

Review Article

Industrial Important Microbial α -Amylase on Starch-Converting Process

Vengadaramana A.

Department of Botany, Faculty of Science, University of Jaffna, Sri Lanka

*Corresponding author

Vengadaramana A.

Email: vengad@jfn.ac.lk

Abstract: In this review properties and industrial application of microbial α -amylase produced by bacteria and fungi are discussed. α -Amylases are among the most important enzymes and are of great significance in industrially and ability to hydrolysing starch and related saccharides. The properties of amyolytic enzymes usually reflect the characteristics of the environment occupied by the living organism. α -Amylases are produced by microorganisms using submerged and solid state fermentation technique. The kinetic properties of α -amylase such as optimum temperature, optimum pH and thermostability are important in the development of fermentation process. Calcium ions are essential for activity and stability of α -amylases.

Keywords: α -amylase, thermostability, fermentation, microbial

Introduction

Starch represents one of the most ubiquitous and accessible energy sources and may be used as a potential substrate for the production of fuels and chemicals by enzymatic and chemical processes [1, 2]. Maize, tapioca, potato and wheat are the major industrial sources of starch, while other significant sources include rye and sorghum [3]. Use of enzyme for starch conversion confers several advantages as opposed to the use of acids. The specificity of enzymes allows the production of sugar syrup with well-defined physical and chemical properties, and in addition the milder enzymatic hydrolysis results in few side reactions and less "browning" [2].

Amylases are among the most important enzymes in present-day biotechnology, and are universally distributed throughout the plant, animal and microbial kingdom. Of great significance are amylases of microbial origin, such as those from bacterial and fungal sources, which have dominated applications in industrial sectors, as they meet process demands, and have successfully replaced the chemical hydrolysis of starch in starch-processing industries [4, 5]. In particular, amyolytic enzymes are used in processes where rapid hydrolysis of starch is required or the high viscosity of starch must be lowered, such as in the textile, glucose syrup, confectionary, brewing, paper and alcohol industries [2]. The commercial production of α -amylases has been limited to only a few selected strains of fungi and bacteria. Microorganisms have bulk production capacity and are easy to manipulate in order to obtain enzymes of desired characteristics, thus making them an attractive and economical source of commercial enzymes [6]. The main source of commercial thermostable α -amylase is strains of the mesophilic bacteria, *Bacillus licheniformis* and *Bacillus amyloliquefaciens* [7]. However, the production of commercially acceptable yields of α -amylases from

microbial sources remains a challenge, and is further compounded by the fact that some strains produce more than one enzyme.

α -Amylases (1,4- α -D-glucan glucanohydrolase EC 3.2.1.1) belong to family 13 of the glucoside hydrolase group of enzymes [8] that catalyze the hydrolysis of the internal α -1,4-glucosidic bond in starch. α -Amylase may be derived from several bacteria, yeasts and fungi [9]. The enzymes are reported to be thermostable, with the half-life of α -amylase and glucoamylase being 0.6 and 4 h, respectively at 70°C. Due to their increased thermostability, these enzymes are potentially useful in the starch industry for production of maltose and glucose [10]. Thermostable α -amylases are generally preferred as their application minimizes contamination risk and reduces reaction time, thus providing considerable energy saving. Hydrolysis carried out at higher temperatures also minimizes polymerization of D-glucose to iso-maltose [11]. Among the genus *Bacillus*, *B.amyloliquefaciens* and *B.licheniformis* are the two species used most frequently in the commercial production of thermostable amylases [12]. The need for enzymes with improved properties has initiated a continuous search for microorganisms producing novel amylases for industrial application [13].

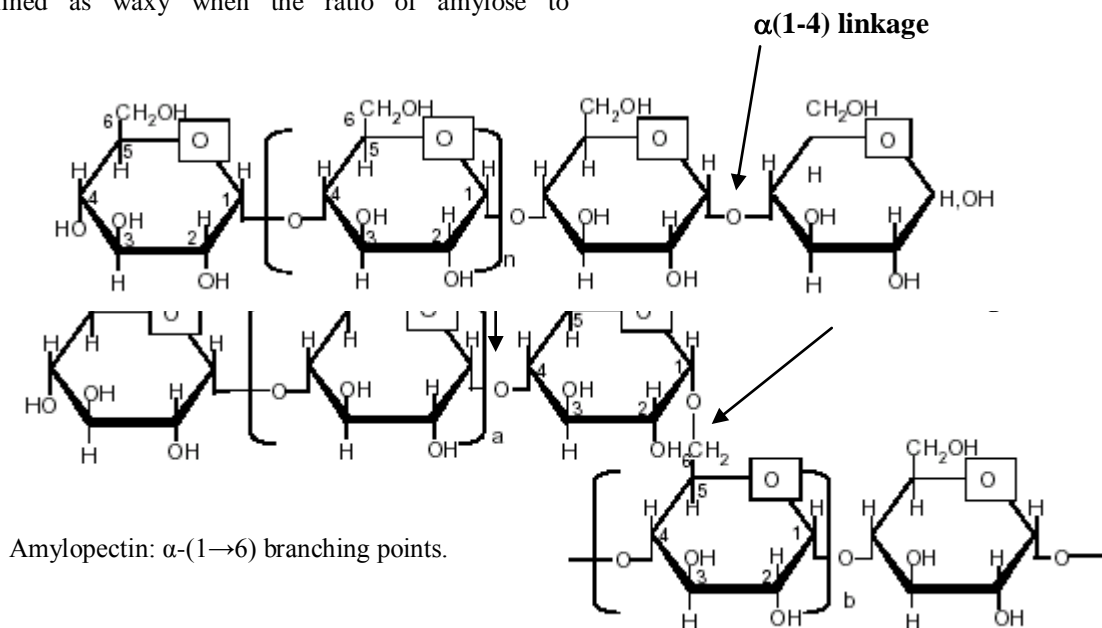
Starch

Native starch is a semi-crystalline material synthesized as roughly spherical granules in many plant tissues including pollen, leaves, stems, roots, tubers, bulbs, rhizomes, fruits, and seeds. Commercially, starch is extracted in pure form variety of sources. Maize is the predominant source, but wheat, rice, potato and sago make significant contribution. Other starch sources include barley, oats, yam and arrowroot. The size, shape and granule size distribution of starch reflects the botanical origin [14, 15].

