



## Analysis of amylase production from different substrate

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### ABSTRACT

Amylases are the enzymes that hydrolyse starch or glycogen and produce, polymers of glucose subunits.  $\alpha$ - amylases are one of the important and widely used enzyme in many sectors such as clinical, medicinal and analytical chemistry. Besides their use in starch saccharification they also find applications in food, baking, brewing, detergent, textile, paper, and distilling industry. Amylase can be derived from several sources, such as plants, animals and microorganisms. Amylase can be produced from economically available agricultural starchy substrate using bacteria and fungi. Different agricultural starchy substrates such as soluble starch, hordium, pearl millet, rice, corn, gram and wheat starch, banana peel, potato peel, coconut oil cake, sesame oil cake, groundnut oil cake, palm kernel cake and olive oil cake were used for production of  $\alpha$ -amylase using different microorganisms.

**Keywords:**  $\alpha$ -amylase, agricultural starchy substrate, microbial source, fermentation

### I. Introduction:

Amylases are one of the most important enzymes and great significance in present day biotechnology and having approximately 25% of the enzyme market (1). Amylases are enzymes which utilize and hydrolyse starch and glycogen as substrate and form polymers of glucose subunits (2, 3). Amylases are classified into three types. On the basis of amylase breakdown starch molecules and produce glucose, {1}  $\alpha$ - amylase (which breaks down the random location along the starch chain), {2}  $\beta$ - amylase (which act on the glucose-glucose bonds and remove two glucose unit at a time and produce maltose and Amyloglucosidase (AMG)), {3}  $\gamma$ - amylase cleaves  $\alpha(1-6)$  glycosidic

linkages, in addition to cleaving the last  $\alpha(1-4)$ -glycosidic linkages at the non-reducing end of amylose and amylopectin resulting in the formation of glucose(2).

The substrate which produces  $\alpha$ -amylase is starch. Starch is a polysaccharide composed of two types of polymers – amylose and amylopectin. Amylose constitutes 20-25% of the starch molecule. It is a linear chain consisting of repetitive glucose units linked by  $\alpha(1, 4)$ -glycosidic linkage. Amylopectin constitutes 75-80% of starch and is characterized by branched chains of glucose units (4). This is very expensive for commercial production of  $\alpha$ - amylase. This expensive products can be replaced in the fermentation medium with the economically available agricultural by-products, like banana peel, potato peel, wheat bran can also be used(5).

Amylase can be derived from various sources such as microorganisms (bacteria and fungi), plants and animals. Microbial production of amylase is more fruitful than that of others sources like plant or animals because, there are 2 major reasons for the increasing interest in microbial sources: {1} The growth of microorganisms is rapid and this will in turn speed up the production of enzyme. Microorganisms are easy to handle then 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. They can be subjected to strain improvement, mutations and other such changes by which the production of  $\alpha$ -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  $\alpha$ -Amylases are desired as they minimize contamination risk and reduce reaction time has saving considerable amount of energy. Also when hydrolysis is carried out at higher temperatures, the polymerization of D-glucose to iso-maltose is minimized (4, 7).

Amylases find potential applications in a number of industrial processes such as food (baking, brewing, dairy industries), fermentation, textile, detergent and paper industries. Microbial amylases have replaced the chemical hydrolysis of starch in starch processing industries. These would also be useful in the pharmaceutical and fine chemical industries. They are mainly employed for starch liquefaction to reduce their viscosity, production of maltose, oligosaccharide mixtures, high fructose syrup and maltotetraose syrup, bioconversion of solid waste(6).

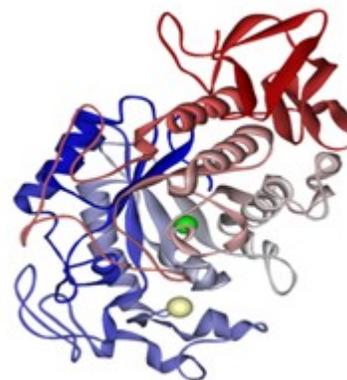
## II. Types of Amylase:

Based on the cleavage site, they are classified into three types:  $\alpha$ - amylase,  $\beta$ - amylase,  $\gamma$ - amylase.

**A.  $\alpha$ -amylase:**  $\alpha$ -Amylase (E.C.3.2.1.1) is a hydrolyse enzyme that catalyses the hydrolysis of internal  $\alpha$ -1, 4-glycosidic linkages in starch to yield products like maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. It is a calcium metalloenzyme, completely unable to function in the absence of calcium(4, 7). Amylases can be divided into two categories, endoamylases and exoamylase. Endoamylases catalyse hydrolysis in a random manner in the interior of the starch molecule. This action causes the formation of linear and branched oligosaccharides of various chain lengths. Exoamylases hydrolyse from the non-reducing end, successively resulting in short end products (8).  $\alpha$ -amylase tends to be faster-acting than  $\beta$ -amylase because it can act anywhere on the substrate. Also found in plants (adequately), fungi (ascomycetes and basidiomycetes) and bacteria (*Bacillus*)(7). In animal, it is a major digestive enzyme, and its optimum pH is 6.7-7.0.

