Al-Farabi Kazakh National University

The Recovery and Purification of Fermentation Products

Lecture 10

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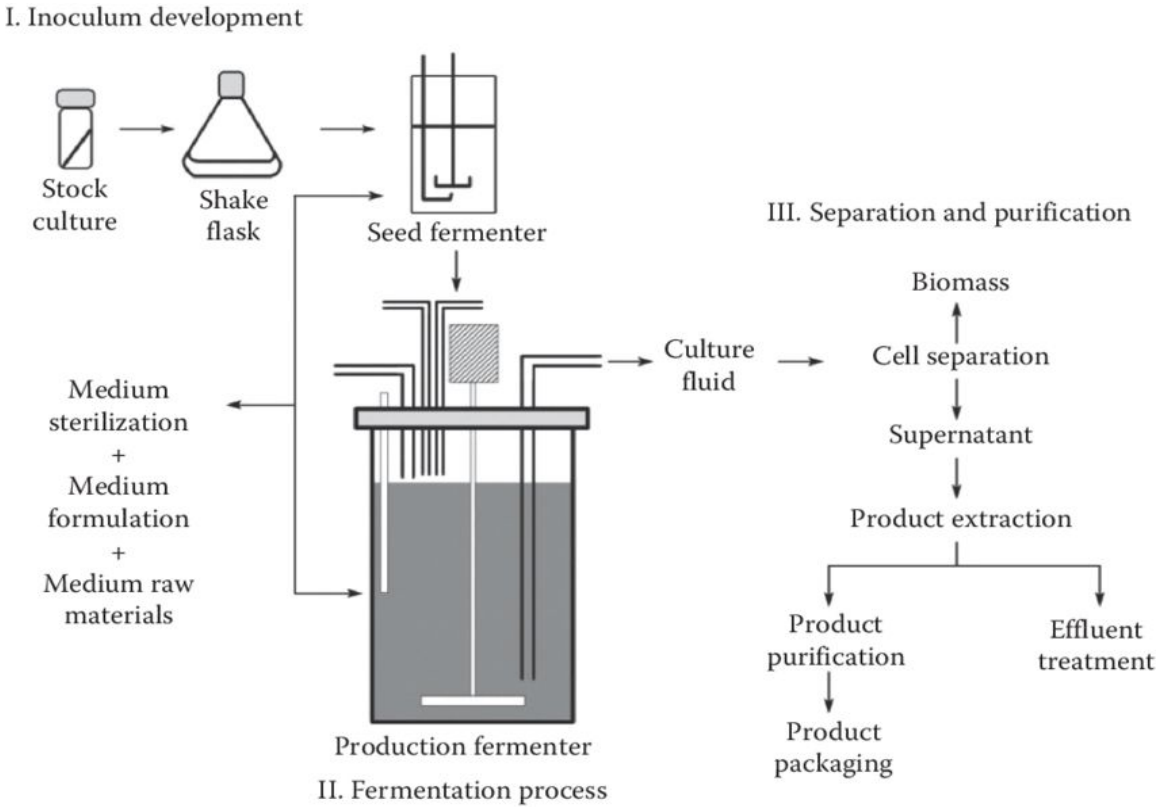
Purification of Products



