Industrial Microbiology

4th year Biology

Dr.Muna S.Al-Delaimi

Downstream processing, what and why?

Downstream processing: is any treatment of culture broth after fermentation to concentrate and purify products. Downstream processing mostly contributes 40-90 % of total cost. It follows a general sequence of steps:

1- *Removal of insolubles*: Is the first step and involves the capture of the product as a solute in a particulate-free liquid. For example separation of cells, cell debris or other particulate matter from fermentation broth containing an antibiotic. Typical operations to achieve this are filtration, centrifugation, coagulation and flocculation.

2- *Primary isolation:* Removing components with properties significantly different from those of the products **(**Large volume, relatively non selective). For most products, water is the chief impurity and isolation steps are designed to remove most of it, reducing the volume of material to be handled and concentrating the product **.** Cell disruption, liquid extraction & precipitation are some of the unit operations involved.

3- *Purification*: Removing components with properties similar to those of the products (Highly selective). Steps in this stage are expensive to carry out and require sensitive and sophisticated equipment. Examples of operations include chromatography, ultra filtration, & fractional precipitation.

4 - ***Final isolation*:** Describes the final processing steps which end with packaging of the product in a form that is stable, easily transportable and convenient.Typical for high-quality products such as pharmaceuticals. Crystallization (followed by centrifugation or filtration) & drying are typical unit operations.

Filtration:

A mechanical operation used for the separation of solids from fluids (Fig.1). Efficiency of filtration depends on Size of organism , Presence of other organisms ,Viscosity of the medium &Temperature . These techniques are generally useful for harvesting filamentous fungi, but are less effective for collecting bacteria. The two main types of conventional filtration commonly used in industry are as follows (Fig.2) :

1- Plate and frame filter (**Filter press**): For small fermentation batches. These systems are used for harvesting microorganisms from fermentations, including the preparation of blocks of baker's yeast, the recovery of protein precipitates and the dewatering of sewage sludge.

2- Rotary-drum vacuum filter: are simple continuous filtration systems that are used in several industrial processes, particularly for harvesting fungal mycelium during antibiotic manufacture, for baker's yeast production and in dewatering sludge during waste-water treatment .A horizontal drum 0.5-3 m in diameter is covered with filter cloth and rotated slowly at 0.1-2 rpm.

Centrifugation:

Is used to separate materials of different density when a force greater than gravity is desired. Centrifugation may be used to separate particles as small as 0.1 μm diameter and is also suitable for some liquid– liquid separations. Its effectiveness , too, depends on particle size, density difference between the cells and the medium, and medium viscosity. Four main types of industrial centrifuge are commonly used:

1. Tubular centrifuges usually produce the highest centrifugal force of 13000–17000***g***.

2. Multichamber bowl centrifuges are capable of operating at 5000–10000***g***.

3. Disc stack centrifuges can operate at 5000–13000***g***

4 . Screw-decanter centrifuges :operate continuously at1500–5000**g** .They are used in sewage systems for the separation of sludge, and for harvesting yeasts and fungal mycelium.

Coagulation & Flocculation:

Coagulation----» Forming semisolid lumps in a liquid

Flocculation -------» Forming woolly cloudlike aggregations

Flocculation is a process of the cells form large aggregates to settle down for easy removal. (Flocculating agents Inorganic salts, Organic polyelectrolyte's, Mineral hydrocolloid's).
" Flocculation is synonymous with , aggregation, and coagulation / coalescence. Some organisms naturally flocculate, which can be enhanced by chemical, physical and biological treatments. Coagulation is the cohesion of dispersed colloids into small flocs; in flocculation these flocs aggregate to form larger masses. The first is induced by electrolytes and the latter by polyelectrolytes, high molecular weight, water soluble compounds that can be obtained in ionic, anionic, or cationic forms. Bacteria and proteins being negatively charged colloids are easily flocculated by electrolytes or polyelectrolytes.

2- Primary product isolation:

- Cell disruption: A lot of biological molecules are inside the cell, and they must be released from it. This is achieved by cell disruption (lysis). Cell disruption is a sensitive process because of the cell wall's resistance to the high osmotic pressure inside them.

