MICROBIAL α-AMYLASES AND THEIR INDUSTRIAL APPLICATIONS: A REVIEW

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Abstract:

The biotechnological potential of α-amylases from microorganisms has drawn a great deal of attention from various researchers worldwide as likely biological catalysts in a variety of industrial processes. The rapid developments in the field of genetic engineering have given a new impetus to the biotechnology. Biotechnology also offers the potential for new industrial processes that require less energy and are based on renewable raw materialsand environmentally healthy practices.This work represents a review ofα-amylase family and the major characteristics, microbial sources, production, properties, industrial applicationsas highly demanded industrial enzyme in various sectors such as food, textiles, detergents, pharmaceuticals, etc. The review intends to explore the potential of these enzymes and to encourage new α-amylase-based industrial technology.

Key words:microbial α-amylase ,enzyme characteristics, production, industrial applications, starch.

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1.Introduction:

Enzymes are globular proteins and like other proteins consist of long chains of amino acids that fold to produce a three-dimensional product. Each unique amino acid sequence produces a specific structure, which has unique properties. Enzymes are responsible for many essential biochemical reactions in microorganisms, plants, animals, and human beings.They differ in function in that they have the unique ability to facilitate biochemical reactions without undergoing change themselves. This catalytic capability is what makes enzymes unique.

Enzymes, biological catalysts with high selectivities, have been used in the foodindustry for hundreds of years, and play an important role in many other industries(washing agents, textile manufacturing, pharmaceuticals, pulp and paper).They without being consumed in the process, can speed up chemical processes that would otherwise run very slowly, or in some cases, not at all [Cavaco-Paulo& Gübitz 2003].After the reaction is complete, the enzyme is released again, ready to start another reaction. Usually most enzymes are used only once and discarded after their catalytic action.All known enzymes are proteins. They therefore consist of one ore more polypeptide chainsand display properties that are typical of proteins. Some enzymes require small non-proteinmolecules, known as cofactors, in order to function as catalysts [Jenkins 2003].

Currently,enzymes are becoming increasingly important in sustainable technology and greenchemistry.Generally they are active at mild temperatures. Above certain temperature the enzyme isdenaturated. Enzymes have a characteristic pH at which their activity is maximal. ExtremepH values influence on the electrostatic interactions within the enzyme, leading toinactivation of enzyme. Other important factors that influence the effect of enzymaticprocesses are the concentration of enzyme, the time of treatment, additives like surfactantsand chelators and mechanical stress [Tavčer 2011]. Enzyme can break down particular compounds. The molecule that an enzyme acts on is known as its substrate, which is converted into a product or products. Some of the most common include amylases which break down starch into simple sugars, proteases which break down proteins, cellulases which break down cellulose, andlipases which split fats (lipids) into glycerol and fatty acids.For each type of reaction in a cell there is a different enzyme and they are classified into six broad categories namely hydrolytic, oxidising and reducing, synthesising, transferring, lytic and isomerising. The essential characteristic of enzymes is catalytic function. Consequently, the original attempt to classify enzymes was done according

to function. The International Commission on Enzymes (EC) was established in 1956 by the International Union of Biochemistry (IUB), in consultation with the International Union of Pure and Applied Chemistry (IUPAC), to put some order to the hundreds of enzymes that had been discovered by that point and establish a standardized terminology that could be used to systematically name newly discovered enzymes. The EC classification system is divided into six categories of basic function:

- EC1 Oxidoreductases: catalyze oxidation/reduction reactions.
- EC2 Transferases: transfer a functional group.
- EC3 Hydrolases: catalyze the hydrolysis of various bonds.
- EC4 Lyases: cleave various bonds by means other than hydrolysis and oxidation.
- EC5 Isomerases: catalyze isomerization changes within a single molecule.
- EC6 Ligases: join two molecules with covalent bonds.

Each enzyme is described by a sequence of four numbers preceded by "EC". The first number broadly classifies the enzyme based on its mechanism.

All α-amylases (EC3.2.1.1) are starch-degrading enzymes that catalyze the hydrolysis and act on internal α-1,4-glycosidiclinkages in starch in lowmolecular weight products, such glucose, maltose andmaltotriose units [Gupta et al. 2003;Kandra 2003; Rajagopalan and Krishnan 2008]. Amylases are among the mostimportant enzymes and are of great significance forbiotechnology, constituting a class of industrial enzymeshaving approximately 25% of the world enzyme market [Rajagopalan and Krishnan 2008; Reddy et al. 2003]. Most of the α-amylases are metalloenzymes, which require calcium ions (Ca^{2+}) for their activity, structural integrity and stability. They belong to family 13 (GH-13) of the glycoside hydrolase group of enzymes [Bordbar et al. 2005].

