PRODUCTION OF AMYLASE

Introduction: Enzymes are used for a various purposes. They are employed in three major fields *viz.*, laboratory, industrial and clinical. In some cases, they may be used in their crude forms. But at other times, they are used in highly purified states (e.g. urease for urea estimation). The enzymes are commercially produced by two methods: (1) Semisolid culture and (2) Submerged culture.

Semisolid culture: The enzyme is producing culture is grown on the surface of a suitable semi-solid substrate. The substrate usually consists of moistened wheat or rice bran, supplemented with nutrient salts. The production medium is prepared by mixing bran with a solution containing desired nutrient salts. The desired pH for optimum growth of the mould is adjusted with acid. Then the medium is steam-sterilized in an autoclave while stirring. This sterilized medium is spread on metal trays up to a depth of 1-10 cm, the total quantity being of the order of thousands of kilograms . Such a transfer is performed under aseptic conditions. Alternatively, the cultivation may be carried out in rotating drums. The fungal spores are inoculated either in the autoclave after cooling or in trays. A series of trays are enclosed in a large vessel. Aeration is ensured by circulation of suitably humidified air over the surface of the culture. It is necessary to keep temperature within narrow limits. Moreover, heat generation occurs during fermentation. Therefore, the trays should be equipped with cooling system. It should be borne in mind that, direct air cooling is not practical since drying of the culture takes place. Subsequently, extraction of enzyme is performed with water. Enzymes produced by a semisolid culture, or a surface culture process are:

Enzyme	*Micro-organism(s)*
α-Amylase	*Aspergillus oryzae*
Glucoamylase	*Rhizopus* spp.
Lactase	*A. oryzae*
Pectinase	*A. niger*
Protease	*A. niger* and *A. oryzae*
Rennet	*Mucor pusillus*

Precautions to be taken:

(i) It is necessary to keep aseptic conditions during the fermentation, since contamin considered a major problem in a semisolid culture.
(ii) Large numbers of spores should be prevented from escaping into the environment.

Advantages:

(i) This method involves comparatively low investment.
(ii) It allows the use of substrates with a high dry matter content. It, therefore, yield enzyme concentration in the crude fermented material.
(iii) It has to be used for cultivating some moulds which are very difficult to grow in fer due to wall growth.

***Submerged culture*:** Now a days submerged culture methods are widely used in the production of enzymes. The fermentation equipment used is the same as in the manufacture of antibiotics. It is a cylindrical tank of stainless steel. The tank is equipped with an agitator, an aerating device, a cooling system and various ancillary equipment (e.g. means of foam control, monitoring of pH,

temperature oxygen tension, etc.). The quantity of production medium taken in fermentation tank is in the range of 1000-30,000 gallons or more.

Amylases: Amylases play the most important part in food technology (e.g. bread-making, beer making etc.). α- Amylases are produced by bacteria and fungi are not identical. These enzymes are employed commercially for the preparation of sizing agents and removal of starch sizing from woven cloth, preparation of starch sizing pastes for use in paper coating, liquefaction of heavy starch pastes which form during heating steps in the manufacture of corn and chocolate syrups, production of bread and removal of food spots in the dry-cleaning industry where amylase functions in conjunction with protease enzymes. In addition these amylases can be employed as a replacement for malt for starch hydrolysis in the brewing industry. Therefore, concentration of α- and β-amylases are prepared and used in various ways. These enzyme preparations must be carefully standardized for activity, according to the purpose for which they are to be used.

The amylases constitute a large group of enzymes. They are characterized by their ability to hydrolyze 1, 4-glucosidic linkages in polysaccharides (e.g. Starch and Glycogen). There aretwo main subgroups: α- amylases and β-amylases. α- Amylases are endo-enzymes. They attack all the linkages between glucose units in the starch molecule. The bond hydrolyzed is that between C-1 and oxygen atom linked to the adjacent glucose group. The process eventually results in complete degradation, through dextrins to glucose. Thus, amylase which is linear starch, is degraded faster than amylopectin, which is branched. α- Amylases vary in their effectiveness, depending on their source. β-Amylases hydrolyze starch and other amylases by splitting off maltose molecules until the action is blocked by the occurrence of either 1, 3linkages or branch points. The residual molecule is then called a limit dextrin.

