Downstream Processing Techniques and Single Use Applications

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Cell culture / Fermentation
Growth of appropriate cells, typically in a bioreactor or fermenter, to produce product of interest

Primary Product Recovery
Primary Product Recovery or Primary Clarification is a:

- Solid : Liquid Separation
- Whole Cells : Liquid Phase containing product + Cellular Debris

Product Transmission is KEY Objective

Separation Efficiency dependent on:
- Amount of Solids
- Particle Size
- Viscosity
Product Location

KEY

<table>
<thead>
<tr>
<th>Key</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td></td>
</tr>
<tr>
<td>Cellular Debris</td>
<td></td>
</tr>
<tr>
<td>Target Molecule</td>
<td></td>
</tr>
<tr>
<td>Host Cell Proteins</td>
<td></td>
</tr>
<tr>
<td>Host Cell DNA</td>
<td></td>
</tr>
<tr>
<td>Viruses</td>
<td></td>
</tr>
<tr>
<td>Endotoxin</td>
<td>Fluid (e.g. media, buffer, water)</td>
</tr>
</tbody>
</table>

Extracellular Expression

Intracellular Expression

Primary Product Recovery

Recovering product that has been produced by cell culture or fermentation

- **Clarification of cell / fermentation broths (removal of whole cells and cellular debris) to recover product (typically soluble)**
  - EXTRACELLULAR EXPRESSION

- **Concentration and recovery of whole cells prior to cell disruption / lysis to release product (soluble or insoluble)**
  - INTRACELLULAR EXPRESSION
Commonly Encountered Cell Types

<table>
<thead>
<tr>
<th></th>
<th>Bacterial</th>
<th>Mammalian</th>
<th>Fungal</th>
<th>GMO Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Example</strong></td>
<td>E. Coli</td>
<td>CHO (Chinese Hamster Ovary)</td>
<td>Penicillium</td>
<td>Pichia Pastoris</td>
</tr>
<tr>
<td><strong>Cell Size</strong></td>
<td>0.5 – 0.8µm</td>
<td>10 - 100µm</td>
<td>3µm</td>
<td>14µm +</td>
</tr>
<tr>
<td><strong>Potential Volumes</strong></td>
<td>30,000 L</td>
<td>100 - 25000 L</td>
<td>100,000 L</td>
<td>30,000 L</td>
</tr>
<tr>
<td><strong>Typical Cell Densities</strong></td>
<td>2 – 5% w/v</td>
<td>1 – 5 x 10^7 cells /ml</td>
<td>40 – 50% w/v (400 – 500 g/l)</td>
<td>40 – 50% w/v (400 – 500 g/l)</td>
</tr>
<tr>
<td><strong>Product Location</strong></td>
<td>Mainly Intracellular</td>
<td>Extracellular</td>
<td>Extracellular</td>
<td>Extracellular</td>
</tr>
<tr>
<td><strong>Example</strong></td>
<td>Antigen Binding Fragments (FAbs)</td>
<td>Monoclonal Antibodies</td>
<td>Antibiotics Industrial Enzymes</td>
<td>Small Peptides and proteins (e.g. Insulin)</td>
</tr>
</tbody>
</table>

Technologies For Primary Product Recovery

- Centrifugation
- Depth filtration
- DFF Capsule
- Hollow fibres
- TFF Capsule
- Ceramic
- Cassettes
- VMF
Biomolecule Purification Strategy

How do we purify the target molecule from this molecular ‘soup’?

Chromatography

A separation method consisting of a:

- **Stationary phase** (sorbent/gel/media/resin or membrane)
- **Mobile phase** (Process fluid stream/buffer)

Properties of mobile phase, stationary phase and molecule determine column residence time
Chromatography Scales

Laboratory

Pilot

Production

<table>
<thead>
<tr>
<th>Key</th>
<th>ID</th>
<th>Typical Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target Molecule</td>
<td>mg/mL</td>
<td></td>
</tr>
<tr>
<td>Host Cell Proteins</td>
<td>ppm or ng/mg</td>
<td></td>
</tr>
<tr>
<td>Host Cell DNA</td>
<td>pg/mL</td>
<td></td>
</tr>
<tr>
<td>Viruses</td>
<td>LRV of virus particles</td>
<td></td>
</tr>
<tr>
<td>Aggregates/ Misfolds</td>
<td>% or ppm</td>
<td></td>
</tr>
<tr>
<td>Endotoxin</td>
<td>EU/mL</td>
<td></td>
</tr>
<tr>
<td>Fluid (e.g. media, buffer, water)</td>
<td>L</td>
<td></td>
</tr>
</tbody>
</table>

