are the most significant category of proteins generated in large numbers. These proteases have many uses in many different industries, some of which are mentioned in Table 6. Proteolytic enzymes have historically been used, sometimes without awareness, in activities such as brewing, baking, and cheese-making. These enzymes are also used significantly in the tanning industry and medicine.

Table 6: Some industrially significant proteolytic enzymes

Protease (General Name)	Application	Source
Microorganisms proteases	Cheese-making Brewing Leather bating Detergent	Fungi and Bacteria
Rennin	Cheese-making	Animal (stomach of unweaned calves); also recombinant
Trypsin	Debriding agent Meat tenderizer	Animal
Papain Collagenase	Brewing Digestive aid Debriding agent	Plant (papaya plant) and Bacterial
Papain	Digestive aid Debriding agent	Animal and Plant (papaya plant)
Bromelains	Anti-inflammatory agent Digestive aid	Plant (pineapple)
Pepsin	Digestive aid	Plant (pineapple) and Animal

Many proteolytic enzymes often used in industrial applications were derived from either plant or animal origins. Microbial proteases are now extensively used in detergent powders, making microbes the primary source of these enzymes. Industrially used microbial proteases are typically extracellular and are generated on a big scale by semisolid or submerged fermentation. The use of "industrial" proteinases in detergent formulations is the most significant use of these enzymes. Recombinant DNA technology has facilitated the synthesis of significant proteolytic enzymes in hitherto unexplored host species. Genetic engineering enables the generation of limitless amounts of proteolytic enzymes, naturally produced in small quantities by the organisms that make them. This method enables the manufacture of certain proteins in organisms categorized as (GRAS). These proteins are naturally generated by microbial species whose safety is uncertain [74].

Proteolytic enzymes of medical significance

Several proteolytic enzymes have significant medicinal uses (Table 6). Typically, these enzymes are manufactured in limited to moderate amounts within the industrial sector and need extensive processing after production. Consequently, most cannot be accurately classified as conventional bulk industrial enzymes [75].

Protease enzymes used in the baking industry

The usage of fungal proteases in the baking industry is limited. Enzymes obtained from several *Aspergillus* species are often used to alter the protein composition of flour, therefore altering the texture of the dough. Gluten is a prevalent protein present in flour. This protein aids in the capture of carbon dioxide produced during fermentation, which then affects the pore structure of leavened bread [76].

Proteases used in the meat and leather industries

Papain is often used as a substance that enhances the tenderness of meat. The proteolytic preparation might be directly applied to the meat or injected into the animal just before it is slaughtered. This method promotes the uniform dispersion of the enzyme. The proteolytic activity of enzymes breaks down the connective tissue collagen and elastin in meat, making it more tender. The main factors that make beef tough are essentially these components. This procedure may also occur spontaneously by refrigerating fresh corpses in cold storage for a few days after they are slaughtered. During this period, various degradative enzymes are secreted when the structural integrity of cells is compromised. This process is called "aging" [77].

Proteolytic enzymes are often used to remove hair and treat leather. Eliminating hair from animal skins may be achieved by subjecting them to a treatment including a lime and sodium sulfide mixture. Nevertheless, these compounds are difficult to handle and can lead to issues with waste management. Proteolytic enzymes are often used as substitutes, either on their own or, more commonly, in conjunction with lower levels of lime. Using alkaline proteases, such as subtilisin, in a lime procedure is necessary because lime creates alkaline conditions [78].

Several proteolytic enzymes are used in the tanning process. The technique of bating makes the leather supple and flexible. Gloves are made with thoroughly tanned leather, but shoe sole leather is not tanned. Historically, trypsin has been used in the baking process. Recently, microbial enzymes derived from different species of *Aspergillus* and *Bacillus* have increased in popularity in this context [79].

Proteases incorporated into detergent products

Detergents incorporate the majority of large-scale proteases. Around the beginning of the twentieth century, detergent formulations first implemented the use of enzymes. Only a handful of these products were successful, primarily because other substances in the detergent mixture or the subsequent washing procedure consistently deactivated the used enzymes, often

derived from animals. Clothing often becomes dirty from colours, biological molecules, soil, and other particles. Biological "dirt" comprises substances derived from protein, lipid, and carbohydrate. Dirt components may originate from people or animals, including skin shedding, blood, and other food or grass sources. Contemporary detergent formulas contain many components that eliminate organic and inorganic filth [81]. Typical ingredients are listed in Table 7.