Starch composition

Pure starch consists predominantly of α -glucan in the form of amylose and amylopectin (Figure 1). Amylose is a roughly linear molecule containing ~99% α -(1-4) and ~1% α -(1-6) bonds with a molecular weight of $\sim 1 \times 10^5$ - 1×10^6 . Amylopectin (molecular weight $\sim 1 \times 10^7$ - 1×10^9) is a much larger molecule than amylose and is heavily branched with ~95% α -(1-4) and ~5% α -(1-6). Each amylose chain is of the order of 1000 glucose units long whereas the unit chains of amylopectin range from ~12 to 120 hydroglucose units. Starches are defined as waxy when the ratio of amylose to

amylopectin is low (~15%), normal when amylose represents ~16-35% and high-amylose (or amylo-) when amylose exceeds ~36%. Waxy mutants are now commonly available for barley, maize and rice and have also been developed recently for wheat and potato. High-amylose mutants, predominantly from maize, are now available commercially [16]. Enzymes used for the industrial starch hydrolysis market are conservatively estimated to occupy 10-15 per cent of the total world enzyme market. This industrial sector is the second biggest consumer of enzymes by market sector [17].



Amylose: α -(1 \rightarrow 4)-glucan; The linear molecule may carry a few occasional moderately long chains linked α -(1 \rightarrow 6).

Figure 1:- Basic structure of amylose and amylopectin [16]

Starch hydrolysis

Starch base raw materials have to be cooked initially, to gelatinize the starch to permit it to be hydrolyzed by acid or more commonly by enzymes [18]. Currently available enzymes cannot rapidly hydrolyze insoluble raw starch. To make the starch readily accessible to enzyme attack, it has to be first gelatinized. This is achieved by making slurry (20-40 percent dry solids) and heating at the appropriate gelatinization temperature (105-110°C). At higher solid levels the water slurry is very thick and viscous. Highest solids give 'paste' which is difficult to handle in industrial circumstances. Gelatinization is the process by which heat treatment is applied to 'disrupt' or 'burst' the starch granules for exposure to enzyme hydrolysis. Disrupted starch in this form will enhance the viscosity of slurry and further complicate the hydrolase action in processing.

Enzymatic hydrolysis of starch

Enzymatic hydrolysis of starch involves two processes.

1. Liquefaction

2. Saccharification

Liquefaction

Traditionally, the process of thinning and dextrinization of starch was carried out using acid. The use of a heat-stable endo α -amylase allows less rigorous conditions to be employed in the liquefaction process. By-product formation becomes less of a problem and refining costs are reduced. The first enzyme to be employed in starch liquefaction was the endo α -amylase produced by *Bacillus subtilis*. The enzyme is utilized in a two-stage enzyme liquefaction process. Starch slurry at 30-40 percent dry solids is thinned at a temperature of 85°C for 1 h at pH 6-6.5. This is followed by a short heat treatment at 140 °C, flash cooled to 85 °C at which point a second enzyme addition is made and the reaction continued until liquefaction is complete. If a thermostable α -amylase is employed, such as from *B.licheniformis* or *B.sterothermophilus*, the heat treatment at 140 °C may be eliminated. The use of thermostable α -amylases has largely replaced acid liquefaction and processing using the heat-stable α -amylase from *B.subtilis* [19].

Saccharification

The second step in the enzymatic hydrolysis of starch is saccharification of liquefied product using a glucoamylase enzyme isolated from *Aspergillus* sp. Because the glucoamylase activity optimum is pH 4.2-4.5 [20], the pH must be reduced to pH 4.5 for this step to proceed efficiently.

Types of starch hydrolyzing enzymes

There are basically four groups of starch-converting enzymes: (i) endoamylases; (ii) exoamylases; (iii) debranching enzymes; and (iv) transferases [21]. Enzymes are used to hydrolyze starches particularly for the production of dextrans and glucose. Although α -amylase and amyloglucosidase are most often used in this respect to make glucose syrup, maltodextrins and crystalline glucose (dextrose), specialist dextrans and maltose may be obtained with β -amylase [16]. Enzymes responsible for starch hydrolysis are presented in Table 1. Thermostable α -amylases are used for the liquefaction of starch at high temperature and

thermolabile α -amylases are used for the saccharification of starch in baking [22].

α -Amylase

α -Amylase (EC 3.2.1.1, 1,4- α -Dglucanohydrolase, endoamylase) hydrolyses starch, glycogen and related polysaccharides by randomly cleaving internal α -1,4-glucosidic linkages. It is widely distributed in various bacteria, fungi, plants and animals and has a major role in the utilization of poly saccharides. α -Amylase (Figure 2) is an important industrial enzyme [22]. Among the various extracellular enzymes, α -amylase ranks first in terms of commercial uses [23]. Spectrum of applications of α -amylase has widened in many sectors such as clinical, medicinal and analytical chemistry. Besides their use in starch saccharification, they also find applications in baking, brewing, detergent, textile, paper and distilling industry [24]. A summary of the properties of the most important commercial α -amylases is shown in Table 2. It can be seen that α -amylases are available for wide temperature and pH range.

Table 1: Starch hydrolysing enzymes [15]

Enzyme	Glycosidic bond specificity	Mode of action	End products
Phosphorylase (1,4- α -D-glucan orthophosphate α -Dglucosyl transferase)	α -(1-4)-glucosyl	Exo	Glucose 1-phosphate
Alpha-amylase (1,4- α -D-glucan glucanohydrolase)	α -(1-4)-glucosyl	Endo	Linear and branched soligosaccharides
Beta-amylase (1,4- α -D-glucan maltohydrolase)	α -(1-4)-glucosyl	Exo Dextrins	Maltose and limit
Amyloglucosidase (Glucoamylase; exo-1,4- α -glucosidase)	α -(1-4)-glucosyl & α -(1-6)-glucosyl	Exo/ Endo	Glucose
Isoamylase (Glycogen 6-glucanohydrolase)	α -(1-6)-glucosyl	Endo	Linear α -(1-4) glucan chains
Pullulanase (limit dextrinase; amylopectin 6-glucanohydrolase)	α -(1-6)-glucosyl	Endo	Linear α -(1-4) glucan chains

Types of α -amylases produced by *Bacillus* species

The genus *Bacillus* produces a large variety of extracellular enzymes, some of which such as the α -amylases are of significant industrial importance. Among these enzymes, the thermostable varieties are more versatile with respect to industrial significance [26]. Thermostable α -amylases have had many commercial applications for several decades. The

advantages for using thermostable α -amylase in industrial processes include the decreased risk of contamination, the increased diffusion rate and the decreased cost of external cooling [27].



Figure 2: Three-dimensional structure of α -amylase of *B. licheniformis* [25]

Bacteria belonging to the genus *Bacillus* have been widely used for the commercial production of thermostable α -amylases. These include α -amylase from *Bacillus coagulans*, *B.sterothermophilus*, *B.caldolyticus*, *B.brevis*, *B.acidocaldarius* and *B.thermoamylolique-faciens* [28]. Among the genus *Bacillus*, *B.amyloliquefaciens* and *B.licheniformis* are

the two species used most frequently in the commercial production of thermostable amylases [29].

