PRODUCTION AND APPLICATIONS OF ENZYMES

1) Amylases

Amylase is an enzyme that catalyses the hydrolysis of starch into sugars. Present in the saliva of humans. Hydrolysis of Starch with amylase will first result in the formation of a short polymer Dextrin and then the disaccharide Maltose and finally glucose. Glucose is not as sweet as Fructose. Thus the next step would be the conversion of Glucose to Fructose by the enzyme Glucose isomerase.

Types of Amylases

1) α- Amylase
2) ß- Amylase
3) γ- Amylase

The amylase production to be carried out in 250 mL conical flasks containing 50 mL medium with the following composition: 5 g/L soluble starch, 5 g/L yeast extract, 2.5 g/L $(NH_4)_2SO_4$, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 3 g/L KH_2PO_4, and 0.25 g/L $CaCl_2 \cdot 2H_2O$ incubated at 50°C under shaking conditions (200 rpm) and inoculated with 2.5% of 24 h old culture. After the specified cultivation time for each set of experimental trials, the culture broths to be centrifuged at 10,000 g for 10 min and the cell free supernatant should be used for enzyme determination.

Applications

• Production of sweeteners for the food industry.

• Removal of starch sizing from woven cloth

• Liquefaction of starch pastes which are formed during the 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 works in conjunction with protease enzymes.

2) Catalases

Catalases involve disproportionation of hydrogen peroxide to water and oxygen effectively, widely distributed in nature and found in bacterial, plant, and animal cells. The active enzymes are important members of the cellular defence system protecting the cell from oxidative damage.

The strain *Acinetobacter* sp. YS0810 was isolated from Qingdao coastal, in China. The strain YS0810 was routinely cultivated aerobically in medium [2% (w/v) peptone, 0.2% (w/v) meat extract, 0.2% (w/v) NH_4Cl, 0.2% (w/v) KH_2PO_4, 0.15% (w/v) KH_2PO_4] at 220 rpm on a rotary shaker at 28°C for 24 h.

3) Peroxidase

Peroxidases are isolated from various sources like plants, animals and microbes. Peroxidases have wide applications in many areas like industrial, medical and food processing. In the present study, Bacillus subtilis was employed for the production of peroxidases. B.subtilis was isolated from soil using the Serial dilution method. Identification of B.subtilis was done by performing various staining techniques and biochemical assays. Pure cultures of B.subtilis were obtained and screened for the production of peroxidases and those cultures which produced the same were selected for further study. B.subtilis produced 0.00045 units of peroxidase per ml of fermentation media. Optimization studies were performed and it was found that the optimum conditions for the production of peroxidase are pH-6, Temperature-370C. Purification of peroxidase enzyme was done using Salt precipitation, dialysis and Ion exchange chromatographic techniques. Quantification of the resultant peroxidase was done by Lowry's method. Kinetics of the peroxidase enzyme were also studied .Enzyme immobilization was done and was found that the peroxidase activity increased after immobilization.

Production:

Media for peroxidase:

50ml of production media was prepared in conical flask and it is autoclaved at 1210c at 15 lbs pressure for 15 minutes. Then a loopful of Bacillus subtilis culture was inoculated into the production media. Finally the bacterial medium was incubated at 37^0C for 48 hours in shaking incubator.

Enzyme extraction:

Taken 50ml of production broth and transferred it into centrifuge tubes. They are centrifuged at 6000 rpm for 10 minutes. Supernatant having crude peroxidase was collected and the pellet was discarded. The supernatant was used for enzyme determination.

Uses:

Peroxidase can be used for treatment of industrial waste waters. For example, phenols, which are important pollutants, can be removed by enzyme-catalyzed polymerization using horseradish peroxidase.

There are many investigations about the use of peroxidase in many manufacturing processes like adhesives, computer chips, car parts, and linings of drums and cans.

Peroxidases are sometimes used as histological markers. Cytochrome c peroxidase is used as a soluble, easily purified model for cytochrome c oxidase.

4) Lipases (Glycerol Ester Hydrolase):

A large number of microorganisms are capable of using natural oils and fats as a carbon source for their growth. The enzyme responsible for the breakdown of the oils and fats prior their digestion by microorganisms are extracellular lipases which catalyse the hydrolysis of triglycerides to free fatty acids, partial glycerides and glycerol.

