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Process Optimization and Industrial Application of Microbial Amylases: A Review

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Abstract: Enzymes are the most important and special types of protein molecules, which can be produced by living organisms to bring about specific biochemical reactions, generally forming parts of the metabolic processes of the cell. They are usually catalyzes a particular biochemical reaction or closely associated responses. Enzymes work by binding to reactant molecules and considered to be faster than chemical catalysts. They are being used in various sectors such as detergent, paper, textile, food and many others industrial applications. Moreover, they play major role in the conversion of starch into maltose, production of beverages like beer, treatment of digestive disorders and production of cheese from milk. Among the enzymes, amylase plays a significant role in all stages of metabolism and biochemical reactions. Certain amylases are used as organic catalysts in numerous processes at an industrial scale. Amylases can be produced from animal, plant and microbial sources. However, microbial enzymes are known to be superior enzymes in various industrial applications. Microorganisms such as bacteria, fungi and yeasts have been globally studied for the biosynthesis of economically viable preparations of various enzymes for commercial applications. To obtain maximum yield of amylase, media optimization plays a significant role throughout the production process. Therefore, the present review focused on the production, process optimization and industrial applications of microbial amylases.

Keyword: Microbial Amylases, Submerged Fermentation, Solid-State Fermentation, Enzymes

1. Introduction

Enzymes are highly efficient biological catalysts which start and speed up thousands of biochemical reactions in living cells [28], and perform all synthetic and degradative responses in living organisms. Enzymes are “green” biological catalysts that have changed the way we prepare our food and improve nutritional value of different food products [1]. Enzymes are the complex protein molecules which are produced by living cells to increase the rate of an immense and diverse set of chemical reactions required for life. They are involved in all processes essential for life such as, DNA replication and transcription, protein synthesis, metabolism, cell regulation and signal transduction often via kinases and phosphatases [9].

Enzymes are highly specific both in the reactions that they catalyze and in their choice of reactants, which are known as substrates. Enzymes have high catalytic rates and work in aqueous solution. An enzyme typically catalyzes a single chemical reaction or a set of closely related reactions [7]. Enzymes are proteins that act as catalysts which can speed up chemical reactions that occur in the cells. An enzyme can add atoms to a molecule, move atoms from a molecule, split a large molecule into smaller molecules, or join together smaller molecules to form a large molecule. Enzymes have unique shapes and reactive sites that allow them to bind with specific molecules namely substrate molecules [18].

Among these, amylases are the most important hydrolytic enzymes that catalyze the hydrolysis of starch into sugars by cleaving the α-1, 4 glucosidal linkage of complex polysaccharides. Amylases are present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Amylases are the most important enzymes and have the great significance for biotechnology, constituting a class of industrial enzymes having approximately 25-30% of the world enzyme market. Presently, amylases become a great commercial value in biotechnological applications ranging from food, textile, paper and pulp, pharmaceuticals, baking and beverages, detergent and leather industries [15]. Due to its potential application in different industrial sectors, the demands of amylase are being increased time to time and have got global attention. Therefore, the objective of this paper was to review about the production, optimization and industrial application of microbial amylases.

1.1. *History of Amylases*

The history of amylases has been investigated in 1811 after the first starch degrading enzyme was found by Kirchhoff in wheat and laid down the foundation for the discovery and research on amylase. This was followed by several reports of digestive amylases and malt amylases [17]. The first amylase was isolated by AnselmePayen in 1833. However, amylase was the first enzyme produced industrially from a fungal source in 1894, which was used as a pharmaceutical aid for the treatment of digestive disorders [14].

2. The Types of Amylase

Amylases are the most important enzyme groups within the field of biotechnology. They are a class of enzymes that are capable of digesting glycosidic linkages in starch components and glycogen molecules [4]. Based on their catalytic efficiency in starch hydrolysis process, amylases are classified as endoamylases and exoamylases [28].

2.1. *Alpha Amylase*

Alpha amylase is a typical calcium containing enzyme which catalyze the hydrolysis of starch and related carbohydrates by randomly cleaving internal α-1, 4-glycosidic linkage to produce glucose, maltose, maltotriose, and other oligosaccharides. It is completely unable to function in the absence of calcium [23]. Alpha Amylases are extracellular enzymes that are important in starch processing industries. The optimal pH of alpha amylases ranges from 2 to 12 and they are thermostable. The application of these enzymes has been established in starch liquefaction, paper, food, sugar and pharmaceutical industries. In the food industry, amylolytic enzymes have a large scale of applications, such as the production of glucose syrups, high fructose corn syrups, maltose syrup, and reduction of viscosity of sugar syrups, reduction of turbidity to produce clarified fruit juice for longer shelf-life, solubilization and saccharification of starch and delay the staling of baked products [10].

