Lec.6 Separation of Bioproducts (Downstream) D. Neihaya Hiekmat

Downstream: means all the necessary steps for the separation and purification of biological products of any type of industrial processes. Separation of bioproducts difficult and expensive and must be done quickly.

These Primary treatments are:

1 - Temperature change: can take place by passing the outputs on the heat exchangers to raise the temperature or cooling it, most contaminated organisms are heat-sensitive, so it least their growth, in addition that cooling increases the stability of most of the sensitive materials.

2- C**hange the pH**: can reduce or raise the pH, which leads to increased stability of the product and inhibition of cell growth.

3- **Add inhibition material:** the inhibitors used and preservative to increase the stability of the material produced and limit the growth of cells used in fermentation as well as other contaminated.

Choosing methods for bioseparation :

Choosing the methods linked with a number of important factors:

1- **Determine the location of biomaterials**: Extracellular products will be in the supernatants, while Intracellular products remain in the cell.

2 - **Concentration of the product in the fermentation media:** most of fermentation products exist in concentrations too low with a mixture of substances soluble and insoluble.

3- **Type of using bioproducts**: if used to human, must be a high degree of purity and this means increasing steps of purification, while the other uses can reduce the purification steps.

Steps of down stream

First- Separation of particles: its include two methods:

1- non-mechanical methods:

A - Settling: one of the traditional methods used to separate the solids from the liquid depending on the weight and size, it descends cells down by gravity, this method used in the alcohol industry and treatment of waste.

B - Flocculation: adding industrial flocculating such as calcium chloride, ammonium compounds and phosphoric acid. This may lead to an equation similar charges such as phosphate, carboxyl negative that makes cells stuck and when neutralized cells tend to the pool and go down to the bottom and thus easily separated by other ways.

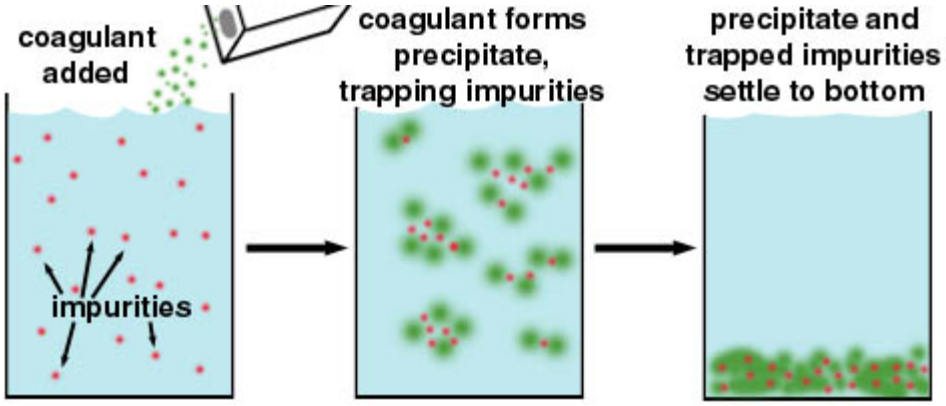

Figure 1. Conceptual illustration of the flocculation process.

C - Flotation: its may be **natural** when the cells raised to the surface crane with solids, or **artificial** by using of materials leads to the formation of foam. Flocculation and foam using combined in the production process of unicellular protein (SCP) and efficiently to separate the biomass.

