Anil Kumar Chawla, PhD, India/Switzerland

- Chairman and Managing Director, Chimera Gentec Pvt Ltd, India/Protaccine Biotec Sarl, Switzerland

Purification of vaccines and biologicals

Anil Kumar Chawla, PhD

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PURIFICATION OF VACCINES & BIOLOGICALS

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VACCINES & BIOLOGICALS

- Vaccines & Biological products include a wide range of products such as vaccines, blood and blood components, allergenics, somatic cells, gene therapy, tissues, and recombinant therapeutic proteins.

- These products are used for a wide range of diseases and conditions, including serious and life-threatening conditions.

Manufacturing Process

VACCINES & BIOLOGICALS

Manufacturing Process

- Cell Bank
- Cultivation
- Harvest
- Purification

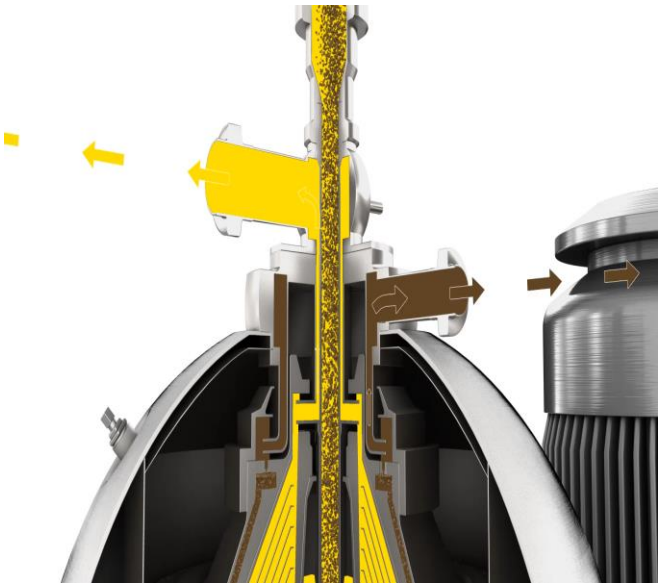
Harvest/Clarification

HARVEST/CLARIFICATION

- The preliminary separation of a protein of interest from a reactor “soup” of process impurities is the first step in a downstream process.

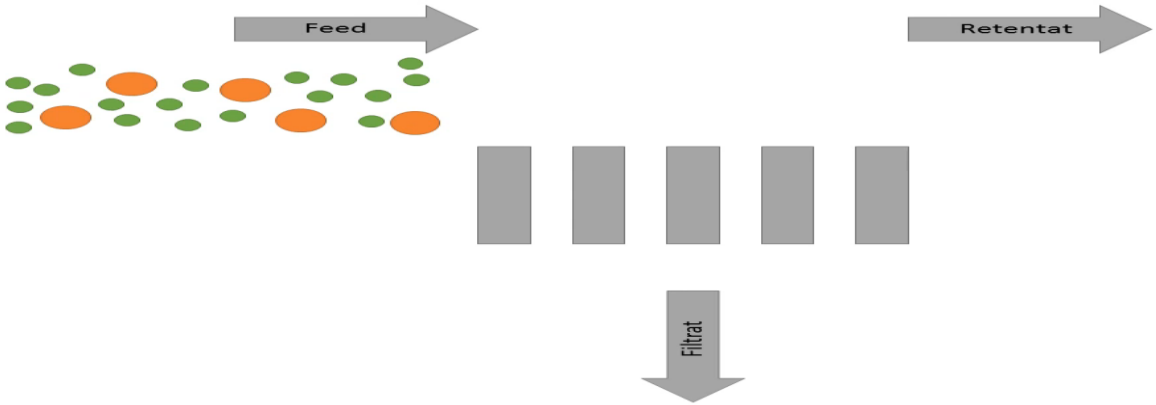
- It is also a primary step that introduces a significant risk of product degradation, bioburden concerns, or process errors.

HARVEST/CLARIFICATION



Method

- Centrifugation
- Depth Filtration
- Microfiltration
- Combination of these (i.e. centrifugation and depth filtration)



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Purpose of Purification

PURPOSE OF PURIFICATION

Quality Assured: A traceable intermediate or Drug substance of assured quality

- Highly pure active drug substance
- Removal of all possible identified impurities
- Capable to produce consistent quality parameters for end product (DS or intermediate)
- Well identified controllable critical parameters
- All possible risks identified for equipment, process and product quality parameters
- Easy and cost effective to operate
- Teams into overall scheme for purification

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HOW MUCH PURE IS CONSIDERED TO BE PURE AND PRODUCT IS SAFE?