2.1.1. *Structural Characteristics of α-Amylase*

The α-amylase catalyzes the initial hydrolysis of starch into shorter oligosaccharides through the cleavage of α-D-1, 4-glycosidic bonds belongs to endo-amylases family. Neither α-1, 6-linkages nor terminal glucose residues can be broken by α-amylase. The end products of α-amylase activity are oligosaccharides with different length of α-configuration and α-limit dextrin, which form a mixture of smaller oligosaccharides consisting of maltose, maltotriose and a number of α-l, 6 and α-1, 4 oligoglucans. Different other enzymes participate in starch breakdown process, but among them, α-amylase is the most important for the initiation of this process [12].

Figure1. *Three dimensional structure of α- Amylase [2]*

2.2. *Beta Amylase*

Beta amylase is an exo-hydrolase enzyme that acts from the non-reducing end of a polysaccharide chain by hydrolysis of α-1, 4-glucan linkages to yield successive maltose units. Since it is unable to cleave branched linkages in branched polysaccharides such as glycogen or amylopectin, the hydrolysis is incomplete and dextrin units remain. Primary sources of β-amylase are the seeds of higher plants and sweet potatoes. During ripening of fruits, β-amylase breaks down starch into maltose resulting in the sweetness of ripened fruit. The optimal pH of the enzyme ranges from 4.0 to 5.5. Beta-amylase can be used for different applications on the research as well as industrial front. It can be used for structural studies of starch and glycogen molecules produced by various methods. Additionally, it is used to produce high maltose syrups and has crucial role in brewing and distilling industry [27].

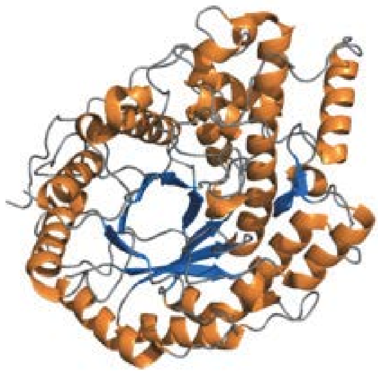

Figure2. *Structure of β- Amylase [2]*

3. Microbial Source of Amylase

Depending on the microbial sources, the level of amylase production greatly differs. Strains isolated from starch- or amylase-rich environments can produce higher amounts of enzyme. Hence, for the commercial production of specific enzymes, effective microbial strains which have the capacity to produce the highest amounts of particular enzymes are desired [18].

Microorganisms are the primary source of amylase, because they are cultured in large quantities in short period of time and genetic manipulation can be done to enhance the enzyme production [2]. In addition, microbial amylases are the most produced and used in industry, due to their cost effectiveness, consistency, short life span and high productivity rate ([13; 6]. Microbial amylases obtained from bacteria, fungi, and yeast has been used predominantly in industrial sectors and scientific research [14].

The use of amylase obtained from fungal and bacterial sources have dominated in different industrial sectors. But bacterial enzymes are the cheapest sources and widely used in different industries such as food, textile, and beverage, as well as in waste treatment [3]. The production of microbial amylases from bacteria depends on the type of strain, composition of medium, method of cultivation, cell growth, nutrient requirements (particularly carbon and nitrogen), incubation period, pH, temperature and metal ions. Therefore, the nature and concentration of nitrogen source in the culture medium affects bacterial growth and amylase production and acts as pH stabilizer [16].

Filamentous fungi have been widely used to produce hydrolytic enzymes for industrial applications [24]. Utilization of the potential amylase producing fungal isolate is vital to be used in starch degradation process [5]. Yeasts are unicellular fungi with ubiquitous distribution in many ecosystems. In recent years, the capability of some yeast species to degrade starch has increased its potentiality in biotechnological applications, such as a source of hydrolytic enzymes. Although yeasts are not among the industrial producers of amylases, the enzyme is widely distributed in many yeast species [29]. Moreover, yeast isolates have the capability to produce cold active amylase [11].

