Microbial enzyme

Commercial microbial enzymes are increasingly replacing conventional chemical catalysts in many industrial processes. Enzymes have several advantages over chemical catalysts, including the ability to function under relatively mild conditions of temperature, pH and pressure. This results in the consumption of less energy and there is usually no requirement for expensive corrosion- resistant equipment. Enzymes are specific, often stereoselective, catalysts, which do not produce unwanted byproducts. Consequently, there is less need for extensive refining and purification of the target product. Also, compared with chemical processes, enzyme-based processes are 'environmentally friendly' as enzymes are biodegradable and there are fewer associated waste disposal problems. Certain enzymes are not restricted to aqueous environments and can operate in two-phase water–organic solvent systems and in non-aqueous organic media, particularly hydrophobic solvents. Operation under such conditions can often improve enzyme performance, especially where substrates have limited water solubility.

Enzyme classification is based on a system originally established by the Commission on Enzymes of the International Union of Biochemistry. There are six main classes, grouped according to the type of reaction catalysed:

1 oxidoreductases (class 1) catalyse oxidation/reduction reactions, the transfer of H atoms, O atoms or electrons;

2 transferases (class 2) catalyse transfer of a group from one molecule to another;

3 hydrolases (class 3) catalyse hydrolysis, the cleavage of bonds by addition of a water molecule;

4 lyases (class 4) catalyse splitting bonds, other than via hydrolysis or oxidation;

5 isomerases (class 5) catalyse structural rearrangements of molecules; and

6 ligases or **synthetases** (class 6) catalyse the formation of new bonds, e.g. C–N, C–O, C–C and C–S, with breakdown of ATP.

The enzymes are biocatalysts, which act in relatively mild conditions of temperature, pH, and pressure. The importance of enzymes in industrial processes can be assessed by the fact that they perform very specific reactions/modification of the substrate without formation of unwanted by-products in comparison to the chemical catalyst. Processes based on enzymes are eco-friendly, require less energy input and do not require reaction vessels made up of expensive corrosion resistant material. In enzymatic processes, the need for extensive refining of the target product is greatly reduced since enzymes catalyse both stereo- and regio-selective modification of substrate in comparison to the chemical catalysts in which side reactions are very common. There are some enzymes which operate in non-aqueous organic media, particularly hydrophobic solvents, more efficiently, especially in conditions where the substrates have limited water solubility. The enzymes are used in different processes (Fig.1) for specific purposes and to reduce the energy and cost inputs to a great extent.