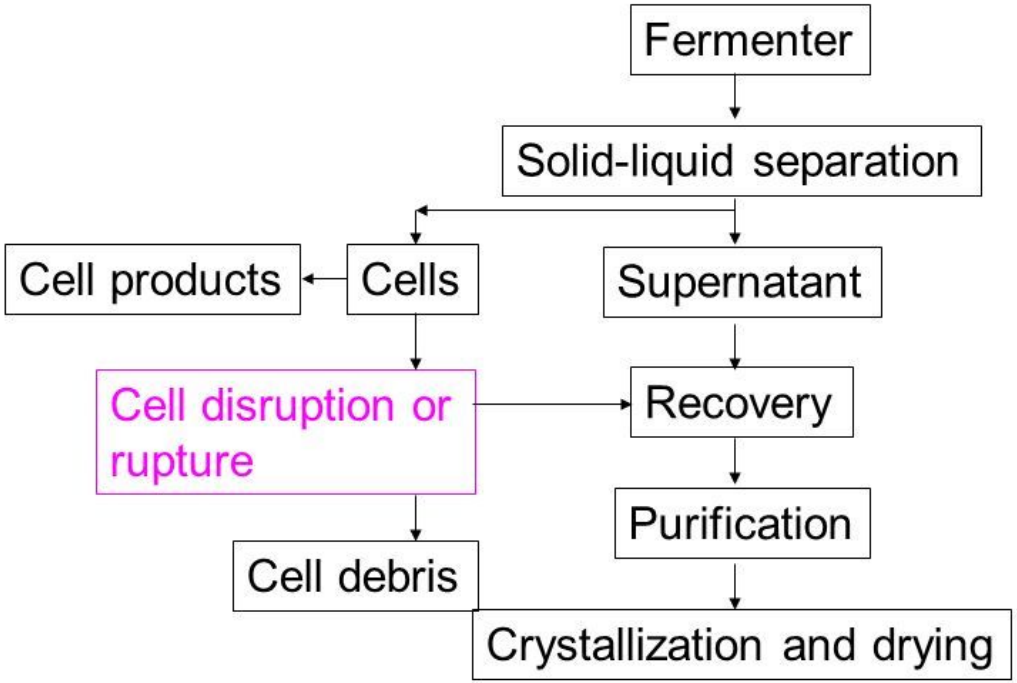
Purification of fermentation products may be difficult and cost

Recovery costs of microbial products may vary from 15% to 70% of total production cost

https://www.researchgate.net/figure/Generalized-schematic-representation-of-a-fermentation-process-Adapted-from-Stanbury_fig2_294881362

Recovery and Purification of Bio-products

- Strategies to recovery and purify bio-products



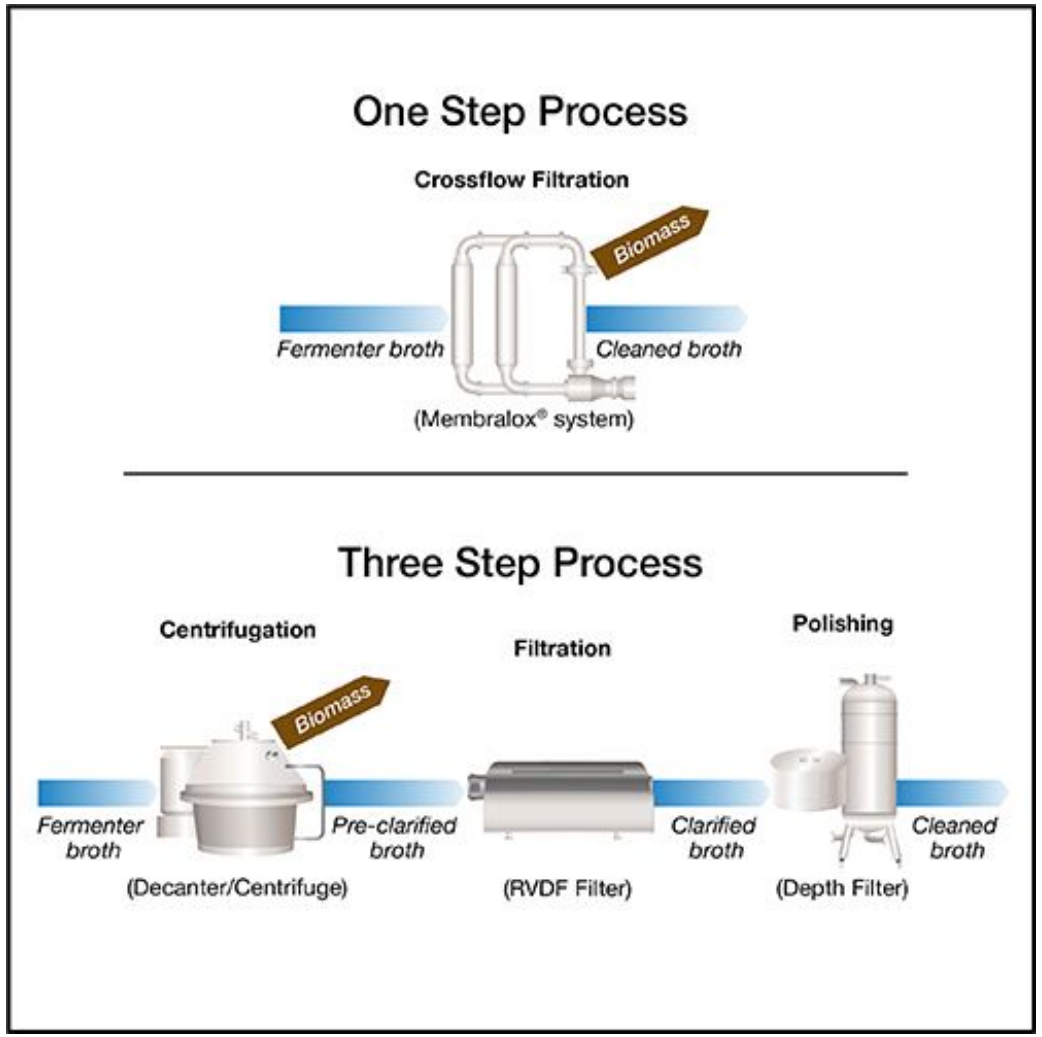
The main objective of the first stage for the recovery of an extracellular product is the removal of large solid particles and microbial cells usually by centrifugation or filtration.

