جامعة الانبار
كلية العلوم
قسم التقنيات الأحيائية

اسم المادة: التقنيات الاحيائية
عنوان المحاضرة :Biotechnology of Enzymes
الاستاذ المساعد الدكتور صفاء عبد لطيف المعيني

Biotechnology of Enzymes

What is the enzyme

- Enzymes are proteins, produced by living cells; they catalyze chemical re actios by lowering the activation energy.

- The molecules at the beginning of the process are called substrates and the enzyme converts these into different molecules, called products.

Examples for industrial enzymes

- The first enzyme produced industrially was the fungal amylase **Taka diastase** which was employed as a pharmaceutical agent for digestive disorders.
- Amylases stand out as a class of enzymes, which are of useful applications in the food, brewing, textile, detergent and pharmaceutical industries. Amylases are enzymes that break down starch or glycogen. The amylases can be derived from several sources such as plants, animals and microbes. Although many microorganisms produce this enzyme, the most commonly used for their industrial application are Bacillus licheniformis, Bacillus amyloliquifaciens and Aspergillus niger

- Proteases are one of the most important groups of industrial enzymes and account for nearly 60% of the total enzyme sale. The major uses of free proteases occur in dry cleaning, detergents, meat processing, cheese making, and silver recovery from photographic film, production

of digestive and certain medical treatments of inflammation and the virulent wounds1 .

- Lipases are one of the highly commercialized enzymes; have an important role found and ranked after proteases and amylases. Lipases are widely used in industrial application such as, detergent industry, pharmaceutical industry, pulp and paper industry, production of biodiesel, dairy and bakery foods, fats and oils. Lipases are fond in animal, plant and microorganisms
- Microbial rennin is also one of the most significant enzymes. It has been used instead of Calf's rennin in cheese production.

Enzymes location

- Enzyme is protein which is synthesized as intra and extra cellular compoundsEnzymes which are produced within the cell or at the cytoplasmic membrane are called as Endocellular enzymes.
- Enzymes which are liberated in the fermentation medium which can attack large polymeric substances are termed as Exocellular enzymes. Eg: Amylases & Proteases

Methods of Enzyme Production

Submerged fermentation

- Submerged fermentation is the cultivation of microorganisms in liquid nutrient broth.
- Industrial enzymes can be produced using this process.
- This involves growing carefully selected micro organisms (bacteria and fungi) in closed vessels containing a rich broth of nutrients (the fermentation medium) and a high concentration of oxygen. As the

microorganisms break down the nutrients, they release the desired enzymes into solution.

- ✓ The fermentor which contains the substrate is operated continuously and the product biomass is continuously harvested from the fermenter by using different techniques then the product is filtered or centrifuged and then dried.
- ✓ Submerged Fermentation (SmF)/Liquid Fermentation (LF) SmF utilizes free liquid substrates, such as molasses and broths.
- ✓ The substrates are utilized quite rapidly; hence need to be constantly replaced/supplemented with nutrients.
- ✓ This fermentation technique is best suited for microorganisms such as bacteria that require high moisture.
- ✓ An additional advantage of this technique is that purification of products is easier.

Solid State Fermentation

- Solid state (substrate) fermentation (SSF) has been defined as the fermentation process occurring in the absence or near-absence of free water.
- Solid state fermentation (SSF) is another method used for the production of enzymes, which involves the cultivation of microorganisms on a solid substrate, such as grains, rice and wheat bran.

This method is alternative to the production of enzyme in liquid by submerged fermentation.

- High volumetric productivity.
- Relatively high concentration of product.
- Less effluent generate.
- Simple fermentation equipment.

Factors Involved in SSF Process

- Selection of Micro-organisms

Filamentous Fungi has shown better results growing in the solid substrate fermentation.

- Substrate
- Process Optimization
- Product Isolation & Purification

Problems in SSF

- Heat Transfer: One of the main difficulty is to control the temperature during the fermentation process.
- Heat is generated during the metabolic activities of microorganisms, since the substrate used has low thermal conductivity heat removal will be slow.

SOLID-STATE FERMENTATION	SUBMERGEDFERMENTATION
Utilizes solid substrates like bran, bagasse and paper pulp.	Utilizes free flowing liquid substrates, such as molasses and broths
Substrates are utilized very slowly, need not to be replaced.	Substrates are utilized quite rapidly, need to be replaced constantly
Best suited for fungi that require less moisture content.	Best suited for bacteria that require high moisture content.
Culture systems involves three phases, solid, liquid and gaseous	Involves two phases ,liquid and gaseous phase.
Inoculum ration is always larger	Inoculum ration is usually small.
System may or may not involve agitation	Agitation is often essential

Improvement of Enzyme production

- Microbial Genetics – High yields can be obtained by Genetic manipulation. Example – Hansenula polymorpha has been genetically modified so that 35% of it's total protein consists of the enzyme alcohol oxidase.
- Optimization of fermentation conditions (Use of low cost nutrients, optimal utilization of components in nutrient solution, temperature and pH)
- New cell breaking methods like Homogenizer, Bead mill, Sonication etc
- Modern purification processes like Counter current distribution, Ion-exchange chromatography, Molecular-sieve chromatography, Affinity chromatography and precipitation by using alcohol, acetone.

References

1- John E. Smith(2010). Biotechnology, fifth edition. CAMBRIDGE UNIVERSITY PRESS.

2- Desmond S.T.Nicholl(2010). An introduction to genetic engineering . CAMBRIDGE UNIVERSITY PRESS.