The amylase family ofenzymes is of great significance due to its wide area ofpotential application. Interestingly, the first enzyme producedindustrially was an amylase from a fungal source in1894, which was used as a pharmaceutical aid for the treatmentof digestive disorders [CruegerW. andCrueger A. 1989]. Amylases find potentialapplication in a number of industrial processes such as inthe food, fermentation, textiles and paper industries.Microbial amylases have successfully replaced the chemicalhydrolysis of starch in starch-processing industries. Theywould be potentially useful in the pharmaceutical and finechemicals industries if enzymes with suitable

propertiescould be prepared [Fogarty and Kelly 1980]. The spectrum of amylaseapplication has widened in many other fields, such as

clinical, medical, and analytical chemistries, as well astheir wide spread application in starch sacccharificationand in the textile, food, fermentation, paper, brewing anddistilling industries [Pandey et al. 2000].

Historically, the application of enzymes in industrial textile processes began around 1857, when malt extract was used to remove size from fabrics before printing [Ciechanska and Kazimierezak 2006; Marcher et al. 1993]. Starch is widely used as a sizing agent, being readily available, relatively cheap and based on natural, sustainable raw materials [Lange 1997]. About 75% of the sizing agents used worldwide are starch and its derivatives [Opwis et al. 1999]. There areseveral processes in the medicinal and clinical areas thatinvolve the application of amylases [Sutton et al. 1999; Chiu and Chandler 1995; Becks et al.1995; Menzel et al. 1998; Chelly et al. 1996; Strandberg et al. 1999]. Due to the increasing demand for these enzymes in various industries, there is enormous interest in developing enzymes with better properties such as raw starch degrading amylases suitable for industrial applications and their cost effective production techniques [Burhan et al. 2003].

They can be obtained from several sources, such as plants, animals and microorganisms. Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry. The amylases of microorganisms have a broad spectrum of industrial applications as they are

more stable than when prepared with plant and animal α- amylases [Tanyildizi et al. 2005]. The major advantage of using microorganisms for the production of *amylases* is the economical bulkproduction capacity and the fact that microbes are easy to manipulate to obtain enzymes of desired characteristics.

α-Amylase has been derived from several fungi, yeasts and bacteria. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors [Gupta et al. 2003].Bacterial amylase, however, is generally preferred overfungal amylase due to several characteristic advantages thatit offers. Strains of *Aspergillus* sp. and *Bacillus* sp.,mainly *Bacillus subtilis*, *B. stearothermophilus*,*B.amyloliquefaciens* and *B. licheniformis*, areknown to be good producers of α-amylase and these have been widely used for commercial production of the

enzyme for various applications [Vihinen and Mantasala 1989; Pandey et al. 2000].Several *Bacillus* sp. and thermostable*Actinomycetes* including *Thermomonospora* and *Thermoactinomyces* are versatile producers of the α-amylases [Ben et al. 1999]. The genus *Bacillus* produces a large variety of extracellular enzymes of which *amylases* and *proteases* are of significant industrial importance. An extremely thermostable α-*amylase* is available from the mesophile*B. licheniformis* [Morgan et al. 1981]. Alkaliphilic*Bacillus* strains often produce enzymes active at alkaline pH, including alkaline α-*amylase*, *protease* and *carboxymethylcellulase* [Horikoshi 1996].

2.Literature Review:

2.1Structural and functional characteristics

O-Glycoside hydrolases (EC3.2.1.-) are a wide spread group of enzymes that hydrolyze the glycosidic bond between two or more carbohydrates, or between a carbohydrate and a non-carbohydrate moiety. A classification system for glycoside hydrolases, based on sequence similarity, has led to the definition of 85 different families. Most of the starch hydrolyzing enzymes belong to the α-*amylase* family or family 13 glycoside hydrolases based on amino acid sequence homology according to the classification of Henrissat (1991). The α-*amylase* family of glycoside hydrolyses, is the largest family of glycoside hydrolases, transferases and isomerases comprising nearly 30 different enzyme specificities [Henrissat 1991]. A large variety of enzymes are able to act on starch. These enzymes are listed in Table 1.

These enzymes can be divide basically into four groups:endoamylases, exoamylases, debranching enzymes and transferases [M.J.E.C.van der Maarel et al. 2002]:

1. Endoamylases: cleave internal α-1,4 bonds resulting in α-anomeric products,
2. Exoamylases: cleave α-1,4 or α-1,6 bonds of the external glucose residues resulting in α- or β-anomeric products,
3. Debranching enzymes: hydrolyze α-1,6 bonds exclusively leaving longlinear polysaccharides, and

4. Transferases: cleave α-1,4 glycosidic bond of the donor molecule and transfer part of the donor to a glycosidic acceptor forming a new glycosidic bond.

