

# Industrially Produced Enzymes and their Applications -An Overview

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## INTRODUCTION

Enzymes may be defined as biocatalysts synthesized by living cells. They are protein in nature (exception-RNA acting as ribozyme), colloidal and thermolabile in character, and specific in their action. All the enzymes are invariably proteins. In recent years, however, a few RNA molecules have been shown to function as enzymes. Each enzyme has its own tertiary structure and specific conformation which is very essential for its catalytic activity. The functional unit of the enzyme is known as holoenzyme which is often made up of apoenzyme (the protein part) and a coenzyme (non-protein organic part). The use of enzymes in the diagnosis of disease is one of the important benefits derived from the intensive research in biochemistry since the 1940's. Enzymes have provided the basis for the field of clinical chemistry.

## Enzymes and Life Processes

The living cell is the site of tremendous biochemical activity called metabolism. This is the process of chemical and physical change which goes on continually in the living organism. Build-up of new tissue, replacement of old tissue, conversion of food to energy, disposal of waste materials, reproduction - all the activities that we characterize as "life."

## Specificity of Enzymes

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will

catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

### 1. Absolute specificity

The enzyme will catalyze only one reaction.

### 2. Group specificity

The enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.

### 3. Linkage specificity

The enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.

### 4. Stereochemical specificity

The enzyme will act on a particular steric or optical isomer.

## Factors Affecting Enzyme Activity

Knowledge of basic enzyme kinetic theory is important in enzyme analysis in order both to understand the basic enzymatic mechanism and to select a method for enzyme analysis. The conditions selected to measure the activity of an enzyme would not be the same as those selected to measure the concentration of its substrate. Several factors affect the rate at which enzymatic reactions proceed - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.

## Enzymes and Their Applications

### Starch Liquifying Amylolytic Enzymes

Diastase	Malt	Digestive aid, Supplement to bread, syrup	$\alpha$ -amylase activity $\beta$ -amylase activity
Takaddiastase	A. Oryzae	Digestive aid	Contains many other enzyme protease, RNase

### Starch Saccharifying Amylolytic Enzymes

Amyloglucosidase	Rhizopus niveus A.niger	Glucose production
Invertase	S.Cervisiae	Confectionaries to prevent crystallization of sugar, Chocolate, High test molasses

### Animal and Vegetable Proteolytic Enzymes

Name of Enzymes	Source	Applications	Notes
Trypsin	Animal stomach	Medical uses, Meat tenderizers, Beer haze removal.	Optimal operating pH of about 7.5-8.5
Pepsin	Animal stomach	Digestive aid , Meat tenderizer& used in the recovery of silver from discarded photographic films .	exhibits maximal activity at pH 2.0 and is inactive at pH 6.5.
Papain	Papaya	Digestive aid, home remedy treatment for jellyfish, bee, Beer haze removal.	Optimal pH 6.0-7.0

### Microbial Proteolytic Enzymes

Name of Enzymes	Source	Applications
Protease	A.niger	Feed, digestive aid
Protease	A.SUBtilis	Removal of gelatin from film, fish soluble.
Varidase	Streptococcus sp.	Medical use

### Other Commercial Enzymes

Penicillinase	B.Subtilis B.Cereus	Removal of of penicillin
Glucose oxidase	P. Chrysogenum	For glucose determination
Hyaluronidase Lipase	Animal, Bacteria, pancreas, mold (Rhizopus)	Medical use , Digestive aid , Flavouring of milk products
Cytochrome C catalase	Yeast (candida)	Medical use Sterilization of milk
Keratinase	Streptomyces fradiae	Removal of hair from hides

### Nucleotic and Other New Enzymes

Name of Enzymes	Source	Applications	Notes
Adenylic acid deaminase	A.Oxyaze	AMP $\longrightarrow$ IMP	IN Takadiastase
Microbial rennet	Mucor sp.	Cheese manufacture	to avoid contamination with unpleasant byproducts of the mold growth
Naringinase Hesperidinase	A.niger	Removal of bitter taste from citrus juice	When naringin is treated with potassium hydroxide or another strong base, and then catalytically hydrogenated it is 300-1800 times sweeter than sugar at threshold concentrations
Glucose isomerase	L.brevis	Glucose $\longrightarrow$ fructose	Optimum pH 6.7
Cellulose	Trichoderma koningi	Digestive aid	Optimum pH 4.6

Amylolytic enzymes represent a group of catalytic proteins of great importance. They were one of the first enzymes to be produced commercially by micro-organisms. Consequently a remarkable number of new starch degrading enzymes have been discovered.

Starch degrading enzymes can be divided into two main groups,  $\alpha$ -1,4-glucanases and  $\alpha$ -1,6-glucanases.