Bacillus licheniformis α -amylase is a highly thermostable enzyme which is widely used in biotechnological processes. Although it is produced by a non-thermophilic bacterium, it remains active for several hours at temperatures over 90 °C under conditions of industrial starch hydrolysis. It is also far more thermostable than the α -amylases from *B. stearothermophilus* and *B. amyloliquefaciens* despite the strong sequence similarities between these three proteins. The most important characteristic of thermophilic organisms is their ability to produce thermostable enzymes with a higher operational stability and longer half-life [30]. As compared with eubacterial enzymes, archaeobacterial amylases from *Pyrococcus furiosus* and *P.woesei* exhibit greater thermostability [31]. Thermostable amylases are commonly important in various starch processing industries [1]. Though the most widely used thermostable amylases are produced by mesophilic microorganisms, thermophilic microorganisms are considered to have enormous potential as sources of thermostable amylases of potential industrial importance [32].

Table 2: Properties of α -amylases [33]

Trivial Name	Source Organism	pH	Temp °C	Commercial Products
(Conventional) Bacterial amylase	<i>B.amyloliquefaciens</i>	5.5-6	50-70	Ban (N)
	<i>B.subtilis</i>	5.5-6	43-70	Aquazyme [®] (N) Dezyme [®] (N) Dex-lo [®] (Gb) Desize [®] 40, 160 and 900 (GCI)
Thermostable Bacterial amylase	<i>B.licheniformis</i>	6-9	76-100	Spezyme [®] AA 20 (GCI) Termamyl [®] (N) Liquozyme [®] 280 L (N) Purafect [®] HP Am L and OxAm (GCI) Desize [®] HT and TEX (GCI)
	<i>B.sterothermophilus</i>	4.5-6	60-80	Maltogenase [®] (N)
Fungal amylase	<i>A.oryzae</i>	4.5-6	35-55	Fungamyl [®] (N) Mycolase [®] (GCI)
Acid amylase	<i>A.niger</i>	4-6	50-60	Hazyme [®] (Gb)

GCI: Genencor International, Gb: Gist-Brocades, N: Novo Nordisk

Large scale production of α -amylase

Statistics [34], indicated that industrial microbial fermentation was responsible for production of 320 tons of α -amylase on an annual basis. Physical factors affect

the large scale fermentation including bioreactors configuration, aeration, agitation, back pressure, medium sterilization, temperature control and inhibitor removal [35, 36, 37]. Industrially important enzymes

have traditionally been obtained from submerged fermentation (SmF) because of the ease of handling and greater control of environmental factors such as temperature and pH [2]. The main problems arising from high cell densities are high oxygen and substrate uptake rates and accumulation of low molecular growth inhibiting metabolites in the cell suspension during the cultivation. To minimize the formation of inhibitors, providing optimal growth conditions is essential. Large-scale production of α -amylase using *Bacillus amyloliquefaciens* and *Aspergillus oryzae* were observed. The use of *Bacillus subtilis* as organism under liquid fermentation culture using two types of fermentors (200 and 600 L) and with a total of 21 production runs resulted to a total volume of 7,400 L of α -amylase. On the other hand, the use of *Aspergillus* sp. under solid substrate fermentation with 48 production runs consisting of 75 trays per run produced 3,600 L of α -amylase.

Bioreactors are at the heart of the fermentation process. These reactors (also known as fermenters) are used for growing cells. The main components of a fermenter are as follows [38]:

- Base components including drive motor, heaters, pumps, gas control etc;
- Vessel and accessories (agitator impeller, aerator, etc);
- Peripheral equipment such as reagent vessels;
- Instrumentation and sensors.

The above components combine to perform the following functions:

- Provide operation free from contamination;
- Maintain a specific temperature;
- Provide adequate mixing and aeration;
- Control the pH of the culture;
- Allow monitoring and/or control of dissolved oxygen;
- Allow feeding of nutrient solutions and reagents;
- Provide access points for inoculation and sampling;
- Use fittings and geometry relevant to scale-up;

Carbon and nitrogen sources on the production of α -amylase

It is a common practice to use carbohydrates as the carbon source in microbial fermentation process. The rate of bacterial α -amylase biosynthesis is controlled by both substrate induction and catabolite repression [32, 39] the composition and concentration of the medium play an important role in the growth and production of extracellular amylase by bacteria, yeast and *Aspergillus* sp [40]. Available carbon and nitrogen sources are the important elements in the optimum production of enzymes and these differ very much from substrate to

substrate (Table 3). It is now recognized that the rate at which the carbon sources is metabolized, can often influence the formation of biomass or production of primary or secondary metabolites. The carbon sources greatly affect the production of thermophilic α -amylases [41, 42]. Undefined carbon sources are known to induce a high level of amylase production in many bacterial strains [43]. Of carbohydrates used, starch is demonstrated to be a good carbon source for the synthesis of amylases in *B.sterothermophilus* [44] and other thermophilic *Bacillus* sp. [41]. Degradation of starch to maltodextrins by many bacteria is catalyzed by α -amylase and is followed by its hydrolysis to glucose by the action of either intracellular or extracellular α -glucosidase [33]. It has been reported that starch was the best inducer for α -amylase production in TA1 strain of *Aspergillus nidulans*, which was comparable with only glucose [45].

Lower levels of nitrogen sources are inadequate for the enzyme production and excess nitrogen is equally detrimental causing enzyme inhibition [46]. Different nitrogen sources have been used for enhancement in the production of α -amylase [47]. The regular use of peptone based fermentation media is not commercially viable for industries. For efficient commercial production, a continuous effort is being made to find cheaper substrate sources.

The expensive products can be replaced in the fermentation medium with the economically available agricultural by products [48]. Oil seed cakes are by-products obtained after oil extraction. Depending upon the extraction methods the chemical composition of oil cake varies. These oil cakes are fairly rich in protein and are traditionally used as feed aquaculture feeds [49]. Several oil seed cakes, because of their abundant, availability and low price, are used as cattle feed [50], fertilizer [51] and in rare cases after proper processing as food for human [52]. Oil seed cakes such as Sesamum and Mustard could completely replace the peptone in the production of α -amylase from *B.licheniformis* ATCC 6346 [53]. α -Amylase formation by *Bacillus subtilis* was stimulated in the presence of Phe and Tyr. While Glu, Met, Pro and Trp were the amino acids that most repressed α -amylase synthesis [54]. Addition of Cys and Gly, inhibited the growth of *Bacillus sterothermophilus*, and that the highest reproductive values were obtained with Phe and Asp [41]. α -Amylase production from *B.licheniformis* ATCC 6346 was improved by the addition of tryptophane [53], while glycine, methionine, proline, glutamic acid and phenylalanine did not influence on the production of enzyme [55].