Table 1. Known activities of Glycoside hydrolase family 13 enzymes

Enzyme	EC number	Main substrate
Amylosucrase	EC: 2.4.1.4	Sucrose
Sucrose phosphorylase	EC: 2.4.1.7	Sucrose
Glucan branching enzyme	EC: 2.4.1.18	Starch, glycogen
Cyclomaltodextrin glycosyltransferase	EC: 2.4.1.19	Starch
Amylomaltase	EC: 2.4.1.25	Starch, glycogen
Maltopentaose-forming alpha-amylase	EC: 3.2.1.-	Starch
Alpha-amylase	EC: 3.2.1.1	Starch
Oligo-1,6-glucosidase	EC: 3.2.1.10	1,6-alpha-D-glucosidic linkages in some oligosaccharides
Alpha-glucosidase	EC: 3.2.1.20	Starch
Amylopullulanase	EC: 3.2.1.41	Pullulan
Cyclomaltodextrinase	EC: 3.2.1.54	linear and cyclomaltodextrin
Isopullulanase	EC: 3.2.1.57	Pullulan
Isoamylase	EC: 3.2.1.68	Amylopectin
Maltotetraose-forming alpha-amylase	EC: 3.2.1.60	Starch
Glucodextranase	EC: 3.2.1.70	Starch
Trehalose-6-phosphate hydrolase	EC: 3.2.1.93	Trehalose
Maltohexaose-forming alpha-amylase	EC: 3.2.1.98	Starch
Maltogenic amylase	EC: 3.2.1.133	Starch
Neopullulanase	EC: 3.2.1.135	Pullulan
Malto-oligosyl trehalase hydrolase	EC: 3.2.1.141	Trehalose
Malto-oligosyl trehalose synthase	EC: 5.4.99.15	Maltose

The α-glycosidic bond is very stable having a spontaneous rate of hydrolysis at room temperature [Wolfenden et al. 1998]. The catalytic mechanism of theα-amylase family is that of the α-

retaining double displacement [M.J.E.C. van der Maarel et al. 2002]. α-Retaining mechanism is the characteristic feature of the enzymes from the α-amylase family. They vary widely in their reaction specificities. The attachments of different domains to the catalytic site or to extra sugar binding subsites around the catalytic site is the prime reason for these differences [M.J.E.C.van der Maarel et al. 2002].

2.2 Starch

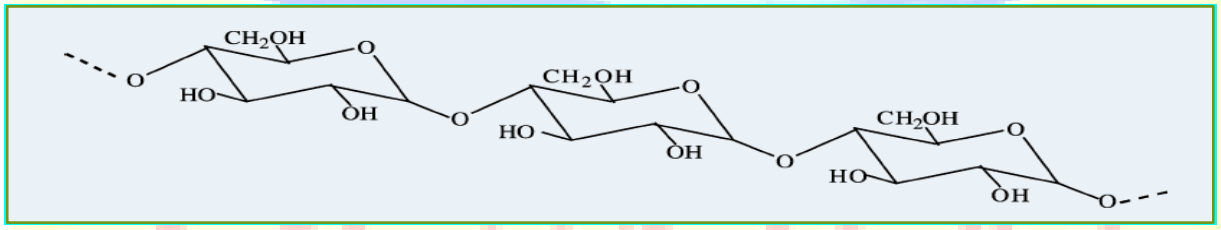
In the green leaves of plants carbon dioxide and water are transformed into glucose andoxygen under the influence of sunlight and with the help of chlorofyl. This process is known as photosynthesis.During the day this starch is deposited as grains in the leaf, the so-called leaf-transition starch.During the night this starch is partially broken down again into sugars which are transported to other areas of the plant. From these sugars the starch arises which is won in the familiar grain shape.The forming of starch is a process which has by far not been clarified yet and during which a number of enzymes play a role.

Starch or amylum is a carbohydrate consisting of a large number of glucose units joined by glycosidic bonds.The majorindustrial sources are maize, tapioca, potato, and wheat, butlimitations such as low shear resistance, thermal resistance,thermal decomposition and high tendency towardsretrogradation limit its use in some industrial food applications[Goyal et al.2005;M.J.E.C.van der Maarel et al. 2002].With the help of a microscope the grain shape reveals from which plant species the starch derives.Native starch, the starch as it occurs in the plant, can not be dissolved in cold water. When we scatter starch, while stirring, into water we get a milky white suspension which can be stirred without much difficulty.When the stirring is stopped the starch sinks to the bottom (sedimentation), during which process a transparent upper layer is formed.When the suspension is heated the white colour disappears at a temperature characteristic for starch. The starch dissolves into an almost transparent solution. This is what we call gelatinized starch. In comparison with the ungelatinized suspension, stirring takes considerably more difficulty.The temperature at which the resistance during stirring noticeably increases, is called the gelatinization temperature.Gelatinizing starch into viscous substances (swellings) is one of the most, if not the most, important characteristic(s) of starch. This phenomenon lies at the basis of the successful application of starch in a large number of sectors.

