DOWNSTREAM PROCESSING

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INTRODUCTION

- A bioprocess is specific process that uses complete living cells or their components (e.g., bacteria, enzymes, chloroplasts) to obtain desired products.
- The upstream process is defined as the entire process from early cell isolation and cultivation, to cell banking and culture expansion of the cells until final harvest.
- The downstream part of a bioprocess refers to the part where the cell mass from the upstream are processed to meet purity and quality requirements.

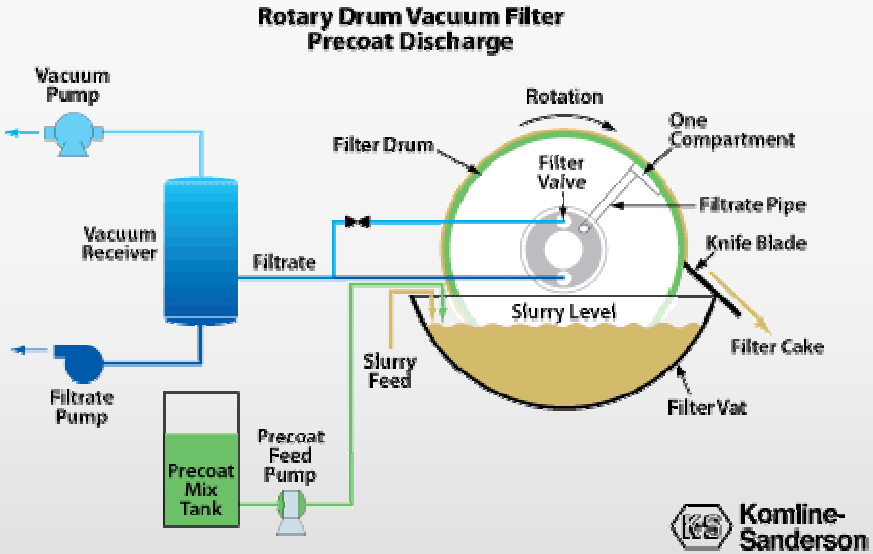
- Downstream processing is usually divided into three main sections:
- Cell disruption,
- purification and
- polishing

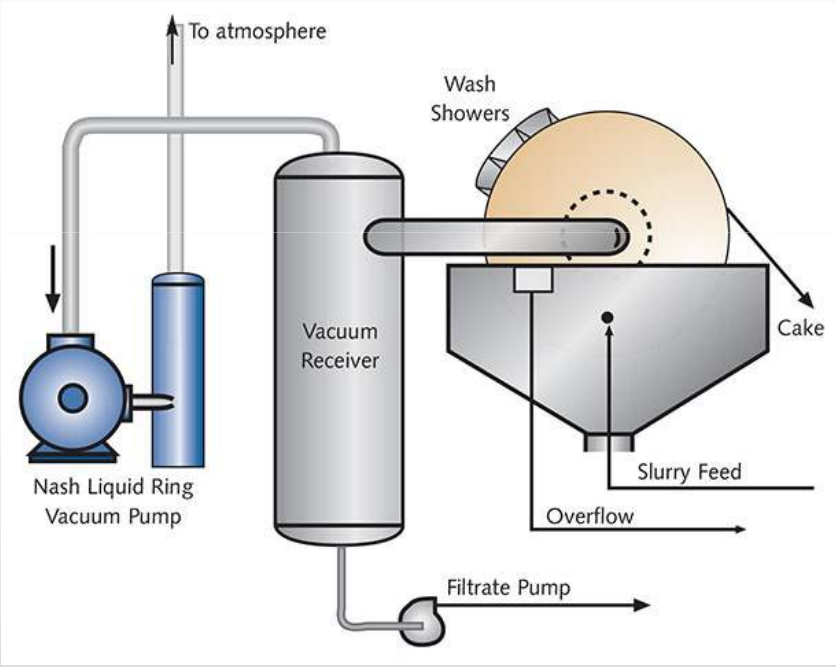
1. Separation of Particles/Cell

- The first step in DSP is the separation of solids, usually cells, from the liquid medium.
- **Filtration**
- It is used for the separation of filamentous fungi and filamentous bacteria, e.g., Streptomyces's, and often for yeast flocks.
- surface filtration, depth filtration, centrifugal filtration, cross-flow filtration, rotary drum vacuum filtration.