Table 3: Various carbon and nitrogen sources used for α -amylases production

Substrate	Organism	Activity (U/g)	Reference
Wheat bran	<i>Bacillus</i> sp. PS-7	464 000	[56]
Spent brewing grain	<i>A. oryzae</i> NRRL 6270	6583	[57]
Maize bran	<i>B. coagulans</i>	22956	[58]
Rice bran	<i>Bacillus</i> sp. PS-7	145 000	[56]
Rice husk	<i>B. subtilis</i>	21 760	[59]
Coconut oil cake	<i>A. oryzae</i>	3 388	[24]
Mustard oil cake	<i>B. coagulans</i>	5 953	[58]
Corn bran	<i>Bacillus</i> sp. PS-7	97 600	[56]
Amaranthus grains	<i>Aspergillus flavus</i>	1 920	[60]
Gram bran	<i>B. coagulans</i>	8 984	[58]

Thermostable starch hydrolyzing α -amylases

The starch industry is one of the largest users of enzymes for the hydrolysis and modification of this useful raw material. The starch polymer, like other such polymers, requires a combination of enzymes for its complete hydrolysis. These include α -amylases, glucoamylases or β -amylases and isoamylases or pullulanases [61]. Gelatinization is achieved by heating the starch with water, and starch is water soluble only at high temperatures which are dependent on the source [62]. For hydrolysis of starch to proceed immediately after gelatinization, hence, among other things avoiding

a lot of cooling time, the enzyme has to be thermostable. Table 4 shows thermostable starch hydrolyzing α -amylases and their properties. The extra thermostability of the thermophilic α -amylase was found to be mainly due to additional salt bridges involving a few specific lysine residues (Lys-385 and Lys-88 and/or Lys-253). These stabilizing electrostatic interactions reduce the extent of unfolding of the enzyme molecule at high temperatures, consequently making it less prone to forming incorrect structures and thus decreasing the overall rate of irreversible thermoinactivation [63].

Table 4: Thermostable starch hydrolyzing α -amylase and their properties

Organism	Enzyme properties		References
	Optimal Tem.(°C)	Optimal pH	
<i>Bacillus amyloliquefaciens</i>	70	7.0	[64]
<i>Bacillus licheniformis</i>	100	6.0-6.5	[65]
<i>Bacillus stearothermophilus</i>	70-80	5.0-6.0	[66]
<i>Bacillus stearothermophilus</i>	70	-	[67]
<i>Bacillus subtilis</i>	70	7.0	[68]
<i>Lactobacillus manihotivorans</i>	55	5.5	[69]
<i>Myceliophthora thermophila</i>	100	5.6	[70]
<i>Pyrococcus furiosus</i>	100	5.5	[71]
<i>Pyrococcus woesei</i>	100	6.5-7.5	[72]
<i>Staphylothermus marinus</i>	65	5.0	[68]
<i>Sulfolobus solfataricus</i>	-	-	[73]
<i>Thermococcus aggregans</i>	100	5.5	[68]
<i>Thermococcus celer</i>	90	5.5	[68]
<i>Thermococcus fumicolans</i>	95	4.0-6.3	[74]
<i>Thermococcus hydrothermalis</i>	85	4.8-7.8	[74]
<i>Thermococcus lanuginosus</i>	60	5.6	[75]
<i>Thermococcus profundus</i>	80	4.0-5.0	[76]

Role of calcium ions on activity and stability of amylases

α -Amylases are metalloenzymes, containing at least one calcium ion per enzyme molecule [77], which is essential for activity and stability (Figure 3). The amount of bound calcium may vary from one to about ten [33, 78]. Calcium ions play an important role in the maintenance of enzymatic activity through allosteric

activation and protein stabilization of α -amylases. Thermal stability of α -amylase by *B. licheniformis* is suggested to be due mainly to the additional salt bridges involved with lysine residues [63]. 1.0 mM Ca^{2+} improved the stability of α -amylase by *Bacillus licheniformis* ATCC 6346 [79]. It is also well established that calcium plays an important role in the thermal stabilization of α -amylases (Table 5). For α -

amylases from *B.licheniformis* and *B.amyloliquefaciens*, calcium is seen to play a role in conformational stabilization, as opposed to having a direct role in the catalytic mechanism [80]. Calcium ions have been implicated in mechanisms involving thermal inactivation of *Bacillus* α -amylases, whereby it has been proposed that the first step involves the

reversible dissociation of calcium ions from the native enzyme, followed by irreversible denaturation at high temperatures [81, 82]. Almost all of the technical α -amylases however need a certain amount of calcium ions in the application, because their thermostability depends on the presence of structural calcium ions [83].

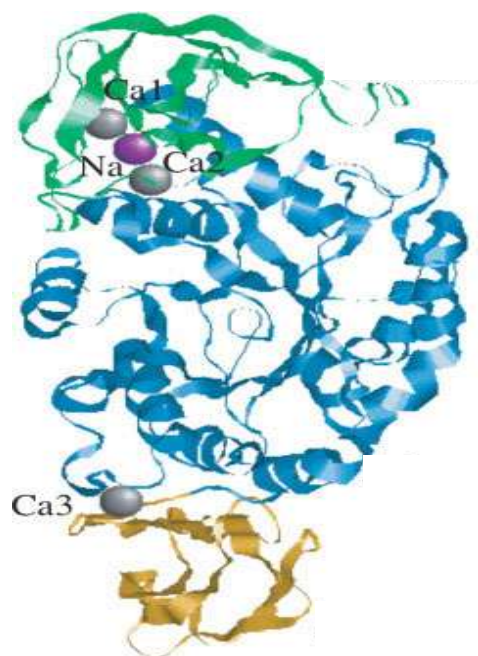


Figure 3: A schematic presentation of the alkaline liquefying α -amylase from alkaliphilic *Bacillus* sp. KSM-1378 structure. The three calcium ions (Ca1-3) and the sodium ion (Na) are represented by gray and purple spheres, respectively [84].

Table 5: Calcium requirement of industrially important starch degrading enzymes [85]

Enzyme	Microorganism	Application Tem. (°C)	Application pH	Minimum Ca ²⁺ dosage (ppm)
Bacterial mesophilic α -amylase	<i>Bacillus subtilis</i>	80-85	6.0-7.0	150
Bacterial thermophilic α -amylase	<i>B.licheniformis</i>	95-105	6.0-7.0	20
Fungal α -amylase	<i>Aspergillus oryzae</i>	55-70	4.0-5.0	50
Amyloglucosidase	<i>A.niger</i>	55-65	3.5-5.0	0
Pullulanase	<i>B.acidopollulicus</i>	55-65	3.5-5.0	0

Calcium has direct role in the stimulation of enzymatic activity of α -amylases from alkaliphilic *Bacillus* species [86, 87, 88]. However, some amylases from archaea such as *Pyrococcus furiosus* are active even in the absence of the metal ion [89, 71]. In some cases, calcium has an inhibitory effect on the enzyme activity, especially at high concentrations (≥ 2 Mm) [72]. The

inhibitory effect is postulated to be due to binding of calcium at the secondary calcium binding site which involves two of the catalytic residues (90, 91).