Chromatography Purification

Process stream composition

Out

Target Molecule >97%
Process-Related Impurities

• Impurities derived from the drug manufacturing process
  – Include Host Cell Proteins (HCPs), host cell DNA
  – Antibiotics, cell culture media components...
  – Column/filter extractables and leachables (protein A)

• All must be removed during downstream processing, using various methods, including chromatography
  – Require different strategies, according to the nature of the impurities, their concentration, and the target protein.

Affinity Chromatography
Protein-A

Target Molecule
Ligand
Impurities

Target Molecule Bound at ~neutral pH
**Affinity Chromatography**

The specific molecule can be eluted by increasing salt concentration or change of pH (acidic)

Target Molecule
High Purity
Reduced Volume

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**Ion Exchange Chromatography**

ANION exchanger
(Q or DEAE)

AETMA or Diethylaminoethyl
Reduce HCP and Viruses

CATION exchanger
(S or CM)

Sulphonic acid or carboxymethyl

= Cl⁻ or Molecule⁻

= Na⁺ or Molecule⁺
Hydrophobic Chromatography (HIC)
Proteins Bind at High Salt Concentration and Elute at Low Salt Concentration

Tangential Flow Filtration

- As permeate (filtrate) volume increases...
- ... permeate flux decreases due to feed viscosity increase, but membrane has reduced fouling by crossflow action.
- Result: TFF allows processing of larger volumes with higher solids loading than DFF.
Flux Versus TMP
Critical for Proper Operation

MEMBRANE CONTROLLED REGION

GEL LAYER CONTROLLED REGION

WATER

PROCESS FLUID

CONSTANT CF (ΔP)

WHERE CF = CROSS FLOW VELOCITY

OPTIMAL

Transmembrane Pressure

Flux Versus TMP
Critical for Proper Operation

Tangential Flow Filtration

Microfiltration (MF)

• Pressure driven process where particulates (e.g. cells or cellular debris - waste) are retained on a basis of their physical size while small particles, small molecular weight species (product) & fluid/water pass through the membrane
• As fluid/water is removed the upstream is ‘concentrated’
• Difiltration in MF is used to ‘wash’ through more product to increase the yield
• 0.1 to 1 micron in general

Ultrafiltration - UF

• Pressure driven process where solutes (e.g. proteins) (Product) are retained on a basis of their molecular size while very small molecular weight species (e.g. salts) & fluid/water pass through the membrane (waste)
• As fluid/water is removed the upstream is ‘concentrated’
• Difiltration in UF is used to exchange buffers to prepare for chromatography to maximize yield
• 0.01 to 0.1 micron
• 5-1000 kDa – MWCO (molecular weight cutoff)
TFF (MF vs. UF)

- Upstream
- Retention
- Tangential or Crossflow

- Downstream
- Passage
- Pore Size Critical

- Feed
- Retentate
- Membrane

- Ultrafiltration
  - Upstream – Product
  - Downstream - Waste

- Microfiltration
  - Upstream – Waste
  - Downstream - Product

Single-Use TFF

- Single-Use Flow Path
- Automated
- Programmable
- Mobile
- Flexible
- Batch Reporting
Tangential Flow Filtration – Microfiltration (MF)

- MF Objectives:
  1. Transmit the product/protein through the membrane to a target yield (i.e. >98% yield).
  2. Retain unwanted waste material/particulate on the upstream side of the membrane.
- Note: Unwanted waste in downstream pool can be removed in successive steps... lost product (retained), is lost yield and is non-recoverable.

Tangential Flow Filtration – Ultrafiltration (UF)

- UF Objectives:
  1. Retain the product on the upstream side to be recovered as yield (i.e. >98% yield).
  2. Prepare (via concentration and diafiltration) the upstream product for chromatography (maximize the efficiency of subsequent chromatography step) or formulation (exchanges product into ideal buffer for formulation operation).
- Note: lost product (passage or poor recovery), is lost yield.
What is Concentration?

Concentration:
• Reduction of initial volume to increase the concentration (i.e. protein) per liter of material which is withheld by the membrane.

