



PRODUCTION OF AMYLASE FROM MICROBIAL SOURCES: A REVIEW

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Received 22nd Jan. 2021; Revised 24th Feb. 2021; Accepted 25th March 2021; Available online 1st April 2021

<https://doi.org/10.31032/IJBPAS/2021/10.4.1038>

ABSTRACT

Amylase is one of oldest enzyme. Amylase is naturally produced in humans by the salivary glands and pancreas. It can also be found naturally in plants and animals. Amylase is an enzyme that converts the hydrolysis of starch into sugar. Amylase divided into three types: 1) α -amylase 2) β -amylase and 3) γ -amylase. α -amylase: Alpha-amylase, is an enzyme EC 3.2.1.1 that hydrolyses alpha bonds of large, it is the major form of amylase found in humans and other mammals. β -amylase the enzymes in the second group, exoamylases, are exclusively cleaved α , 1-4. glycoside bonds such as beta-amylase or cleavage glycoside bonds α , 1-4 and α , 1-6. Amyl glucosidase or glucoamylase and α -glucosidase. γ -amylase: γ -amylase cuts glycosidic α (1-6) compounds, in addition to purifying the final α (1-4) glycosidic links in the non-limiting structure of amylose and amylopectin, express glucose. Unlike other types of amylase, γ -amylase works best in acidic environments and contains 3 pH high. Two major groups of microorganisms play an important role in amylase production, namely: bacteria and fungi. Starch is a substrate used in the production of amylase. In between two fermentation processes used in the production of amylase (e.g., immersed boiling and solid state fermentation), this has great benefits as it saves costs,

produces less pollution, and is higher a lot of product. In this review, various applications, production and factors affecting amylase activities are discussed.

Keywords: Enzyme, amylase, production, fermentation, SSF, SmF

INTRODUCTION

Amylase is perhaps the most seasoned catalyst known, the historical backdrop of amylase started in 1815 when Kirchhoff established the framework for the revelation of amylase, Kirchhoff performed an investigation, which changed over four pieces of water, two pieces of starch, and malt into a starch glue. This glue started to condense into sweet syrup; his outcomes demonstrated that gluten could change over a bigger amount of starch into sugar. In 1831, Erhard Friedrich Leuchs portrayed the hydrolysis of starch by spit, due to the presence of a protein in salivation, "ptyalin", an amylase [1]. In 1833, Anselme Payen and Jean-François Persoz further portray and confine diastase (amylase) in powder structure from grain malt, demonstrating it to be heat-labile [2]. Compounds are organic impetuses which are a vital segment of organic responses. The utilization of substance impetuses has been followed for an extremely long time. **α -amylase:** Alpha-amylase, is an enzyme EC 3.2.1.1 that hydrolyses alpha bonds of large, it is the major form of amylase found in humans and other mammals. **β -amylase** the enzymes in the second group, exoamylases, are exclusively cleaved α , 1-4. glycoside bonds such as

beta-amylase or cleavage glycoside bonds α ,1-4 and α ,1-6. [29] Amyl glucosidase or glucoamylase and α -glucosidase. **Γ -amylase:** Y1-amylase cuts glycosidic α (1-6) compounds, in addition to purifying the final α (1-4) glycosidic links in the non-limiting structure of amylose and amylopectin, express glucose. Unlike other types of amylase, γ -amylase works best in acidic environments and contains 3 pH high [25]. Two major groups of microorganisms play an important role in amylase Synthetic catalysis however generally utilized was very awkward. The impediments that this technique presents incorporate a requirement for high temperature and weight for catalysis and moderate explicitness [28].

These restrictions were overwhelmed by the utilization of compounds. Catalysts work at milder conditions when contrasted with that needed by synthetic impetuses for activity. Additionally, catalysts are exceptionally explicit and catalyze responses quicker than compound impetuses [1]. Compounds are currently being utilized in different areas of industry. They are utilized in cleansers, paper industry, material industry, food industry, and numerous other modern applications.

Catalysts have been being used since old occasions [2] and they have been utilized in saccharification of starch, creation of refreshments like a lager, treatment of stomach related problems, and creation of cheddar from milk [4]. Among the numerous catalysts that are generally utilized α -Amylase has been in expanding request because of its pivotal part of starch hydrolysis and the utilizations of this hydrolytic activity. The accompanying segments expound on the sorts of amylases and their jobs in enzymatic responses.

