Topic: **ENZYMES: AMYLASES, PROTEASES, RENNIN**

Subject: Botany

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Dr. Saumya Srivastava
Assistant Professor,
P.G. Department of Botany,
Patna University,
Patna- 800005

Email id: sonata906@gmail.com

Some of the important commercially produced enzymes are-

Enzymes used in industry: amylases, proteases, catalases, isomerases, penicillin acylases.

Enzymes used for analytical purposes: glucose oxidase, galactose oxidase, alcohol dehydrogenase, hexokinase, etc.

Enzymes used in medicine: asparaginase, proteases, lipases, and streptokinase.

The prospects of enzyme application have improved due to developments in the areas of microbial genetics, optimization of fermentation conditions, release of enzymes from cells by means of new cell breaking methods, modern purification processes and development of processes for immobilization of enzymes, and continuous enzyme production in special reactors causing investment cost to be minimized.

AMYLASES

It is widely used in the production of sweeteners for the food industry. Also, used in brad and beer making etc. For this, mainly concentrates of α and β amylases are prepared.

Amylases are characterized by their ability to hydrolyze 1,4- glycosidic linkages in polysaccharides (starch and glycogen).

Starch $\xrightarrow{\text{Amylase}}$ Sugar

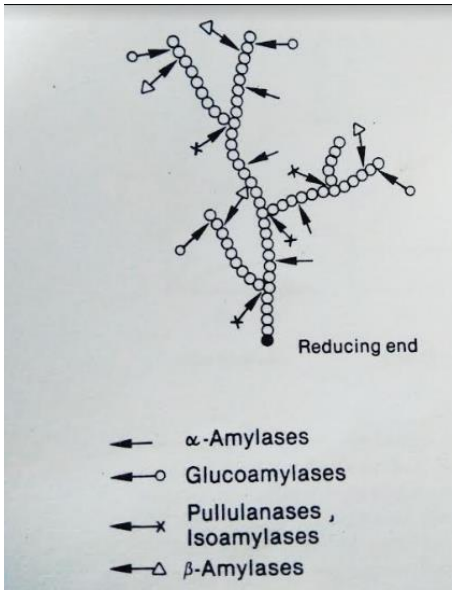

Fig. 1. Mechanism of starch hydrolysis by amylases

α amylases are endo-enzymes which attack all linkages between glucose units in starch; results in complete degradation from dextrins to glucose. Thus, amylose (a linear starch) is degraded faster than amylopectin (branched starch). β amylases are exo-enzymes which splits off maltose molecule and results in dextrin.

Production of α amylases

α amylases produced by use of fungi (*Aspergillus niger* and *A. oryzae*) are called fungal α amylases, whereas produced by use of bacteria (*Bacillus amyloliquefaciens* and *B. licheniformis*) are known as bacterial α amylases.

Fungal α amylases can be produced by both semi-solid and submerged cultures. In semi solid culture it is grown on wheat bran supplied with nutrient salts, whereas sugmerged culture has following medium-

Corn starch……24 g/L
Corn steep liquor……...36 g/L
KCl……0.2 g/L
Na_2HPO_4 ……. 47 g/L
$CaCl_2$…… 1.0 g/L
$MgCl_2.6H_2O$…….0.2 g/L

Bacterial α amylases are produced only by submerged culture. Medium is as follows-

Ground soyabean meal…….1.85%
Autolysed brewer's yeast…….1.5%
Distiller's dried solubles……0.75%
N-Z amine…….0.65%
Lactose…….4.75%
$MgSO_4.7H_20$…….0.04%
Hodag KG 1 antifoam……0.05%
H_2O……90.4%
Temp. 30-40^oC; pH 7 (as amylase is denatured below 6)

Production of α amylase begins when bacterial count reaches 10^9-10^{10} cell after about 10-20 hrs, and continues for 100-150 hrs. Preservation of liquid preparation of bacterial α amylases is done by 20% NaCl. Most active preparation have 2% active amylase protein, and solid preparation have 5% active amylase protein.

α amylases find application in starch industry, milling, alcohol making, increasing the proportion of fermentable carbohydrates in baked goods, brewing industry; paper, textile and feed industry; sugar industry, and also in laundry and detergent industry. β amylases are now used for production of maltose syrup.

Glucoamylases act on starch by splitting glucose units from the non reducing end. Maltose is broken down only slowly, while 1,6-bonds in the branched polysaccharides are hardly attacked. Thus, glucose, maltose and limit dextrins are the end products of glucoamylase action. Finds application in fructose syrup production.

Enzymes which can split the side chains of amylopectin via hydrolysis of α-1,6- bonds are important in commercial processes and includes pullulanases and isoamylases.

PROTEASES

After amylases, the second most important industrial enzymes are the proteases. These are used primarily in the detergent industry and in the dairy industry. Other areas include pharmaceutical industry, leather industry, in the manufacturing of protein hydrolysates, food industry, film industry, waste processing etc.

They are commercially produced both from bacteria and fungi. Proteases in the market include alkaline, neutral, and acid proteases.

Alkaline proteases

Many bacteria and fungi excrete alkaline proteases. Enzymes used in detergents are mainly proteases from *Bacillus* strains (Bacillopeptidases). Have many features of value for use as detergent enzymes like stability at high temperature, stability in the alkaline range (pH 9-11), stability in association with chelating agents and perborates etc.

Neutral proteases

Are quickly inactivated by alkaline proteases, therefore, have limited applications, but find uses in leather industries and in food industry for manufacture of crackers, bread, and rolls.

Acid proteases

In this category are rennin like proteases from fungi which are chiefly used in cheese production. In addition, other acid proteases are also in market having pH optimum 2-4. These are used in medicines, in the digestion of soy protein for soy sauce production, and to break down wheat gluten in the baking industry.

RENNIN

This milk coagulating enzyme rennin is known from long time. In modern processes, starter cultures are added which convert lactose to lactic acid and lower the pH. Commonly used strains are *Streptococcus lactis, S. cremoris, Lactobacillus helveticus* etc. These organisms are cultured industrially and the culture selected depends upon the desired curd structure and flavor, a minimum gas production during fermentation, a low sensitivity to bacteriophages, and the extent of acid production.

Rennet is an extract from the fourth stomach of 3 to 4 week old calves which have been raised on milk. The purified enzyme is called rennin, chymase, or chymosin.

Only 3 strains of fungi are used worldwide in production, and are subdivided into 2 groups.
Type I *Mucor pusillus* var. Lindt …… ….Solid surface culture

Type II *Endothia parasitica* …….Submerged culture (1st rennin marketed)

Mucor miehei…… ….. Submerged culture

Suggested readings

Industrial microbiology- A. H. Patel

Enzymes in Industry (Production and Applications)- Wolfgang Aehle