Shree H. N. Shukla Institute of Pharmaceutical Education and Research, Rajkot

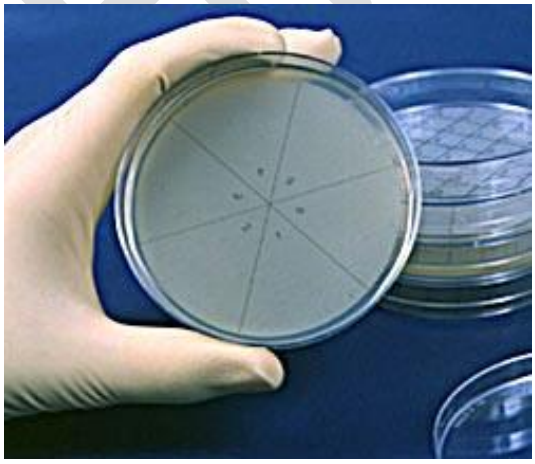
B. Pharm
Semester-V

Subject Name: Pharmaceutical Biotechnology
Subject Code: BP505TT

Chapter-5
FERMENTATION METHOD

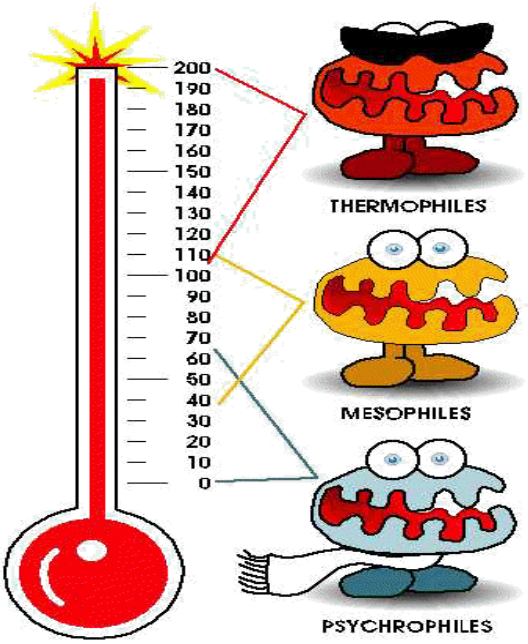
Introduction

- The fermentation industry is composed of five major bio-ingredient categories.
- They are:
  - Proteins & amino acids.
  - Organic acids.
  - Antibiotics.
  - Enzymes.
  - Vitamins & hormones.
- **Fermentation industry is driven by:**
  - The cost and availability of feed-stocks.
  - The efficiency of industrial microorganism.
  - Fermentation condition and optimization.
  - Down stream process and end-product recovery efficiency.
  - Fermentation by-product utilization.
  - Utility consumption and labor cost.
- Optimum balance of the media is mandatory for cells propagation and for the maximum production of target metabolite (end-product).
- Media compositions:
  - Carbon source.
  - Nitrogen source.
  - Minerals.
  - Growth factors.
  - Precursors (mutants).



Industrial microorganisms

- Microbial screening.
  - Wild strains.
- Microbial yield improvement
  - Mutation.
  - Recombinant DNA.
  - Genetically engineered.
- Microbial selection.
- Industrial microorganism



Types of fermentation

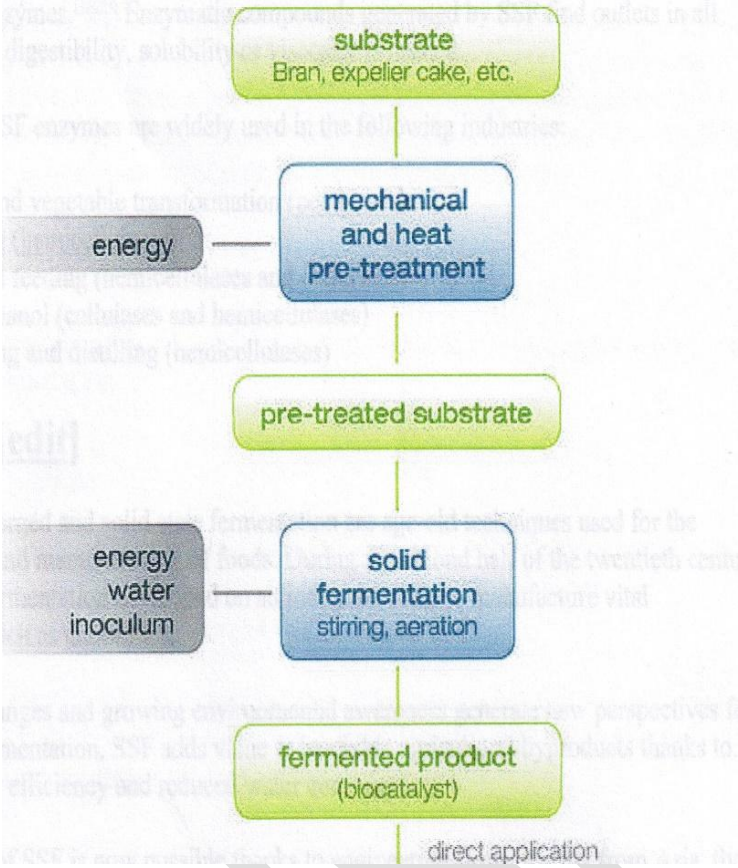
- **Solid State fermentation (SSF).**
- **Liquid State fermentation (LSF) Surface culture & submerged culture**