PRODUCTION OF AMYLASE:

1) The growth of microorganisms is rapid and this will in turn speed up the production of enzyme. Microorganisms are easy to handle when compared to animals and plants. They require lesser space and serve as more cost-effective sources.

2) Microorganisms can be easily manipulated using genetic engineering or other means. They can be subjected to strain improvement, mutations and other such changes by which the production of α -Amylase can be optimized. Also, the microorganisms can be tailored to cater to the needs of growing industries and to obtain enzymes with desired characteristics like thermostability for example. Thermostable α -Amylases are desired as they minimize contamination risk and reduce reaction time, thus saving considerable amount of energy. Also, when

hydrolysis is carried out at higher temperatures, the polymerization of D-glucose to iso-maltose is minimized [9].

Amylase has been produced by main two methods;

- **Submerged fermentation.**
- **Solid state fermentation.**

Submerged fermentation:

Submerged fermentation is a method of producing biomolecules that involves submerging enzymes and other reactive compounds in a liquid such as alcohol, oil, or nutrient broth. Submerged Fermentation (SmF)/Liquid Fermentation (LF) uses free-flowing liquid substrates like molasses and broths for fermentation. The procedure is used for a number of reasons, the majority of which are related to industrial production. The growth of microorganisms in a liquid broth is referred to as submerged fermentation. The method often necessitates a large amount of oxygen. When the microorganisms interact with the nutrients in the broth, they break them down, resulting in the formation of enzymes. In the fermentation broth, bioactive compounds are secreted.

Solid State fermentation:

Solid State Fermentation (SSF) is a fermentation process used by a variety of industries, including pharmaceuticals, food, and textiles, to manufacture microorganism metabolites using a solid support rather

than a liquid medium. It's known as microbe growth in the absence of a free-flowing aqueous process. For the processing of value-added products such as antibiotics, single-cell protein, PUFAs, enzymes, organic acids, biopesticides, biofuel, and aromas, the SSF is a viable alternative to submerged fermentation. Grain brans, de-oiled oil seed cakes, and other materials are among the materials used as a support. Initially, fungi were primarily used in this form of fermentation (as these microorganisms were considered to be very optimally active in very low water activity). Later, a variety of bacterial species and yeasts were used in the fermentation process.

Organism used in SSF:

SSF can also be made with bacteria and yeasts, which require a higher moisture content for efficient fermentation, but with a lower yield.

SOURCES OF AMYLASE:

Animal sources:

Ptyalin, salivary α -amylase (α -1,4- α -D-glucan-4-glucanohydrolase; EC 3.2.1.1) one of the most important enzymes in the saliva. The enzyme was expressed by titling by Leuchs in 1831 [7]. It consists of two families of isoenzymes, one of which is glycosylated and the other does not contain carbohydrates. The molecular weight of the glycosylated form is approximately 57 kDa, that of the non-glycosylated form is

approximately 54 kDa. Saliva amylases hold 40% to 50% of complete protein saliva and most of the enzyme associated with parotid hunger (80% of number) [7]. Calcium metalloenzyme containing hydrolyzes α -1,4 links starch in glucose and maltose. It is known to be involved mainly in the initiation of digestion starch in the oral cavity. However, Salivary α -amylase has also been shown to have an important function in viral interactions [9].

Microbial sources:

In addition to the widespread distribution of enzyme, microbial sources, particularly fungous similarly bacterium enzyme, that is employed in industrial production owing to such advantages as prices efficiency, consistency, period and easy method modification and potency [10]. Fungal amylases are widely utilized in the preparation of oriental food [12]. In between bacteria, *Bacillus* sp. is widely utilized in the assembly of volatile α -amylase to fulfill business requirements. *B. subtilis*, *B. stearothermophilus*, *B. licheniformis* *a*, and *B. amyloliquefaciens* are renowned have been sensible producers of α -amylase and these are widely utilized in business production of a catalyst for varied applications [13]. Similarly, threadlike fungi have widely used the production of amylases for hundreds of years. As these fungi are removed to be the leading

producers of foreign proteins, they are extremely exploited within the production of assorted enzymes including as well.