In the next stage, the broth is fractionated or extracted into major fractions further more precise chromatographic techniques and crystallization to obtain a product which is highly concentrated and essentially free from impurities.

The major problem currently faced in product recovery is the large-scale purification of biologically active molecules

The choice of recovery process is based on the following criteria:

1. The intracellular or extracellular location of the product.
2. The concentration of the product in the fermentation broth.
3. The physical and chemical properties of the desired product (as an aid to select separation procedures).
4. The intended use of the product.
5. The minimal acceptable standard of purity.
6. The magnitude of biohazard of the product or broth.
7. The impurities in the fermenter broth.
8. The marketable price for the product.



During fermentation, the microorganisms multiply in industrial bioreactors, and the enzyme is typically excreted into the culture medium (extracellular).

After fermentation, extraction, and purification are important steps for enzyme recovery from biomass.

https://www.pall.com/en/food-beverage/enzyme-filtration/fermentation-broth-clarification.html

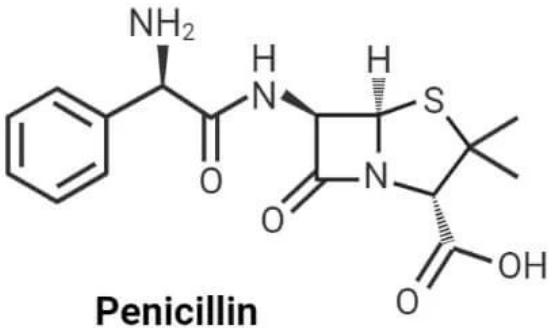
Possible problems in fermentation and product purification

- time of harvest,
- pigment production,
- ionic strength,
- culture medium constituents.

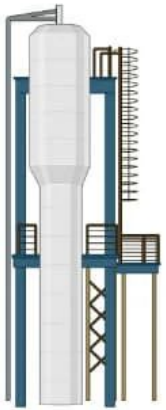
Large volumes of supernatants containing extracellular enzymes need immediate processing while harvesting times and enzyme yields might not be predictable. This can make recovery programs difficult to plan.

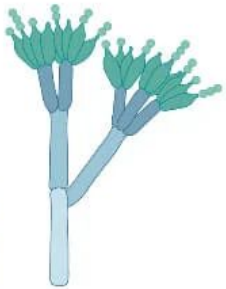
Changes in fermentation conditions may reduce pigment formation.

FIGURE 10.2 Recovery and Partial Purification of Penicillin G



Penicillin





Penicillium

1. Harvest broth from fermenter
2. Chill to 5–10°C
3. Filter off *P. chrysogenum* mycelium using rotary vacuum filter
4. Acidify filtrate to pH 2.0–2.5 with H_2SO_4
5. Extract penicillin from aqueous filtrate into butyl acetate in a centrifugal counter-current extractor (treat/dispose aqueous Phase)
6. Extract penicillin from butyl acetate into aqueous buffer (pH 7.0) in a centrifugal counter-current extractor (recover and recycle butyl acetate)
7. Acidify the aqueous fraction to pH 2.0–2.5 with H_2SO_4 and re-extract penicillin into butyl acetate as in stage 5
8. Add potassium acetate to the organic extract in a crystallization tank to crystallize the penicillin as the potassium salt
9. Recover crystals in a filter centrifuge (recover and recycle butyl acetate)
10. Further processing of penicillin salt