The use of  $\alpha$ -amylase in detergents formulations has also increased dramatically with growing awareness about environment protection. Enzymes are

environmentally safe and enhance the detergents ability to remove tough stain. There are many such applications of the enzyme which is the driving force behind the research to produce this enzyme in an optimum, safe and convenient manner (4)



**Figure: 1 Structure of  $\alpha$ -amylase (9)**

**B.  $\beta$ -Amylase:**  $\beta$ -Amylase (EC 3.2.1.2) is an exo-hydrolase enzyme that acts from the nonreducing end of a polysaccharide chain by hydrolysis of  $\alpha$ -1, 4-glucan linkages to yield successive maltose units. Primary sources of  $\beta$ -Amylase are the seeds of higher plants and sweet potatoes. During ripening of fruits,  $\beta$ -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(4). Many microbes also produce amylase to degrade extracellular starches. Animal tissues do not contain  $\beta$ -amylase although it may be present in microorganisms contained within the digestive tract (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. In the industry it is used for fermentation in brewing and distilling industry. Also, it is used to produce high maltose syrups (4).

**C.  $\gamma$ - Amylase:**  $\gamma$ -Amylase (EC 3.2.1.3) cleaves  $\alpha$  (1-6) glycosidic linkages, in addition to cleaving the last  $\alpha$  (1-4) glycosidic linkages at the nonreducing end of amylose and amylopectin, unlike the other forms of amylase, yielding glucose.  $\gamma$ - Amylase is most efficient in acidic environments and has an optimum pH of 3(5).

**TABLE 1:** Amylase production from agricultural by-product by bacteria using fermentation method at optimum condition

Bacteria	Carbon source	Optimum condition			Method	Amylase Activity	Reference
		pH	Incubation time(hrs)	Incubation temperature(°C)			
<i>Bacillus subtilis</i>	Banana peel	7	24	35	SSF	6.97IU/mL/min	1
<i>Bacterium mura</i>	Wheat	7	24	37	SFF	27IU/ml	9
<i>Anoxybacillus amylolyticus</i>	potato peel	5.6	24	60	SmF	1000 U/gds	10
<i>Bacillus subtilis</i>	corn steep liquor	7	48	40	SSF	45 U/mg.	11
<i>Halomonas meridian</i>	Soluble starch	7.2	24	37	SSF	100U/g	12
<i>Bacillus coagulans</i>	Wheat bran	7	72	50	SSF	23192U/g	13
<i>Bacillus licheniformis</i>	wheat bran	7	48	40	SmF	240 IU/ml/Min	14
<i>Bacillus cereus</i>	wheat bran	5	72	55	SSF	94U/g	15
<i>Bacillus amyloliquefaciens</i> ATCC 23842	wheat bran and groundnut oil cake (1:1)	5	72	37	SSF	62 470 U/g	16
<i>Bacillus licheniformis</i> GCUCM-30	Pearl millet	7.5	48	40	SFF	936 U/G	5
<i>Bacillus subtilis</i>	Starch	7	24	37	SSF	228 U/ml.	17

**TABLE: 2 Amylase production from agricultural by-product by fungi using fermentation method at optimum condition.**

Fungi	Carbon source	Optimum condition			method	Amylase activity	Reference
		pH	Incubation time (days)	Temperature (°C)			
<i>Aspergillus niger</i>	wheat bran	6.2	5	28	SSF	0.08U/ml/min	6
<i>Penicillium fellutanum</i>	starch	6.5	3	30		94 U/ml,	18
<i>Penicillium chrysogenum</i>	Wheat bran	5	7	28	SSF	160 U/MI	19
<i>Thermomyces lanuginosus</i>	Wheat bran	6	5	50	SSF	534 U/g	20
<i>Penicillium janthinellum</i>	wheat bran	5	4	35	SSF	300 U/gds	21
<i>Pycnopus</i>	Wheat	7	4	37	SmF	1.5U/mg	22

<i>sanguineus</i>	bran						
<i>Aspergillus oryzae</i>	wheat bran and groundnut oil cake	5	3	30	SSF	9196 IU/gds	23
<i>Penicillium expansum</i>	loquat kernel	6	5	30	SSF	1012 U/g	24
<i>Thermomyces lanuginosus</i>	Wheat bran	6	5	50	SSF	534 U/g	17

IU=International Unit, Gds=gram dry substrate.

**Table: 3** Applications of amylase.

No.	Industry	Application	Reference
1	Bread and baking Industry	Bread softness and higher volume, flour adjustment, better colour.	25
2	Starch liquefaction and saccharification	Starchhydrolysis, such as glucose and fructose. Starch is converted into high fructose corn syrups.	26
3	Detergent Industry	starch stain removal, Digests starch containing foods to water soluble dextrin	25
4	Beverage Industry	as sweeteners for soft drinks	27
5	Textile desizing	Used in removal of starch sizing agent from woven fabric	28
6	Pulp and paper Industry	Modification of starches for coated paper, Protect the paper against mechanical damage during processing, Improves the quality of the finished paper, Size enhances the stiffness and strength in paper, Improves the erasibility and good coating for the paper.	29

### Conclusion:

Amylase is the enzyme which catalyses the reaction of conversion of starch to sugar. Different carbohydrate sources can be used for the production of amylase. The substrate which gives the highest yield is combination of wheat bran and groundnut oil cake (1:1) and the organisms used for this production was *Bacillus amyloliquefaciens* ATCC 23842. Fungi can also be used to produce amylase in high amount that is *Aspergillus oryzae* using combination of wheat bran and groundnut oil cake as substrate. Wheat bran, banana peel, agro- industrial waste mainly used as substrate because they serve as green alternatives of carbon sources, cheap and easily available. So amylase can be used in different industries for serving different purposes.

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