Effect of metal ions on activity and stability of α -amylases

Apart from calcium, other ions such as sodium and chloride have been implicated as allosteric activators of α -amylases. Sodium ions rather than calcium ions have been to retain the structure and function of an α -amylase from *Bacillus sp.* Strain KSM-K38 [92, 93] and various other α -amylases [66, 94]. *Aspergillus tamari* producing α -amylase was not significantly influenced by metal ions, except by Fe^{3+} , Pb^{2+} , Cu^{2+} and Hg^{2+} , which strongly inhibited its activity but α -amylase activity was slightly enhanced by Ba^{2+} . Of the cations, Na^+ , Ca^{2+} , and Mg^{2+} , showed stimulatory effect on α -amylase of *Bacillus licheniformis* CUMC 305, whereas Hg^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Ag^+ , Fe^{2+} , Co^{2+} , Cd^{2+} , Al^{3+} and Mn^{2+} showed inhibitory effect [95]. Cu^{2+} has been reported to inhibit the activity of amylases from *B.circulans* [96], *B.coagulans* [58] and *B.licheniformis* [95]. The inhibition of *Bacillus sp.* Strain SMIA-2 α -amylase, by Co^{2+} , Cu^{2+} and Ba^{2+} ions could be due to competition between the exogenous cation and the protein-associated cation, resulting in decreased metalloenzyme activity [97]. 0.1 M Na^+ stabilized α -amylase from *B. licheniformis* ATCC 6346, however, 0.1 M Na^+ could not continue to keep the α -amylase in the active state at higher temperatures, above 70 °C. This could be due to the breakdown of the Na^+ - α -amylase bond with the increase in temperature [79]. Presence of Ca^{2+} and Na^+ ions together increased the stability of α -amylase from *B. licheniformis* ATCC 6346 more than when they were present alone and α -Amylase activity was strongly inhibited by Cu^{2+} , Hg^{2+} and Mn^{2+} but less affected by Mg^{2+} and Ba^{2+} [79].

Applications of α -amylases

The range of technical applications of α -amylases is very wide (Table 6). α -Amylases are used in textile desizing [98, 6], clarification of haze formed in beer and fruit juices, or for the pre-treatment of animal feed to improve digestibility [99]. α -Amylases are also used as a partial replacement for the expensive malt used in the brewing industry [99] and for the production of low viscosity, high molecular weight starch for coating of paper, and in the de-linking and drainage improvement within the pulp and paper industry [6]. Amylase applications have further expanded into other fields such as clinical, medical and analytical chemistry [6]. Because of the industrial importance of amylases, there is ongoing interest in the isolation of new bacterial strains, such as alkaline amylases suitable for new industrial applications, such as alkaline amylases for the detergent industry and amylases capable of producing high levels of a specific maltooligosaccharide from starch [100]. Earlier the α -amylase from *Bacillus amyloliquefaciens* had been used but it is replaced by the α -amylase of *Bacillus stearothermophilus* or *Bacillus licheniformis* (99). The enzymes from the *Bacillus* species are of special interest for large-scale biotechnological processes due to their thermostability (101). In the light of modern biotechnology, α -amylases are now gaining importance in biopharmaceutical applications. Still, their application in food and starch based industries is the major market and thus the demand of α -amylases would always be high in these sectors [6].

Table 6: Industrial application of α -amylase.

α -Amylase used in industrial products	References
Glucose and high fructose syrup	[2]
Oligosaccharides	[3]
Baking industry	[99]
Cyclodextrin	[1]
Dtergent	[102]
Textile	[98, 6]
Brewing industry	[99]
Paper industry	[6]

Conclusion

α -Amylases are produced by animal, plant and micro-organisms. However, enzymes from fungal and bacterial sources have more preferable in industrial sectors. The production of microbial α -amylase is dependent on the nature of strain, composition of medium, nutrient requirement, metal ions, pH, temperature, type of fermentation and condition of the microbial existing environment. Most of α -amylases are known to be calcium ion-dependent enzymes, therefore calcium ions are used for increase the production and thermal stability of the enzymes. The characters of α -

amylases such as stability, durability, reusability are essential for industrial application.

References

- [1] Crabb W, Mitchinson C; Enzymes involved in the processing of Starch to sugars. Trends Biotechnol, 1997; 15(9): 349-352.
- [2] Nigam P, Singh D; Enzyme and microbial systems involved in starch processing. Enz Microb Technol., 1995; 17: 770-778.