Table 7: The primary components of contemporary detergent formulations [80]

The detergent compound	Influence
Enzymes	Removal of biological dirt, which is mainly protein
Sodium carbonate and silicate	Maintenance of an alkaline Ph
Polycarboxylates	Helps disperse dirt particles in water
Sodium tripolyphosphate	Used to soften water
Silicones	Controls foaming
Perfume	Imparts an appropriate scent to fabrics
Soap and various surfactants	Removal of dirt particles, especially hydrophobic molecules from fabrics
Sodium perborate	Removal of dyes and stains from fabrics

Soaps and surfactants effectively eliminate the bulk of dirt particles from clothes. Perborates provide a bleaching effect, eliminating colors and stains, including those derived from tea and coffee. Proteolytic enzymes can analyze protein-based substances, including blood, eggs, and gravy. The washing procedure often leads to denaturation and aggregation of proteins. This makes them considerably more challenging to eliminate from fabric fibers. Using proteolytic enzymes that remain active throughout the washing process significantly enhances the breakdown and elimination of persistent stains. Phosphates, such as sodium tripolyphosphate, work to reduce the hardness of water, maximizing the effectiveness of detergents [82].

Proteolytic enzymes were widely used in detergent formulations in the 1960s. This fact was seen in a substantial surge in global protease sales during this timeframe. During the late 1960s, the use of proteolytic enzymes in detergents sparked significant debate. The frequent occurrence of allergic responses to the powder components primarily caused this. During that period, unrefined protease products were added to the detergent as fine powder, creating a substantial amount of dust. Allergic responses often occur due to inhaling enzymes and other components found in detergents. Production staff responsible for handling and packaging enzyme-containing goods were most susceptible to experiencing these negative allergy responses. Several end-users also had a variety of adverse reactions, including respiratory issues and rashes [83].

By the early 1970s, enzymes had been eliminated from most detergent formulations. This was shown in a sudden decline in global bulk proteinase sales. However, further scientific examinations indicated that incorporating enzymes alone was a secure industrial procedure. New granulation methods were developed to decrease dust generation using dry enzyme preparations. At first, enzyme granules were combined with melted wax and then cooled by spraying. The procedure, called prilling, produced granules with a uniform distribution of wax and enzymes. Another procedure included the production of granules from a paste containing just enzymes. These granules were then covered with wax, guaranteeing that they remained intact and reduced dust development. The use of granulation technology enabled the extensive reintroduction of enzymes in detergents while minimizing the potential health hazards linked to the creation of dust particles [84].

Subtilisin Carlsberg was one of the first proteolytic enzymes effectively used in detergent formulations. Subtilisin Carlsberg is a monomeric serine protease synthesized by *Bacillus licheniformis*. The protein comprises 274 amino acids and has a molecular weight of 27,500 Daltons. The polypeptide is a single-domain molecule with an active site composed of serine 221, aspartate 32, and histidine 64. The enzyme has the characteristic Michaelis-Menten kinetics and a wide product selectivity range. The protein has optimal activity at pH levels ranging from 8 to 10 and is stable at temperatures over 50 °C. The subtilisin gene has been replicated and activated using alternative manufacturing methods. The compound's structure and mechanism are very simple, making it easy to study. Its significant relevance in the industry has led to much research [85].

The potential applicability of alkaline proteases generated by various bacteria has been investigated for use in detergent formulations. The activities shown by certain alkalophilic species of *Bacillus* have become particularly significant in this context. Like subtilisin, these enzymes are serine proteases of a single chain. Many exhibits include general features that make them as appropriate for detergent usage as subtilisin Carlsberg [86].

Proteases used in cheese manufacture

Rennin, also known as chymosin, is a proteolytic enzyme in high demand in several industries. This protease is used in the cheese production process. The first stage of cheese-making includes the enzymatic coagulation of milk. Rennin facilitates the controlled breakdown of milk kappa casein by restricted proteolytic cleavage. This disrupts the stability of casein micelles and stimulates their aggregation, resulting in the formation of curds. The residual liquid, known as whey, is extracted, and the curd undergoes further processing, producing cheese or other dairy products [87].