https://thebiologynotes.com/microbial-production-of-penicillin/

Removal of Microbial Cells and Other Solid Matter

Normally separated from the harvested broth by filtration or centrifugation

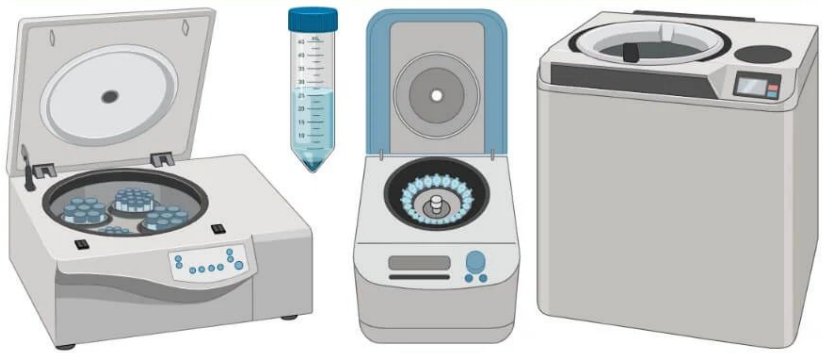
Foam separation E. coli using lauric acid, stearyl amine t-octyl amine as surfactants, it was shown that up 90% of the cells were removed in 1 minute and 99% 10 minutes

It is common practice to use filter aids when filtering is slow. Kieselguhr (diatomaceous earth) is the most widely used material.

Centrifugation

A centrifuge may be expensive when compared with a filter it may be essential when

- Filtration is slow and difficult.
- The cells or other suspended matter must be obtained free of filter aids.
- Continuous separation to a high standard of hygiene is required.



https://microbenotes.com/centrifuge-and-centrifugation/

Cell Disruption

Disruption: the cell envelope is physically broken, releasing all intracellular components into the surrounding medium

Methods: mechanical and non mechanical

Mechanical

- Ultrasonication (sonicators) bacteria, virus and spores suspensions at lab-scale

Electronic generator→ ultrasonic waves →mechanical oscillation by a titanium probe immersed in a cell disruption.

Non Mechanical Cell Disruption

- Chemicals: use chemicals to solubilise the components in the cell walls to release the product.

Chemical requirements:
- products are insensitive to the used chemicals.
- the chemicals must be easily separable.

Types of chemicals:
- Surfactants (solubilizing lipids): sodium sulfonate, sodium dodecyl sulfate.
- Alkali: sodium hydroxide, harsh
- Organic solvents: penetrating the lipids and swelling the cells. e.g. toluene.

Bacteria were treated with acetone followed by sodium dodecyl sulfate extraction of cellular proteins.
For example: sodium sulfonate: NaHSO3, sodium dodecylsulfate: Sodium dodecyl sulfate (or sulphate) (SDS or NaDS) ($C_{12}H_{25}NaO_4S$)

Cont…

- Enzymes: to lyse cell walls to release the product.
gentle, but high cost, i.e. lysozyme (carbohydrase) to lyse the cell walls of bacteria.

- Osmotic shock
Osmosis is the transport of water molecules from high- to a low-concentration region when these two phases are separated by a selective membrane.

Water is easier to pass the membrane than other components.
When cells are dumped into pure water, cells can swell and burst due to the osmotic flow of water into the cells.

Separation

- The separation of a component from a liquid mixture by treatment with a solvent in which the desired component is preferentially soluble is known as liquid-liquid extraction.
- Polar liquids mix with each other and dissolve salts and other polar solids.
- The solvents for nonpolar compounds are liquids of low or nil polarity.
- The dielectric constant is a measure of the degree of molar polarization of a compound.

Liquid-liquid extraction:

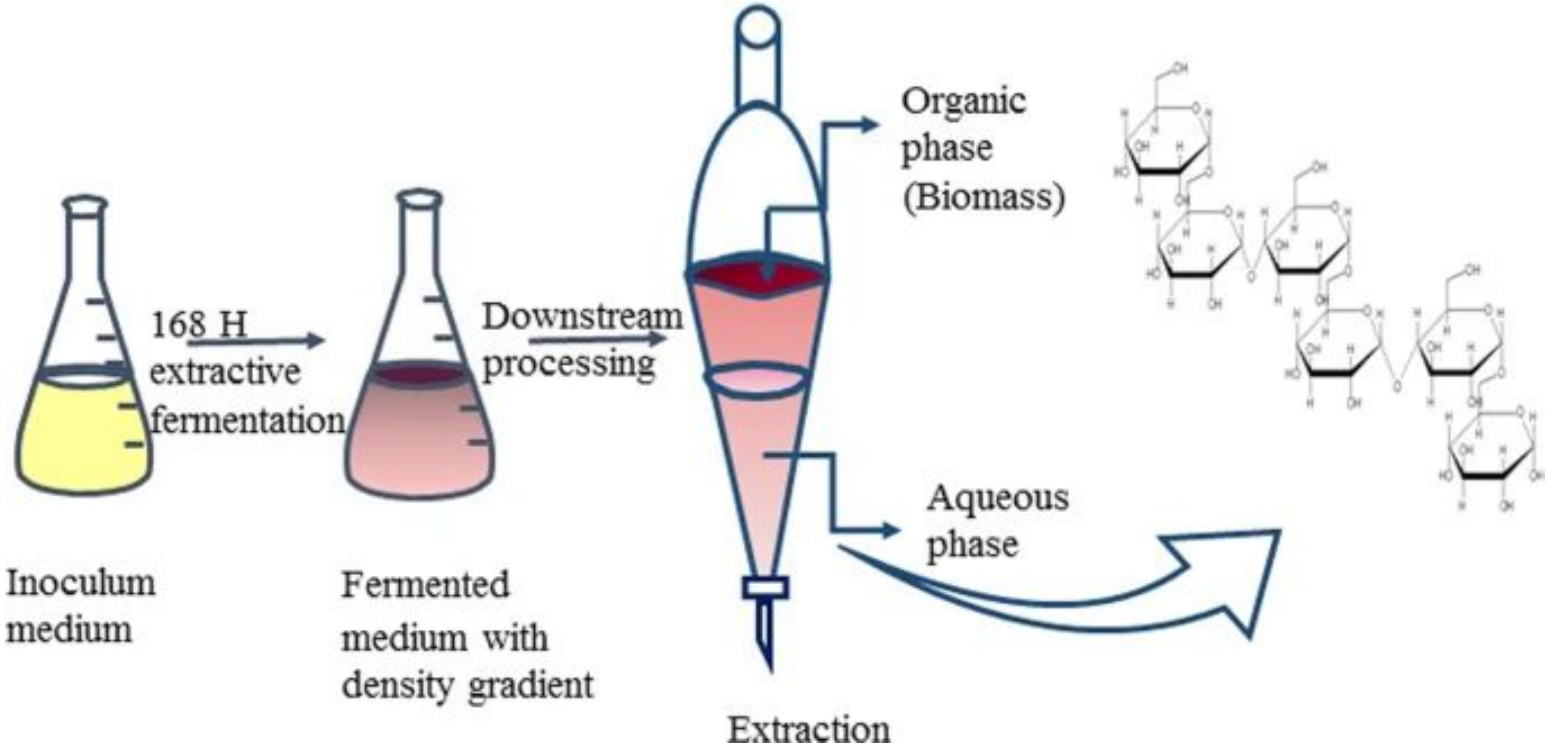
Difference of solubility in two immiscible liquid

Applicable: separate inhibitory fermentation products such as ethanol and acetone-butanol from fermentation broth.
For example: antibiotics (i.e. solvent amylacetate)

Requirements of liquid extractants:
nontoxic, selective, inexpensive, immiscible with fermentation broth and
high distribution coefficient:

$$KD=YL/XH$$

YL and XH are concentrations of the solute in light and heavy phases, respectively.



Precipitation

Reduce the product solubility in the fermentation broth by adding chemicals.

Applicable: separate proteins or antibiotics from fermentation broth.

Methods:

- salting-out by adding inorganic salts such as ammonium sulfate, or sodium sulfate to increase high ionic strength (factors: pH, temperature). The solubility of hemoglobin is reduced with increased amount of ammonium sulfate.

- added salts interact more stronger with water so that the proteins precipitate.

- inexpensive

- Isoelectric (IE) precipitation: Precipitate a protein at its isoelectric point.

Adsorption

Adsorb soluble product from fermentation broth onto solids.