4. Process Optimization for Amylase Production

Optimization is the process which is used to maintain a balance between the medium components and minimize the utilization of unused products at the end of the processing. 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 [15]. Various factors such as microbial sources, process optimization and method of fermentation strongly impacts on the desired product of the enzyme. Different physical and chemical parameters such as incubation period, pH, temperature, carbon source and nitrogen source and minerals of microbial fermentation process play great role in enzyme production. The formation of clear zones around the colonies refers to the hydrolysis process of amylase production. The enzymes maintain high stability over a particular setting of temperature and pH. Temperature is said to be the prime aspect for almost all enzyme activities. It is also essential for the enzymes to be active and stable even at peak temperature levels [20].

4.1. Effect of Temperature, pH and Incubation Period

Optimum temperature ranges are required for maximum amylase production. On the other hand, optimum hydrogen ion concentration plays a vital role for the stability and maximum enzyme production. Incubation time play a very crucial role in bacterial metabolic activity and growth. Production of amylase may not take a lot of time, but considerable increase in its yield is noted after its submission to an incubation period of several minutes. Enzyme activity increases when incubation time increase till it reaches the optimum duration. In most cases, the production of enzyme begins to decline if the incubation time is further increased. This could be due to the depletion of nutrients in the medium or release of toxic substances [26].

4.2. Effect of Carbon and Nitrogen Sources

Different carbon and nitrogen sources present in the fermentation medium are the major factors affecting microorganism's growth and amylase production as well. The effect of different carbon source such as glucose, galactose, maltose, sucrose, lactose, fructose and dextrose can be used as carbon sources. On the other hand, organic and inorganic nitrogen sources like peptone, casein, urea, yeast or beef extract, tryptone or soybean meal, ammonium sulphate, ammonium nitrate and ammonium chloride, can be used as sole nitrogen source for the growth and production of amylase [20].

4.3. Effect of Inoculums Size

For the production of amylase, inoculums size plays a significant role. It is one of the most important parameter which affects the enzyme activity by affecting substrate utilization rate. It is analyzed that by increasing the overall size of the inoculums, its production is enhanced. An accurate inoculums size is not usually followed [20].

5. Industrial Applications of Microbial Amylases

Applications of bacterial amylases at industrial level have inspired the interest to explore several microbes as bio-resources for their amylolytic activity. Amylases have potential application in a number of industrial processes such as in the food, textiles, paper industries, bread making, glucose and fructose syrups, detergents, fuel ethanol from starches, fruit juices, alcoholic beverages, sweeteners, pharmaceutical, chemicals and digestive aid. The potential industrial applications of enzymes are determined by the ability to screen new and improved enzymes, their fermentation and purification in large scale, and the formulations of enzymes. Bacterial α-amylases are now also used in areas of clinical, medicinal, and analytical chemistry. It is extensively used in pharmaceutical industries in digestive tonics, for hydrolysis of starch to produce different sugars like glucose and maltose which have several applications. The most widespread applications of α-amylases are in the starch based food and non-food industry, which are used for starch hydrolysis in the starch liquefaction process that alters starch into fructose and glucose syrups [19].

5.1. Food Industry

The beginning of current biotechnology has got several modifications in the food industry. The application of enzymes in food industry is different. Amylases are commonly employed in processed food industry including baking, beverages, starch syrups etc. Amylases are used in the clarification of juices to increase the production of clear juice [22]. In addition, α-amylase is added to the dough and hence it plays a vital role in bread making process. This makes the starch to break down into small dextrins, which allows yeast to work constantly throughout the dough fermentation process. Hence amylase can increase bread volume and crumb texture. Furthermore, small oligosaccharides and sugars can increase the Maillard reactions responsible for the browning of the crust and the development of an attractive baked flavor [19].

Amylases play a significant role to increase the rate of fermentation and the reduction of the viscosity of dough, resulting in improvements in the volume and texture of the product. Moreover, it also produces additional sugar in the dough, which improves the taste, crust color and toasting qualities of the bread. Besides generating fermentable compounds, amylases also have an anti-staling effect in bread baking; improve the softness retention of baked goods and increasing the shelf life of these products [10; 18].

5.2. Starch Processing Industry

Starch is an important food ingredient and used as a substrate for the production of many industrial products. Starch is the second main polysaccharide food store in nature and it is the principal constituent of majority of staple foods and used in various food and non-food industries. Starch is widely used in many industries including food, alcohol, paper, textiles etc. Enzymatic starch hydrolysis was the first industrial enzyme-driven process, established since the 1840s in France [8]. Starch is a widely used renewable resource. It is present as a storage compound in the leaves, tubers, seeds and roots of many plants. The starch is usually modified chemically or enzymatically to a wide variety of derivatives. However, the degradation of starch in industrial sector is usually initiated by α-amylases which are very common enzymes in micro-organisms [9].