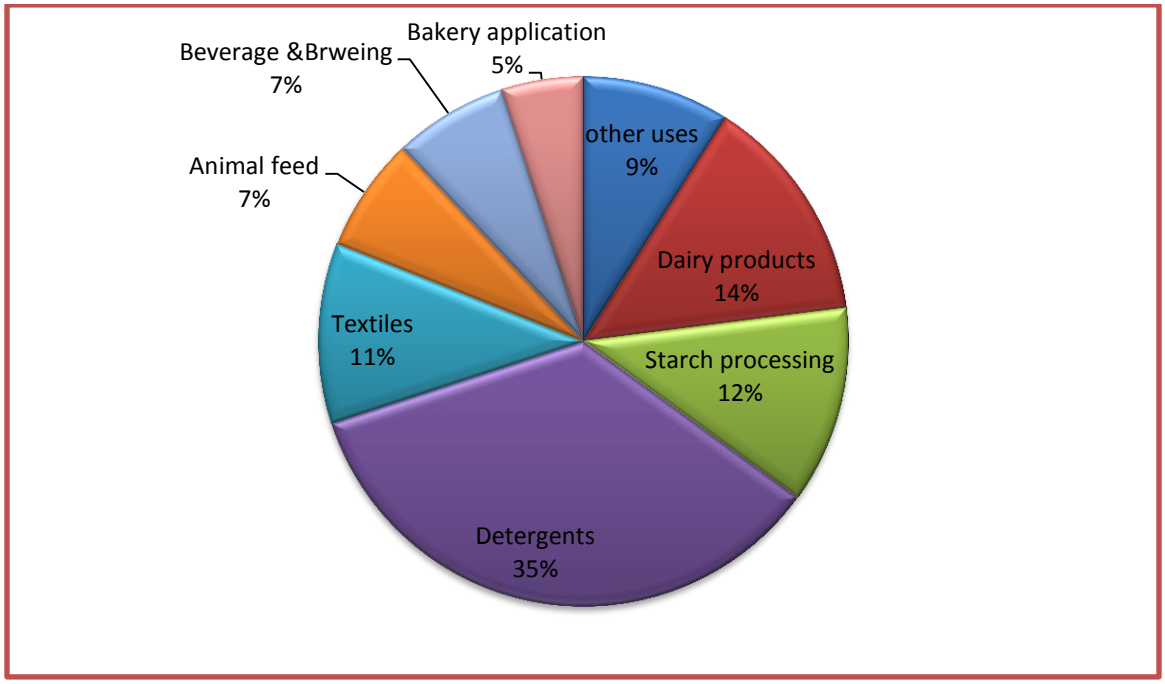

Fig. 1 Applications of bulk microbial enzymes

Amylases

Higher plants store carbohydrates in the form of starch (granules) which is composed of 20-30% amylose (linear polymer of 500-20,000 α-1,4 linked D-glucose units) and 70-80% amylopectin (branched polymer formed by joining of linear polymer of 24-30 α-1,4 linked D-glucose units by α-1,6 glycosidic bond). Starch hydrolyzing enzymes are referred as **amylases**, and are mainly used in the production of sweeteners for the food industry. Enzymatic hydrolysis of starch first produces short-chain polymers of glucose called dextrins, then the disaccharide maltose, and finally glucose. Starch saccharification process involves use of α-amylases, β-amylases, glucoamylases, pullulanases and isoamylases glucose isomerases.

The commercially important amylases of microbial origin that split α-1, 4 and/or α-1, 6 bonds in starch molecule have been classified into six groups as shown in **Table (1)** and the specific glycosidic bond hydrolysed by amylases .

Table 1: Classification of amylases on the basis of glycosidic bond hydrolysis.

Class	Glycosidic bonds hydrolyzing properties	Examples
1	Hydrolyse α-1,4 bond and bypass α-1,6 linkages	α-Amylase(endoacting amylases)
2	Hydrolyse α-1,4 bond and cannot bypass α-1,6 linkages	β-Amylase(exoacting amylases producing maltose as a major end product)
3	Hydrolyse α-1,4 and α-1,6 linkages	Amyloglucosidase (glucoamylase)and exoacting amylase
4	Hydrolyze only α-1,6 linkages	Pullulanase and other debranching enzymes
5	Hydrolyse preferentially α -1,4 linkages in short chain oligosaccharides produced after hydrolysis of amylose and amylopectin by other amylases	α-Glucosidases
6	Hydrolyse starch to a series of nonreducing cyclic D-glucosyl polymers (cyclodextrins or Sachardinger dextrins)	Bacillus macerans amylase (cyclodextrin producing enzyme)

α-Amylases:

α-Amylases (1,4- α-glucan-glucanohydrolases) are extracellular enzymes which hydrolyze α-1,4-glycosidic bonds present in the interior of starch and thus are endoacting enzymes. α-Amylases are produced by many bacteria and fungi and are classified on the basis of their starch-liquefying and/or saccharogenic effect, pH optimum, temperature range, and stability. Saccharogenic amylases produce free sugars upon starch hydrolysis, whereas starch-liquefying amylases breakdown the starch polymer but do not produce free sugars. ***Bacillus subtilis*** Marburg, ***B. subtilis*** var. amylosaccharaticus, and ***B. natto*** produces saccharogenic α-amylase, where as B. amyloliquefaciens produces liquefying α-amylase. α-Amylases contain a large proportion of tyrosine and tryptophan in enzyme protein and most of them require calcium as a stabilizer. This enzyme is extensively used in different industry (Table 2).

Table 2: Uses of α-amylase in different industries

Industry	Application
Starch Processing	Liquefaction of starch in the production of sugar syrup
Milling	Modification of α-amylase-deficient strains
Baking	Generation of fermentable sugars in flour, and improvement of crust colour
Brewing	Starch hydrolysis during wort preparation from barley
Paper	Liquefaction of starch without sugar production for sizing of paper
Textile	Continuous desizing at high temperatures
Feed	Treatment of barley for poultry and calf
Biological detergents	Starch removal from food stains
Sugar industry	Breakdown of starch from cane juice to improve filterability

α-Amylase producing bacteria:

Bacillus subtilis, B. subtilis var**. *amylosaccharaticus***, ***B. natto, B. cereus, B. amyloliquefaciens, B. coagulans, B. polymyxa, B. stearothermophilus, B. cladolyticus, B. acidocaldarius, B. subtilis var. amylosaccharticus, B. licheniformis,*** Lactobacillus, Micrococcus, Pseudomonas, Arthrobacter, Escherichia, Proteus, Thermonospora, and Serratia are some α-amylase producing bacteria. However, ***Bacillus amyloliquefaciens*** and ***B. licheniformis*** are mainly produced for the industrial production of α-amylase.

α-Amylase producing fungi

Aspergillus, Penicillium, Cephalosporium, Mucor, Candida, Neurospora and Rhizopus are some α-amylase producing molds and ***Aspergillus oryzae*** is one of the mold used as source for the industrial production of α-amylase.