- [3] Gozmn-Maldonado H, Paredes-Lopez; Amylolytic enzymes and products derived from starch: a review. *Crit Rev Food Sci Nutr*, 1995; 35: 373-403.
- [4] Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R; *Advances in Microbial amylases*. *Biotechnol Appl Biochem*, 2000; 31: 135-152.
- [5] Reddy NS, Nimmagadda A, Sambasivarao KRS; An overview of the microbial α -amylase family. *Afr J Biotechnol* 2003; 2: 645-648.
- [6] Gupta R, Gigras P, Mohaptra H, Goswami VK, Chauhan B; Microbial α -amylase: a biotechnological perspective. *Process Biochem*, 2003; 38: 1599-1616.
- [7] Kelly CT, Tigue MM, Doyle EM, Fogarty WM; The raw starch-degrading alkaline amylase of *Bacillus* sp. IMD 370. *J Ind Microbiol*, 1995; 15: 446-8.
- [8] Henrissat B, Bairoch A; Updating the sequence-based classification of glycosyl hydrolases. *J Biochem*, 1996; 316: 695-696.
- [9] Fabiana GM, Veridiana L, Rosane MP; A thermostable maltose-tolerant α -amylase from *Aspergillus tamarii*. *J Basic Microbiol*, 2004; 44(1): 29-25.
- [10] Mishra RS, Maheshwari R; Amylases of thermophilic fungus *Thermomyces lanuginosus*: their purification, properties, action on starch and response to heat. *J Biosci* 1996; 21: 653– 672.
- [11] Kunamneni A, Kumar SK, Singh S; Response surface methodological approach to optimize the nutritional parameters for enhanced production of α -amylase in solid state fermentation by *Thermomyces lanuginosus*. *Afr J Biotechnol*, 2005; 4(7): 708-716.
- [12] Fogarty WM, Kelly CT; In *Microbial enzyme and Bioconversions*. Academic press New York, 1980: 115-169.
- [13] Kochhar S, Dua RD; Thermostable liquefying α -amylase from *Bacillus amyloliquefaciens*. *Biotechnol Lett*, 1990; 12: 393-396.
- [14] Morrison WR, Karkalas J; Starch, in: *Methods in Plant Biochemistry* (P. M. Dey, ed.). London Academic Press 1990; 2: 323-352.
- [15] Tester RF, Karkalas J; Starch, in: *Biopolymers, Polysaccharides II: Polysaccharides from Eukaryotes*, (A. Steinbüchel series ed.; E. J. Vandamme, S. De Baets and A. Steinbüchel volume eds), Weinheim: Wiley-VCH. 2002; 6: 381-438.
- [16] Tester RF, Karkalas J, Qi X; Starch structure and digestibility Enzyme-Substrate relationship. *World's Poultry Sci J*, 2004; 60: 186-195.
- [17] Hashim OS; An alkaline active maltooligosaccharide forming α -amylase from *Bacillus halodurans*. Doctoral thesis, Department of Biotechnology, Lund university, Lund 2004; 22-23.
- [18] Leach HW; Gelatinization of starch. In *starch: Chemistry and technology. Fundamental Aspects* ; Whilster, R.L., Paschall, E.F., eds.; Academic Press, New York, 1965; 1: 289-307
- [19] Bentley IS, Williams EC; Starch conversion, In T. Godfrey and S. West (ed.), *Industrial enzymology*, The Macmillan Press Ltd., London, United Kingdom. 1996; 2: 341-357.
- [20] Hashida M, Bisgaard-Frantzen H; Protein engineering of new industrial amylases. *Trends Glycosci Glycotechnol*, 2000; 68: 389-401.
- [21] Marc JEC, Bart van der Veen, Joost CM, Hans Leemhuis, Dijkhuizen L; Properties and applications of starch-converting enzymes of the α -amylase family. *J Biotechnol*, 2002; 94: 137-155.
- [22] Shaw J, Pang L, Chen S, Chen I; Purification and properties of an extracellular α -amylase from *Thermus* sp. *Bot Bull Acad Sin*, 1995; 36: 195–200.
- [23] Babu KR, Satyanarayana T; Parametric optimization of extracellular α -amylase production by thermophilic *Bacillus coagulans*. *Folia Microbiol*, 1993; 38: 77–80.
- [24] Ramachandran S, Patel AK, Nampoothiri KM, Chandran S, Szakacs G, Soccol CR, Pandey A; α -Amylase from a fungal culture grown on oil cakes and its properties. *Bra Arch Biol Technol*, 2004; 47: 309–317.
- [25] Pradeep NV, Anupama, Ankitha K, Pooja J; Categorizing Phenomenal features of α -Amylase (*Bacillus* species) using Bioinformatic Tools. *Adv Lif Sci Technol*, 2012; 4: 27-31.
- [26] Bolton DJ, Kelly CT, Fogarty WM; Purification and characterization of the α -amylase of *Bacillus flavothermus*. *Enz Microb Technol*, 1992; 20: 340-343.
- [27] Lin LL, Chaya CC, Hsu WH; Production and properties of a raw-starch-degrading amylase from the thermophilic and alkaliphilic *Bacillus* sp. TS-23. *Biotechnol Appl Biochem*, 1998; 26: 61-68.
- [28] Cambell, LL; Crystalization of α -amylase from thermophilic bacterium. *J Amer chem soci* 1955; 76: 52-56.

- [29] Forarty WM, Kelly CT; Amylases, amyloglucosidases and related glucanases. In Microbial Enzymes and Bioconversion: Economic Microbiol Academic Press, London. 1980; 5: 115-170.
- [30] Niehaus F, Bertoldo C, Kahler M, Antranikian G; Extremophiles as a sources of novel enzymes for industrial application. Appl microbiol Biotechnol, 1999; 51: 711-729
- [31] Brown S, Costantino H, Kelly R; Characterization of amyolytic enzymes associated with the hyperthermophilic archeobacterium *Pyrococcus furiosus*. Appl Environ Microbiol, 1990; 56: 1985-1991.
- [32] Antranikian G; Physiology and Enzymology of Thermophilic Anaerobic Bacteria Degrading Starch. FEMS Microbial Rev, 1990; 75: 201-218.
- [33] Vihinen M, Mantsala P; Micronbial amyolytic enzymes. Crit Rev Biochem Mol Biol, 1989; 24(4): 329-418.
- [34] Eveleigh DE; The microbial production of industrial chemicals. Scintific American, 1981; 245:155-178.
- [35] Jem KJ; Scale-down techniques for fermentation. Biopharm, 1989; 3: 30-39
- [36] Trilli A; Scale-up of fermentation. in industrial Microbiology and Bioreclinology. ed. A.L. Demain & N.A. Solomon. Amer Soci Microbiol, 1986; 377-307.
- [37] Votruba J, Sobotka M; Physiological similarity and bioreactor scale-up. Folia Microbiol, 1992; 37: 331-345.
- [38] Rengasayee, V., Jerry Thomas, N. and Siddharth, B. Optimisation of xylanase enzyme production in *aspergillus* spp. A project report in partial fulfillment for the award of the degree of bachelor of technology in chemical engineering. Sri venkateswara college of engineering, pennalur anna university : chennai 600025, 2005.
- [39] Laoide BM, Mc Connelcis DJ; Sequences involved in Modulating Excretion of *Bacillus subtilis*.Effect of Sporulation Mutations and Catabolites Repression Resistant Mutations on Expression. J Bacteriol, 1989; 171: 2443-2450.
- [40] Zhou Y, Ru-Zhang J; Recognition Sequence Specificity of Signal Peptidase I and the Role of Signal Peptide in Secretion of Protein in *Bacillus subtilis*. Sci in China, 1990; 34: 1082-1091.
- [41] Srivastava RAK, Baruah JN; Culture Conditions for production of Thermostable amylase by *Bacillus sterothermophilus*. Appl Environ Microbiol, 1986; 52: 179-184.
- [42] Kumar SU, Rehana F, Nand K; Production of an extracellular thermostable calcium-inhibited α -amylase by *Bacillus licheniformis* MY 10. Enz Microbiol Technol, 1990; 12: 714-716.
- [43] Fogarty WM, Griffin PJ, Joyce AM; Enzymes of *Bacillus* species. Pro Biochem, 1974; 9: 11-24.
1. [44] Welker NE, Cambell LL; Effect of carbon sources on formation of alpha-amylase by *Bacillus stearothermophilus*. J Bacteriol, 1963; 86: 681-686.
- [45] Agger T, Petersen JB, Connor SM, Murphy RL, Kelly JM, Nielsen J; Physiology characterization of recombinant *Aspergillus nidulans* strains with a genotypes expressing *A. oryzae* α -amylase. J Biotechnol, 2002; 92: 279-285.
- [46] Aiyer PV; Effect of C:N ratio on alpha amylase production by *Bacillus licheniformis* SPT 27. Afr J Biotechnol, 2004; 3(10): 519-522.
- [47] Pedersen H, Nielsen J; The influence of nitrogen sources on the α -amylase productivity of *Aspergillus oryzae* in continuous cultures. Appl Microbiol Biotechnol, 2000; 53: 278-281.
- [48] Ghosh SB, Chandra AK; Nutritional requirements and cultural characteristics of *Bacillus apiarius* CBML-152 for the production of thermostable alpha-amylase. Zbl Microbiol, 1984; 139: 293-304.
- [49] Singh K, Garg SK, Kalla A, Bhatnagar A; Oilcakes as protein sources in supplementary diets for the growth of *Cirrhinus mrigala* (Ham) fingerlings:laboratory and field studies. Biore Technol, 2003; 86: 283-291
- [50] Norton CL, Eston HD; Dry calf starters for dairy calves. Cornell Univ Agric Expt Sto Bull, 1974; 835: 32.
- [51] Salgado MLM; Coconut poonac as manure. J Trop Agri (Ceylon), 1940; 95:3-7.
- [52] Rastogi MK, Singh C, Krishnamurti CR; Protein hydrolysates from indigenous sources. Proceedings of the Symposium on Proteins, Mysore, India 1960: 318-325.