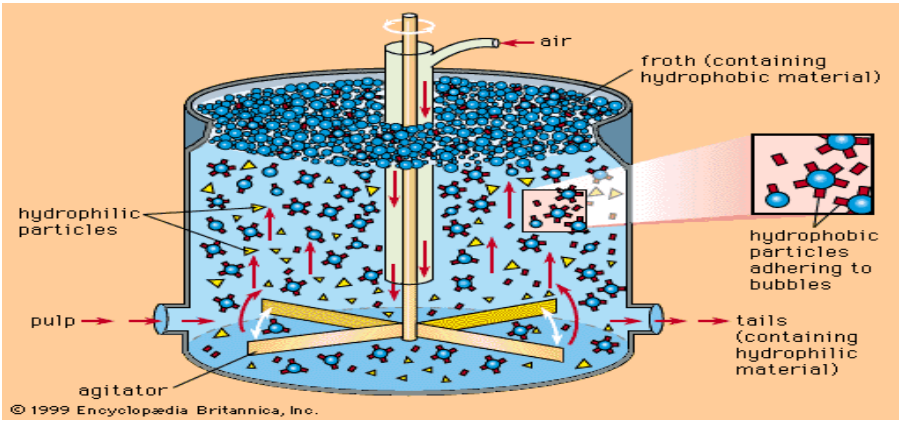

Figure 2. The flotation machine.

2- Mechanical methods:

A - Filtration: To separation parts of suspended solids from liquids through membranes as a result of differential pressure and concentration, and is widely used to separate filamentous molds and bacteria from the fermentation medium and also to separate the yeasts.

B - Centrifugation: Centrifuge used to separate bacteria, and can be used efficiently in the case of significant differences in density between the minutes of solid and liquid portion.

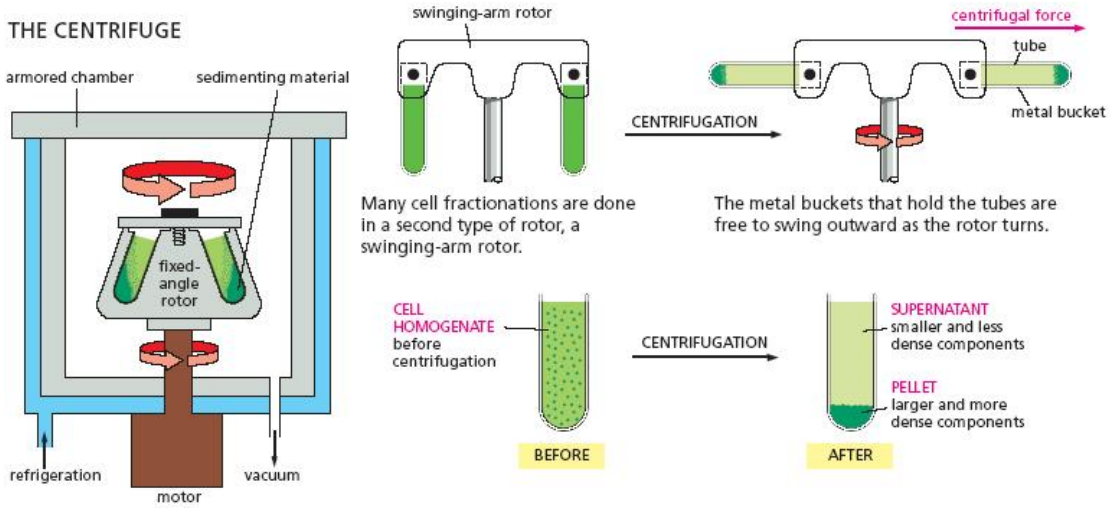

Figure 3.Centrifugation process

Second: Cell disruption

It is used to extract some bioproducts appear within cells, and is divided into physical and chemical methods:

A-Physical methods:

- **Homogenization:** used for filamentous organisms such as fungi and animal tissues using a blender.

- **Freezing and thawing**: This method does not use for separation of intracellular enzymes because of resisting the cells to cracking.
- **Agitation with abrasives**: It is one of the most effective ways to physically break down the cells on a large scale, and the factors affecting them are the type of device, the speed of the flow of the medium, the particle size and the increase of temperature.

- **Crushing:** includes the use of milling operations (Ball, Colloid and rotor-stator) mill.

- **Pressure:** the cells are exposed to high pressure followed by a lower, and this generates a break of the cells as a result of fragmentation.

Ultra sonication: Here it composed cavities inside the cells and this breaks it, but the problem is the occurrence of high heat in the device.

