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CONTENT

PRODUCTION AND APPLICATION OF α-AMYLASE ENZYME IN TEXTILE INDUSTRY

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Abstract: *Amylases are important hydrolase enzymes which have een widely used in industry. Such enzymes hydrolyze the starch molecules into polymers composed of glucose units. Among amylases α-amylase is in maximum demand due to its wide range of applications. α-Amylase can be obtained from plants, animals and microorganisms. However, α-amylase from fungal and bacterial sources has dominated application in industrial sectors. A large number of microbial α-amylase has applications in different industrial processes such as food, textile, pharmaceutical, paper, fermentation and detergent industries. The production of α-amylase has generally been carried out using submerged fermentation. This review focuses on the production of bacterial and fungal α-amylases, their physical and chemical parameters, and the use of these enzymes in textile industry.*

Keywords: enzymes, α-Amylase, production, applications, textile industry.

PROIZVODNJA I PRIMENA ENZIMA α-AMILAZA U TEKSTILNOJ INDUSTRIJI

Apstrakt: *Amilaze su važni hidrolazni enzimi koji se široko koriste u industriji. Takvi enzimi hidrolizuju molekule skroba u polimere sastavljene od jedinica glukoze. Među amilazama α-amilaza je u maksimalnoj potražnji zbog širokog spektra primene. α-Amilaza se može dobiti od biljaka, životinja i mikroorganizama. Međutim, α-amilaza iz gljivičnih i bakterijskih izvora dominira primenom u industrijskim sektorima. Veliki broj mikrobnih α-amilaza ima primene u različitim industrijskim procesima kao što su prehrambena, tekstilna, farmaceutska, papirna, fermentacijska i deterdžentska industrija. Proizvodnja α-amilaze je generalno sprovedena korišćenjem submerzne fermentacije. Ovaj pregled se fokusira na proizvodnju bakterijskih i gljivičnih α-amilaza, njihovih fizičkih i hemijskih parametara i upotrebu ovih enzima u tekstilnoj industriji.*

Ključne reči: enzimi, α-amilaza, proizvodnja, primena, tekstilna industrija.

1. INTRODUCTION

Enzymes are very effective and specific biocatalysts. Tyey are globular proteins, and like the other proteins, they contain long linear chains of amino acids. Every individual amino acid sequence creates a unique structure, with properties specific to it. The main sources of enzymes are collected from several primary sources, such as plants, microbes and animal tissue [1, 2]. Enzymes are rapidly becoming very important, especially in the spheres of sustainable technology and green chemistry.

One of the sectors of industry that holds a major share in the global pollution is textile industry. Therefore use of enzymes on textiles play a key role as an alternative process for textile processing and have become an integral part of the textile processing industry [3]. The process of use of enzymes is energy saving and does not require any special equipment for heat resistance, pressure or corrosion. Their efficiency, high biodegradability and the mild conditions of working mark their use in a wide range of industrial applications. Enzymes work only on renewable raw materials, so can be handled without any risks [2].

Enzyme usage has been a tradition in textile manufacturing, specifically in the modern industries. Starch and its derivatives make up for 75% of the worldwide usage of sizing agents [5]. Starch is widely used as a sizing agent, being readily available, relatively cheap and based on natural, sustainable raw materials. Use of amylase for removing of starch sizes is among the oldest applications of enzymes [2, 6]. Among the many enzymes that are widely used α-amylase has been in increasing demand due to its role of starch hydrolysis [7]. Amylases are among the most important enzymes and are of great significance for biotechnology.

α-Amylases (E.C.3.2.1.1) are enzymes that catalyses the hydrolysis of internal α-1,4-glycosidic linkages in starch in low molecular weight products, which constitute a mixture of shorter oligosaccharides with varying length with an α-configuration and α-limit dextrins [8, 9, 10]. Today a large number of microbial α-amylases are available commercially and they have a broad spectrum of industrial applications as they are more stable than when prepared with plant and animal α-amylases [11]. In the recent past, there has been extensive research on microbial production of α-amylase. The most widely used source among the bacterial species is the *Bacillus spp*., *B. amyloliquefaciens* and *B. licheniformis* [12]. Fungal sources of α-amylase are confined to mostly to *Aspergillus* species and to only few species of *Penicillium* [13]. The fungal α-amylases are preferred over other microbial sources due to their more accepted GRAS (GenerallyRecognized As Safe) status [14].