Solid State Fermentation (SSF)

- SSF process can be defined as microbial growth on particles without presence of free water.
- Particles are a solid culture substrate such as rice or wheat bran saturated with water and inoculated with (mold, yeast, bacteria) in controlled room temperature.
- It is ideal for growing filamentous fungi.
- It has been used in Asia and developing nations.
- It is more cost effective (smaller vessels lower water consumption, reduced waste water treatment costs, lower energy consumption, and less contamination problems).

Applications:

- Potentially many high value products such as extra-cellular enzymes, primary metabolites, and antibiotics could be produced in SSF.
- It is estimated that nearly a third of industrial enzyme produced in Japan is made by SSF process.
- Production of organic and ethanol from starchy substrates.
  Digestibility of fibers and lignocelluloses materials for both human and animal consumption



Liquid State fermentation (LSF)

[Submerged culture]

- Submerged culture is performed in tanks which can reach in size for over 100,000 gallons.
- It is ideal for the growing unicellular organisms such as bacteria and yeast.

LSF methods:

- **Batch fermentation**.
- **Fed-batch fermentation**.
- **Continuous fermentation.**
- **Semi-continuous fermentation.**

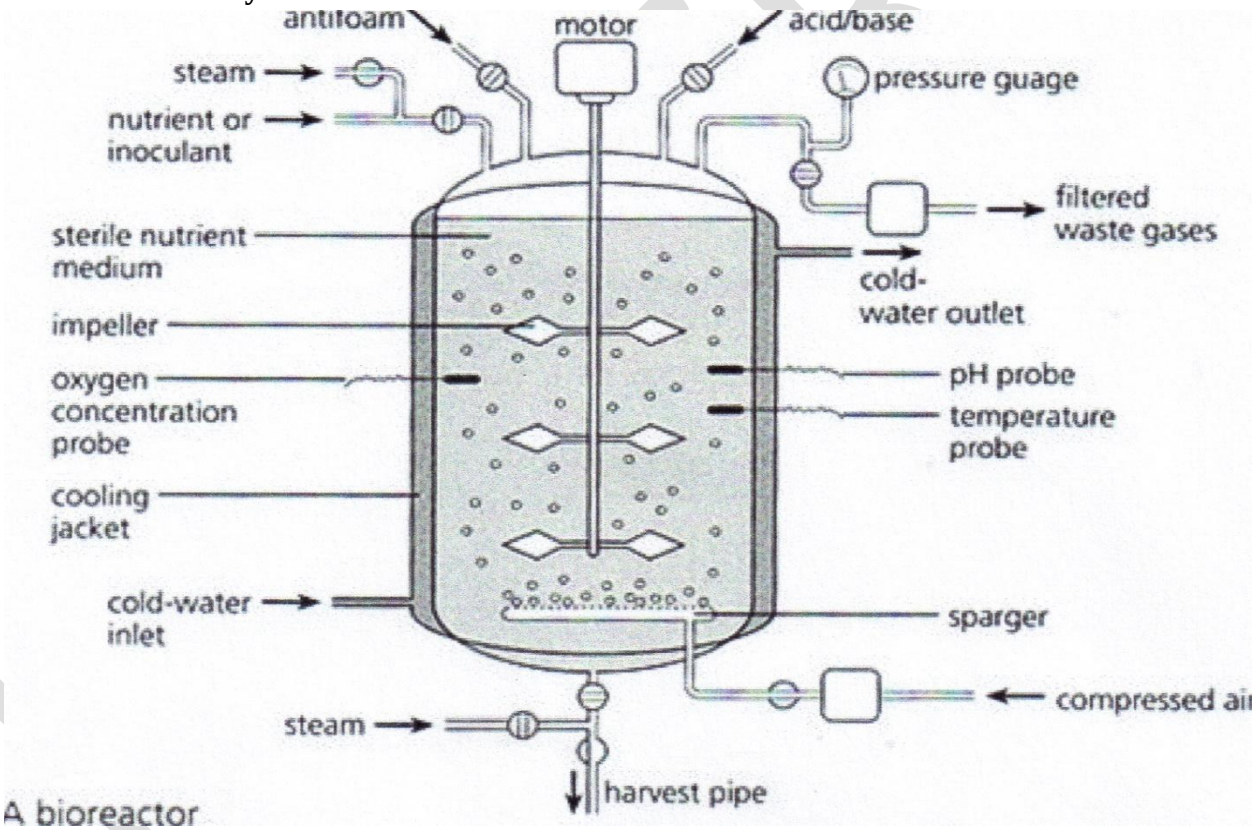
Batch fermentation

- Considered to be a closed system.
- The sterilized media in the fermenter is inoculated with the microorganism.
- Incubation is allowed under the optimum conditions (aeration, agitation, temperature).
- During entire fermentation nothing is added except air, antifoam and acid/base.

Fed-Batch fermentation

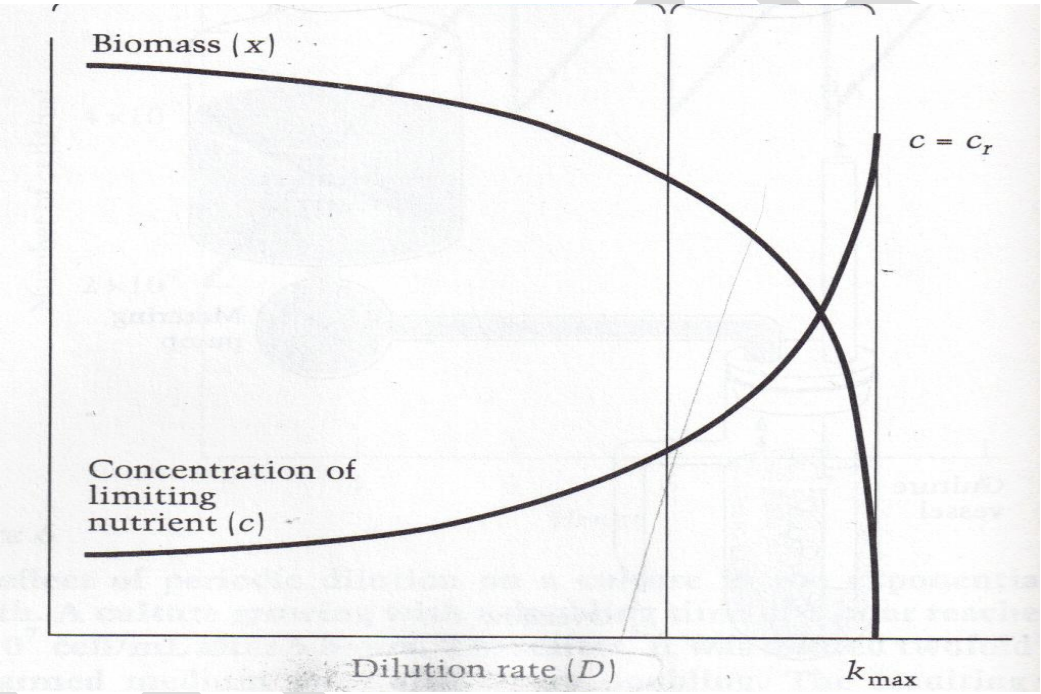
- It is enhancement of batch fermentation.
- Continue adding the nutrients (feeding) in a small doses during the fermentation.
- The method in controlling nutrients feeding process is by measuring methods.
- The main advantage of fed-batch fermentation is the elimination of catabolite repression (feed-back inhibition).

