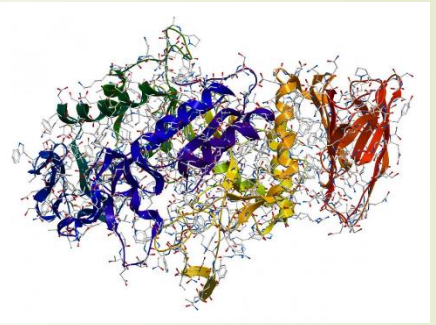


CEN-CHE 422
ENYZME ENGINEERING

Methods of Separation and Purification of Enzymes-I

The strategy followed in separation:

Where will the enzyme be used ?
Laboratory or industry?

Enzyme Source?
Bacteria, yeast, plant

Cost?

The medium (in which enzymes are produced) is complex (sequential steps)
Separation cost > Production cost
ChemEng classical separation processes (special techniques)

When enzymes are purified:

- High efficiency should be ensured
- Should not degrade
- Must be of high purity (not contain other molecules)

Purification of enzymes are important for:

- Characterization of structures
- Determination of catalytic mechanisms
- Understanding substrate specificity
- Use as a biocatalyst in biotransformation.

Consecutive steps in separation:

Separation of insoluble product and solids removal of water
Removal of contaminating chemicals
Purification of the product
Drying

In terms of process economy, water should be separated as early as possible and scaled down.

Fermentation

Centrifugation/Filtration — *Cell removal /concentration*

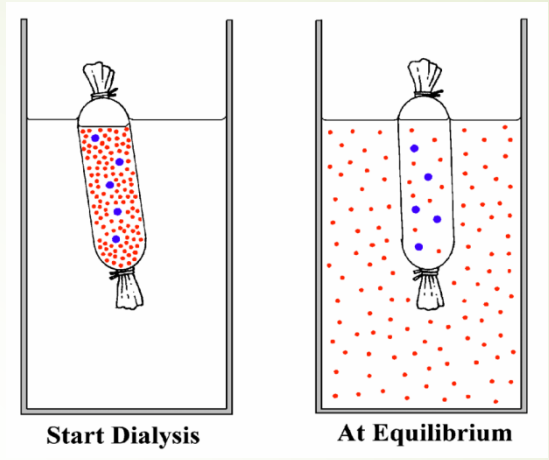
Cell disruption

Centrifugation — *Separation of cell debrits*

Protein precipitation → *Salt (NH4)2SO4, Solvent (acetone) Low pH High temperature*

Ultrafiltration

Dialysis



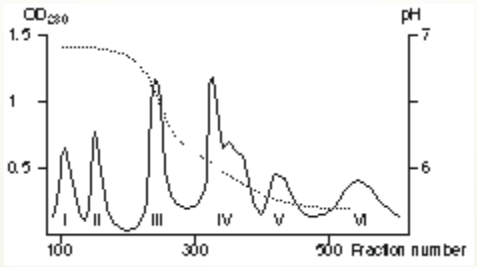
Ion Exchange Chromatography

Gel Filtration Chromatography

Hydrophobic interaction chromatography

Affinity chromatography

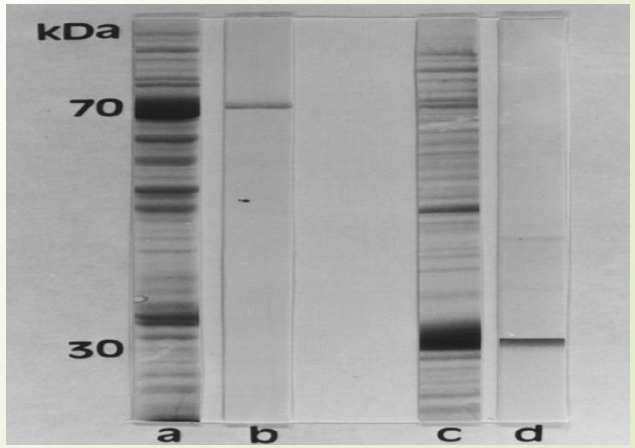
Can be applied singly or sequentially



Product

At each step :

- ***protein conc. is measured***
- ***Enzyme activity is measured***
- ***Gel electrophoresis is performed***



Protein Prufication Methods

Property	Method
Size, mass or density	Centrifugation, Filtration, Gel filtration, Ultrafiltration, Microfiltration, Nanofiltration, Reverse Osmosis, Dialysis
Charge	Ion Exchange Chromatography, Chromofocusing, Electrophoresis Isoelectricfocusing
Hydrophobicity	Hydrophobic Chromatography
Spesifik binding sites	Affinity chromatography

(Cell Disruption)

Non-Mechanical Methods
Enzymatic Lysis
Chemical Treatment (Detergent, solvent, acid, base)
Physical Process (Osmotic shock, freezing-heating (Throwing)

Mechanical Methods
Solid shear stress
Liquid shear stress

Cell disruption methods

Enzymatic Lysis: Bacteria wall, lysozyme enzyme, expensive
Gram (+) bacteria such as B. subtilis are incubated with lysozyme.
For Gram (-) bacteria such as E.coli, EDTA is added to the lysozyme.

Breaking with osmotic shock and ice crystals:
slow cooling-mild heating, Gram (-) bacteria

Sonication (Ultrasonic vibration): Bacteria, heat released problem

French Press:

Cells are passed through a thin orifice at high pressure.
The resulting mechanical shear force breaks the cell

Ball mill:

20-50 mesh balls; grinding chamber 80% ball;
high shear stress created by the balls when rotating at high speed
Ball diameter and amount, feed concentration, disc rotation speed
heat