- [53] Vengadaramana A, Balakumar S, Vasanthi A; Improving α -amylase production by *Bacillus licheniformis* ATCC 6346 with local nitrogen sources and amino acids supplementations. Arch Appl Sci Res, 2011; 3(6):87-97.
- [54] Sema A, yavuz ensari N, Uyar F, Otludil B; The effect of amino acids on production and transport of α -amylase through bacterial membrane. J Starch/starke, 2000; 52: 290-295.
- [55] Vengadaramana A, Balakumar S, Vasanthi A; Supplementation of Carbohydrate to Enhance the α -amylase Production by *Bacillus licheniformis* ATCC 6346 in Presence of Seed Cakes. Malaysian J Microbiol, 2012; 8(4):242-247.
- [56] Sodhi HK, Sharma K, Gupta JK, Soni SK; Production of a thermostable α -amylase from *Bacillus* sp. PS-7 by solid- state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production. Process Biochem, 2003; 40: 525–534.
- [57] Francis F, Sabu A, Nampoothiri KM, Ramachandran S, Ghosh S, Szakacs G, Pandey A; Use of response surface methodology for optimizing process parameters for the production of α -amylase by *Aspergillus oryzae*. Biochem Eng J, 2003; 15: 107–115.
- [58] Babu KR, Satyanarayana T; α -Amylase production by thermophilic *Bacillus coagulans* in solid-state fermentation. Process Biochem, 1995; 30: 305–309.
- [59] Baysal Z, Uyar F, Aytekin C; Solid-state fermentation for production of α -amylase by a thermotolerant *Bacillus subtilis* from hot-spring water. Process Biochem 2003; 38: 1665–1668.
- [60] Vishwanathan P, Surlikar NR. Production of α -amylase with *Aspergillus flavus* on *Amaranthus* grains by solid-state fermentation. J. Basic Microbiol., 2001; 41:57–64.
- [61] Poonam N, Dalel S; Enzyme and microbial systems involved in starch processing. Enz Microbiol Technol, 1995; 17: 770-778.
- [62] Rakshit SK; Utilization of starch industry wastes. In : Martin, A.(Ed.), Bioconversion of waste materials to industrial products, second ed. Blackie Academic and Professional London, 1998.
- [63] Tomazic SJ, Klibanov A; Why is one *Bacillus* α -amylases more resistant against irreversible thermoinactivation than another?. J Biol Chem, 1988; 263(7): 3092-3096.
- [64] Underkofler L; Microbial enzymes. In: Miller, B., Litsky, W.(Eds), McGraw-Hill, New York. Industrial microbial, 1976; 128.
- [65] Viara N, Elena P, Elka I; Purification and characterization of a thermostable α -amylase from *Bacillus licheniformis*. J Biotechnol, 1993; 28: 277-289.
- [66] Vihinen M, Mantsala P, Characterization of thermostable *Bacillus sterothermophilus* α -amylase. Biotechnol Appl Biochem, 1990; 12: 427-435.
- [67] Jeayoung K, Takashi N, Ryu S; Thermostable raw starch digesting amylase from *Bacillus sterothermophilus*. Appl Environ Microbiol, 1989; 55: 1638-1639.
- [68] Canganella F, Andrade C, Antranikian G; Characterization of amylolytic and pullulytic enzymes from thermophilic archaea and from a new *Feravidobacterium* species. Appl Microbiol Biotechnol, 1994; 42: 239-245
- [69] Aguilar G, Morlon-Guyot J, Trejo-Aguilar B, Guyot JP; Purification and characterization of an extracellular α -amylase produced by *Lactobacillus manihottivorans* an amylolytic lactic acid bacterium. Enz Microbiol, 2000; 27: 406-413
- [70] Ramkrishna S, Samir K, Chakrabarty S; Immobilization of α -amylase from *Mycelophthora thermophila* D-14 (ATCC 48104). Enz Microbiol Technol, 1993; 15: 801-804.
- [71] Laderman KA, Davis BR, Krutzsch HC, Lewis Ms, Griko YV, Privalov PL, Anfinsen CB; The purification and characterization of an extremely thermostable α -amylase from the hyperthermophilic archaeobacterium *Pyrococcus furiosus*. J Biol Chem, 1993; 268: 24394-24401.
- [72] Koch R, Spreinat A, Lemke K, Antranikian G; Purification and properties of a hyperthermoactive α -amylase from archaeobacterium *Pyrococcus woesei*. Arch Microbiol, 1991; 155 : 572-578.
- [73] Haseltine C, Rolfsmeier M, Blum P; The glucose effect regulation of α -amylase synthesis in the hypothermophilic archaeon *Sulfolobus solfataricus*. J Biotechnol, 1996; 178: 954-950
- [74] Estelle L, Ladrat C, Ann GGeorges B, Francis D; Thermostable amylolytic enzymes of thermophilic microorganisms from deep-sea hydrothermal vents. Animal biology CR Acad Sci Paris 1997; 320: 893-898.
- [75] Jensen B, Olsen J; Physicochemical properties of a purified α -amylase from the thermophilic fungus *Thermomyces lanuginosus*. Enz Microb Technol, 1991; 14: 112-116.