Among carbohydrate polymers, starch is currentlyenjoying increased attention due to its usefulness in differentfood products. Starch contributes greatly to the texturalproperties of many foods and is widely used in food and industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent and water retention agent [JaspreetSinghaet al. 2007]. Starch is a polymer of glucose linked to another one through the glycosidic bond. Two types of glucose polymersare present in starch: amylose and amylopectin (Fig.1). Amylose and amylopectin have different structures and properties. Amylose is a linear polymer consisting of up to6000 glucose units with α-1,4glycosidic bonds. Amylopectin consists of short α-1,4 linked to linear chains of 10–60 glucose units and α-1,6 linked to side chains with 15–45 glucose units. Granule bound starch synthase can elongate maltooligosaccharides to form amylose and is considered to be responsible for the synthesis of this polymer. Soluble starch synthase is considered to be responsible for the synthesis of unit chains of amylopectin.

α -Amylase is able to cleave α-1,4glycosidic bonds present in the inner part of the amylose or amylopectin chain [Muralikrishna and Nirmala2005; Tester et al. 2004;M.J.E.C.van der Maarel et al. 2002].

A. Structure of amylose



B. Structure of amylopectin

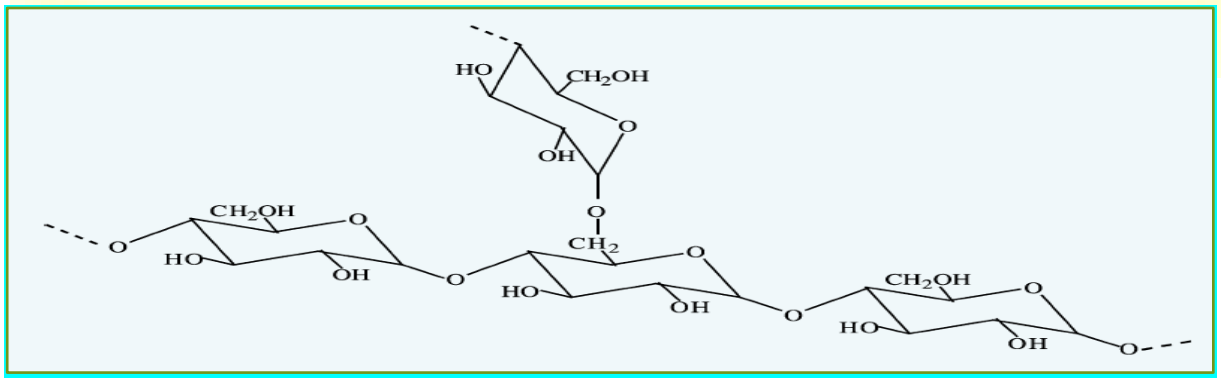


Figure 1. Types of glucose polymers present in starch: amylose (A) and amylopectin (B). FromMuralikrishna and Nirmala(2005).

Endoamylases are able to cleave α,1-4 glycosidicbonds present in the inner part (endo-) of theamylose or amylopectin chain. α-*Amylase* (EC3.2.1.1) is a well-known *endoamylase*. It is foundin a wide variety of microorganisms [Pandey et al.2000]. The end products of α-amylase action areoligosaccharides with varying length with an α-configuration and α-limit dextrins, which constitutebranched oligosaccharides,wich is one of the most important commercial enzyme processes.Saccharide composition obtained after hydrolyze of starch ishighly dependent on the effect of temperature, the conditions ofhydrolysis and the origin of enzyme. Specificity,thermostability and pH response of the enzymes are criticalproperties for industrial use [Kandra 2003].

α-*Amylase*find application in all theindustrial processes such as in food, detergents, textiles and inpaper industry, for the hydrolysis of starch [Gupta et al. 2003; Konsula and Liakopoulou-Kyriakides 2004; Tanyildizi et al.2005].*Exoamylases* act onthe external glucose residues of amylose or amylopectinand thus produce only glucose (*glucoamylase*and α-*glucosidase*), or maltose and β-limit dextrin (β-*amylase*).

A number of reviews exist on amylases and theirapplications, however, none specifically covers α-amylases at length. α-Amylases are one of the most popularand important form of industrial amylases and thepresent review highlights the various aspects of microbial α-amylases.

2.3 Microbialα-amylase production

Commercial sources of enzymes are obtained from three primary sources, i.e., animal tissue, plants and microbies. These naturally occurring enzymes are quite often not readily available in sufficient quantities for food applications or industrial use. However, by isolating microbial strains that produce the desired enzyme and optimizing the conditions for growth, commercial quantities can be obtained. This technique, well known for more than 3,000 years, is called fermentation. The enzymes are inducible, i.e., produced only when needed, and they contribute to the natural carbon cycle. Several methos, such as submerged fermentation (SmF) and solid-state

fermentation (SSF) have been successfully used for α-*amylase* production from various microorganisms. Agro-industrial residues such as wheat bran, spent brewing grain, maize bran, rice bran, rice husk, coconut oil cake, mustard oil cake, corn bran, etc., are generally considered the best substrates for processes [Sodhi et al. 2005; Francis et al. 2003; Babu and Satyanarayana 1995; Baysal et al. 2003; Ramachandran et al. 2004; Vishwanathan and Surlikar 2001]. In addition, the utilization of these agroindustrial wastes, on one hand, provides alternative substrates and, on the other, helps in solving pollution problems, which otherwise may cause their disposal [Pandey et al. 1999].

