Preparation of Enzymes

FE 361 Enzymology

06.11.2020

Enzyme Extraction/ Isolation

- Highly purified and good quality of hygiene is required for industries such as healthcare,, agriculture, food etc.
- Enzyme extraction and purification techniques are taking attention.

- For a correct method selection for enzyme extraction and location, location of the enzyme in the cell is important.

Choice of Enzyme Sources for Enzyme Extraction

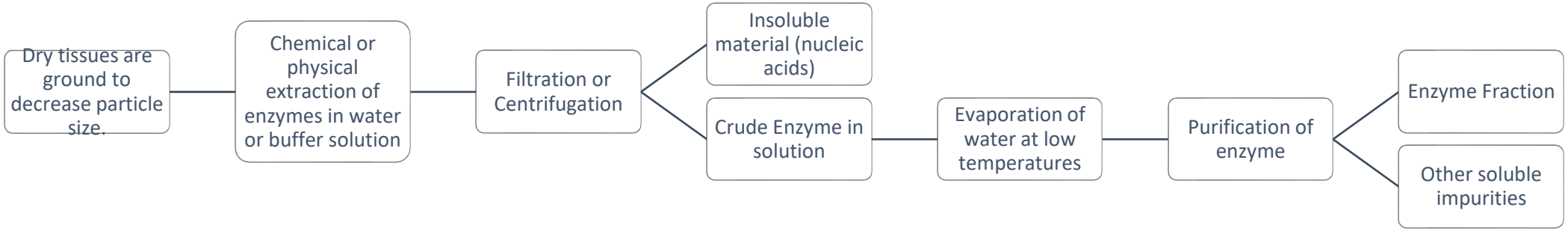
- Selection of right source: The required enzyme should occur in large amounts in the source
- Availability of the source: The source should be available. If not, some DNA transferations can be needed.
- Location of the enzyme: The location of the enzyme in the cell is important to select the extraction method.

Sources of Enzymes

- Biologically active enzymes may be extracted from any living organism.
  - Plant tissue
  - Animal tissue
  - Microorganisms (mostly fungi and yeast)

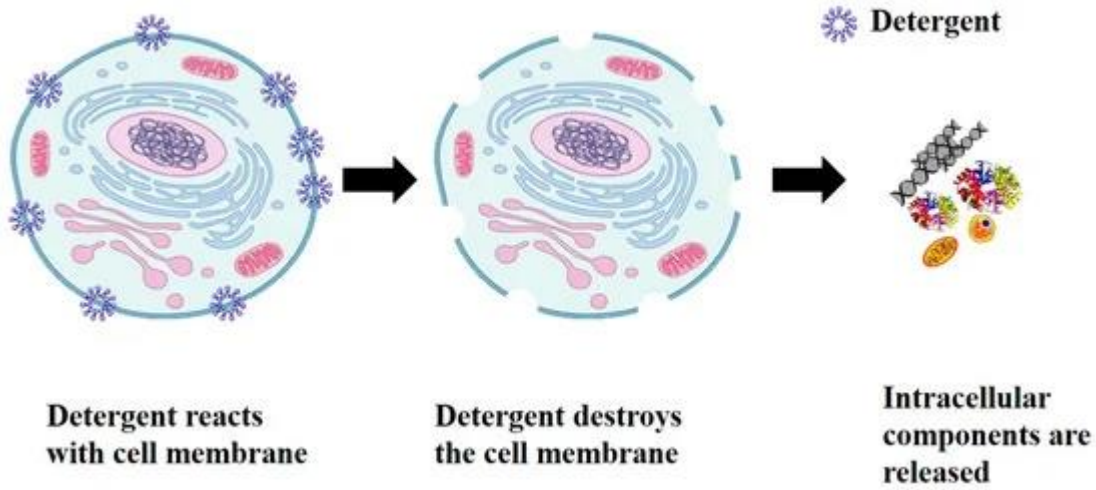
  Enzymes can be classified into two groups due to their location in the living cell:
  - Extracellular (outside the cell)
  - Intracellular (inside the cell, in cytoplasm or in organelle)