Approaches: physical adsorption (activated carbon), ion exchange (carboxylic acid cation exchange resin for recovering streptomycin)

Adsorption capacity: mass of solute adsorbed per unit mass of adsorbent

Affected by properties of adsorbents:

- functional groups and their numbers,
- surface properties by properties of solution: solutes, pH, ionic strength and temperature

Difference of Affinity of product in the solid and liquid phase.
Applicable: soluble products from dilute fermentation

Separation

Microfiltration: μm, bacterial and yeast cells.

Ultrafiltration: macromolecules (2000 <MW< 500,000)

Dialysis: removal of low-MW solutes: organic acids (100<MW<500) and inorganic ions (10<MW<100).

Reverse osmosis: a pressure is applied onto a salt-containing phase, which drives water from a low to a high concentration region. MW < 300.

The common features of the above methods:
Use membrane
Driving forces: pressure

*MW- molecular weight

Chromatography

To separate the solutes based on the different rate of movement of the solutes in the column with adsorbent materials.

Principles:
Chromatographic processes involve a stationary phase and a mobile phase.
Stationary phase can be adsorbent, ion-exchange resin, porous solid, or gel usually packed in a cylindrical column.

Mobil phase is the solution containing solutes to be separated and the eluant that carriers the solution through the stationary phase.

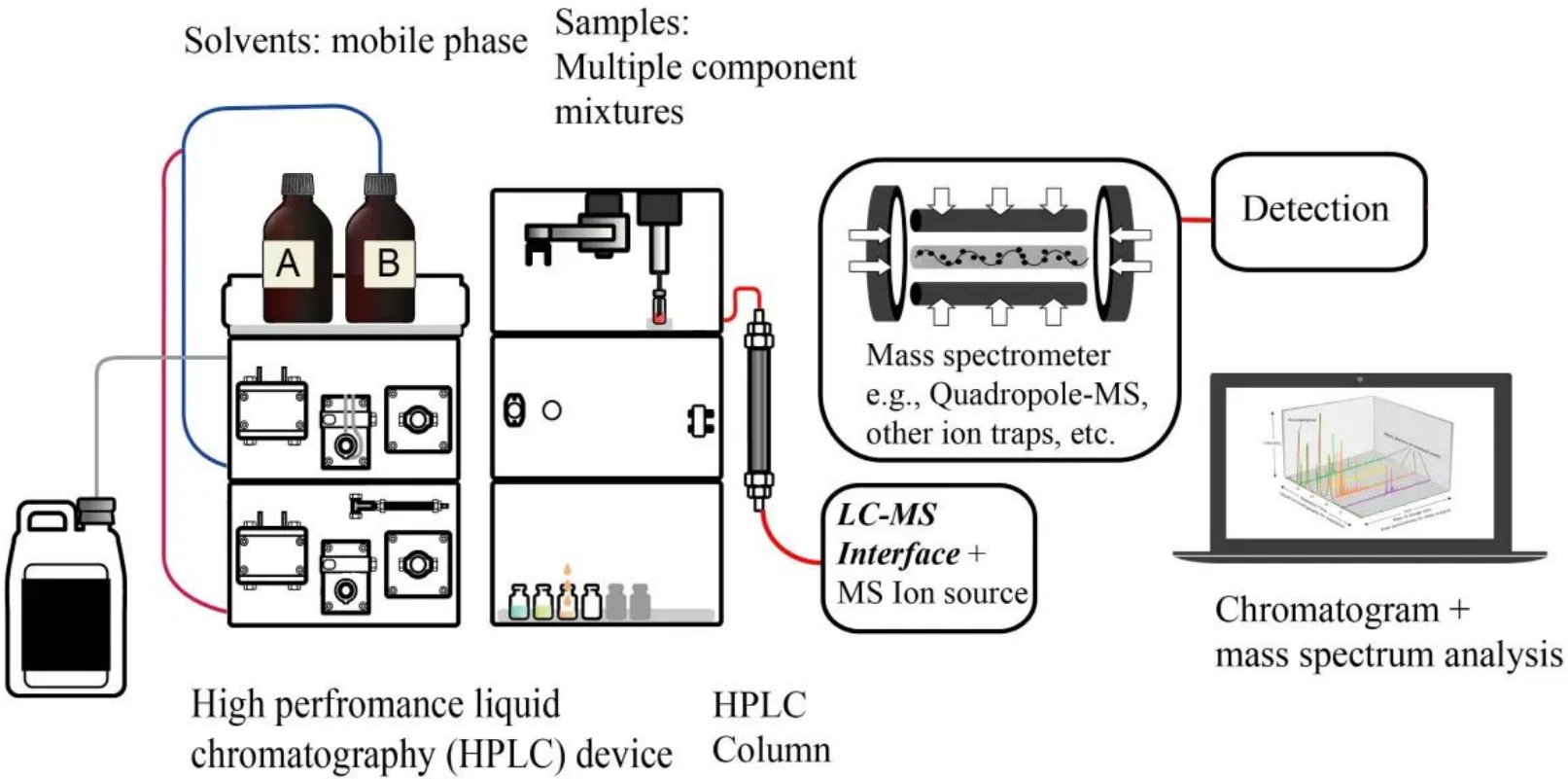
Applicable for protein, organics separation.