DIFFERENT TECHNIQUES USED FOR PURIFICATIONS

Cell Disruption – Mechanical

CELL DISRUPTION

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

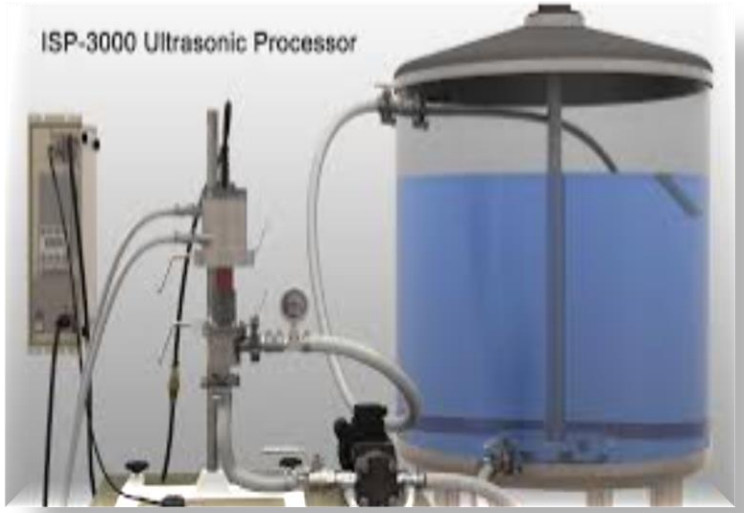
Methods: Mechanical and non mechanical

- **Mechanical**

✓ **Ultrasonication** (sonicators) bacteria, virus and spore suspensions at lab-scale

Electronic generator: ultrasonic waves

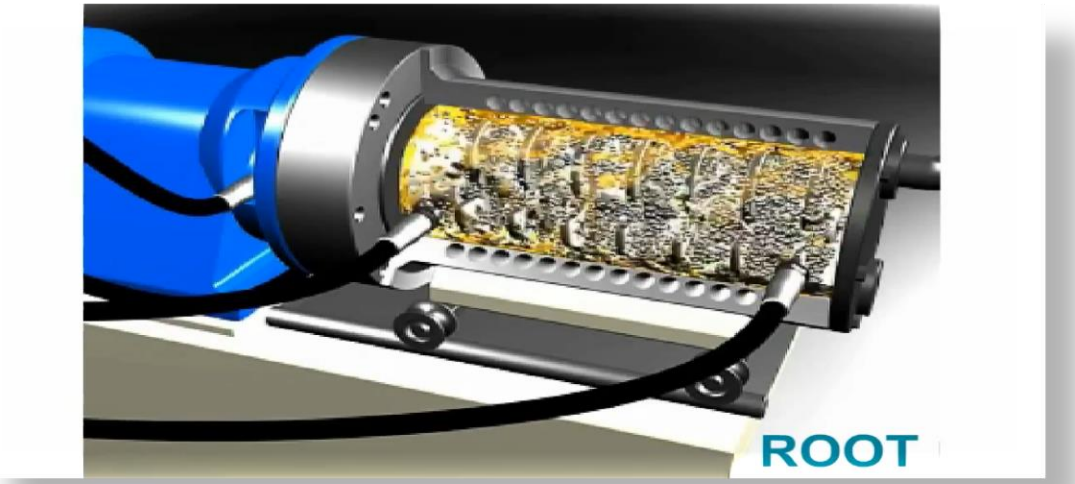
✓ **Mechanical oscillation**: by a titanium probe immersed in a cell disruption vessel



CELL DISRUPTION CONTI…

Mechanical conti…

Milling: continuous operation at low temperature



- Bacteria and fungi
- Large scale

Principle :

A grinding chamber filled with about 80% validated glass beads.

High shearing and impact forces from the beads break the cell wall.

CELL DISRUPTION CONTI…

Mechanical conti…

Ball Mill: solid

- ✓Frozen cell paste, cells attached to or within a solid matrix.
- ✓Large scale disruption of microorganism.

CELL DISRUPTION CONTI...

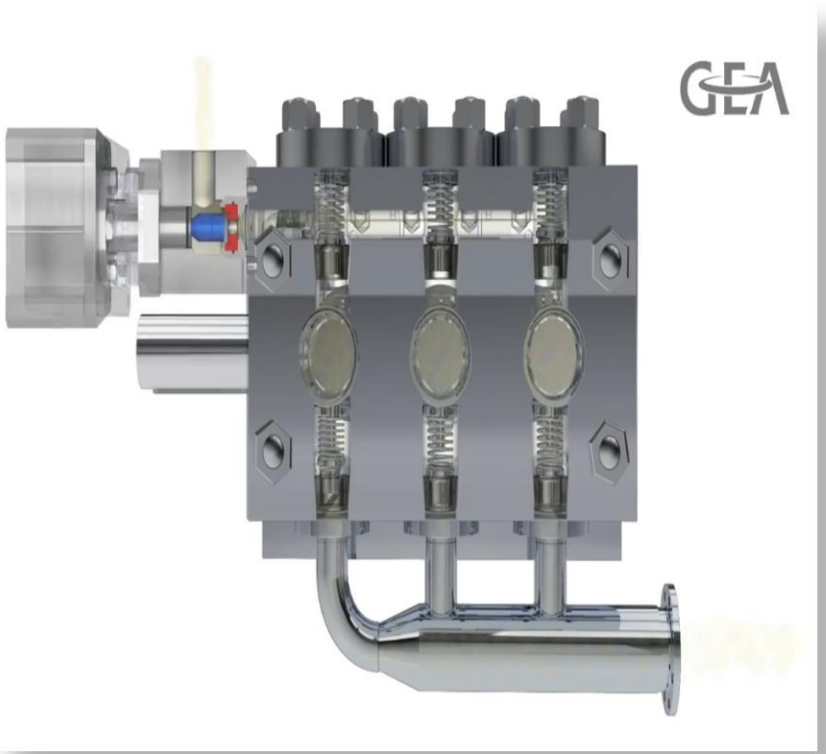
Mechanical conti...