Various agro-industrial residues (agrosubstrates) used for microbial α-*amylase*productionare shown in Table 2.

Table 2. Various agro-industrial residues (agrosubstrates) used for α-*amylase*production

Substrate	**Organism**	**Activity, U/g**	**Reference**
Wheat bran	*Bacillus* sp. PS-7	464 000	[Sodhi et al. 2005]
Spent brewing grain	*A. oryzae* NRRL 6270	6 583	[Francis et al. 2003]
Maize bran	*B. coagulans*	22 956	**[**Babu and Satyanarayana 1995]
Rice bran	*Bacillus* sp. PS-7	145 000	[Sodhi et al. 2005]
Rice husk	*B. subtilis*	21 760	[Baysal et al. 2003]
Coconut oil cake	*A. oryzae*	3 388	[Ramachandran et al. 2004]
Mustard oil cake	*B. coagulans*	5 953	**[**Babu and Satyanarayana 1995]
Corn bran	*Bacillus* sp. PS-7	97 600	[Sodhi et al. 2005]
Amaranthus grains	*Aspergillusflavus*	1 920	[Vishwanathan and Surlikar 2001]
Gram bran	*B. coagulans*	8 984	**[**Babu and Satyanarayana 1995]

α-*Amylase* may be derived from several bacteria, yeasts andfungi,but for commercial applications α-*amylase* ismainly derived from the genus *Bacillus*. Bacterial *amylase*, however, is generally

preferred overfungal *amylase* due to several characteristic advantages thatit offers. Strains of *Aspergillus sp*. and *Bacillus sp*.,mainly *Bacillus amyloliquefaciens* and *B. licheniformis*, areemployed for commercial applications. Thermostableα-*amylases*

are generally preferred as their application minimizescontamination risk and reduces reaction time, thusprovidingconsiderable energy saving.Hydrolysiscarriedoutat higher temperatures also minimizes polymerization of D-glucoseto iso-maltose.

Most reports about fungi that produce α-*amylase* havebeen limited to a few species of mesophilic fungi, mostlyto *Aspergillus*and *Penicillium*[Gupta et al. 2003; Kathiresan and Manivannan 2006].Filamentous fungi, suchas *Aspergillusoryzae* and *Aspergillusniger*, produceconsiderable quantities of enzymes that are used extensively in the industry [Djekrif-Dakhmouche et al. 2005; Hernandez et al. 2006; Jin et al. 1998; Kammoun et al. 2008].The thermophilic fungus *Thermomyceslanuginosus* is anexcellent producer of *amylase* [Jensen et al. 2002; Kunamneni et al. 2005].

The fungal α-amylases are preferred over othermicrobial sources due to their more accepted GRAS (GenerallyRecognized As Safe) status [Gupta et al. 2003].

Optimization of various parameters and manipulation of media are one of the most important techniques used for the overproduction of enzymes in large quantities to meet industrial demands [Tanyildizi et al. 2005]. Growth of myceliumis crucial for extracellular enzymes like α-*amylase*[Carlsen et al. 1996]. Various physical and chemical factors have been known to affect the production of α-*amylase* such as temperature, pH, period of incubation, carbon sources acting as inducers, surfactants, nitrogen sources, phosphate, different metal ions, moisture and agitation with regards to SSF and SmF, respectively.

There is a very huge demand to improve the stability ofthe enzymes to meet the requirements set by specificapplications, especially with respect to temperature andpH.

The optimum temperature depends on whether the culture is mesophilic or thermophilic. Among the fungi, most *amylase* production studies have been done with mesophilic fungi within the temperature range of 25-37 °C [Ramachandran et al. 2004; Francis et al. 2003]. The temperature optimum for the activity of α-*amylase* isrelated to the growth of the microorganism [Vihinen et al.1989]. Thermostabilities are affectd by many factors like

presence of calcium, substrate and other stabilizers[Vihinen et al. 1989].

pH is one of the important factors that determine the growth of microorganisms as they are sensitive to the concentration of hydrogen ions preseny in the medium. α-*Amylases* are generally stable over a wide range of pHfrom 4 to 11 [Fogarty et al. 1979; Vihinen et al. 1989;Hamilton et al.1999; Khoo et al.1994], however, α-*amylases* with stability in a narrowrange have also been reported [Coronado et al. 2000; Fogarty 1983]. Fungi of *Aspergillus* sp. Were found to give significant yields of α-*amylase* at pH=5.0-6.0 in SmF[Hayashida and Teramoto1986; Carlsen et al. 1996; Djekrif-Dakhmouche et al. 2005]. Bacterial cultures *Bacillus* sp. required an initial pH of 7.0 [Tanyildizi et al. 2005; Syu and Chen 1997;Haq et al. 2005].