The commercial use of α-*amylase* generally does not require purification of the enzyme, but enzyme applications in pharmaceutical and clinical sectors require high purity amylases. Enzymes application comes with many benefits when being compared to the non-enzymatic processes. They can be used in catalytic concentrations with low temperatures and at pH-values near to neutral [15]. Modern production processes in the textile industry can cause breaking of the warp thread. To strengthen the thread, sizing agents are used which strengthen the thread by forming a layer on it and can be removed after the fabric is woven [7, 14]. The enzymatic desizing of cotton with α-amylases is state-of-the-art since many decades [16]. Starch is a polysaccharide composed of two types of polymers: amylose and amylopectin. Amylose constitutes 20-25% of the starch molecule (linear chain consisting of repetitive glucose units linked by α-1,4-glycosidic linkage). Amylopectin constitutes 75-80% of starch (branched chains of glucose units). The amylose is bioconverted to 100% into glucose whereas the amylopectin is bioconverted to 50% into glucose and maltose by the α-amylase [7]. The hydrolysate composition is dependent on the effect of the origin of enzyme, temperature, pH, and the conditions of hydrolysis. Amylases bring about complete removal of the size without any harmful effects on the fabric. The advantage of enzymatic desizing over traditional desizing are:

- there is no adverse effect on cellulose, resulting in strength retention;
- process time of desizing can be reduced;
- neutralisation is not required;
- saving of energy;
- feel of fabric is much softer;
- the use of acid in the conventional method increases the BOD and COD of the process considerably compared to that of bio desizing [17, 18].

2. α-AMYLASE

The α-amylase (α-1,4-glucan-4-glucanohydrolase) can be found in microorganisms, plants and higher organisms. The α-amylase (E.C.3.2.1.1) is a hydrolase enzyme belongs to a family of *endo*-amylases that catalyses the initial hydrolysis of internal α-1,4-glycosidic linkages in starch in low molecular weight products. The end products of α-amylase action are a mixture of maltose, maltotriose, and branched oligosaccharides of 6–8 glucose units that contain both *α*-1,4 and *α*-1,6 linkages [9, 10]. α-Amylases are one of the most popular and important form of industrial amylases. Specificity, thermostability and pH response of the enzymes are critical properties for industrial use [19]. α-Amylases from most bacteria and fungi are quite stable over a wide range of pH from 4 to 11. Optimum temperature of a-amylases is usually related to growth of the producer micro-organism. In general, microbial α-amylases display the highest specificity towards starch. α-Amylase find application in all the industrial processes such as in food, detergents, textiles and in paper industry, for the hydrolysis of starch [14].

3. STARCH

In the green leaves of plants carbon dioxide and water are transformed into glucose and oxygen under the influence of sunlight and with the help of chlorophyll. This process is known as photosynthesis. Starch is a carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. Native starch, the starch as it occurs in the plant, can not be dissolved in cold water. Gelatinizing starch into viscous substances (swellings) is one of the most important characteristics of starch. This phenomenon lies at the basis of the successful application of starch in a large number of sectors. Starch is a polymer of glucose linked to another one through the glycosidic bond. Two types of glucose polymers are present in starch: amylose (*Figure 1*) and amylopectin (*Figure 2*).

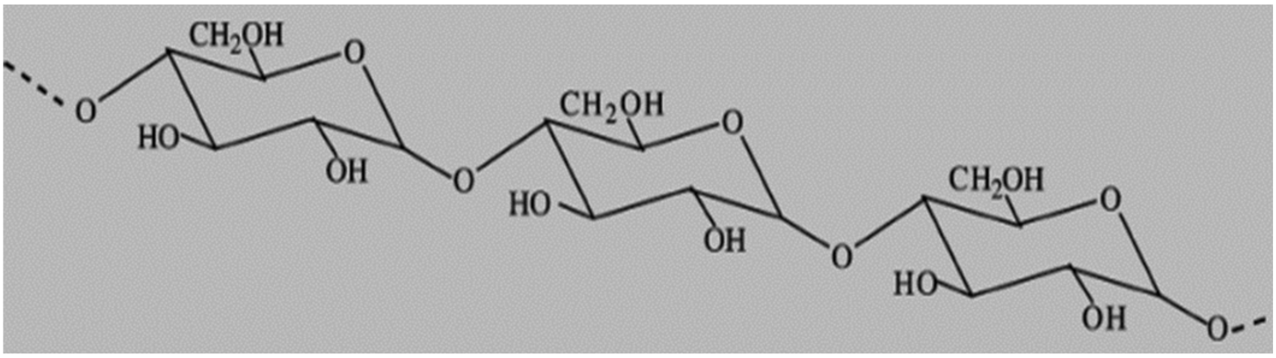

Figure 1. Structure of amylose [21]