Rennin is derived from the abomasum of young calves. The extraction process typically entails extended treatment of dehydrated strips of the calf stomach with a saline solution that includes boric acid. The resultant enzymatic extract, rennet,

comprises various proteolytic and other enzymatic activities. Rennin constitutes a maximum of 2-3% of these formulations. Rennin, also known as chymosin, is an aspartic proteinase enzyme that is produced in the form of preprotein. The hydrophobic leader sequence, responsible for guiding the newly produced molecule through the endoplasmic reticulum, is removed, resulting in the formation of prorenin, the inactive form of the enzyme renin. Prorennin is activated by autocatalysis in the presence of acidic conditions in the stomach, producing the active enzyme. Rennin has a molecular weight of 35,000 Da and comprises 323 amino acids. There are two naturally occurring isoforms of the enzyme, chymosin A and chymosin B. Both demonstrate optimal acidity at a pH of around 4.0. Calf rennin acts as a catalyst to break a specific peptide link, the Phel05-Met106 bond, in the x-casein molecule. This process leads to the precipitation of proteins and the production of curds. The precise nature of rennin towards its casein substrate makes this enzyme suitable for cheese-making processes. Additional enzymatic breakdown of casein would lead to the creation of cheeses of lower grade that are not enjoyable to taste [88]; [89].

The pace at which young calves are killed directly reflects the demand for veal in the market. The volatility of this demand impacts the accessibility and cost of rennet. Several alternative sources have been thoroughly evaluated to find an appropriate substitute protease. Pepsin preparations derived from various slaughterhouse animals have shown minimal efficacy in this respect. However, they are sometimes used in conjunction with rennet preparations. Of all the microbial species examined so far, only a few can cause curd formation, which is considered good. Various species from the Mucor genus of heat-loving fungus were discovered to provide satisfactory substitute functions to calf rennin. The fermentation technique allows for the cost-effective and abundant production of Mucor enzymes. Like renin, these enzymes facilitate the partial breakdown of the casein molecule and effectively operate within the parameters of the cheese-making process. Most *Mucor* enzymes are thermostable, meaning they can withstand high temperatures without being deactivated. A different method includes introducing the rennin cDNA into genetically modified microbial species to produce recombinant rennin. The chymosin cDNAH has been successfully produced in many systems, including *E. coli*, *Aspergillus nidulans*, Saccharomyces cerevisiae, and Trichoderma reesei. The FDA authorized recombinant rennin, a heterologous protein generated in *E. coli* K-12 using recombinant DNA technology, as a food component in the USA in 1990. The recombinant rennet undergoes the formation of inclusion bodies inside *E. coli*. These inclusion bodies may be recovered, and active rennin can be obtained by solubilizing and then renaturing them. The recombinant product has indistinguishable biological characteristics from those of the natural renin. Moreover, this preparation has a purity level above 60%, much higher than the usual 2-3% range in native rennet preparations. Alternative recombinant systems that can create significant amounts of chymosin as an extracellular protein would effectively rival the suggested *E. coli* production methods in the commercial market [90];[91].

Proteolytic activity is believed to play a role in the formation of flavours in cheese. Several peptides are recognized for their ability to provide a distinct taste to food items. Peptides, referred to as "bitter," have been extracted from several varieties of cheese. Most bitter peptides include many hydrophobic amino acids, so their existence in cheese is typically undesirable. Confirming the presence of peptides that provide a pleasant taste to cheese products is still pending [92].

LIPASES

Lipases are enzymes that facilitate the breakdown of lipids. Unlike catalysts that facilitate the breakdown of other biopolymers by hydrolysis, these enzymes are manufactured on a very limited scale in the industry. This is due to a low industrial demand despite their use for many specialized purposes. These enzymes are often used to enhance the production of flavors in food products. Lipases appropriate for the task are synthesized by a limited range of microorganisms, such as certain strains of *Bacillus*, *Aspergillus*, and *Mucor* [93].