α- Amylases are produced by the use of fungi (i.e. *Aspergillus niger* and *A. oryzae*) as well as bacteria (i.e. *Bacillus amyloliquefaciens* and *B. licheniformis*). Therefore, α- amylases are called either fungal α- amylases or bacterial α- amylases according to the nature of the microbes used for their production.

Fungal amylases: Fungal amylases, as employed in the "Amylo" process to hydrolyze starch for yeast-alcohol production, is not separated from the fungal mycelium. Thus, to prepare the mash for the yeast, the grain is first soaked in water and then heated to solubalize its starch. The resulting mash is acidified, inoculated with Mucor or Rhizopus fungal species, and incubated approximately one day before further inoculation with the yeast. However, in a modification of this process, the yeast is added simultaneously with the fungus to bring on quicker alcohol formation.

Fungal amylase production (in which the enzyme is separated from the mycelium and the mycelium is discarded) utilizes strains of *Aspergillus oryzae* for the stationary culture with wheat bran and strains of *Aspergillus niger* for the submerged aerated-agitated culture. As regards these alternative processes, the wheat bran stationary culture has been employed extensively for the fungal amylase. For this process, the wheat bran, spread in relatively thin layers in trays (or even in rotary drum fermentors), is moistened with water or dilute acid, sterilized and inoculated with spores of fungus such as *Aspergillus oryzae.* After growth, the fungus plus bran can be dried at 500C or less and then ground to serve as an amylase preparation. However, more often the amylase is extracted by water from the wheat bran culture, precipitated from the aqueous solutions by the addition of alcohol and dried at 550C or less. The submerged fermentation of amylase production have recently become economically feasible and, although strains of both the *Aspergillus niger* and *A. oryzae* have been studied extensively for their possible use in

submerged fermentation. *Aspergillus niger* growing in starch-salt medium has now been commercialized. **Fungal α- amylases** are produced by the above mentioned two species of the fungus. These fungi are grown on wheat bran (semisolid culture). It is also possible to produce fungal α- amylases by submerged-culture, employing the following medium:

Component	Amount (g./litre)
Corn starch	24
Corn-steep liquor	36
KCl	0·2
Na_2HPO_4	47
$CaCl_2$	1
$MgCl_2 . 6H_2O$	0·2

Submerged-culture Medium for fungal α- amylases

There is a problem for aeration and agitation because of a very high viscosity of the medium due to the presence of mycelia body. Amylases biosynthesis is inhibited when the medium contain glucose.

Bacterial amylases: Various bacteria elaborate amylases (e.g. *Bacillus amyloliquefaciens* and *B. licheniformis*)., but only those from *Bacillus subtilis* and *Bacillus diasticus* have been commercially under those conditions in which fungal amylase or amylases from other sources, hydrolyze starch less well. For instance, bacterial amylase has an optimum temperature 550C, and the enzyme is relatively heat resistant. As a result, the bacterial enzyme finds particular application in situations in which starch hydrolysis must be conducted at higher temperatures.

Strains of *Bacillus subtilis* are specially selected for amylase with high starch liquefying and dextrinizing activity and consequently, this amylase produces relatively less fermentable sugars when acting on starch. High yields of bacterial amylase are obtained when *Bacillus subtilis* is grown in stationary culture. The amylase is constitutive and, surprisingly, a medium is employed which contains high level of crude protein; a high carbohydrate level in the medium stimulate protease production and depresses amylase production. Nevertheless, some of the soluble protein of the medium may first be partially hydrolyzed by boiling in dilute acid or by enzymatic treatment. The pH of the medium is near neutrality, and the fermentation proceeds for approximately 6 days (ranging from 3 to 6 days) at an incubation temperature 25-300C. *Bacillus subtilis* in this stationary liquid culture produces a heavey surface-pellicle growth, which apparently is associated with high amylase yield, and fresh sterile air is circulated over the pellicle to improve aeration. At harvest, the culture is filtered or centrifuged, the recovered aqueous portion is concentrated by evaporation to yield an amylase concentrate, then salt and an antiseptic are added. As an alternate recovery procedure, the amylase can be precipitated from the aqueous solution by addition of cold acetone, ethanol, isopropanol, or ammonium sulphate. In addition to their amylase content, these preparations also demonstrate some protease activity which is produced by *Bacillus subtilis* concurrently with the amylase.