Batch fermenter system

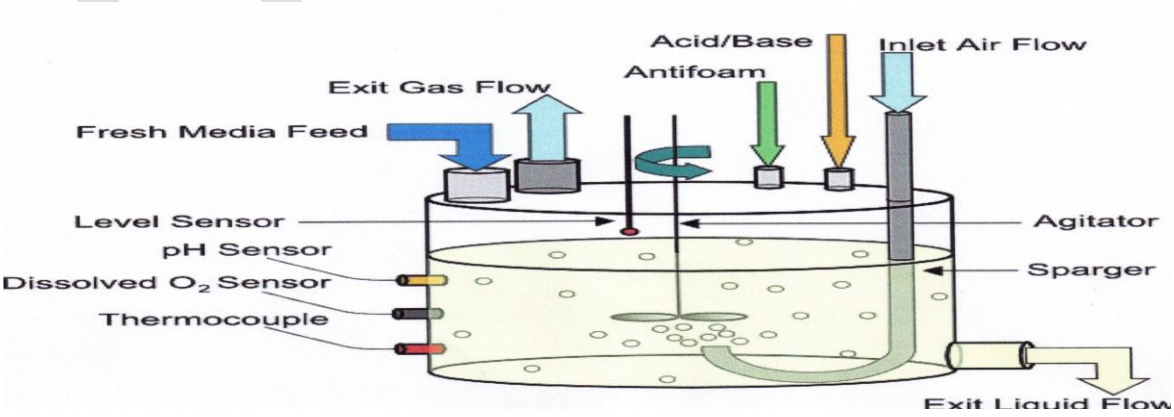


Continuous fermentation

- It is an open system.
- Continuously sterile nutrient is added and the converted nutrient is taken out from the fermentor.
- In continuous process cell loss as a result of outflow must be balanced by growth of the microorganism.
- **Effect of flow rate on substrate concentration**



Continuous fermenter system



Important factors for continuous fermentation

- The system must be stable for at least 500 hours.
- Maintaining sterile conditions for all period of fermentation time.
- The composition of nutrients must be constant all the time.
- Maintaining the strain stability for constant high production yield (concerning about reverse mutation).

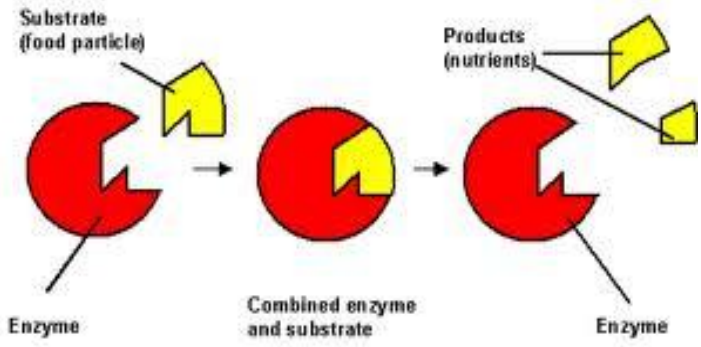
Semi-continuous fermentation

- Semi-continuous fermentations, in which a fraction of a fermentation is replaced with fresh media at regular intervals.