Thermophilic anaerobic bacteria Clostridium thermosulfurogenes gave maximum titres of α-*amylase* at pH=7.0 [Swamy and Seenayya 1996].Infungal processes, the buffering capacity of some mediaconstituents sometimes eliminates the need for pHcontrol [Chahal 1983]. The pH values also serves as a valuableindicator of the initiation and end of enzyme synthesis[Friedrich et al. 1989]. It is reported that *A. oryzae* 557 accumulated α-*amylase*in the mycelia when grown in phosphate orsulphate deficient medium and was released when themycelia were replaced in a medium with alkaline pH(above 7.2) [Yabuki et al. 1977].

Industrial enzymes produced in bulk generally requirelittle downstream processing and hence are relatively crudepreparations. The commercial use of α-*amylase* generally doesnot require purification of the enzyme, but enzyme applicationsin pharmaceutical and clinical sectors require high purity*amylases*.Laboratoryscale purification for α-amylase includes various combinationsof ion exchange, gel filtration, hydrophobicity interactions andreverse phase chromatography. Alternatively, α-*amylase*extraction protocols using organic solvents such as ethanol,acetone and ammonium sulfate precipitation [Glymph and Stutzenberger 1977; Hamilton et al.1999; Khoo et al. 1994] andultrafiltration have been proposed [Moraeset al. 1999]. These conventionalmulti-step methods requires expensive equipments at each step,making them laborious, time consuming, barely reproducibleand may result in increasing loss of the desired product [Arauza et al. 2009].

Purification processes in downstreamprocessing after fermentation strongly depend on the

market, processing cost, final quality, and available technology.Most enzymes are purified by chromatographictechniques after crude isolation by precipitation andmembrane separations. The need for large-scale cost effectivepurification of proteins has resulted in evolutionof techniques

that provide fast, efficient and economicalprotocols in fewer processing steps [Amritkar et al. 2004].Purificationtechniques that produce homogeneous preparation ofamylases in a single step are given in Table 3.

Table 3. Methods of one-step purification of α-amylases

Method	Adsorbent	Yield/ %	Purificationfold	Reference
Affinity adsorption chromatography	*β-cyclodextrin-iminodiacetic acid-Cu*$^{2+}$	95	_	[Liao and Syu 2005]
Expanded bed chromatography	*Alginic acid-cellulose cell beads*	69	51	[Amritkar et al. 2004]
High speed counter current chromatography	*PEG4000-aqueous two-phase system*	73.1	_	[Zhi et al. 2005]
Magnetic affinity adsorption	*Magnetic alginate microparticles*	88	9	[Safarikova et al. 2003]
Substitute affinity method	*Insoluble corn starch at 4 °C*	78	163	[Najafi and Kembhavi 2005]

2.4Industrial applications of α-amylase

The history of the industrial production of enzymes datesback to the time when Dr. Jhokichi Takamine began theproduction of digestive enzyme preparation of α-*amylase* from *A. oryzae*in 1894known as »Taka diastase«, which was used as a digestiveaid.

Amylases are among the most important hydrolyticenzymes for all starch based industries, and the commercialisationof amylases is oldest with first use in1984, as a pharmaceutical aid for the treatment ofdigestive disorders.

Such enzymes hydrolyze the starch moleculesinto polymers composed of glucose units. *Amylases*have found applications in starch processing, desizing of textiles,paper sizing, as

detergent additive, and bread improvement, pharmaceutical industries, ethanol, and otherfermentation processes [Haki and Rakshit 2003; Lowe 2002; Gomes et al.2005].

The global market for enzymes was about $2billion in 2004. It is expected to have an average annualgrowth rate of 3.3 %. The share of carbohydrases comprising*amylases, isomerases, pectinases and cellulases*is about 40 % [Riegal and Bissinger 2003].The food and beverage sectors utilize90 % of the carbohydrases produced. Today, *amylases* have the major world market share ofenzymes [Aehle and Misset 1999].In Table 4are shown applications of *amylases*in various sectors of industry.In this light, microbial amylaseshave completely replaced chemical hydrolysis in thestarch processing industry.

Table 4. Uses of amylases in various sectors of industry

Sector	Uses	Reference
Food industry	Production of glucose syrups, crystalline glucose; Production of high fructose corn syrups; Production of maltose syrups; Reduction of viscosity of sugar syrups; Reduction of haze formation in juices; Solubilization and saccharification of starch for alcohol fermentation in brewing industries; Retardation of staling in baking industry;	-[M.J.E.C. van der Maarel et al. 2002] -[Riegal and Bissinger 2003] -[Gupta et al. 2003] - [Haki and Rakshit 2003] - [Lowe 2002] -[Gomes et al. 2005].
Detergent industry	Used as an additive to remove starch based dirts	
Paper industry	Reduction of viscosity of starch for appropriate coating of paper	
Textile industry	Warp sizing of textile fibers	
Pharmaceutical industry	Used as a digestive aid	

A comprehensiveaccount on commercial applications of α-*amylases* isquoted by Godfrey and West (1996).Various applicationsof α-*amylase* are dealt here in brief.