Bacillus subtilis amylase can also be produced in highly aerated submerged culture by employing a special highly starchy medium. However, the ability to produce amylase is a

somewhat unstable characteristic of this organism, and culture degeneration, which is difficult to detect, may occur during the fermentation. This culture degeneration, however, is not as serious a problem with the stationary, pellicle-forming *Bacillus subtilis* fermentation. **Bacterial α-amylases** are produced by above mentioned two bacterial species (*Bacillus amyloliquefaciens* and *B. licheniformis*). Bacterial amylase is produced only by submerged-culture using the following medium:

Component	*Amount (%)*
Ground soya bean meal	1·85
Amber BYF (autolyzed brewers' yeast fractions, Amber Laboratories)	1·50
Distillers' dried solubles	0·76
N-Z amine (enzymic casein hydrolyzate, Sheffield Chemical Co.)	0·65
Lactose	4·75
$MgSO_4 . 7H_2O$	0·04
Hodag KG-1 antifoam (Hodag Chemical Corp.)	0·05
Water	90·40

Submerged-culture Medium for Bacterial amylase

A temperature in the range of 300C to 400C is satisfactory. The optimum pH for the fermentation medium is 7.0. It is necessary to maintain the pH near neutrality, since the amylase is denatured below 6. Calcium carbonate is used as the buffer to maintain neutral pH. The production of α- amylase begin when the bacterial count reaches 109 – 1010 cells per millilitre after about 10-20 hours, and continues for another 100-150 hours. Preservation of liquid preparations of bacterial α- amylase is done by 20% sodium chloride. The most active solid preparations contain 5% active amylase protein. The different steps involved in the purification of endocelluar enzymes are schematically shown in **Figure 1**

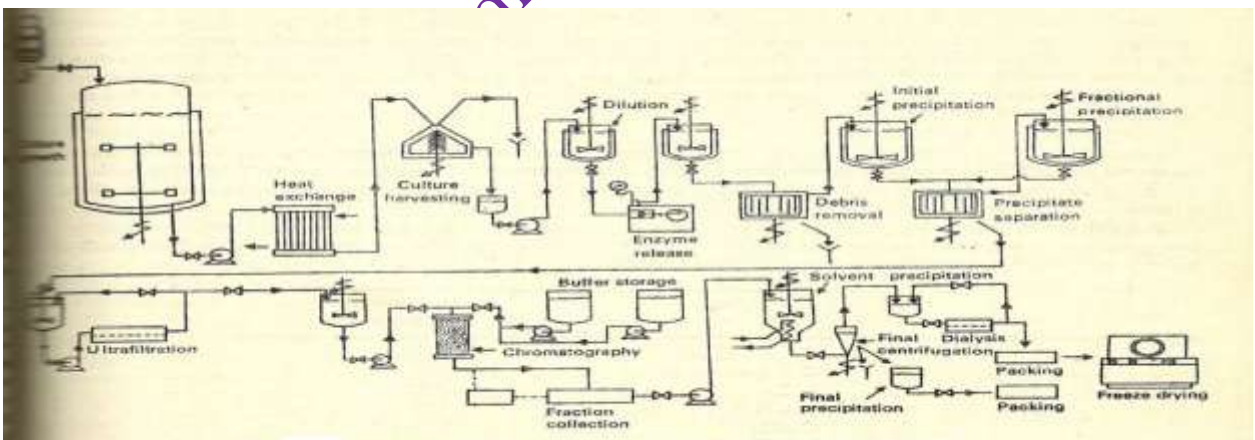

Fig. Flow sheet for production of purified endocellular enzymes.