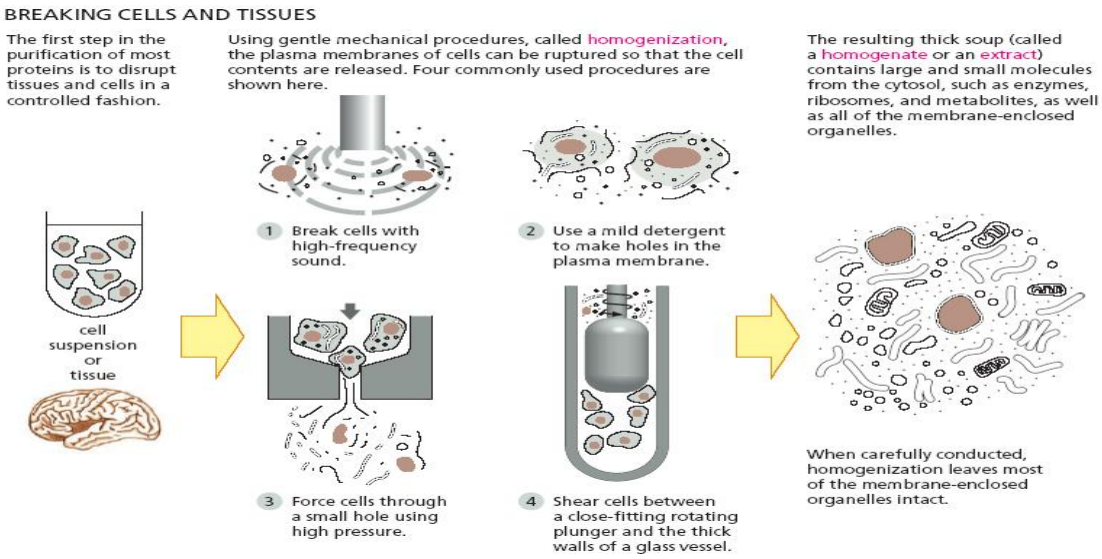

Figure 4. Disruption of cells

B - Chemical methods:

• **Alkali treatment**: used in the analysis of plant cells, bacteria, enzymes, molds, and the advantages of it simple and low costing.

• **Acid treatment**.

• **Detergents:** such as Sodium dodecyl sulfate (SDS) where works to break down the cell wall.

• **Enzyme treatment**: many enzymes that can affect the cellular walls and analyzes it, but its expensive way.

• **Solvents**: It weakens the cell membrane because of the dissolved fat in the membranes.

• **Osmotic shock**: intended to make a sudden change in salt concentration, which causes cracking animal cells on a large scale either bacteria. There are other methods such as the **use of urea**.

Third: Extraction methods

These methods Used for products that secrete outside or inside the cells, and this is done by mixing the solution who has a product with a solvent, which the product dissolves well in it, and returns after the product easily either by sedimentation (using salts such as ammonium sulfate or organic solvents) or evaporation or other methods.