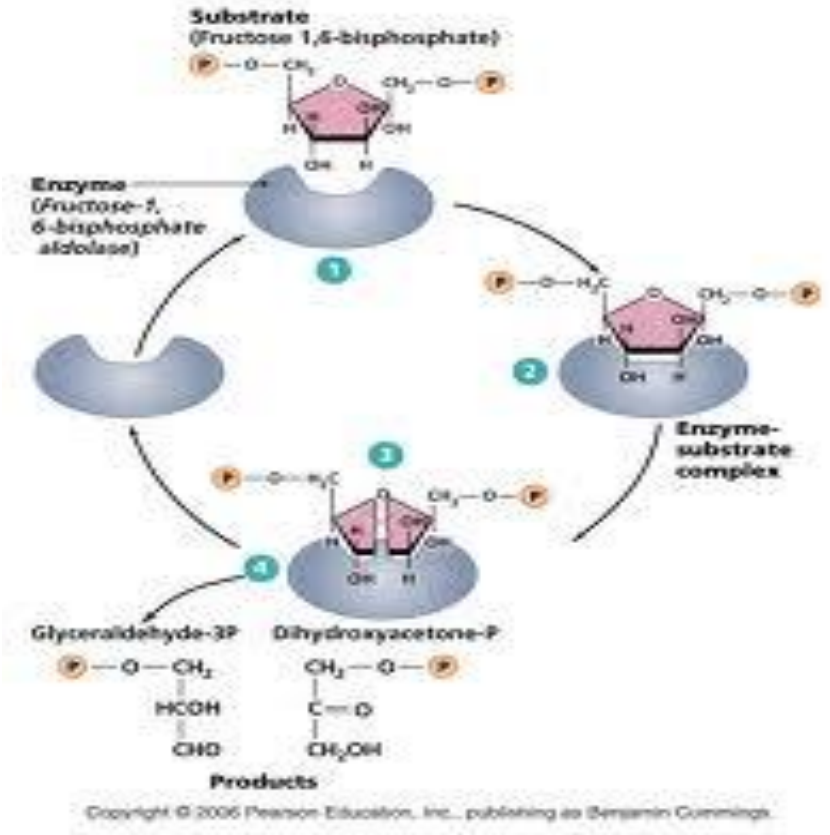
Enzymes

- Enzymes are active biological molecules responsible for thousands of metabolic process that sustain life.
- Most enzymes are proteins, although some catalytic enzymes are RNA molecules.
- In enzyme reactions, the molecules at the beginning of the process, called **substrates** and converted into different molecules that called **end-products.**
- Enzymes are very specific as which reaction they catalyze and the substrate that involved in the reaction.
- Depends on enzyme activity, the bioconversion to end-products can be faster and reached the equilibrium state rapidly.

Enzyme/substrate interaction



How enzymes break down food into nutrients



Factors Effects Enzymes Catalytic Activity

- **Temperature:** The optimum is generally 40-60^{0}C. Some enzymes exhibit an optimum at almost 100^{0}C.
- **Value of PH:** The optimum generally in the range from 5-7. Extreme values of 1.5-10.5 have been found.
- **Activation:** Many chemical activates the catalytic enzymes activity, Such as inorganic ions.
- **Inhibitors:** Many chemical inhibits the catalytic enzymes activity
- There are two types of enzymes inhibition: *Irreversible inhibitors* (competitive inhibition) and *reversible inhibitors* (uncompetitive inhibition).
- **Substrate inhibition:** High concentration of substrate may inhibit the catalytic activity of an enzyme.

- **End-product inhibition**: In the case of multi enzyme system (catalytic inhibition).

Activators
(Cofactors and Coenzymes)

- Some enzymes do not need any additional components to show full activity.
- Cofactors can be either inorganic (metals) or organic compounds (flavin and heme).
- Coenzymes include NAD^+, $NADP^+$ and ATP.
- These coenzymes transfer chemical group between enzymes.
- The chemical groups carried by the hydride ion (H^+) carried by NADH or NADPH.

$NAD^+ + 2H^+ + 2e^-$ NADH / $NADP^+ + 2O^-_2 + 2H^+$ NADPH + $2O_2$

- Or phosphate groups carried by ATP.

ATP +H_2o ADP+ P_1 (-7.3kcal/ mole) / ATP +H_2o AMP + PP_1 (-14kcal/mole

Enzymes inhibitors and activators mechanism

Classification of enzymes

- The International of Biochemistry and Molecular Biology developed a nomenclature for enzymes (EC number).
- Each enzyme is classified by sequence of four numbers preceded by EC. (E.C. 5.3.1.18 Glucose isomerase)
- **The top-level classification is:**

- EC1 *Oxidoreductases* (catalyze oxidation/reduction reactions).

- EC2 *Tranferases* (transferee a functional group).

- EC3 *Hydrolases* (catalyze the hydrolysis of various bonds).

- EC4 *Lyases* (cleave bonds by mean of hydrolysis /oxidation).

- EC5 *Isomerases* (isomerization within same molecule).

- EC6 *Ligase* (join two molecules with covalent bonds).

Enzymes production

- **Constitutive enzymes:** The microorganism produce the enzyme in minimal fermentation media.
- **Inducible enzymes:** The microorganism require adding inducible agents in the media to produce the target enzyme.
- **Extracellular enzymes:** The microorganism secrete the enzyme in the fermentation media.
- **Intracellular enzymes:** The microorganism produce theenzyme inside the cell.
- Enzymes are usually sold based on the activity
- (u/ml or u/gm).
- If the efficiency of enzymes are considered, their cost, is based on active enzyme protein u/mg protein (specific activity).
- The commercial exploitation of enzymes range from high-volume but low cost (industrial enzymes) to low volume, but high cost (enzymes for medical, scientific and analytical use).

- Workers handling industrial enzymes should use protective clothing and eye protection.
- Food enzymes if foods processing have bees shows to be safe through many years of manufacturing practice.