A- Mechanical methods: When the target material is intracellular, the means microorganisms are disrupted mainly by mechanical disruption of the cells. Equipment for cell disruption includes:

i) French press (pressure cell) and high-pressure homogenizers:

In these devices, the cell suspension is drawn through a check valve into a pump cylinder at very high pressure (Disruption of yeast -----» 650 bar ,*E. coli* generally needs 1100–1500 bar) followed by an instant expansion through a special exiting nozzle. The sudden pressure causes an explosion of the cell. A problem with this method of cell disruption is that all intracellular materials are released.

ii) Ultrasonic disruption: This method of cell lysis is achieved with high frequency sound that is produced electronically and transported through a metallic tip to an appropriately concentrated cellular suspension. It is expensive and is used mainly in laboratories.

B- Non-mechanical methods: This can be accomplished by :

A-Autolysis: It has the advantages of lower cost and uses the microbes' own enzymes, so that no foreign substances are introduced into the product. Has been used for the production of yeast extract and other yeast products

B- Osmotic shock : by first allowing the cells to equilibrate internal and external osmotic pressure in a high sucrose (20% w/v) medium, and then rapidly diluting away the sucrose (in water at $4^{o}C$). The resulting immediate overpressure of the cytosol is assumed to damage the cell membrane.

C- Addition of chemicals (EDTA, Triton X-100), enzymes (hydrolyses, β-glucanases), antibiotics (penicillin, cycloserine).

D- Heat shock(thermolysis):The cells are exposed to heat which result in disintegration of the cells.It is economical method but the product has to be heat stable.

- Liquid Extraction:

The process of removing a compound or a group of compounds from a mixture or from cells into a solvent phase is called extraction.
Also known as solvent extraction or liquid-liquid extraction this procedure is widely used in industry. It is used to transfer a solute from one solvent into another in which it is more soluble. It also can be used to separate soluble solids from the mixture with insoluble material by treatment with a solvent.
Liquid – liquid extraction is basically of 2 types:

a- extraction of low molecular weight products---- extraction lipid soluble
b- extraction of high molecular weight products ---- extraction of protein

- Precipitation: Formation of a solid in a solution during a chemical reaction. Solid formed is called the precipitate and the liquid remaining above the solid is called the supernatant.

***Organic solvent precipitation:** By adding an organic solvent to an aqueous fermentation broth, the dielectric constant will decrease causing the solubility to decrease. **e.g:** Xanthan gum is recovered from an aqueous fermented broth containing the gum by adding to the broth organic solvent.

***Insoluble salt precipitation**: Usually used in extraction of amino acid and organic acid. **e.g.** Citric acid extraction and purification process.

3-Purification:

- Chromatography is a separation technique based on the different interactions of compounds with two phases, a mobile phase and a stationary phase, as the compounds travel through a supporting medium.

The **mobile phase** of chromatography refers to the fluid that carries the mixture of substances in the sample through the adsorptive material.

The **stationary or adsorbent phase** refers to the solid material that takes up the particles of the substance passing through it.

Supporting medium a solid surface on which the stationary phase is bound.

The separating molecules based on their size, charge, hydrophobicity & specific binding to ligands (Table:1). The equipments are as follows:

1-Adsorption chromatography:

Is the oldest type of chromatography. Separates according to the affinity of the protein, or other material, for the surfaces of the solid matrix, that in which the stationary phase is an adsorbent

2-Gel filtration chromatography:

Involves separation on the basis of molecular size , although molecular shape can also influence separation performance. The stationary phase consists of gel-forming hydrophilic beads containing specifically sized pore that trap and delay molecules small enough to enter them. It is useful for desalting protein preparations.

3-Ion-exchange chromatography:

Relies on charge-charge interactions between the protein of interest and charges on a resin (bead). Resins is Organic or inorganic polymer used to exchange cations (positive ions) or anions (negative ions) from a solution phase**.**

Can be subdivided into cation (positive ions) exchange chromatography, in which a positively charged protein of interest binds to a negatively charged resin; and anion (negative ions) exchange chromatography, in which a

negatively charged protein of interest binds to a positively charged resin. Once the protein of interest is bound, the column is washed with equilibration buffer to remove unattached entities.