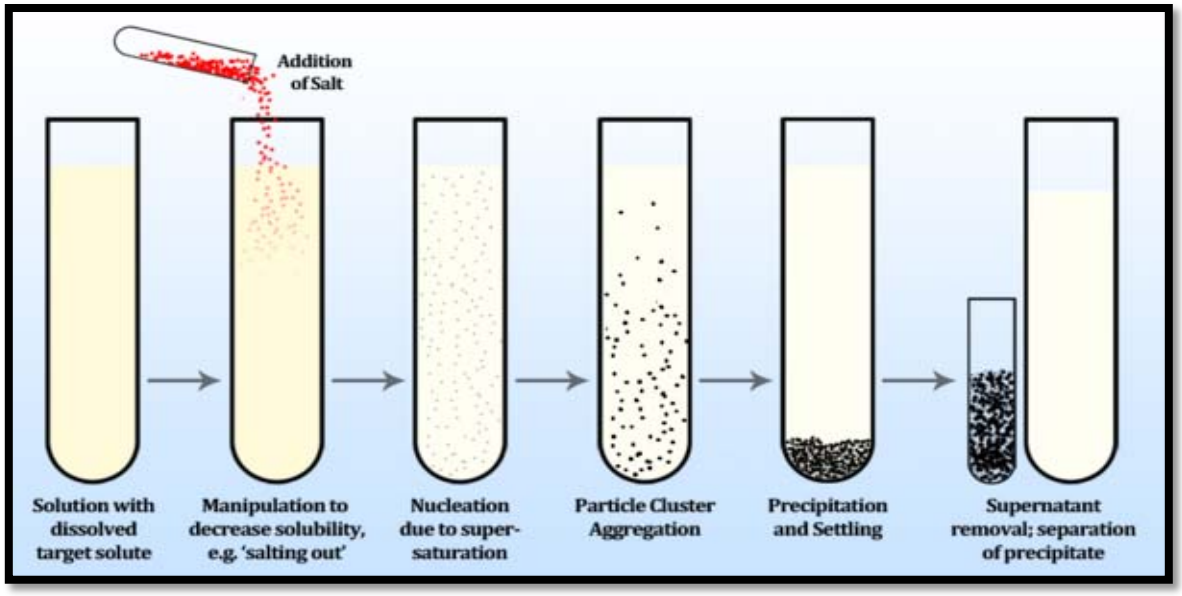

Figure 5.precipitation with salt

Fourth: Concentration methods

This process is done because most of the bioproducts are usually at low concentration, and they are:

A- Evaporation: This method is used with extracts obtained by solvent extraction.

B- Membrane filtration: Dissolved substances are concentrated through remove water from the product without making the evaporation, and the pressure on membrane must be higher than the pressure of the solution to push the water through it.

C- Adsorption resins: It's a porous polymers have effective groups working on a modification of polarimetric without ionization, where its compounds adsorbed in solutions and relive by extraction with organic solvents.

D- Ion-exchange resins: There are two types of it: **artificial polymers** such as polystyrene and polymethyl acrylate that separate only small molecules such as amino acids, but **natural ion exchangers** separate the larger one such as cellulose and dextran.

Fifth: Purification methods

A - Crystallization: used mainly in the purification of compounds of the low molecular weight, such as using of butyl acetate and Ethyl Acetate to pure antibiotics (penicillin and streptomycin), respectively, also its used for the purification of organic acids.

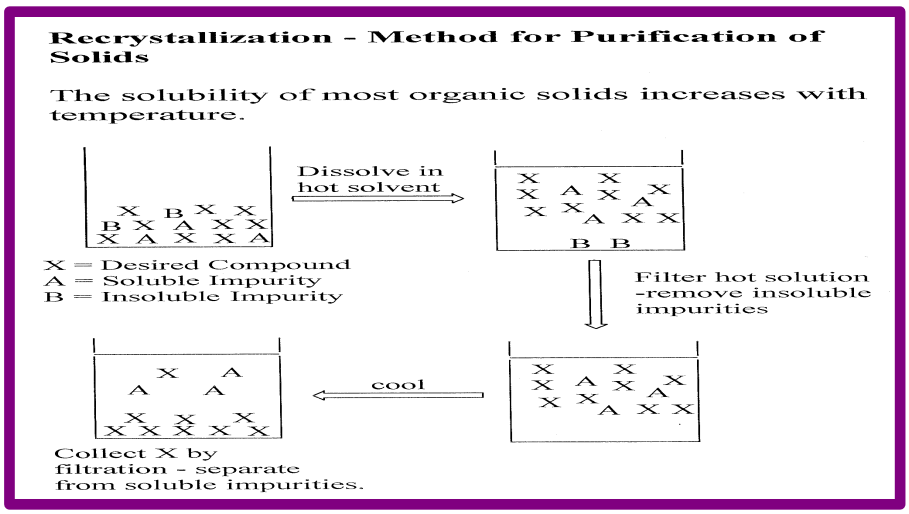

Figure 6. Crystallization process

B - Chromatography methods: chromatographic methods are used in the purification vital in the later stages often they include several types including:

1- **Gel-filtration chromatography**: Its used materials with specific qualities like granules spherical (Beads) with Pore homogeneous diameters allow the passage of molecular weights through them, purification mainly effected on molecular weight and shape.