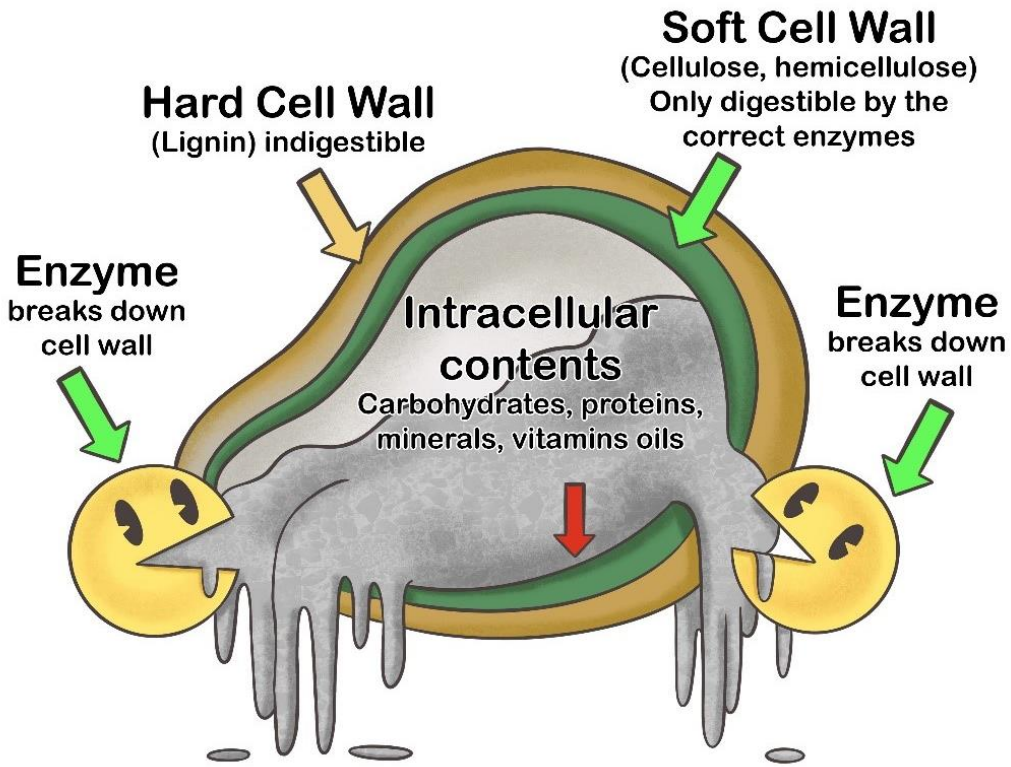
Enzyme Extraction



Extraction of Enzymes By Chemical Methods

1. Alkali treatment: Extraction of the enzymes from the cell wall of the sources. Different alkalis sucha as NaOH, KOH etc. are used.
2. Lysozyme treatment: Mostly suitable for gram positive bacteria. Lysozyme (an enzyme) with the help of osmotic treatment degrade the cell Wall and cell membrane.
3. EDTA: EDTA chelates the ions in the cell and thus cell lysis occur.
4. Detergents: Under low ionic conditions, detergents interact on the ionic and wan der waals forces with the lipoprotein and phospholipids in the membrane which results in the solubilization of the cell. Thus cell lysis occurs.
5. Osmotic shock: Osmotic pressure due to the presence of solutes inside and outside the cell. A technique which adopts the hyper- hypo conditions for cell lysis.


Detergent
Detergent reacts with cell membrane
Detergent destroys the cell membrane
Intracellular components are released


Soft Cell Wall
(Cellulose, hemicellulose)
Only digestible by the correct enzymes
Hard Cell Wall
(Lignin) indigestible
Enzyme
breaks down cell wall
Intracellular contents
Carbohydrates, proteins, minerals, vitamins oils
Enzyme
breaks down cell wall

Extraction of Enzymes by Physical Methods

1. Ultrasonication: Ultrasonic cavitation cause the formation of shock waves that cam mediates the cell lysis.
2. Homogenization: Mechanical technique to distrupt the cell Wall and cell membrane.
3. Freezing: A cold shock at -15 to -25 C. Cell Wall and membrane lysis occurs.

Isolation of Enzymes

- When the enzyme is extracted from the cell, the resultant cell free extract may contain other components (contaminants) such as nucleic acids, lipids, sugars etc.
- Removal of these contaminants is essential for proper isolation of the enzyme.
  - pH
  - Temperature
  - detergents

Seperation methods of high purity enzymes:

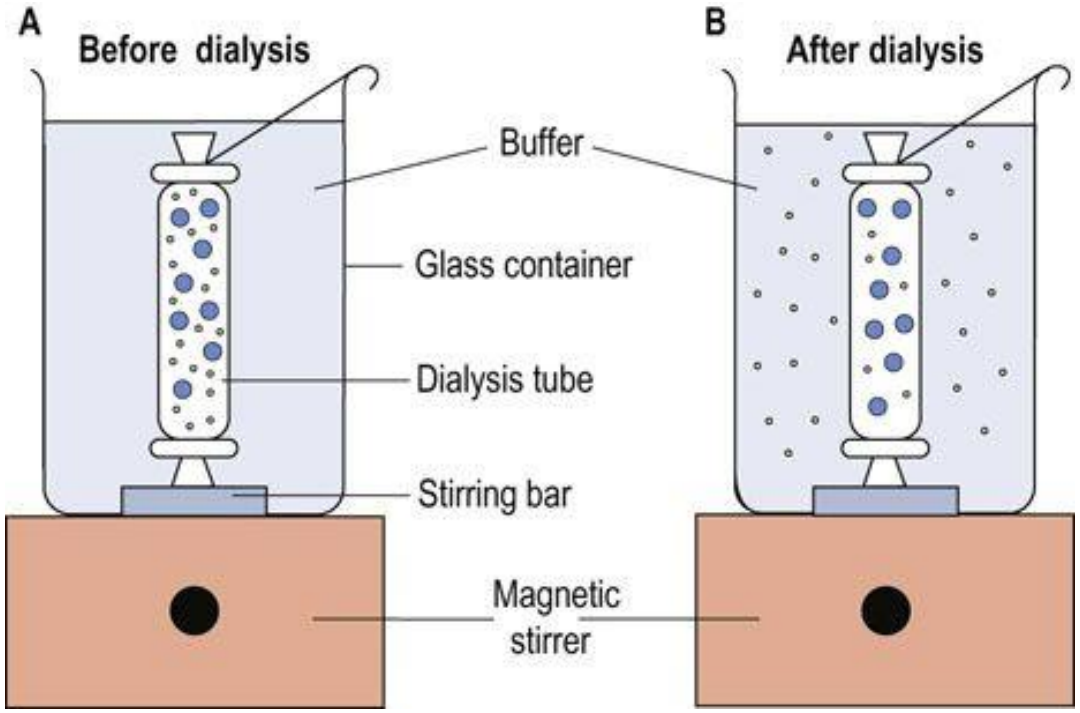
- Seperation methods can be classified based on the enzyme property such as;

- Molecular size
- Solubility of the enzyme
- Electrical charge
- Difference in adsorption properties
- Biological affinity for other molecules

Methods based on Molecular Size

- Membrane separation: The aim of separation is to concentrate aquous solutions of macromolecules and to remove small inorganic ions such as solutions.
- Membranes are freely permeable to small molecules but impermeable to large molecules.
- Two techniques of membrane seperation:
  1. Dialysis
  2. Ultrafiltration

1. Dialysis



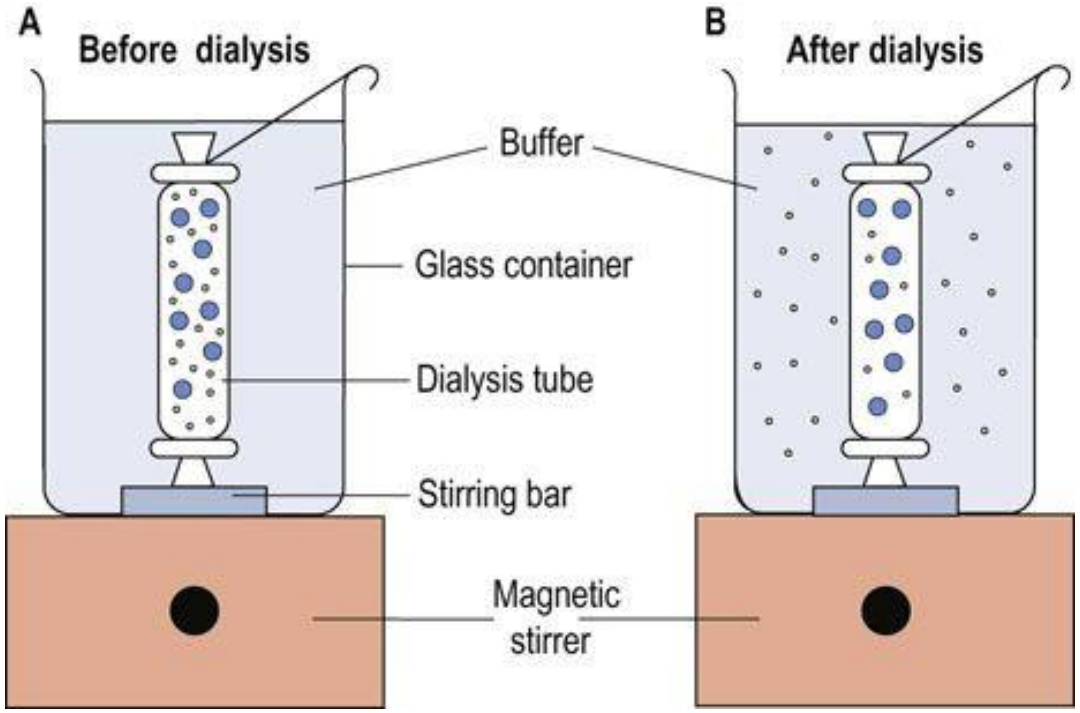
An old method for removal of low molecular weight solutes.