ROTARY DRUM VACUUM FILTERS

- The filter is in form of a rotating drum with a partial vacuum on the inside of the drum.
- A portion of the drum rotates through the medium and the cells are sucked to form a coating on the drum; the cells are continuously scraped off to prevent blocking of the filter.


Rotary Drum Vacuum Filter
Precoat Discharge
Vacuum Pump
Vacuum Receiver
Filtrate
Filtrate Pump
Precoat Mix Tank
Precoat Feed Pump
Slurry Feed
Rotation
Filter Drum
Filter Valve
One Compartment
Filtrate Pipe
Knife Blade
Slurry Level
Filter Cake
Filter Vat
Komline-Sanderson


To atmosphere
Wash Showers
Vacuum Receiver
Cake
Slurry Feed
Overflow
Nash Liquid Ring Vacuum Pump
Filtrate Pump

CENTRIFUGATION

- It may be used to separate bacteria and, usually, protein precipitates. But difficulties arise due to small differences in the densities of the particles and the medium.
- In addition, equipment cost, power consumption, temperature, etc. are the other disadvantages.

FLOCCULATION

- Small bacterial cells, which are difficult to separate even by centrifugation, can be recovered as follows. Flocculation, i.e., sticking together of cells, can be induced by inorganic salts, mineral hydrocolloids and organic polyelectrolytes.
- Since sedimentation rate of a particle increases with size, flocculated cells can be recovered by centrifugation.

FLOATATION

- In cases, where flocculation is not effective, very fine gas bubbles can be created by sparging, release of overpressure or electrolysis.
- In any case, the gas bubbles adsorb to and surround the cells, raising them to the surface of medium in form of foam (floatation); long chain fatty acids or amines promote stable foam formation.
- The cells collected in the foam are readily recovered.

2. Disruption of Cells

- When the product is intra-cellular, one more step is added after cell separation. This is cell disruption, to release the product contained within.
- Cells are broken by mechanical, physical or chemical methods.
- Disruption of microbial cells is usually difficult due to their small size, strong cell wall and high osmotic pressure inside cells.

Mechanical Cell Disruption

- This approach uses shear, e.g., grinding in a ball mill, colloid mill, etc., pressure and pressure release, e.g., homogenizer, and ultrasound.

