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Amylases as a Tool for Industrial Application: A Review

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Amylases are main enzymes used in industry which hydrolyze the starch molecules into polymers composed of glucose units. Amylases have potential application in industrial processes such as food, fermentation and pharmaceutical industries. The production of α-amylase is essential for conversion of starches into oligosaccharides. Starch converting enzymes are used in the production of malt dextrin, modified starches, or glucose and fructose syrups. A large number of microbial α-amylases have applications in different industrial sectors such as food, textile, paper and detergent industries. This review focuses on the role of these enzymes as industrial applications.

Key words: Amylases, Glucoamylase, Pullulanase, Industries.

Enzymes that participate in the hydrolytic degradation of starch are referred as amylolytic enzymes or amylases. Amylases are classified as α-amylase, β-amylase, gluco-amylase, pullulanase and inso-amylase. Basing on property, these enzymes are classified into two categories, endoamylases and exoamylases[1]. Endoamylases catalyse hydrolysis in a random manner in the interior of the starch molecule and causes the formation of linear and branched oligosaccharides of various chain lengths. Exoamylases hydrolyse from the non-reducing end resulting in short end products. A large array of amylases involved in the complete breakdown of starch. Enzymatic degradation of starch yields glucose, maltose and other low molecular weight sugars. The initial step in starch hydrolysis entails disruption of the starch granule[1]. Solubilisation of the granules facilitates subsequent catalytic degradation. Gelatinisation is normally achieved by heating the starch to temperatures often in excess of 100°C for several minutes. α- Amylase may be added immediately prior to the heating step, in order to render more efficient the process of granule disruption. Once the granules have been disrupted, additional α-amylase is added in order to liquefy the starch slurry. This process reduces the viscosity of the starch solution[2].

α- Amylase

Amylases are among the most important enzymes and are of great significance for biotechnology, constituting a class of industrial enzymes having approximately 25% of the world enzyme market[3,4]. α-Amylase is an endo-acting enzyme, catalyzing the random hydrolysis of internal α-1,4 glycosidic linkages present in the starch substrate. However, α - amylases which are in most demand hydrolyses the α-1, 4 glycosidic

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bond in the interior of the molecule[1]. These enzymes are incapable of hydrolyzing α-1, 6 glycosidic linkages present at branch points of amylopectin chains. The α-amylase family consists of a large group of starch hydrolases and related enzymes are known as glycosyl hydrolases[5]. Two of the more commonly used bacterial α-amylases are those isolated from *Bacillus amyloliquefaciens* and *Bacillus licheniformis*. *Bacillus* amylases exhibit a pH optimum close to neutrality and are stabilized by the presence of calcium ions. á-amylase produced by *Bacillus licheniformis* is particularly suited to industrial applications because of its thermal stability. This enzyme consists of 483 amino acids and has a molecularweight of 55.2 kDa. Its pH optimum is 6.0 and its temperature optimum is 90°C. Most other α-amylases, including those produced by *B. amyloliquefaciens*, are rapidly inactivated at temperatures above 40°C[6].