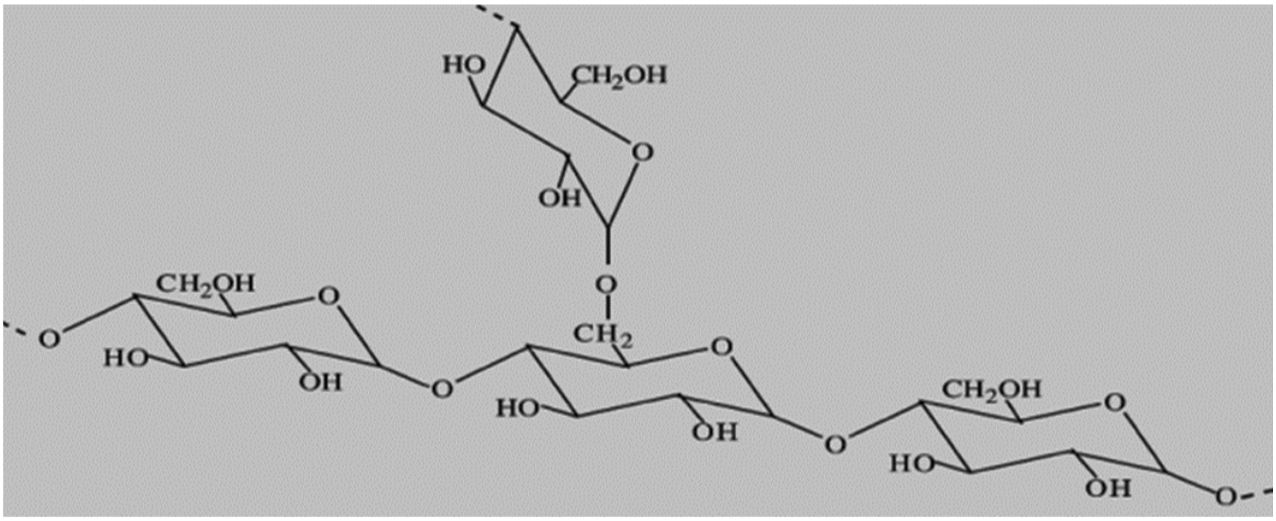

Figure 2. Structure of amylopectin [21]

Amylose and amylopectin have different structures and properties. Amylose is a linear polymer consisting of up to 6000 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 [20]. α-Amylase is a well-known *endo*-amylase. α -Amylase is able to cleave α-1,4 glycosidic bonds present in the inner part of the amylose or amylopectin chain [10, 21]. *Exo*-amylases act on the external glucose residues of amylose or amylopectin and thus produce only glucose (glucoamylase and α-glucosidase), or maltose and β-limit dextrin (β-amylase).

4. MICROBIAL α-AMYLASE PRODUCTION AND PURIFICATION

The 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 [20]. The enzymes are inducible, i.e., produced only when needed. Several methos, such as submerged fermentation (SmF) and solid-state fermentation (SSF) have been successfully used for α-amylase production from various microorganisms. Submerged fermentation has been traditionally used for the production of industrially important enzymes because of the ease of control of different parameters such as pH, temperature, aeration and oxygen transfer and moisture [22]. The optimization of fermentation conditions, are important in the development of fermentation processes due to their impact on the economy [23]. Agro-industrial residues such as wheat bran, rice bran, maize bran, rice husk, coconut oil cake, mustard oil cake, corn bran, etc., are generally considered the best substrates for processes [20, 24].

α-Amylase can be produced by different species of microorganisms, but for commercial applications α-amylase is mainly derived from the genus *Bacillus* [25]. α-Amylases produced from *Bacillus licheniformis*, *Bacillus stearothermophilus*, and *Bacillus amyloliquefaciens* find potential application in a number of industrial processes such as in food, fermentation, textiles and paper industries [26].

Thermostability is a desired characteristic of most of the industrial enzymes. Thermostable enzymes isolated from thermophilic organisms have found a number of commercial applications because of their stability. *Bacillus subtilis, Bacillus stearothermophilus, Bacillus licheniformis,* and *Bacillus amyloliquefaciens* are known to be good producers of thermostable α-amylase [27].