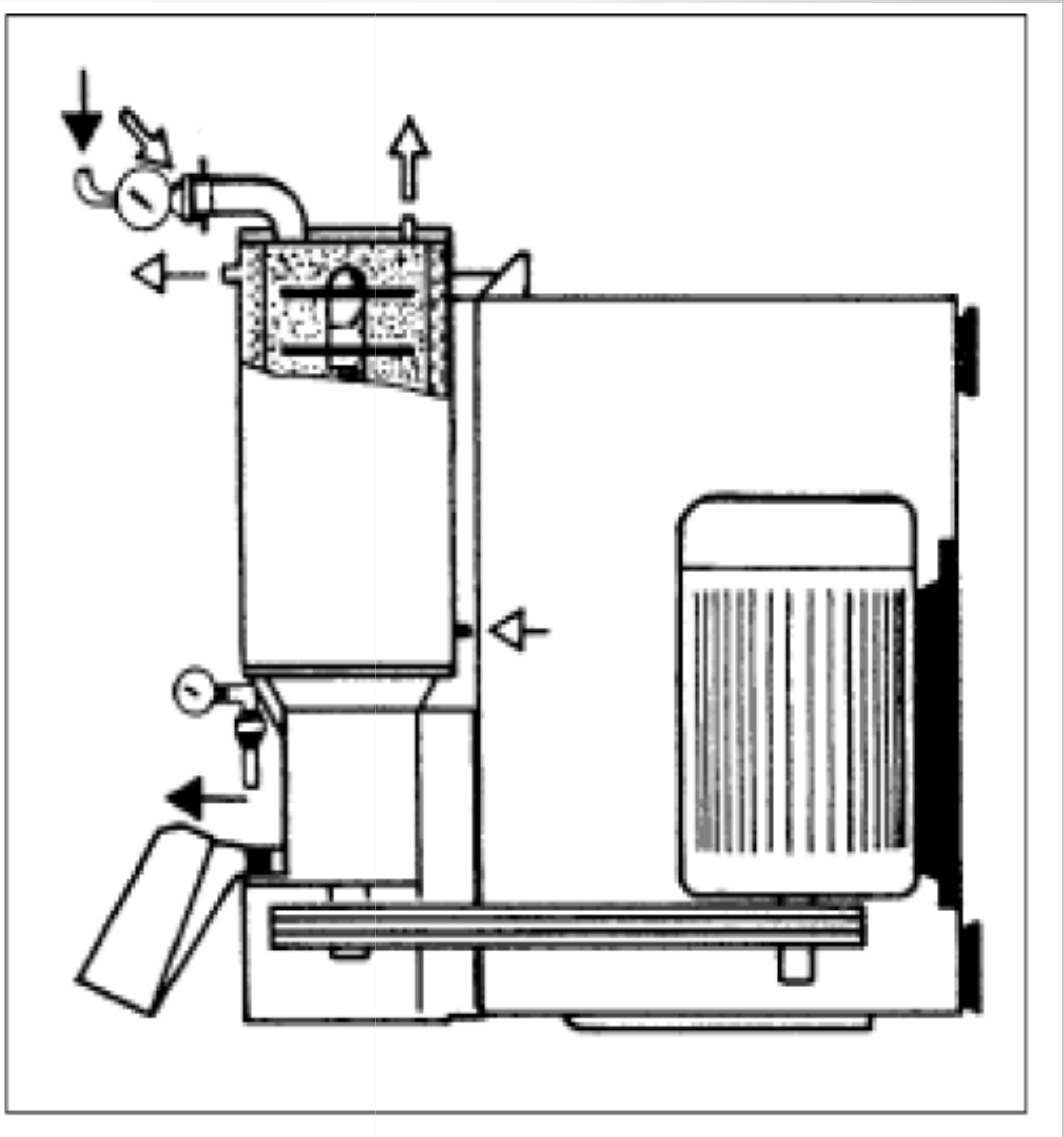
ULTRASOUND

- A common laboratory-scale method for cell disruption applies ultrasound (typically 20–50 kHz).
- In principle, the high-frequency is generated electronically and the mechanical energy is transmitted to the sample via a metal probe that oscillates with high frequency.
- The probe is placed into the cell-containing sample generating high-frequency oscillation ultimately breaking open the cells.
- Disadvantage - Heat generation, High noise levels


SONOPULS HD 2070

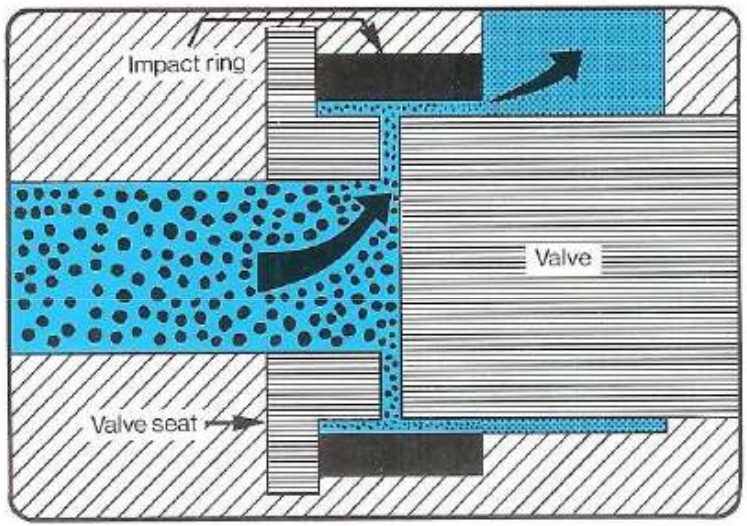
BEAD MILLS

- Mills consist of either a vertical or a horizontal cylindrical chamber with a motor-driven central.
- The chamber is filled to the desired level with steel or 0,1-2 mm abrasion- resistant glass beads (ballotini) which provide the grinding action.
- The charge of grinding beads is retained in the chamber by a sieve-plate.
- The horizontal configuration of the mill is known to give a better efficiency of disruption relative to the vertical one.
- Advantages: continuous operation possible
- scale up possible (up to 600-1000 litre grinding space)
- Disadvantages:
- Large energy consumption