2.4.1Food industry

Amylases are extensively employed in processed-foodindustry such as baking, brewing, preparation of digestive aids,production of cakes, fruit juices and starch syrups [Couto and Sanromán2006]. For decades, microbial α-*amylases* have been widely used inthe baking industry [Hamer 1995; Si 1999].Theseenzymes can be added to the dough of bread to degrade thestarch in the flour into smaller dextrins, which are subsequentlyfermented by the yeast.Besides generatingfermentable compounds, α-*amylases* also have an anti-stalingeffect in bread baking, and they improve the softness retentionof baked goods, increasing the shelf life of these products [Gupta et al.2003; M.J.E.C. van der Maarel et al. 2002;Sahlstrom and Brathen 1997].The addition of α-*amylase* to the doughresults in enhancing the rate of fermentation and the reductionof the viscosity of dough, resulting in improvements in thevolume and texture of the product.

The most widespread applications of α-*amylases* are inthe starch industry, wich are used for starch hydrolysis in thestarch liquefaction process that converts starch into fructoseand glucose syrups [Nielsen and Borchert2000; Gupta 2003].The hydrolysis of starch may be carried out using either acid or enzyme as catalyst.Acid conversion has, however, many limitations: it is non-specific, lacks ways ofcontrolling saccharide composition, require high refining coasts and is lessenvironmentally friendly. The application of enzymes for this process has avoidedthese limitations [Crabb and Shetty 1999] Conversion of starch into sugar, syrups anddextrins forms the major part of the starch processingindustry.*Amylases* are also used for the clarification ofbeer or fruit juices, or for the pretreatment of animal feed toimprove the digestibility of fiber [Gavrilescu and Chisti2005; Ghorai et al. 2009; M.J.E.C. van der Maarel et al. 2002].

2.4.2Textile industry

Amylases are used in textile industry for desizing process.Sizing agents like starch are applied to yarn before fabricproduction to ensure a fast and secure weaving process. In textile weaving, starch paste is applied for warping.This gives strength to the textile at weaving. It alsoprevents the

loss of string by friction, cutting andgeneration of static electricity on the string by givingsoftness to the surface of string due to laid down warp.

After weaving the cloth, the starch is removed and thecloth goes to scouring and dyeing. The starch on cloth isusually removed by application of α-*amylase*[Hendriksen et al. 1999].

Starchis a very attractive size, because it is cheap, easily available inmost regions of the world, and it can be removed quite easily.Starch is later removed from the woven fabric in a wet-processin the textile finishing industry.

The enzymatic desizing of cotton with α-*amylases* is state-of-the-art since many decades [Marcher et al. 1993]. The amylose is bioconverted to 100% by the α-*amylase* into glucose whereas the amylopectin is converted to 50% into glucose and maltose. Bio-desizing is preferred due to their high efficiency and specific action. *Amylases* bring about complete removal of the size without any harmful effects on the fabric besides eco friendly behavior.

The α-*amylases* remove selectively the sizeand do not attack the fibres [Ahlawat et al. 2009;Feitkenhauer2003; Gupta 2003].*Amylase* from *Bacillus*stain was employed in textile industries for quite a long time.

2.4.3 Paper industry

The use of α-*amylase*in pulp and paper industry is in the modification of starchesfor coated paper, i.e. for theproduction of low-viscosity, high molecular weight starch. As for textiles, sizing of paper with starch is performed to protect thepaper against mechanical damage during processing [Gupta et al. 2003; M.J.E.C. van der Maarel et al. 2002;Bruinenberget al. 1996].The coating treatment serves improves the quality ofthe finished product, enhances stiffness, and elasticity of paper [Gupta et al. 2003; Bruinenberg et al. 1996].Because starch isadded to paper at a temperature range of 45- 60 °C, and the viscosity of the naturalstarch is too high for paper sizing partial degradation of this polymer is essential.α-*Amylase*is employed for this purpose [Gupta et al. 2003].

2.4.4Detergent applications

The demand for α-*amylase* for use in laundry and automatic dishwashing is veryhigh.The use ofenzymes in detergents formulations enhances the detergentsability to remove tough stains and making the detergentenvironmentally safe.These enzymes are used in detergents for laundry andautomatic dishwashing to degrade the residues of starchy foodssuch as potatoes, gravies, custard, chocolate, etc. to dextrinsand other smaller oligosaccharides [Mukherjee et al. 2009; [Olsen and Falholt 1998].Removal of starch from surfaces is also

important in providing a whiteness benefit, since starch can bean attractant for many types of particulate soils.90% of all liquid detergents contain these enzymes [Gupta et al. 2003].

The oxidativestability of amylases is one of the most important criteria fortheir use in detergents where the washing environment is veryoxidizing [Kirk et al. 2002].

