Industrial Microbiology

4th year Biology

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Microbial Enzymes

Enzymes occur in every living cell, hence in all living microorganisms. Each single strain of organism produce a large number of enzymes, hydrolyzing, oxidizing or reducing, and metabolic in nature. But the absolute and relative amounts of the various individual enzymes produced vary markedly between species and even between strains of the same species. Hence, it is customary to select strains for the commercial production of specific enzymes which have the capacity for producing highest amounts of the particular enzymes desired.

Special **characteristics of microbial enzymes** include their capability and appreciable activity under abnormal conditions, mainly of:

1- **Temperature**: certain microbial enzymes are categorized as thermophilic, Microorganisms with systems of thermostable enzymes that can function at higher than normal reaction temperatures would decrease the possibility of microbial contamination in large scale industrial reactions of prolonged durations.

2- **pH:** Neutral, acidophilic or alkalophilic.

Phases of enzymes production

The enzymes production process can be divided into the following **phases**:

1- Selection of an enzyme.

The criteria used in the selection of industrial enzymes include specificity, reaction rate, PH, temperature, stability and affinity to substrate.

2- **Selection of production strain**.

- Extracellular enzymes are much simpler than in cases of enzymes produced intracellular.
- The production strain most is (**G**enerally **R**ecognized **A**s **S**afe) GRAS, especially in food processes.
- The organism should be able to produce high amount of desired enzymes.

3- Construction of an overproducing strain by genetic engineering.

Most enzymes are produced by microorganisms with GRAS-status, usually the production organisms and often the individual enzymes as well as have been genetically engineered for maximal productivity and optimized enzymes properties.

4- **Optimization of culture medium and production conditions**.

This covers media composition and their optimal economical concentration, cultivation types, and process conditions.

5- **Optimization of recovery process** (and purification if needed).

6- Formulation of stable enzyme product.

Enzymes should be stored at 4-8C^{o}, which will minimize activity loss to less than 5% over 36 months.

Depending on the **site of reaction catalyzed**, enzymes are divided into two types:

1. **Intracellular enzymes** : Microorganisms produce enzymes that function inside their cells**.**
2. and they may also produce enzymes that are secreted and function outside the cells **(extracellular enzymes)**

#	**Intracellular enzymes**	**Extracellular enzymes**
1.	More difficult to isolate	Easier to isolate
2.	Cells have to be broken apart to release them	No need to break cells – secreted in large amounts into medium surrounding cells
3.	Have to be separated out from cell debris and a mixture of many enzymes and other chemicals	Often secreted on their own or with a few other enzymes
4.	Often stable only in environment inside intact cell	More stable
5.	Purification/downstreaming processing is difficult /expensive	Purification/downstreaming processing is easier / inexpensive

Methods of fermentation:

1- **Submerged fermentation** is the cultivation of microorganisms in liquid nutrient broth. Industrial enzymes can be produced using this process. This involves growing carefully selected microorganisms (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.

2- **Solid state fermentation (SSF)**: is involves the cultivation of microorganisms on a solid substrate, such as grains, rice and wheat bran. This method is an alternative to the production of enzymes in liquid by submerged fermentation.

SSF has many **advantages** over submerged fermentation. These include:

High volumetric productivity, relatively high concentration of product, less effluent generated and simple fermentation equipment.

Surface

1- Requires much space

2- Requires much hand labor
Uses low pressure air blower
Little power requirement

3- Minimum control necessary
Little contamination problem

4- Recovery involves extraction with aqueous solution, filtration or centrifugation and or perhaps precipitation