The widest applications of α-amylases are in the starch industry, which are used for starch hydrolysis in the starch liquefaction process that converts starch into fructose and glucose syrups. Chemical processing of starch has disadvantages such as requirement of high temperature, low pH, and lower yield of glucose, unpleasant taste of compounds and synthesis of undesired color; whereas enzyme mediated hydrolysis has the abilities to solve the above mentioned conditions. The enzymatic conversion of starch to syrups is widely used in the starch industry, which includes: gelatinization, involves the dissolution of starch granules to form a viscous suspension. The next process includes liquefaction, which involves partial hydrolysis of starch to reduce viscosity; and the third is saccharification, involving the production of glucose and maltose as end products via further hydrolysis [18].

5.3. Detergent Industry

Currently, amylase plays vital role in local detergent industries [21].The use of enzymes in detergents has increased with the changing methods of dishwashing and laundry. Amylases are supplemented in liquid laundry and dishwashing detergent formulations to remove residues of starch based food products. Consumers choose to use cold water and mild conditions which needs the detergent to work in those activities. The enzymes are environmentally safe and work at mild conditions. Alpha amylase is used to digest the starch containing food particles into smaller water soluble oligosaccharides. Hence removal of starch is also important to maintain the whiteness of clothes. The stability of α-amylase at low temperature and alkaline pH contributes to its extensive use in detergents [26].

5.4. Paper Industry

Due to its various advantages, the demand of microbial source enzymes has increased time to time in paper and pulp industry. The uses of enzymes in this industry reduce energy consumption, processing time and quantity of chemicals required for processing. The primary application of amylase in paper industry is to produce high molecular weight starch with low viscosity through modification of starch of coated paper. The coating treatment makes the smooth and strong paper to enhance the writing quality. Amylase is very important to degrade polymer partly in a batch or continuous process and maintain the starch viscosity for paper sizing. Starch is a good sizing agent for the finishing of paper, improving the quality and erasability. Paper sizing improve the quality of the finished product and improve the strength and stiffness in papers. Low temperature active amylases are crucial to reduce starch viscosity for proper coating of paper [25; 18].

5.5. Fuel Alcohol Industry

Ethanol is the most utilized liquid biofuel. For ethanol production, starch is the most abundant substrate due to its low price and easy availability as a raw material. During this process, starch is solubilized and subjected into two enzymatic steps to obtain fermentable sugars. The first step is bioconversion of starch into ethanol through liquefaction and saccharification process. The second step is the hydrolysis of starch into sugar using an amylolytic enzyme such as α-amylase, followed by fermentation. After that sugar is converted into ethanol using an ethanol fermenting microorganism like Saccharomyces cerevisia [18].

5.6. Textile Industry

Textile industries are extensively using alpha amylases to hydrolyze and solubilize the starch, which then wash out of the cloth for increasing the stiffness of the finished products. In this industry, the starch is usually removed by the application of alpha amylase for desizing process. That means alpha amylase is used as desizing agent for removing starch from the grey cloth before its further processing in bleaching and dyeing. Hence, removal of starch increases uniform wet processing. In textile weaving, starch paste is used to give strength and prevents loss of strings. After weaving, amylase is added that specifically catalyze hydrolysis of starch to water soluble dextrins. Amylase efficiently removes starch without damaging the fabric. The enzymatic desizing of cotton with amylases is state of-the-art since many decades. Many garments especially the ubiquitous ʿJean’ are desized after mashing and the desired fabrics are finally laundered and rinsed [19].

6. Conclusion

Amylases are among the most important and widely used industrial enzymes that catalyze the hydrolysis of starch into sugars by cleaving glycosidic bonds which is found in starch and glycogen molecules. Due to their cost effectiveness, consistency, short life span and high productivity rate, microbial amylases particularly obtained from bacterial and fungal sources are the most desirable and commonly used in various industries. Hence, to meet the required amounts or demands of many industries, media optimization plays a crucial role for the overproduction of enzymes in large quantities. In the bright of current biotechnology, microbial amylases are primarily used in food, textile, paper, beverage, pharmaceutical, detergent and starch processing industries. Out of them, the demands of amylase in food and starch based industries are becoming high. Although microbial amylases have been widely studied by many researchers, there is a need to obtain efficient enzymes to maximize enzyme production.

Conflicts of Interest

The authors declare that they have no competing interests.

Data Availability

Data will available on request.

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