Alkaliphilic *Bacillus* strains often produce enzymes active at alkaline pH,including alkaline α-*amylase*[Horikoshi, 1996]. When alkaline α-*amylase* is usedas a component of detergents, the chelating agents usually contained in detergentseasily remove calcium, which is essential for its stability. Thus there is a search forCa free α-*amylase*[Nonaka et *al*, 2003].

2.4.5 Beverage alcohol and Fuel Ethanol production

In beer industries microbial amylases are used to aid cereal amylase in theproduction of fermentable sugar. Ethanol is the most utilized liquid biofuel. Over the past decades, there has been anincreasing interest in fuel ethanol as a result of increased environmental concern andhigher crude oil prices.Ethanol fuels can be derived from renewable resources such

as agricultural crops and by products. For the ethanolproduction, starch is the most used substrate due to its lowprice and easily available raw material in most regions of theworld [Chi et al. 2009]. The bioconversion of starch into ethanolinvolves liquefaction and saccharification, where starch isconverted into sugar using an amylolytic microorganism orenzymes such as α-*amylase*, followed by fermentation, wheresugar is converted into ethanol using an ethanol fermentingmicroorganism such as yeast *Saccharomyces cerevisiae* [Moraeset al. 1999].

Enzymes such as α-*amylase*,*glucoamylase* and *cellulases* are important to produce fermentable sugars to produceethanol [Kirk et al. 2002].

2.4.6Treatment of starch processing waste water

Starch is also present in waste produced from foodprocessing plants. Starch waste causes pollutionproblems. Biotechnological treatment of food processingwaste water can produce valuable products such asmicrobial biomass protein and also purifies the effluent [Friendrich et al. 1987; Kingspohn et al., 1993].

2.4.7 Other applications

The spectrum of amylase application has widened in many other fields, such asclinical, medical, and analytical chemistries [Pandey et al.,2000; Cherry et al,2004]. To some extent *amylases* are also used asdigestive aids to supplement the diastaticactivity of flour and to improve digestibility of some of theanimal feed ingredients[Kumar et al. 1995].

There are several processes in themedicinal and clinical areas that involve the application

of *amylases*. The application of a liquid stable reagent,based on α-*amylase* for the Ciba Corning Expressclinical chemistry system has been described [Becks et al. 1995]. Aprocess for the detection of higher oligosaccharides,which involved the application of *amylase* was alsodeveloped [Giri et al. 1990].

3. Future Directions:

As evident from the foregoing review, *amylases* areamong the most important enzymes used in industrialprocesses. Although, the use of α-*amylase* in starch based industries has beenprevalent for many decades and a number of microbial sourcesexist for the efficient production of this enzyme, but only a fewselected strains of fungi and bacteria meet the criteria forcommercial production

The continued development of new enzymes through modernbiotechnology may, for example, lead to enzyme products withimproved cleaning effects at low temperatures. This could allowwash temperatures to be reduced, saving energy in countrieswhere hot washes are still used.

Today, white biotechnology is geared towards creating new materials and biobased fuels from agricultural waste and providing alternative biobased routes to chemical processes. These efforts could lead to the development of improved enzymes such as *amylases*, *hemicellulases*or*cellulases* that could be used in the industries.

Today, the application of biotechnology to industrial processes holds many promises for sustainable development. New and exciting enzyme applications are likely to bring benefitsin other areas: less harm to the environment; greater efficiency;lower costs; lower energy consumption; and theenhancementof a product's properties.

The use of enzymes not only make the process less toxic (by substituting enzymatic treatments for harmful chemical treatments) and eco-friendly, they reduce costs associated with the production process, and consumption of natural resources (water, electricity, fuels), while also improving the quality of the final product. It seems that in the future it will be possible to do every process using enzymes.

4. Conclusion:

Amylases are important in many industrial processes and are one of the most widely used enzymesrequired for the preparation of fermented foods. Apartfrom food and starch industries, in which demand forthem is increasing continuously, they are also used invarious other industries such as paper and pulp, textile,*etc.*To alleviate the ever-growing demand for fuel energy the production of fuel ethanolfrom plant material is the focus of research. To this effect the application of amylolyticenzymes in the production of the fermentable sugar from starchy crops isindispensable. Starch degrading enzymes like *amylase* have received great deal ofattention because of their perceived technological significance and economic benefits.

Pollution free processes are gaining ground all over the world. Enzymes are not only beneficial from ecological point of view but they are also saving lot of money by reducing water and energy consumption which ultimately reduce the cost of production.

Anumber of microbial sources exist for the efficientproduction of this enzyme, but only a few selected strainsof fungi and bacteria meet the criteria for commercialproduction. In order to achieve the efficient, large-scaleproduction, the structural and functional relationships ofα-

amylases have to be known in detail. This will lead toimproving the stability of the existing enzymes anddiscovery of many new ones.

5.REFERENCES:

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