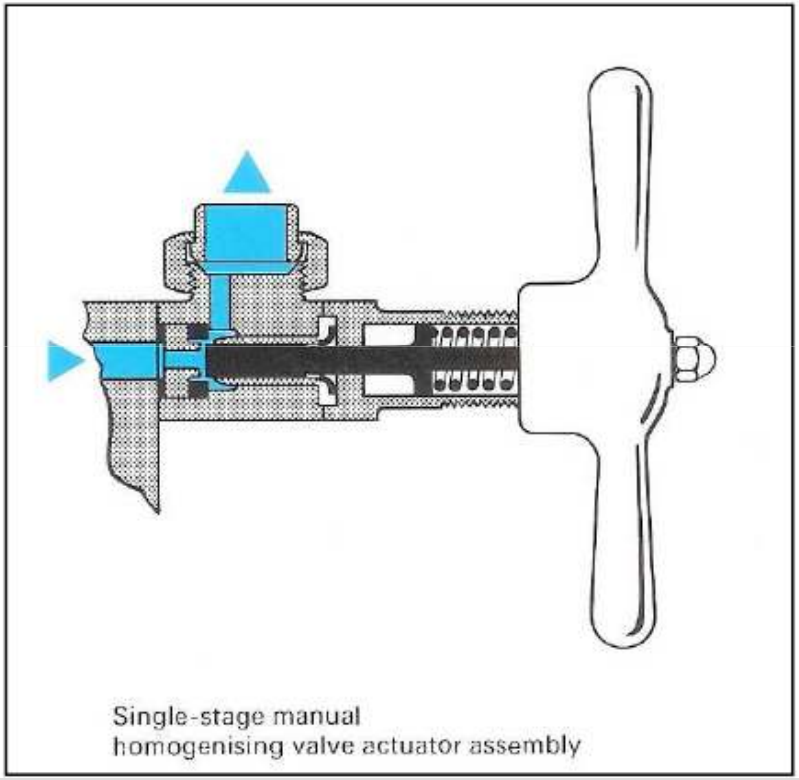


HIGH PRESSURE HOMOGENISATORS

- The high-pressure homogenizer consists of a positive displacement piston pump.
- The cell suspension is drawn through a check valve into the pump cylinder and, on the pressure stroke (extreme high pressure, 200 - 600 - 1000 bar), is forced through an adjustable discharge valve.
- The discharge pressure is controlled by a handwheel assembly, which, through a springloaded valve rod.
- During disruption, the cell suspension passes between the valve and its seat and impinges on an impact ring.



High-pressure homogenizer discharge valve unit.



Single-stage manual homogenising valve actuator assembly

PHYSICAL METHODS OF CELL DISRUPTION

Drying

- The cells may be dried, e.g., by adding the cells into a large excess of cold acetone and subsequently extracted using buffer or salt solutions.
- Drying induces changes in cell wall structure, which facilitate extraction. This method is widely used.
- Hot air drying
- Slow vacuum drying of pastes (esp. bacteria)
- lyophilisation (freeze drying) of cell suspensions is particularly useful for labileenzymes.
- Solvent drying dehydration with water-miscible solvents at low temperatures (e.g. acetone, gives acetone powder)

FREEZING – THAWING

- The freeze-thaw method is commonly used to lyse bacterial and mammalian cells.
- The technique involves freezing a cell suspension in a dry ice/ethanol bath or freezer and then thawing the material at room temperature or 37 °C.
- This method of lysis causes cells to swell and ultimately break as ice crystals form during the freezing process and then contract during thawing.

CHANGE OF PHYSICAL CONDITIONS

- **Heat shock** – in aqueous medium (0,5-1 hour at 60-80-100 °C, mind the heat denaturation)
- **Osmotic shock** – with neutral compounds (sugars, sugar alcohols, glycerol) not with salts.
- "Saturate" the cells with sugar, than transfer them into pure water.
- **Solvent treatment**
- Drying with acetone than dissolve the cell membranes with ether
- Autolysis of yeasts with toluene
- **Detergent treatment**
- They penetrate into the cell membrane and destroy its structure.
- Both cationic and anionic detergents
- Bile acids

ENZYMATIC METHODS

Specific enzymes hydrolyzing the cell wall:

bacteria	- lysozyme
yeasts	- mannanase (Yeast Lyase, *Cytophaga sp.*)
moulds	- chitinase, cellulase
plant cells	- cellulase, pectinase

3. EXTRACTION

- The process of recovering a compound or a group of compounds from a mixture or from cells into a solvent phase is called extraction.

LIQUID-LIQUID EXTRACTION

- It employs two immiscible liquids into which the product is differentially soluble.
- The extraction may be performed in a single step, by multi-stage parallel-flow extraction.

AQUEOUS MULTIPHASE EXTRACTION

- It is used for separation of enzymes from cells/cell debris.
- The enzymes are extracted in an aqueous polyethylene glycol-dextran mixture, which form separate phases.
- Recovery of enzymes from these phases is rather easy and free from some of the difficulties encountered in centrifugation.