### AMYLASE

Amylase is an exoenzyme which has the capacity to hydrolyse starch and polysaccharides into disaccharides and monosaccharides. This enzyme ( $\alpha$ -1,4-glucan-glucanhydrolase) acts on starch components which contain at least three  $\alpha$ -1,4 linked glucose units as an endolase, i.e., in an essentially random manner, with the production of reducing sugars.

Two types of microbial  $\alpha$ -amylase have been recognized, termed as "liquefying" and "saccharifying"  $\alpha$ -amylases. The main difference between them is that the saccharifying enzymes produce a higher yield of reducing sugars than the liquefying enzymes.

They can also be classified according to their source of production into:

1. Bacterial  $\alpha$ -Amylase
2. Fungal  $\alpha$ -Amylase.

### Bacterial $\alpha$ – Amylase

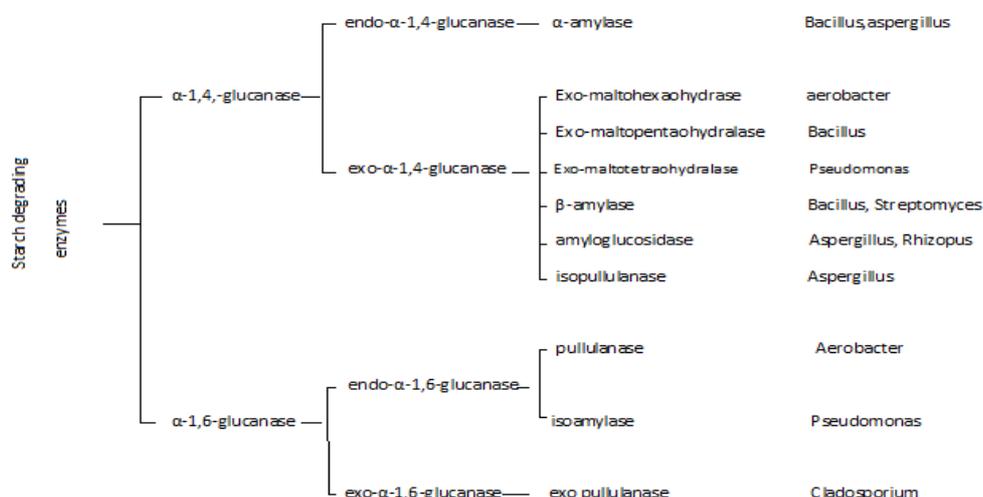
This is mainly produced from *Bacillus subtilis* and *Bacillus diastolicus*. Strains of *Bacillus subtilis* are especially selected for amylase with high starch liquefying activity and

consequently this amylase produces relatively less fermentable sugars when acting on starch. *Bacillus subtilis* amylase can also be produced in highly aerated submerged cultures by employing a special highly starchy medium. Beta-amylases yield beta-limit dextrins and maltose. Gamma-amylases yield glucose. Amylases are used as digestants. Glucose and maltose are also produced depending on the degree of hydrolysis. Because of this starch degrading ability of **Bacterial Alpha Amylase**, it is widely used in pharmaceutical industry in various digestive aid preparations. Due to the presence of **Bacterial Alpha Amylase** starch in the consumed food is better digested, this increases the overall digestibility of food. Such digestive aid preparations are used for the treatment of patients whose digest power is reduced due to illness. Many such a commercial formulation of digestive aid either as syrup or as tablet has been seen in many drug stores.

### Fungal $\alpha$ -amylase

Strains of *Aspergillus oryzae* may be utilized for stationary culture for the production of fungal  $\alpha$ -amylase and strains of *A. niger* for submerged aerated agitated cultures. It is also utilized to increase the amount of fermentable sugar available for fermentation. The activity of fungal amylase is effective in the temperature range of 40°C to 60°C, with its optimum temperature is 50°C. Significantly important, amylase finds a variety of applications in different industries.

### Classification of Starch – Degrading Enzymes of Industrial Interest



**Commercial Applications of Amylase**

INDUSTRY	APPLICATIONS	ENZYME	SOURCE
Baking&milling	Reduction of dough viscosity, acceleration of fermentation process, increase in loaf volume, improvement of crumbscore& softness, maintainence of freshness & softness.	Amylase	Fungal
Beer	Mashing	Amylase	Fungal/Bacterial
Cereals	Precooked baby foods, Breakfast foods.	Amylase	Fungal
Chocolate Cocoa	Manufacture of syrup	Amylase	Fungal/Bacterial
Confectionery, Candy	Sugar recovery from scrap candy	Amylase	Fungal/ Bacterial
Corn syrup	manuafacture of high malatse syrup	Amylase	Fungal,Bacterial
Distilled beveragesqu	Mashing	Amylase	Fungal/bacterial
Flavours	Clarification(starch removal)	Amylase	Fungal
Pharmaceutical&clinical	Digestive aids	Amylase	Fungal
Textiles	Desizing of fabrics	Amylase	Bacterial
Vegetables	Liquefying purees & soups	Amylase	Fungal
Wine	Clarification	Amylase	Fungal

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