Homogenization

- ✓ Suspension, large scale
- ✓ To pump a slurry (up to 1500 bar) through a restricted orifice valve.

The cells disrupt as they are extruded through the valve to atmosphere pressure by

- high liquid shear in the orifice
- sudden pressure drop upon discharge



CELL DISRUPTION CONTI…

Non-mechanical

- **Chemicals**: chemicals to solubilize the components in the cell walls to release the product.

Chemical requirements:

✓ Products are insensitive to the used chemicals.

✓ 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.

Cell Disruption – Non-Mechanical

CELL DISRUPTION CONTI...

Non-mechanical conti...

- **Enzymes**:

✓ to lyse cell walls to release the product.

✓ Gentle, but high cost e.g 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 through 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.

CELL DISRUPTION CONTI…

Challenge:

Damage to the product

- Heat denaturation
- Oxidation of the product
- Unhindered release of all intracellular products
- Renaturation process using specialized sets of buffer conditions

SEPARATION OF SOLUBLE PRODUCTS

Precipitation

SEPARATION OF SOLUBLE PRODUCTS

Precipitation

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

Applicability: separate proteins or antibiotics from fermentation broth.

SEPARATION OF SOLUBLE PRODUCTS CONTI…

Precipitation

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 tetanus or diphtheria toxin is reduced with increased amount of ammonium sulfate.

✓ Added salts interact more strongly with water so that the proteins precipitate.

✓ Inexpensive

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

e.g. The IE of cytochrome c_M (without histidine tag) is 5.6

(Cho, et.al., 2000, Eur. J. Biochem. 267, 1068±1074).

Adsorption

SEPARATION OF SOLUBLE PRODUCTS CONTI...

Adsorption

Adsorb soluble product from fermentation broth onto solids.

- **Physical adsorption** (Silica for rHBsAg), 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 adsorbent:

- ✓ Functional groups and their numbers
- ✓ Surface properties by properties of solution
- ✓ Solutes
- ✓ pH
- ✓ Ionic strength
- ✓ Temperature

Membrane Separation

SEPARATION OF SOLUBLE PRODUCTS CONTI...

Membrane separation:

- **Microfiltration:** 0.1 - 10 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).

The common features of the above methods:

- *Use of membrane or cassettes*
- *Driving forces: pressure*

Chromatography

SEPARATION OF SOLUBLE PRODUCTS CONTI…

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 eluent that carriers the solution through the stationary phase.
- ✓ Applicable for protein, organics separation.

SEPARATION OF SOLUBLE PRODUCTS CONTI..

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 solute 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.

SEPARATION OF SOLUBLE PRODUCTS CONTI…

Chromatography Mechanism:

- Similarity to adsorption: interaction of solute-adsorbent
- Difference with adsorption:

✓ Chromatography is based on different rates of movement of the solutes in the column

✓ Adsorption is based on the separation of one solute from the other constituents by being captured on the adsorbent.

QUALITY RISK MANAGEMENT

QUALITY RISK MANAGEMENT

- Controlling critical process parameters
- Age of purification equipment, mediums and cassettes
- Automation versus manual control of purification
- In-process checks for start and stops
- In-process checks for rejections and abandoning the process
- Assigning risk score or robustness score to purification process
- Trending critical equipment and process parameters
- Re-validation after changes or time-lapse

CRITICAL AND MAJOR OBSERVATIONS- WHO INSPECTIONS

CRITICAL AND MAJOR OBSERVATIONS – WHO INSPECTIONS

- Control of biological burden during purification
- Storage of purification equipment and mediums
- Dedication of purification mediums to single product
- Use of disposable accessories where ever possible
- Monitoring of clean room parameters during purification process
- Improving purification processes based on historical trend data
- Improving the monitoring based on historical trend data
- Replacing aseptic processes with sterile filtrations where ever possible
- Avoiding aggregate formation and product degradation.

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Thank You