4. CONCENTRATION

- Some concentration of the product may occur during the extraction step. Further concentration may be achieved by the following:
- (i) evaporation, (ii) membrane filtration, (iii) ion exchange methods, and (iv) adsorption methods.

EVAPORATION

- It is generally used in cases of solvent extraction using various devices, e.g., continuous flow evaporators, falling film evaporators, thin film evaporators, centrifugal thin film evaporators and spray-dryers.

MEMBRANE FILTRATION

- It generally achieves both concentration and separation of the products, usually, based on the size of molecules.
- The different processes of membrane filtration are as follows: microfiltration, ultra-filtration, reverse osmosis, and electro-dialysis.
- Micro- and ultra-filtrations work as sieves and separate molecules of different sizes, but reverse osmosis can separate molecules of similar size.

Ion Exchange Resins

- These are polymers having firmly attached ionizable groups (anions or cations), which ionize under a suitable environment.
- These may be solid, e.g., dextran, cellulose, polyamine, acrylate, etc., or liquid, e.g., a solvent carrying a functional group like phosphoric acid mono- or di-ester, etc.
- The product is recovered from the ion exchangers by ion displacement.

Adsorption Resins

- These are porous polymers without ionization. Most compounds are adsorbed to the resins in non-ionized state.
- The porosity of the resin determines the surface available for adsorption. These resins may be apolar (e.g., styrene-divinyl beneze), polar (e.g., sulfoxide, amide, etc.), or semi-polar (e.g., acrylic ester).
- The products are recovered from such resins by solvent (organic) extraction, changed pH, etc.

5. PURIFICATION

- The final step in the recovery of a product is purification, which aims at obtaining the product in a highly purified state.
- Purification is achieved by the following procedures: (i) crystallization and (ii) chromatographic procedures.
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CRYSTALLIZATION

- It is mainly used for purification of low molecular weight compounds like antibiotics, e.g., penicillin G is usually extracted from the fermentation broth in butyl acetate and cystallized by the addition of potassium acetate in ethanolic solution.
- Crystallization is the final stage in purification of products like citric acid, sodium glutamate, etc.

CHROMATOGRAPHY

- These are used for the purification of low molecular weight compounds from mixtures of similar molecules, e.g., homologous antibiotics, and of macromolecules, especially enzymes, which are similar in properties.
- The different chromatographic procedures are as follows: (i) adsorption, (ii) ion exchange, (iii) gel filtration, (iv) hydrophobic, (v) affinity, (vi) covalent and (vii) partition chromatography.

- Adsorption chromatography separates molecules due to their differential affinities for the surface of a solid matrix, e.g., silica gel, alumina, hydroxyapatite (all inorganic) or an organic polymer.
- Gel filtration uses molecular sieves composed of neutral cross-linked carriers e.g., polymers like agarose, dextrans, of different pore sizes. Molecules smaller than the pore size enter the carrier and are retained; they are later eluted (in order of molecule size) and collected.
- Hydrophobic enzymes and proteins.

- Affinity chromatography uses molecules, called effectors to which the product has high and specific affinity.

TABLE 14.14. A list of some effectors used in affinity chromatography and the molecules purified by them

Effector (ligand in column)	*Purified molecule*
Antibodies	Specific antigens
Antigens	Specific antibodies
Enzymes	Specific inhibitors, coenzymes, cofactors, and substrate (under specific conditions)
Enzyme inhibitors	Specific enzymes
Hormones	Specific hormone receptors
Lectins	Glycoproteins, polysaccharides
Nucleic acids	Complementary base sequences
Transcription factor eIF-4E	5′-Capped mRNAs (nearly all eukaryotic mRNAs)
Vitamins	Vitamin carrier proteins

6. DRYING

- Drying makes the products suitable for handling and storage.
- It should be accomplished with a minimum rise in temperature due to heat sensitivity of most products.
- Addition of sugars or other stabilizers improves the heat tolerance of some products like enzymes and pharmaceutical preparations.
- The most common approaches to drying are as follows: (i) vacuum drying, (ii) spray drying, and (iii) freeze drying.

7. POLISHING

- The last part is product polishing in which the purified product is packed into a form which is stable, easily portable and convenient to use.
- Product sterilization and removal of trace contaminants are then performed to ensure the safety of the product.
- Products are purified to achieve the demands of the market/customers (standards, regulations, legal measures).

THANK YOU