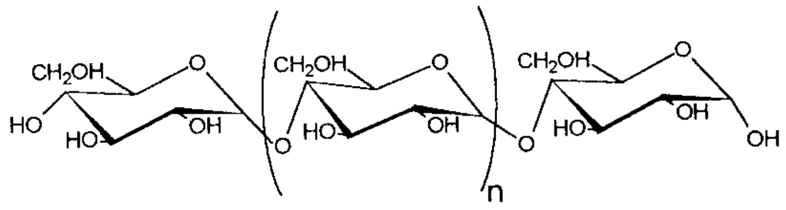

Fig. 1. Structure of Amylase

Glucoamylase

Glucoamylases are exoacting enzymes that cleave both α-1-4 and α-1-6 bonds A number of glycoamylases are known to hydrolyse both α-1-4 and α-1-6 glycosidic linkage, their activity towards the latter is very low[7]. The action of this enzyme liberates one molecule of β-d-glucose at a time, causing the complete conversion of polysaccharides to glucose. This process is described as the saccharification of starch[6]. These enzymes are used for the production of high-glucose syrup from starch-originated polysaccharides. In industrial production, the glucoamylase from *A. niger* is generally used. The glucose syrup produced by this method is often used by the food and related industries. A further application entails conversion of some of the glucose to fructose by enzyme isomerase, thus producing high fructose-syrup. Other applications include the production of different antibiotics, amino acids, ethanol and organic edible acids[8] .

Pullulanase

Pullulanase, debranching enzyme cleaves α-1-6 bonds in starch. It is used in the reduction of high-glucose syrup from polysaccharides. As pullulanse is able to hydrolyze α-1-6 linkages in dextrins it is used in combination with other amylolytic enzymes (β-amylase, α-amylase, α-glucosidase and glucoamylase) in starch saccharification. Wind[9] reported that for industrial purposes, the pullulanase from *B. acidopullulyticus* is often used in combination with the glucoamylase from *A. niger.* In addition to α-1-6 bonds, type II pullulanase cleaves α-1-4 bonds and is able to saccharify starch directly into maltotetraose, maltotrise, and maltobiose. Pullulanase is usually referred to as amylopullulanase[10].

Applications of amylase enzymes

Amylases are used in industries such as the starch, textile, detergent and baking industries[11].

Detergent industry

The use of enzymes in detergents formulations enhances the detergents ability to remove tough stains and making the detergent environmentally safe. Amylases are the second type of enzymes used in the formulation of enzymatic detergent, and 90% of all liquid detergents contain these enzymes[1,12]. Amylases have activity at lower temperatures and alkaline pH, maintaining the necessary stability under detergent conditions and the oxidative stability of amylases is one of the most important criteria for their use in detergents where the washing environment is very oxidizing[13,14]. Removal of starch from surfaces is also important in providing a whiteness benefit, since starch can be an attractant for many types of particulate soils. Examples of amylases used in the detergent industry are derived from *Bacillus* or *Aspergillus*[12]. Amylases are used as ingredients in modern compact detergents. The advantage of enzyme application in detergents is due to much milder conditions than with enzyme free detergents[1]. Amylases are used as detergent additives represent the largest application in industries both in terms of volume and value. The use of these enzymes in detergents affords numerous advantages such as energy saving because they require lower temperature and the reduction or replacement of other components that may be more harmful to the environment[15]. The major component of these enzymes is proteases, but other and very different hydrolases are introduced to provide various benefits, such as the efficient removal of specific stains[14]. Some of these enzymes are obtainable from renewable sources are biodegradable and act without risking aquatic life or having a negative effect upon residual water treatments[15].

Fuel alcohol production

The bioconversion of starch into ethanol involves liquefaction and saccharification, where starch is converted into sugar using an amylolytic microorganism or enzymes such as α-amylase, followed by fermentation, where sugar is converted into ethanol using an ethanol fermenting microorganism such as yeast *Saccharomyces cerevisiae*[16, 17]. Among bacteria, α-amylase obtained from thermoresistant bacteria like *Bacillus licheniformis* or from engineered strains of *Escherichia coli* or *Bacillus subtilis* is used during the first step of hydrolysis of starch suspensions[18].

Starch liquefaction and saccharification

α-amylase involved in hydrolysis of starch into glucose and fructose[1]. The enzymatic conversion of starch to high fructose corn syrup is a well-established process. Because of their high sweetening property, these are used in huge quantities in the beverages industry as sweeteners for soft drinks. Enzymes used in the starch industry are also subjected to improvements[19]. The first step in the process is the conversion of starch to oligomaltodextrins by the action of α-amylase. α-amylases with optimised properties such as enhanced thermal stability, acid tolerance and ability to function without the addition of calcium have been developed in the industry [2,19].

