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FACULTY OF ENGINEERING & TECHNOLOGY

Production of amylase

- Amylases are important hydrolase enzymes which have been widely used since many decades.

- These enzymes randomly cleave internal glycosidic linkages in starch molecules.

- To hydrolyze them and yield: (dextrins) (oligosaccharides)

- α-Amylase is a hydrolase enzyme that catalyses the hydrolysis of internal α-1, 4- glycosidiclinkages in starch

- To yield products like glucose and maltose.

- It is a calcium metalloenzyme i.e. it depends on the presence of a metal co factor for its activity.

- The optimum pH for activity is found to be 7.0

•**Amylase Production Methods**

•There are mainly two methods which are used for production of α-Amylase on a commercial scale. These are: 1) Submerged fermentation and 2) Solid State fermentation.

•Solid State fermentation is a fairly new method while the Submerged fermentation is a traditional method of enzyme production from microbes which has been in use for a longer period of time.

•Submerged fermentation (SmF) employs free flowing liquid substrates, such as molasses and broths. The products yielded in fermentation are secreted into the fermentation broth.

•The substrates are utilized quite rapidly; hence the substrates need to be constantly replenished. This fermentation technique is suitable for microorganisms such as bacteria that require high moisture content for their growth. SmF is primarily used for the extraction of secondary metabolites that need to be used in liquid form. This method has several advantages.

•SmF allows the utilization of genetically modified organisms to a greater extent than SSF. The sterilization of the medium and purification process of the end products can be done easily. Also the control of process parameters like temperature, pH, aeration, oxygen transfer and moisture can be done conveniently. Solid state fermentation is a method used for microbes which require less moisture content for their growth.

•The solid substrates commonly used in this method are, bran, bagasse, and paper pulp.

•The main advantage is that nutrient-rich waste materials can be easily recycled and used as substrates in this method.

•Unlike SmF, in this fermentation technique, the substrates are utilized very slowly and steadily. Hence the same substrate can be used for a longer duration, thereby eliminating the need to constantly supply substrate to the process.

•Other advantages that SSF offers over SmF are simpler equipments, higher volumetric productivity, higher concentration of products and lesser effluent generation.

•For several such reasons SSF is considered as a promising method for commercial production of enzymes.

Purification of α-Amylase

•Enzymes used for industrial applications are usually crude preparations and require less downstream processing. Whereas the enzymes used clinical and pharmaceutical industry need to be highly purified. Also when used for study of structure function relationships and biochemical properties the enzymes have to be in purified form.

•Purification methods commonly employed are precipitation, chromatography and liquid-liquid extraction depending on the properties of the enzyme desired. A combination of the above methods is used in a series of steps to achieve high purity.

•The number of steps involved in purification will depend on the extent of purity that is desired. The crude extracellular enzyme sample can be obtained from the fermented mass by filtration and centrifugation. In the case of intracellular enzymes, raw corn starch may be added followed by filtration and subsequent steps.
•The crude amylase enzyme can be precipitated and concentrated using ammonium sulphate precipitation or organic solvents.

•The precipitated sample can be subjected to dialysis against water or a buffer for further concentration. This can be followed by any of the chromatographic techniques like ion exchange, gel filtration and affinity chromatography for further separation and purification of the enzyme.

Production of Pectinase Enzyme

•Pectinases enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples.

•Pectinases have also been used in wine production since the 1960s. Enzymes were used industrially because of their high catalytic power, specific mode of action, stereo specificity, eco-friendly use, reduced energy requirement. The commercial application of pectic enzymes was noted in 1930 for the preparation of wines and fruit juices.

•The chemical nature of the plant tissues was apparent only in 1960's. In 1995 was one billion dollars, of which 75 million dollars. By 2005 the whole world market for industrial enzymes is expected to be 1.7-2 billion dollars.

•They are of prime importance for plants as they help in cell wall extension and softening of some plant tissues during maturation and storage. They also aid in maintaining ecological balance by causing decomposition and recycling of waste plant materials.

Isolation of micro organisms: Bacterial species were isolated on NAM media from the soil enumerated for 20 days with fruit peels as a sole of carbon source by the serial dilution in the range of 10-1 to10-6. Few drops were spread from each sample on media for growth.

Biochemical tests: Some biochemical and staining methods ware used for the identification of the bacteria these included gram staining method, endospore staining, catalase test, Simmon citrate agar test, MR-VP tests etc

Screening: Various screening tests were performed by inoculating bacteria on mannitol agar media, Amylase screening test also performed and results obtained positive for pectinase screening test.

Fermentation and media: A basic liquid medium was used for the production of pectinase having compostion (g/ml), Pectin (1.0), Ammonium dihydrogen sulphate (0.14), Potassium dihydrogen phosphate (0.2), Potassium hydrogen phosphate (0.6)/Magnesium sulphate (0.02) at pH 7.2 . The production media was incubated for 48 h for submerged fermentation.

Enzyme extraction and purification: Culture medium is centrifuged and supernatant was used as crude enzyme source. The crude enzyme was precipitated by 60 % ammonium sulfate saturation, incubated for night at 40 C and centrifuged at 5000 rpm for 20 min and palates were dissolved in T.E. buffer at pH 7. Dialysis was also performed against T. E. buffer overnight at 4 0 C.

Ion exchange chromatography: Dialyzed buffer is applied to the agarose column (1×30 cm.) equilibrated with TE buffer (pH 7.0). Elution was carried out by liner gradient of NaCl (0.1M - 0.6M). About 5 ml of fraction collected and activity was observed.

Determination of protein: Total protein is determined by the method of Lowery et al. (1951) by using BSA as standard.

Enzyme assay Pectinase activity was measured by estimation of glucose by DNS method using pectin as substrate. Standard graph prepared by concentration of standard glucose solution. One unit of Pectinase activity was defined as the amount of enzyme which liberated 1µm glucose per min.