This method is based on the properties of a semi-permeable membrane separating the protein solution from the dialysis buffer.

Membrane allows free passage of molecules below a certain molecular weight while macromolecules can not penetrate the pores of the membrane.

Driving force of dialysis is the difference in concentrations of the solutes on the two sides of the membrane. The small molecules will leave the bag, until their concentrations are same on both sides of the membrane. At this point, equilibrium concentration is obtained.

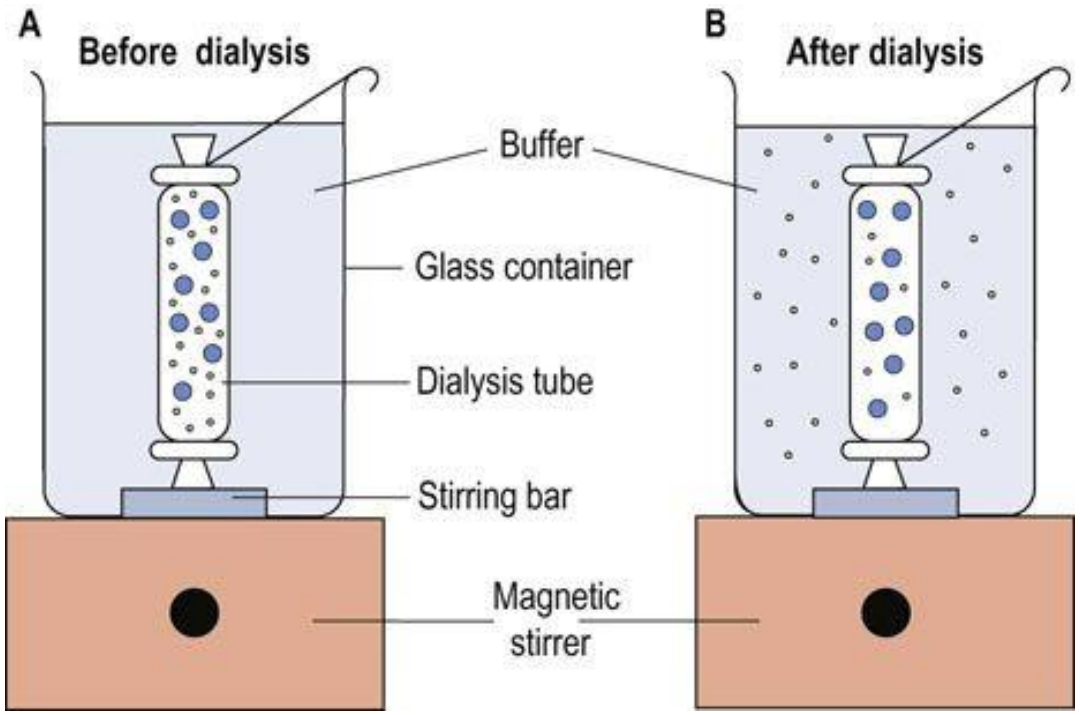
1. Dialysis



Stirring increases the rate of movement of molecules. Further reduction of solute concentration in protein solution can be achived by:

1. Changing the dialysis buffer
2. By increasing the ratio of membrane area to the volume of the solution

1. Dialysis



External Methods affecting the diffusion:

Diffusion depends on temperature and viscosity of the solution.

If temperature increases, diffusion increases. Howeveri at high temperatures the enzyme is denaturated. Dialysis should be conducted at 4-8 C.

Increasing viscosity decreases the rate of diffusion. So that, high viscous solutions should be diluted before dialysis.

Mostly used membranes are cellulose sheets. They are available with a range of permeability and varying diameter.