Textile applications

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 which serves as the strengthening agent to prevent breaking of the warp thread during the weaving process. The α-amylases remove selectively the size and do not attack the fibres[20,1]. Amylase from *Bacillus* stain was employed in textile industries for quite a long time. In textile weaving, a starch paste is applied for warping which gives strength to the textile at weaving and also prevents the loss of string by friction, cutting, generation of static electricity on the string by giving softness to the surface of string due to laid down warp[21]. After weaving the cloth the starch has to be removed, it is at this point of the process that α-amylase is introduced to help with the removal of the starch[21,14]. Kirk *et al.* [14] reported that enzymes are used in seven various unit operations in textile wet processing and the manufacturing of denim.

Paper industry

The use of α-amylases in the pulp and paper industry is for the modification of starch of coated paper, i.e. for the production of low-viscosity, high molecular weight starch[1]. Examples of amylases obtained from microorganisms used in paper industry includes Amizyme, Termamy, Fungamyl and α-amylase(G9995)[22]. Starch has too high viscosity for paper sizing and this can be modified by degrading the polymer with α-amylases in a batch or continuous processes. Starch is a good sizing agent for the finishing of paper and improving the quality besides being a good coating for the paper. The size enhances the stiffness and strength in paper [22,1].

Enzymes for the food industry

Amylases are extensively employed in processed-food industry such as baking, brewing, preparation of digestive aids, production of cakes, fruit juices and starch syrups[23]. The enzyme application in the food industry are many and diverse ranging from texturising to flavouring. In food applications the enzymes are applied to processed food products as processing agents upstream from the final product. Optimisations of enzymes are advanced for existing applications and in the use of recombinant protein production to provide efficient mono-component enzymes that do not have potential detrimental side-effects[14]. Malt and microbial α-amylases enzymes have been widely used in the baking industry[24]. Enzymes are used in bread and rolls to give these products a higher volume, better colour, improve flavour and as an anti-staling agent. Malt preparations have led the way and created opportunities for many enzymes to be used commercially in baking. The α-amylases used in baking have been cereal enzymes from barley malt and microbial enzymes from both fungi and bacteria[25]. Supplementation of flour with exogenous fungal α-amylase has a higher activity and enhances the rate of fermentation and reduces the viscosity of the dough. There is an improvement in the volume and texture of the product, which also generates additional sugar in the dough, subsequently improving the taste, crust colour and toasting qualities of the bread[26]. Currently the use of enzymes in the baking industry is moving towards the further understanding of bread staling and the mechanisms behind the enzymatic prevention of staling when using á-amylases and zylanases[27]. α-amylases also have an anti-staling effect in bread baking, and they improve the softness retention of baked goods, increasing the shelf life of these products[1,28,19]. Thermostable maltogenic amylase of *Bacillus stearothermophilus* is used commercially in the bakery industry[19]. Amylases are also used for the clarification of beer or fruit juices, or for the pretreatment of animal feed to improve the digestibility of fiber[1,30,19].

Future perspectives

Amylases are important in many industrial processes. A number of microbial sources exist for the efficient production of this enzyme, but only a few selected strains of fungi and bacteria meet the criteria for commercial production. Amylases have immense applications in microbiological and biotechnological industries. There is lot of necessary to design newer amylases with well documented, desired selectivity and substrate tolerance.

Most of the studies performed so far have been concentrated with the screening, isolation, production, purification, characterization and applications of amylases enzymes in increasing demands in industry. Some reports are available on the applications of amylases in other clinical and pharmaceutical industries. Study of the molecular aspects of amylases and engineering of enzymes that are more robust with respect to their pH and temperature kinetics by the techniques of protein engineering and site directed mutagenesis should receive increased attention in the coming times. Future studies on amylase enzymes should be devoted to the understanding of the regulatory mechanism of the enzyme secretion at the molecular level and the mechanism of action of different amylases enzyme.

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