PARAMETERS AFFECTING ENZYME ACTIVITY

Carbon sources:

Carbon sources such as galactose, glycogen, and Inulin have been reported as suitable substrates for amylase production by *B. licheniformis* and *Bacillus* [11]. Starch and glycerol were known to increase enzyme production in *B. subtilis* IMG22, *Bacillus sp.*, PS-7, and *Bacillus sp.* 1-3 [18]. Soluble starch has been found as a leading substrate production of alpha-amylase by *B. stearothermophilus* [19]. *Bacillus sp.* it was noted that the high given raw starch digests amylase in the area containing lactose (1%) and yeast extraction (15%). Agricultural waste is used in both liquid and solid fermentation to reduce the cost of fermentation media. Waste contains carbon and nitrogen resources needed for physical growth and transformation. These nutrient sources include orange peel, peanuts for peers' starch, potatoes, corn, tapioca, wheat, and rice as flour [16].

Nitrogen sources:

Soybean is a wonderful supply of N for alpha enzyme by true bacteria sp. 1-3. Tanyildizi *et al* rumored that organic compounds increased accelerator activity whereas the discharge of yeast failed to show a sway on alpha enzyme production

[17]. Remains of true bacteria *stearothermophilus* and *B. Amylolyticus* secreted high alpha enzyme in a neighborhood infused with I Chronicles organic compound, 0.5% yeast extract, and zero.5% malt6ose below intense vibration conditions [20]. L-asparagine was is rumored to be one of the foremost promising sources of N sources of alpha enzyme production by *Thermomyces lanuginosus* [19].

Temperature

The effect of heat on amylase production is related to body growth [3]. Therefore, the maximum temperature depends on whether the culture is mesophilic or thermophilic. In the middle of the mold is a lot of amylase production studies were performed with mesophilic mold within a temperature of 25-37°C [22]. A raw amylase-damaging starch was made by *Aspergillus ficum* at 30°C by Hayashida in 1986. Amylase production in High levels has been reported between 50-55°C by fungalophilic fungal cultures such as *Talaromyces Emersonii* etc. [21].

pH:

pH is one of the important factors that determine the growth and morphology of microorganisms as they are sensitive to the concentration of hydrogen ions in between [19]. Previous studies have shown that mold is needed acidic pH and bacteria require a neutral pH to grow properly. pH

is known for incorporating and secreting pH alpha-amylase [24]. *Aspergillus fungus* sp. as *A. oryzae*, *A. ficuum*, and *A. Niger* have been found to provide a high yield of alpha-amylase at a pH of 5.0 to 6.0 in SmF [23] Alpha-amylase species that produce yeast such as *Cerevisiae* and *Sekyere* have shown high enzyme production at pH 5.0 [22].

DURATION OF FERMENTATION

This is an important factor in the fermentation process. If the process is done shorter than the time limit the maximum yield cannot be obtained. Enzyme activity increases with increasing incubation time until you reach the best time. In most cases, enzyme production begins to decline as the incubation period increases further. This can be caused by nutrient depletion in the middle [31, 32] or the release of toxic substances. *Bacillus subtilis*, a well-known producer of alpha amylase was studied to compare between different hours of fermentation and studies have shown a high yield of alpha amylase after 48 hours of fermentation [33]. The duration of the incubation period was compared with the amylase product from *Penicillium fellutanum* separated from the mangrove rhizosphere soil. The culture when incubated at 96 h, showed a significant activity of 136 U / ml. This was a double increase in activity compared to a 24 h incubation (68 ± 2.3 U / ml) [12]. In an

attempt to produce α -Amylase from *A. oryzae* using coconut oil cake as a substrate, the main activity was found to be 1752 U / gds which resulted in 72 h incubation [32]. The positive α -Amylase activity of 62 470 U / g was obtained after 72 hours of fermentation during the production of *Bacillus amyloliquefaciens* [34]

APPLICATION

Amylase has been used in various industries. Like Alcohol industries, Chocolate industries, Paper industries, Bread Industries, Textile Industries, Biofuel Industries, Detergent industries [35].

CONCLUSION

Amylase is known as one of the oldest enzymes. And it was discovered by Kirchoff, 1815. It has been divided into three types: α , β , and Γ amylase. Mainly amylase is used in industries like the paper industry, chocolate industry, textile industry, bread industry, the alcohol industry and Bio-fuel industry, Detergent industry, etc.

ACKNOWLEDGEMENT

It's our privilege and honour to express our sincerest gratitude to the Parul University, Vadodara, Gujarat for providing me all the necessary support and facilities including state of the art infrastructural facilities with advanced technological scientific laboratories and everything else that was required to carry out this work.

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