Lipase enzymes are often added to milk and milk products to enhance certain taste attributes by breaking triglycerides into free fatty acids. Dairy products, such as cheese, contain certain fatty acids. The lipolytic enzymes catalyze the hydrolysis process. Triglynes undergo additional metabolism, resulting in the production of several metabolites, including ketones. These ketones, together with the short-chain fatty acids, contribute to the taste profile of the meal. While the present global lipase market may be relatively small, there is a projected steady growth in the demand for these enzymes in the future. Lipases have many applications, including generating modified fats and oils and using them in organic chemical synthesis. The desire to make detergent preparations more ecologically sustainable may lead to the use of lipases in detergents. Several other possible industrial uses of these enzymes are being actively researched in laboratories [94]

SAFETY ASPECTS

The presence of allergic responses to proteases included in detergent formulations raises concerns about the safety of industrial enzyme preparations. The importance of this subject becomes clearer when one acknowledges the vital function enzymes play in the food processing sector. Thorough research and literature studies conducted by reputable agencies, including the FDA, the World Health Organization, and the Food and Agricultural Organization, consistently affirm that enzymes used in the food processing sector are intrinsically non-toxic and pose no harm when consumed. Often, enzymes added to facilitate a certain outcome during food processing are deactivated or eliminated later in the production process. Enzymes used in the food industry or for medical reasons must be sourced from non-toxic sources to prevent the potential presence of hazardous contaminants in enzymatic preparations. Therefore, microbial sources must be recognized as (GRAS). Additionally, all components that create the microbial fermentation medium must be non-toxic. The manufacturing process must use measures to prevent the introduction of potentially dangerous or harmful compounds into the product during subsequent processing stages. Similarly, enzymes derived from plants or animals must be sourced from edible and non-toxic

sources [95]; [96].

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التكنولوجيا الحيوية للبروتين: أهمية وتطبيقات الإنزيمات الصناعية المحللة للبوليمرات.

مصطفى محمد عمر

كلية الزراعة ،جامعة كركوك ،كركوك ،العراق.

الخلاصة

الكائنات الحية الدقيقة هي المصدر الرئيسي للأنزيمات الصناعية، حيث يعد الجنسان ***Bacillus*** و***Aspergillus*** المنتجين الرئيسين لهذه الأنزيمات. أُسْتُخْدِمت سلالات ***Bacillus*** تاريخيًا لإنتاج الأنزيمات ذات الأهمية التجارية نظرًا لوضعها على أنها ***GRAS*** (معترف بها عمومًا على أنها آمنة) وتوزيعها على نطاق واسع في البيئة الطبيعية. قد توفر الخميرة، ولا سيما ***Saccharomyces***، كثير من الأنزيمات المهمة في الصناعة. تُستخدم تقنيات الغربلة الأولية والثانوية في تحديد الكائنات الحية الدقيقة التي لها القابلية على إنتاج الأنزيم المستهدف، وقد أُسْتُخْدِمت تقنيات الهندسة الوراثية مثل تكوين توليفات جديدة من الحامض النووي، وكذلك الطفرات الوراثية لزيادة الغلة. تُستخدم الكائنات الحية الدقيقة المحبة للحرارة للحصول على الأنزيمات ذات ثباتية عالية اتجاه الحرارة، التي تتميز بكونها نشطة للغاية في درجات

حرارة تزيد عن 80 درجة مئوية. تمكن هذه الأنزيمات من إجراء كثير من العمليات الصناعية التي تعتمد على تحفيز الإنزيم في درجات حرارة أعلى، مما يتيح معدلات استجابة أفضل. ومع ذلك، فإن معظم الأنزيمات المحبة للحرارة لم تخضع لتوصيف شامل وغير مدرجة في قائمة المعترف بها عمومًا على أنها آمنة. تبلغ المبيعات العالمية مما يسمى "***Bulk Enzymes***" نحو 600 مليون دولار أمريكي سنويًا، وَيُعْزَى 66٪ منها إلى مستحضرات تحلل البروتين المختلفة. ينتج أنزيمات ***Proteases***، نحو 350 مليوناً في المبيعات السنوية. تمثل أنزيمات ***Lipase*** الآن جزءً صغيرًا من إجمالي مبيعات الأنزيمات، ولكن من المتوقع أن تزداد الحاجة إليها. تُستخدم كميات هائلة من الأنزيمات في التكنولوجيا الحيوية المختلفة، مثل قطاعات التخمير وصناعة الخبز وصناعة الأجبان، وقد سهلت تصميم طرق تكنولوجية حيوية أخرى تنتج منتجات متنوعة ذات أهمية تجارية لتحسين الطعم والنكهة وخصائص المظهر للمواد الغذائية، وتعتيق اللحوم، وتصفية العصائر، ويتم تضمينها في تركيبات المنظفات المختلفة.

الكلمات المفتاحية: الانزيمات الصناعية، الاميلز، البروتييز، التطبيقات الصناعية.