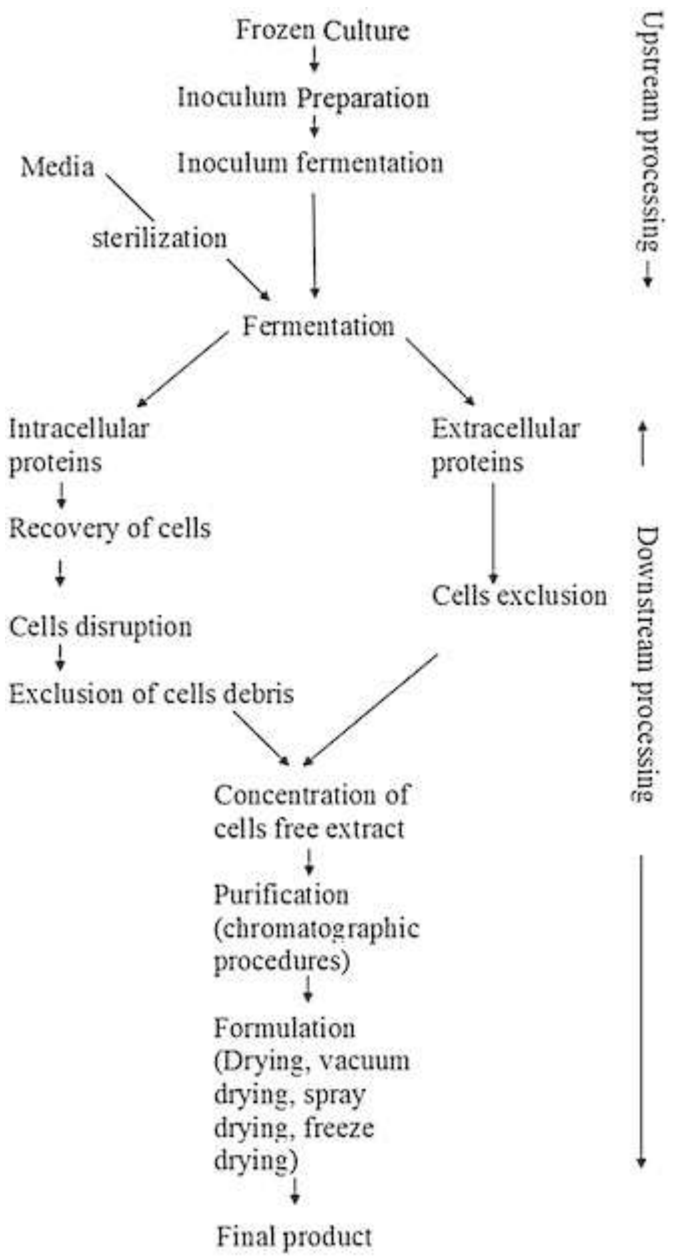
Submerged

1- Uses compact closed fermentor

2- Requires minimum of labor
Requires high pressure air
Needs considerable power for air compressors and agitations

3- Requires careful control
Contamination frequently a serious problem

4- Recovery involves filtration centrifugation, and or precipitation



The maximum enzyme production is usually in stationary phase of microbe growth, so a batch or fed-batch processes are usually used. The medium must be chosen to stimulate the microbe into synthesizing the correct enzyme .For example to stimulate a microbe to synthesis amylase enzymes , a medium with starch but no sugars is used. In downstream processing, the remaining mixture contains enzymes, waste materials, nutrients and cells.Then enzyme is extracted by downstream processing, nature of the downstream processing depends on two considerations: Whether enzyme is intracellular or extracellular

Enzymes are widely used commercially, for example in the detergent, food and brewing industries. Protease enzymes are used in 'biological' washing powders to speed up the breakdown of proteins in stains like blood and egg. Pectinase is used to produce and clarify fruit juices. Problems using enzymes commercially include:

- they are water soluble which makes them hard to recover
- some products can inhibit the enzyme activity (feedback inhibition)

Immobilized enzymes

Enzymes can **be immobilized by** fixing them to a solid surface. This has a number of commercial advantages:

- the enzyme is easily removed
- the enzyme can be packed into columns and used over a long period
- speedy separation of products reduces feedback inhibition
- thermal stability is increased allowing higher temperatures to be used
- higher operating temperatures increase rate of reaction

There are **four principal methods of immobilization** currently in use:

- adsorption onto an insoluble substance
- covalent bonding to a solid support
- encapsulation behind a selectively permeable membrane
- entrapment within a gel