- [76] Kwak Y, Akeba T, Kudo T; Purification and characterization of enzyme from hyperthermophilic archaeon *Thermococcus profundus*, which hydrolysis both α -1-4 and α -1-6 glucosidic linkages. J Ferment Bioeng, 1998; 86: 363-367.
- [77] Vallee BL, Stein EA, Sumerwell WN, Fisher EH; Metal content of α -amylase of various origins. J Biol Chem, 1959; 234: 2901-2905.
- [78] Janecek S, Balaz S; α -amylases and approaches leading to their enhanced stability. FEBS Lett, 1992; 304: 1-3.
- [79] Vengadaramana A, Balakumar S, Arasaratnam V; Stimulation of thermal stability of α -amylase from *Bacillus licheniformis* ATCC 6346 by treating with cations. Cey J Sci (Bio Sci), 2012; 41 (1): 35-44
- [80] Fitter J, Hermann R, Dencher NA, Blume A, Hauss T; Activity and stability of a thermostable α -amylase compared to its mesophilic homologue: mechanism of thermal adaptation. Biochem, 2001; 40: 10723-10731.
- [81] Lecker DN, Khan A; Theoretical and experimental studies of the effects of heat, EDTA, and enzyme concentration on the inactivation rate of α -amylase from *Bacillus* sp. Biotechnol Prog, 1996; 12: 713-717.
- [82] Tanaka A, Hoshino E; Calcium binding parameter of *Bacillus amyloliquefaciens* α -amylase determined by inactivation kinetics. Biochem J, 2002; 364 (3): 635-639.
- [83] Chiang JP, Alter JE, Sternberg-Elkhart M; Purification and characterization of thermostable α -amylase from *Bacillus licheniformis*. Starch/Strake, 1979; 31: 86-92.
- [84] Tsuyoshi S, Kazuaki I, Tadahiro O, Hiroshi H, Tohru K, Katsuya O, Susumu I; Ancestral Sequence Evolutionary Trace and Crystal Structure Analyses of Alkaline α -Amylase From *Bacillus* sp. KSM-1378 to Clarify the Alkaline Adaptation Process of Proteins. PROTEINS: Structure, Function, and Bioinformatics 2007; 66:600-610.
- [85] Hans S; Enzymes in food processings, A reprint from Biotechnology, second, completely revised ed. Weinheim 1995.
- [86] Kim TU, Gu BG, Jeong JY, Byun SM, Shin YC; Purification and characterization of a maltotetraose-forming alkaline α -amylases from an alkaliphilic *Bacillus* strain, GM8901. Appl Environ Microbiol, 1995; 61: 3105-3112.
- [87] Duedhal-Olesen L, Kragh K, Zimmerman W; Purification and characterization of a malto-oligosaccharide forming amylase active at high pH from *Bacillus Clausii* BT-21. Carbohydr Res, 2000; 329: 97-107.
- [88] Mc Tigue MA, Kelly CT, Doyle EM, Fogarty WM; The alkaline amylase of the alkaliphilic *Bacillus* sp. IMD 370. Enz Microb Technol, 1995; 17: 570-573.
- [89] Dong G, Vieille C, Savchenko A, eikus JG ; Cloning, sequencing and expression of the gene encoding extracellular α -amylase from *Pyrococcus furiosus* and biochemical characterization of the recombinant enzyme. Appl Environ Microbiol, 1997; 63: 3569-3576.
- [90] Boel E, Brady L, Brzozowski AM, Derewenda Z, Dodson GG, Jensen VJ, Petersen SB, Swift H, Thim L, Woldike HF; Calcium binding in α -amylase: an x-ray diffraction study at 2.1-Å resolution of two enzymes from *Aspergillus*. Biochem, 1990; 29: 6244-6249.
- [91] Tanaka A, Hoshino E; Secondary Calcium-binding parameter of *Bacillus amyloliquefaciens* α -amylase obtained from inhibition kinetics. J Biosci Bioeng, 2003; 96 (3):262-267.
- [92] Hagihara H, Kazuki I, Yasuhiro H, Endo K, Ikawa-Kitayama K, Ozaki K, Kawai S, Ito S; Novel α -amylase that is highly resistant to chelating reagents and chemical oxidants from the alkaliphilic *Bacillus* isolate KSM-K38. App Environ Microbiol, 2001; 67: 1744-1750.
- [93] Nonaka T, Fujihashi M, Kita A, Hagihara H, Ozaki K, Ito S, Miki K; Crystal structure of calcium-free α -amylase from *Bacillus* sp. Strain KSM-K38 (AmyK38) and its sodium ion binding sites. J Biol Chem, 2003; 278: 24818-24824.
- [94] Kobayashi T, Kanai H, Hayashi T, Akiba T, Akaboshi R, HoriKoshi K; Haloalkaliphilic maltotriose-forming α -amylase from the archaeobacterium *Natronococcus* sp. Strain Ah-36. J Bacteriol, 1992; 174: 3439-3444.
- [95] Krishanan T, Chandra AK; Purification and characterization of alpha-amylase from *Bacillus licheniformis* CUMC305. Appl Environ Microbiol, 1983; 44: 270-274.
- [96] Takasaki Y; Production of maltohexaose by α -amylase from *Bacillus circulans* G6. Agricul Biol Chem, 1982; 46: 1539-1547.

[97] Leveque E, Janeck S, Haye B, Belarbi A; Thermophilic archeal amylolytic enzymes. *Enz Microbiol Technol*, 2000; 26: 3-14.

[98] Nielsen AD, Pusey ML, Fuglsang CC, Westh P; A proposed mechanism for the thermal denaturation of a recombinant *Bacillus halmapalus* α -amylase – the effect of calcium ions. *Biochem Biophys Acta*, 2003; 1652 (1): 52-63.

[99] Van der Marrel MJ, van der Veen B, Uitdehaag JC, Leemhuis H, Dijkhuizen L; Properties and applications of starch-converting enzymes of the α -amylase family. *J Biotechnol*, 2002; 94: 137-155.

[100] Mc Tighe MA, Kelly CT, Doyle EM, Fogarty WM; The alkaline amylase of the alkalophilic *Bacillus* sp. IMD 370. *Enz Microb Technol*, 1995; 17: 570-573.

[101] Prakash O, Jaiswal N. α -Amylase: an ideal representative of thermostable enzymes. *Appl Biochem Biotechnol.*, 2010; 160 (8): 2401-2414.

[102] Grant WD, M watha WE, Jonnes BE; Alkaliphiles: ecology, diversity and applications. *FEMS Microbiol Rev*, 1990; 75: 255-270.