Production of α-Amylase

Bacterial α-amylase

The bacterial α-amylase is inducible and repressible by glucose produced by the hydrolysis of starch. It is assumed that a basal level of the enzyme is produced constitutively, which hydrolyses the starch generating the low molecular weight inducers. The production media for amylase includes 5% starch, 0.5% NH_4NO_3, 0.28% sodium citrate, 0.13% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.01% $CaCl_2 \cdot 2H_2O$, 0.5% peptone. 0.2% yeast extract and the fermentation is carried out either in batch or in fed-batch manner around pH 6.8. Mostly thermophilic strains are being used for the production of the amylase for applications at elevated temperatures. The temperature of the fermentation process depends on the strain used. In the fermentation process, amylase is produced during the growth of the bacteria and formation of spores.

β-Amylases:

β-Amylases (α-1,4-glucan-maltohydrolases) are the exoacting enzymes hydrolyzing the α-1,4-glycosidic bonds from the non-reducing ends producing maltose and limit dextrin as the major product and are unable to hydrolyse the α-1,6 branching present in amylopectin. This enzyme is mainly present in plants but some microbes are known to produce this enzyme which includes: *Bacillus polymyxa*, *B. cereus*, *B. megatarium*, *Streptomyces* sp., *Pseudomonas* sp. and *Rhizopus japonicus*. β-Amylase has been produced on starch waste by a hyper-amylolytic strain of *B. megaterium* B6 mutant UN12 in Submerged fermentation (SmF) and Solid state fermentation (SSF) . The starchy wastes from arrowroot, arum, maize, potato, pulse, rice, rice husk, tamarind kernel, cassava, water chestnut, wheat and wheat bran are used as substrate. This enzyme is mainly used in the

production of maltose syrup and also digestion of barley starch during beer production.

Glucoamylases:

Glucoamylases (α-1,4-glucan-glucohydrolases) hydrolyse starch from the non reducing end producing glucose, maltose, and limit dextrins. *Aspergillus niger*, *A. oryzae*, *A. awamori*, *Rhizopus niveus*, *R. delemar*, *R. formosaensis*, and *R. javanicus* are the strains employed for the production of glucoamylases. This enzyme is mainly used for the production of fructose syrup and its production is carried out in submerged fermenter. Starch or dextrin induces the production of glucoamylases, therefore, starch is generally added in the production media. The production of the enzyme is carried at 28-30 ºC for 3-5 days depending on the strain. Glucoamylase also catabolite repressible by glucose, glutamic acid and lactose.

1, 6-Glycosidic bond hydrolyzing enzymes:

The amylopectin is a branched polymer of glucose chain linked by α-1,6 glycosidic bonds. Pullulanases and isoamylases hydrolyse this bond resulting production of straight chain maltodextrin. Pullulan is obtained from *Pullularia pullulans* which is neutral glucan polymer consisting of α-1,4 linked maltotriose unit joined to each other by α-1,6 glycosidic bond. Pullulanases are the enzymes that hydrolyse the α-1,6 glycosidic bond of the pullulan and amylopectins but the isoamylases only hydrolyse the α-1,6 glycosidic bond of amylopectin. *Aerobactor aerogens*, *Bacillus acidopullulyticus*, *B. polymyxa*, *Pseudomonas* s*accharophila*, *Streptococcus* sp., *Strptomyces* sp., are some of the strains used for pullulanase production. Isoamylases are obtained from *Agrobacterium*, *Erwinia*, *Staphylococcus*, *Serratia*, *Nocardia*, *Bacillus*, *Pediococcus*, *Lactobacillus*, and *Leuconostoc*. Pullulanases and isoamylases are used in the starch hydrolysis process.

Application of amylases

Amylases were the first enzymes to be produced industrially and share 20% of the present enzyme market. Amylases are used in food, feed, textile, and pharmaceutical industries. In the food sector they are mainly used for liquefaction of starch, manufacture of maltose, high fructose containing syrups, oligosaccharide mixture, maltotetraose syrup, and high molecular weight branched dextrins. These products are used for various food preparations (cake, candies, etc) adding characteristic high or low sweetness and maintain texture. The use of amylase has replaced the chemical hydrolysis of the starch as the latter used to yield undesirable by-products and is also uneconomical. Amylase is also used for removal of starch sizer from textile (desizing) making the fabric ready for scouring and dyeing.

Ethanol production from starchy substrates has been improved by using amylases or coculturing the amylolytic strains with ethanol producing microbes in starch based media. This technique has also been used to digest the starch in the brewing process. Amylases are also used for processing waste containing starch generated from food processing plants ultimately reducing the pollution load of effluent and producing microbial biomass protein. Alkaline amylases are used in detergents and dish bar for removing the starch stains on cloths and utensils respectively. Amylases are also ingredient of the digestive syrups used for treating digestive disorders. Amylase treated flour is used for preparing animal feed and have improved digestibility.