Then the bound protein of interest is eluted off using an elution buffer of increasing ionic strength or of a different pH. Either weakens the attachment of the protein of interest to the bead and the protein of interest is bumped off and eluted from the resin.

Ion exchange resins are the cheapest of the chromatography media available and are therefore almost always used as a step in biopharmaceutical protein production purification.

4- Affinity chromatography:

It is particularly powerful and highly selective purification technique, separates proteins on the basis of a reversible interaction between a protein and a specific ligand coupled to a chromatography matrix (The technique involves specific chemical interactions between solute molecules, such as proteins, and an immobilized ligand (functional molecule). To elute the target molecule from the affinity medium the interaction can be reversed, either specifically using a competitive ligand, or non-specifically, by changing the pH, ionic strength or polarity.

Table(1): Affinity chromatography ligands

Protein	***Ligand***
Antigen	***Antibody***
Antibody	***Hapten***
Glycoproteins	***Lectins***
Metaloprotein	***Metal ions***
Hormone	***Receptor***

5-High performance liquid chromatography (HPLC):

A type of automated chromatography in which the mobile phase is a liquid which is forced under high pressure through a column packed with a sorbent.

6-Hydrophobic chromatography :

Relies on hydrophobic interaction between hydrophobic regions of a solute protein and hydrophobic functional groups of the support particles. Adsorption takes place in high salt and elution in low salt concentrations.This technique provides good resolution and, like ion-exchange chromatography has a very high capacity as it is not limited by sample volume**.**

4-Finishing steps (final isolation):

- Crystallization

Is the final purification method for those materials which can stand heat. The solution is concentrated by heating and evaporation at atmospheric pressure to produce a super saturated. Many fermentation products will not however stand heat and the initial water removal is made by heating at reduced pressure or by lowering the temperature to form crystals which can be centrifuged off leaving concentrated liquor. The product's solubility can be reduced by adding solvents, salts, polymers, and polyelectrolyte, or by altering pH.

-Drying

Involves the transfer of heat to the wet material and removal of the moisture as water vapor. Usually, this must be performed in such a way as to retain the biological activity of the product.

Parameters affecting drying are:

1-The physical properties of the solid–liquid system,

2-Intrinsic properties of the solute,

3-Conditions of the drying environment and heat transfer parameters

Drying can be considered under two heads:

(i) Liquid-phase moisture removal:

Liquid-phase moisture removal involves drying by heat.

The most important methods for drying are:

1-vacuum tray drier. 2-Drum dryers. 3-Spray drying

(ii) solid-phase moisture removal (freeze-drying):

Freeze-drying (lophylization): is a process of drying in which water is sublimed from the product after it is frozen. It is a drying process applicable to manufacture of certain pharmaceuticals and biological that are thermolabile (enzymes, bacteria, and antibiotics) or otherwise unstable in aqueous solutions for prolonged storage periods, but that are stable in the dry state.

The term "lyophilization" describes a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase

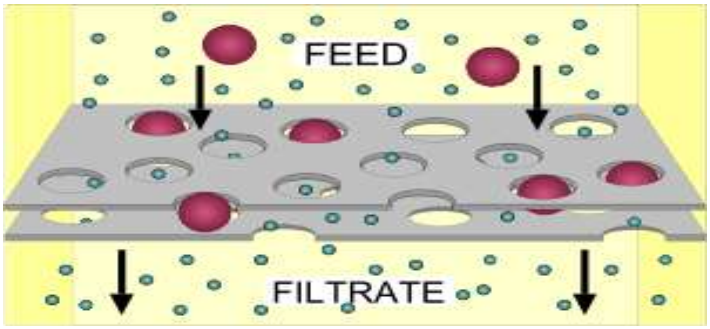

Fig.1*: oversize particles in the feed cannot pass through the lattice structure of the filter, while fluid and small particles pass through, becoming filtrate*.

Fig.2: *Plate and frame filter(right) & Rotary-drum vacuum filter(left).*