Production:

Olive oil is being used as the substrate for the lipase enzyme. Stable emulsion of olive oil was prepared by vigorous mixing of the oil with a solution of an emulsion stabilizer Triton X-100 in 50 mM potassium phosphate buffer. The fermentation should be carried out at 29°C for 96 h supplied with 1% carbon source. The type of oil used proved to be important both lipase production and fungal growth.

Recovery:

The mycelia were filtered and the culture filtrate was subjected to lipase assay. Lipase determination can be done either by stirring method or by standing method.

Uses:

Miyoshi Oil and Fat Co. Japan are using Candida cylindracae lipase to hydrolyse oils for the production of soaps. Microbial liapases can also be used as catalysts for inter-esterification reactions. Some Italian cheese is manufactured using lipases. Microbial lipases are also used in the formulation of clothes, washing products etc.

5) Proteases:

This group of enzymes catalyse the hydrolysis of the protein molecule. The enzyme is actually a mixture of proteinases and peptidases. These hydrolyse polypeptide fragments as amino acids.

Production:

A high yielding strain is selected and inoculated in special culture media containing 2-6% of carbohydrate, protein and mineral salt. It is incubated for 3-5 days at about 37°C with adequate aeration. The filtrate is concentrated, and the enzymes are used in this form from the culture is purified and absorbed onto some inert material such as saw dust. It is always kept in mind that such strains are suitable for enzyme production which can give rise high yield of proteases and comparatively low yield of other enzymes. Many different media such as those containing wheat bran, soybean cake, alfalfa meal, are proved to be better for protease production.

Commercial Applications of Proteases:

The numerous legitimate applications of the **'proteases'** are as given under :

(1) Primarily extensively in the **'detergent industry'.**

(2) In the **'silk-industry'** proteases help in the liberation of the silk fibres from the naturally occurring protenaceous material wherein they are actually imbedded.

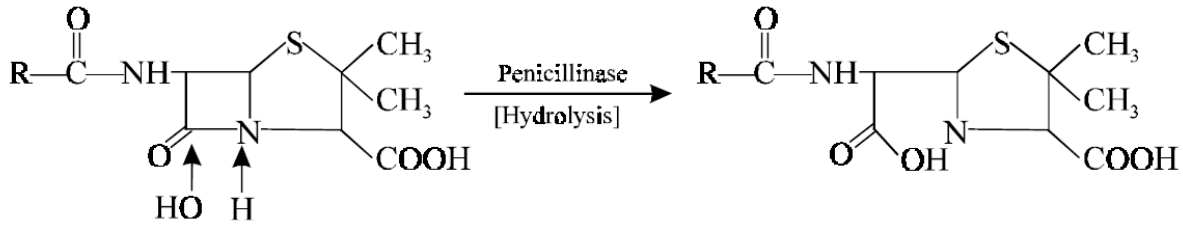
(3) Proteases (*e.g.*, papain) are also employed as a meat tenderizer.

(4) As an active and vital component in most **spot-remover** preparations for removing food spots in the **dry-cleaning** industry.

(8) Other areas in which proteases are employed include: food industry, brewing industry, film industry, waste-disposal (processing) management.

6) Penicillinase

Penicillinase is a *bacterial enzyme* that invariably inactivates most but not all *penicillins.* Importantly, this is regarded as an *extracellular type enzyme* produced adaptively by members of the coliform group of bacteria, by most *Bacillus* species, and certain strains of *Staphylococcus.* The enzyme exclusively carries out the hydrolysis of **penicillin** to **penicilloic acid** *i.e.,* a dicarboxylic acid as depicted below.



Penicillin Penicilloic Acid

It has been duly observed that a rather large segment of penicillin-resistant pathogenic strains of *Staphlococcus aureus* invariably comprise of this specific enzyme; and perhaps it overwhelmingly contributes a *major factor of penicillin resistance during infection.* Importantly, this enzyme causes an extremely rapid degradation of **penicillin** particularly in **penicillin fermentations** in case a specific contaminant which produces the enzyme incidentally gains an access to and be able to grow simultaneously in the **fermentation broth.**