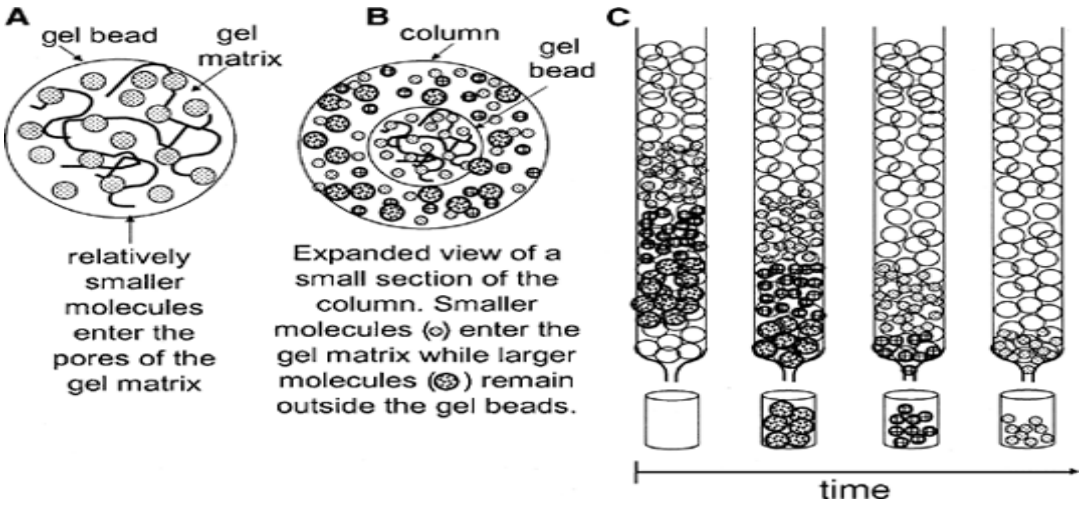

Figure 7. Schematic representations of the principle of gel filtration chromatography.

2- **Adsorption Chromatography**: used to separate many proteins and enzymes in addition to the purification of some antibiotics such as streptomycin, where adsorbed material to be purified on special solids packed with special columns, these materials may be organic material

or inorganic, such as carbon activated, silica gel, and aluminum oxide. It's obtained after the end of the process by washing the column using types of solutions.

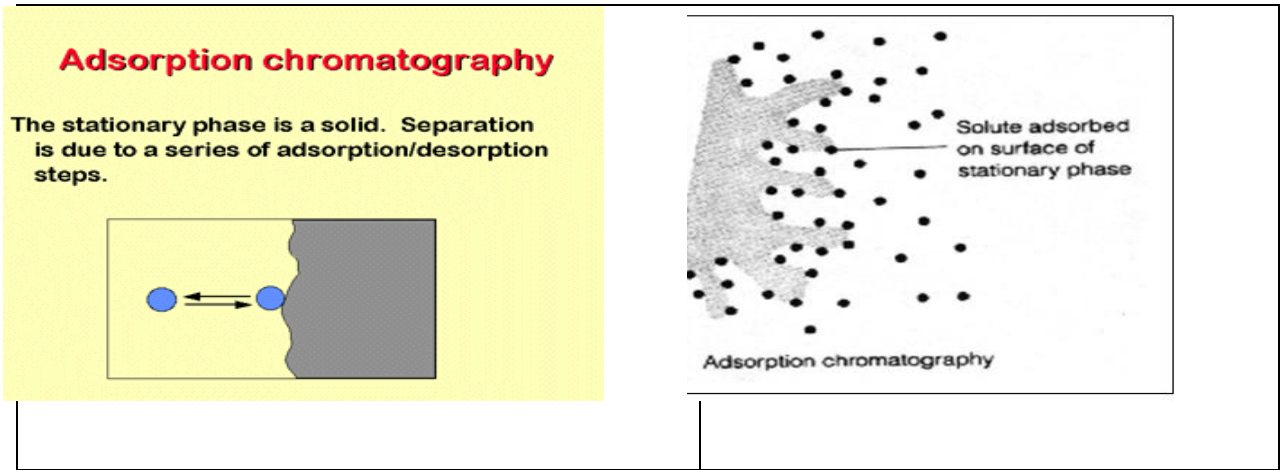

Figure 8. Schematic representations of the principle of adsorption chromatography