Chromatography

Method: A solution containing several solutes is injected at one end of the column followed by the eluent carrying the solution through the column.
Each solutes in the original solution moves at a rate proportional to its relative affinity for the stationary phase and comes out at the end of the column as a separated band.

Mechanism:
Similar to adsorption: interaction of solute-adsorbent
Different to adsorption:
- Chromatography is based on different rate of movement of the solute in the column
- Adsorption is based on the separation of one solute from other constituents by being captured on the adsorbent.

Liquid Chromatography



https://www.nebiolab.com/complete-guide-on-liquid-chromatography-mass-spectrometry-lc-ms/

Electrophoresis

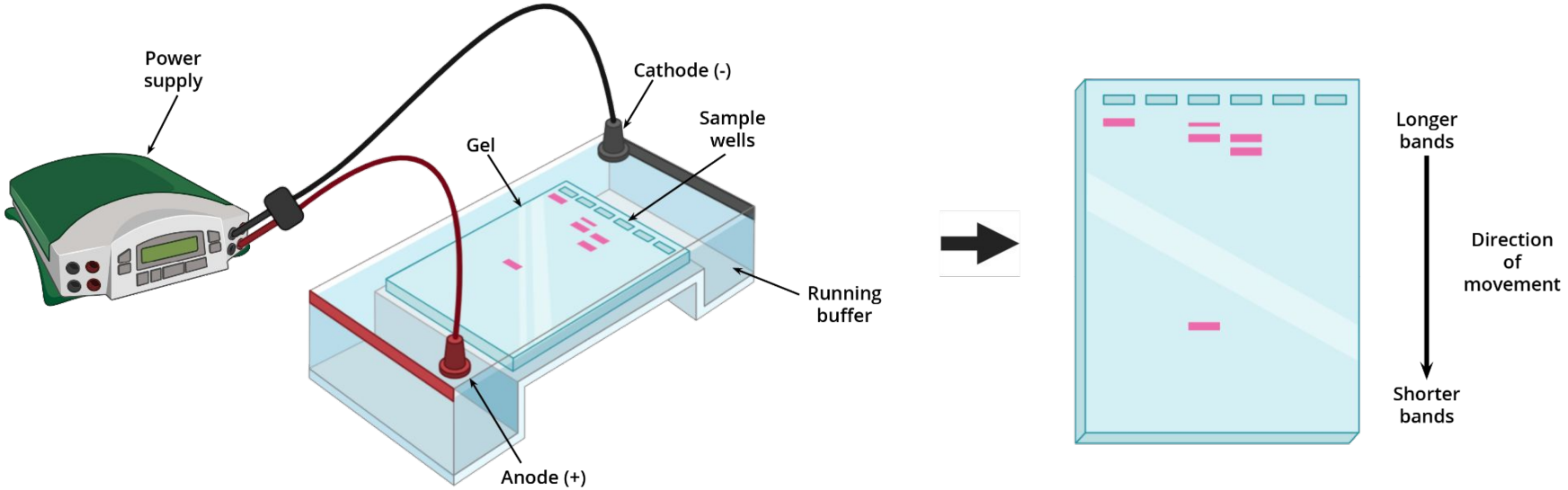
To separate charged solutes based on their specific migration rates in an electrical field.

Positive charged solutes are attracted to anode
and negative charged solutes to cathode.

Factors: electric field strength, electric charge of the solutes, viscosity of liquid and the particles size.

Applicable for protein separation.

Gel Electrophoresis



https://www.aatbio.com/catalog/gel-electrophoresis