Concentration speeds up chromatography by reducing the volume to be processed.

What is Diafiltration?

Diafiltration (DF):
• Exchange of buffer in which product is held to alter conditions (i.e. salt or pH conditions).

Diafiltration places the product (i.e. protein) in the ideal buffer conditions to optimize chromatography.
Continuous Versus Discontinuous DF

<table>
<thead>
<tr>
<th>Diafiltration Volumes</th>
<th>Continuous</th>
<th>Discontinuous 2X</th>
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<tbody>
<tr>
<td></td>
<td>Percent removal (100% permeable)</td>
<td>Percent removal (100% permeable)</td>
</tr>
<tr>
<td>1</td>
<td>63</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>86</td>
<td>75</td>
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<tr>
<td>3</td>
<td>95</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>98.2</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td>99.3</td>
<td>96.9</td>
</tr>
<tr>
<td>6</td>
<td>99.7</td>
<td>98.4</td>
</tr>
<tr>
<td>7</td>
<td>99.9</td>
<td>99.2</td>
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TFF Diafiltration

- Upstream
- Downstream
- Retentate
- Permeate
- Diafiltration Buffer
- Ultrafiltration
  - Upstream – Product
  - Downstream - Waste
- Membrane
  - Pore Size Critical
- Passage
  - Device (i.e. cassette or hollow fiber)
TFF Downstream Examples

TFF Example 1 — Post-Protein A or Low pH VI
- TFF diafiltration to modify buffer and pH to optimize load conditions for Ion Exchange chromatography.

TFF Example 2 — Post-Cation Exchange
- TFF diafiltration to lower conductivity of MAb eluted in 300mM NaCl from Cation Exchange chromatography from 30-60 mS/cm to 5 mS/cm to optimize load conditions for Anion Exchange chromatography.

TFF Example 3 — Prep for Formulation
- TFF diafiltration to exchange to neutral buffer to enable formulation of final product.

Viral Clearance by Solvent/Detergent Inactivation
- Organic Solvent tri-(n-butyl) phosphate (TnBP)
- Detergent (Tween 80, Triton X-100)
- Generally Done following Protein-A capture
- Effective for lipid enveloped viruses
- Solvent enhances aggregation reaction between viral lipid coating and detergent
Viral Clearance by Low pH Viral Inactivation

- Lower pH ~3.5 to 4.0 depending on protein of interest
- Done following Protein-A capture
- Denature enveloped viruses
- Target protein should be resistant to denaturation from low pH for at least 2 hours.

Viral Clearance by Direct Flow Filtration

- Broad capability based on size exclusion
- Specific ‘Robust’ step
- Biological activity of product is maintained
- Viral components are removed
- Non-contaminating
- Easily validated
**Single-Use Viral Filtration or Low pH Inactivation**

- Single-Use Flow Path
- Automated
- Programmable
- Mobile
- Flexible
- Batch Reporting

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**Sterile Filtration – Bulk Fill**

<table>
<thead>
<tr>
<th>Primary Objectives</th>
<th>Process stream composition</th>
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<tbody>
<tr>
<td>Transmission of target molecule</td>
<td>Key</td>
</tr>
<tr>
<td>Removal of any bacterial contaminant</td>
<td></td>
</tr>
</tbody>
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**Purified Product**

- Bioburden CFU / 100mL
Single-Use Applications for Formulation & Filling

Mixing for formulation prior to bulk, bulk filling, storage, sterile packaging, transportation, sterile filtration, sterile transfer, final filling, validation

Bulk Fill System
Single-Use
Bulk Fill System
Single-Use

- Single-Use
- Automated
- Programmable
- Mobile
- Flexible
- Batch Reporting

Single-Use Applications

- **Process safety, robustness and automation**
  - Fully automated process control, monitoring and reliability in manufacture
  - Reproducible process performances, scalable solutions

- **Single-use flow paths**
  - Eliminates risk of batch or cross contamination
  - Eliminates cleaning requirements
  - Reduces validation time and costs

- **Ease-of-use / Flexibility**
  - Installation, operation, disassembly
  - Ready-to-use solutions, with reduced pre-use conditioning

- **Process economics**
  - Significant savings in capital, materials, labor & facility operating costs
  - Increases productivity and enhanced resource allocation
Downstream Processing Techniques and Single Use Applications

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