3 - **Ion-exchange Chromatography:** depends on the exchange of ions, purified material may be charged positive or negative exchange with similar ions present on the support materials (resins or cellulose materials), which are either negative charges exchangers (carrying positive charge) or positive charges exchangers (carrying a negative charge). This method depends on the ion exchange and net charge density, pH and ionic concentration of the solvents used.

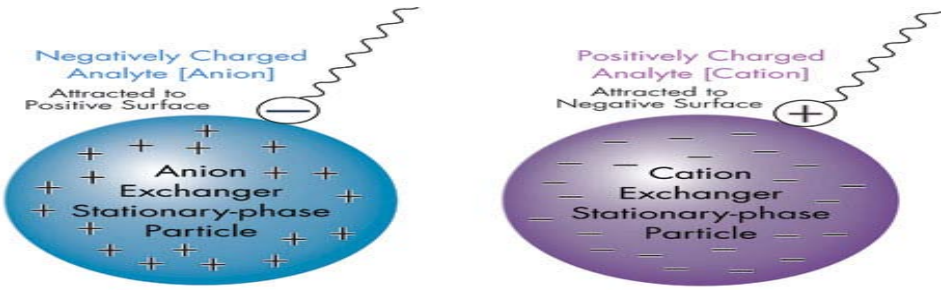

Figure 9. Ion Exchange Chromatography Principle

4 - Affinity chromatography: it's used for the purification of proteins; antiviral and interferon depending on its ability effective connect to Ligand which linked to the matrix through Spacer arm, also its recovery the materials from the column either by change pH or change the solution ionic strength regulator.

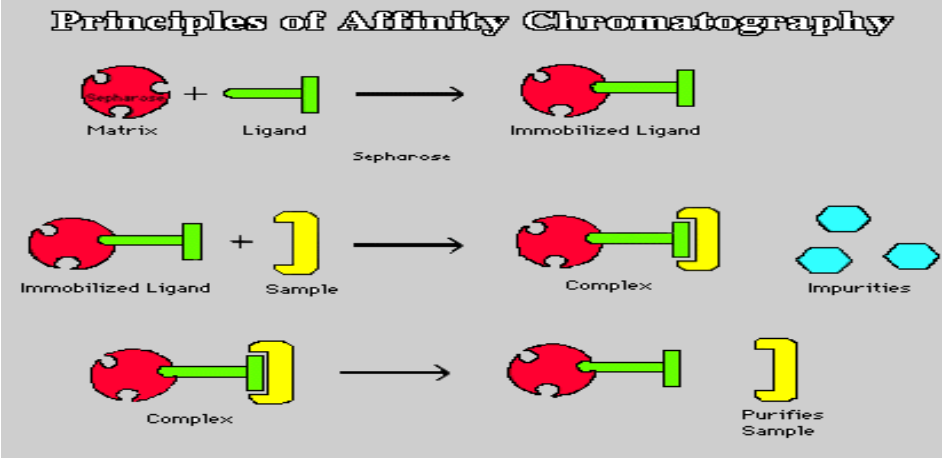

Figure 10. Cartoon illustration of steps for affinity chromatography.

Sixth: Drying :

The purpose of this last step to increase the duration of persistence, storage and suitability for trading, and the important methods of drying:

1- **Vacuum drying:** This process is done either by batch way using Chamber dryers or continuous manner using Rotary drum dryer, heat transfer occurs mainly through contact with hot surfaces of the product and it evaporated and dried.

2- **Spray drying:** Heat transmitted in a way influenced by moving stream of hot air and cause drying without touching the product like enzymes, acids, antibiotics and food.

3- **Freeze drying (Lyophilization):** Water here sublimates from the block frozen. This method is used for drying vaccines, hormones, enzymes, sensitive and precious materials used for diagnosis.