Enzymes produced by some halophilic bacteria such as *Chromohalobacter sp.*, *Halobacillus sp.*, *Halomonas meridiana*, and *Bacillus dipsosauri* have optimal activity at high salinities and could therefore be used in many harsh industrial processes [28].

The fungal source used predominantly for commercial production of α-Amylase are the strains of *Aspergillus spp*. *Aspergillus oryzae, A. niger* and *A. awamori* [12]. Genetically modified organisms are also being used for production of α-amylase. *Bacillus amyloliquefaciens UNG-16* was subjected to mutation by both the chemical and radiation method [29].

Enzymes used for industrial applications generally require less downstream processing and are usually crude preparations. The commercial use of α-amylase generally does not require purification of the enzyme. Purification methods commonly employed are ion exchange, gel filtration, precipitation, liquid-liquid extraction and reverse phase chromatography depending on the properties of the enzyme desired [30, 31]. These conventional multi-step methods requires expensive equipments at each step. However, liquid–liquid extractions consist of an interesting purification alternative. Liquid-liquid extraction is the transfer of certain components from one phase to another when immiscible or partially soluble liquid phases are brought into contact with each other. This purification has been successfully carried out on a large scale for more than a decade. Advantages of using this system are lower viscosity, lower cost of chemicals and shorter phase separation time [32]. From the fermented mass by filtration and centrifugation can be obtained the crude extracellular enzyme sample [33].

5. APPLICATION OF α-AMYLASE IN TEXTILE INDUSTRY

The textile industry is one of the largest contributors to environmental pollution from desizing of fabrics, bleaching chemicals and dye. In such industries, enzymes are used to allow the development of environmentally friendly technologies in fiber processing [34]. The main classes of enzymes involved in cotton pre-treatment and finishing processes are hydrolase and oxidoreductase. The group of hydrolase includes amylase, cellulase, cutinase, protease, pectinase and lipase and the group of oxidoreductase includes catalase, laccase, peroxidase, and ligninase [35]. Applications of enzymes in textiles industries are shown in *Table 1*.

Table 1. Uses of enzymes in textile industry

Enzyme	Microorganisms	Use
Amylase	*Bacillus* sp., *B. licheniformis*	Desizing
Cellulase	*Aspergillus niger, Penicillium funiculosum*	Cotton softening, denim finishing
Cutinase	*Pseudomonas mendocina*	Cotton scouring
Protease	*Aspergillus niger, B. subtilis*	Removal of wool fiber scales, degumming of silk
Pectate lyase	*Bacillus* sp., *Pseudomonas* sp.	Bioscouring
Lipase	*Candida Antarctica*	Removal of size lubricants
Catalase	*Aspergillus* sp.	Bleach termination
Laccase	*Bacillus subtilis*	Bleaching, fabric dyeing
Ligninase	*Trametes versicolor, Phlebia radiata*	Wool finishing

α-Amylase is gaining increased attention due to its starch hydrolyzing properties and widely used applications on the industrial front. Enzymes have replaced the previously used chemical methods of hydrolysis in various industrial sectors to make the process easie and environment friendly [7, 25].

Amylases are used in textile industry for desizing process. Sizing agents like starch are applied to yarn before fabric production to ensure a fast and secure weaving process. Desizing involves the removal of starch from the fabric. α-Amylase is employed to cleave starch particles randomly into water soluble components that can be removed by washing. This also reduced the discharge of waste chemicals to the environment. The α-amylases remove selectively the size and do not attack the fibres [14, 25, 36]. Before the discovery of amylases, desizing used to be carried out by treating the fabric with acid, alkali or oxidizing agents at high temperatures and the chemical treatment was not totally effective in removing the starch, and also resulted in a degradation of the cotton fibre. [37].

6. CONCLUSION

α-Amylase can be produced using microbes by submerged fermentation (SmF) or solid state fermentation (SSF) which employs waste products of other processes. SmF is primarily used for the extraction of secondary metabolites that need to be used in liquid form. SmF allows the utilization of genetically modified organisms to a greater extent than SSF.

The prospects of industrial uses of microbial enzymes have increased greatly in 21st century and continuously increasing as enzymes have significant potential for many industries. Enzymes of microbial origin have significant potential in textile industry, and consequently in the development of green environment.

The search for new microorganisms that can be used for amylase production is a continuous process. Nowadays amylases are commercialized and preferred for desizing due to their high efficiency and specificity, completely removing the size without any harmful effects on the fabric [38, 39]. There is still considerable potential for new and improved enzyme applications in future textile processing. More recently, many authors have presented good results in developing α-amylase purification techniques.

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