5 August 2018, Furama Resort Da Nang, Vietnam

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GaBI Educational Workshops

1st ASEAN Overview Workshop on
GMP for BIOLOGICALS/BIOSIMILARS

5 August 2018, Furama Resort Da Nang, Vietnam

Harvest process in commercial biologicals manufacturing

Yusdy Pan, MSc, PhD

5 August 2018

GaBi
GENERICS AND BIOSIMILARS INITIATIVE
Building trust in cost-effective treatments

PPCI
Pro Pharma Communications International

asean

HARVEST PROCESS IN COMMERCIAL BIOLOGICALS MANUFACTURING

YUSDY PAN PH.D.
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AGENDA

- Small synthetic vs large biologics modalities
- Conventional harvest process in biologics manufacturing
- New harvest technologies for high cell density cell culture process

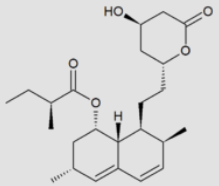
Disclaimer:

This presentation do not represent what we do at Amgen, but represent current technologies across industry with examples of vendors for each technology. Amgen does not provide any endorsement for vendors listed in this presentation

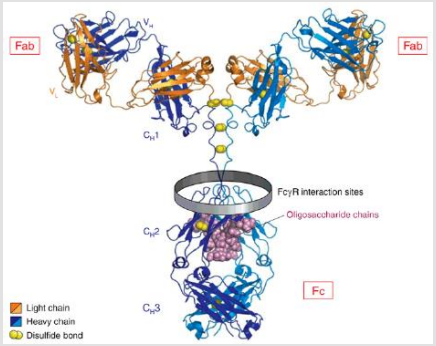
BIOLOGICS ARE MORE COMPLEX THAN SMALL SYNTHETIC MOLECULE THERAPEUTICS

Small Synthetic Molecules	Biologics
Chemical ingredients in simple structures	Proteins produced by living systems
Relatively stable	Variable; sensitive to conditions
Defined structure and easy to characterize	Heterogeneous structures and difficult to characterize

courtesy of D. Fenton



Lovastatin, the first statin to be marketed
MW: 404.55



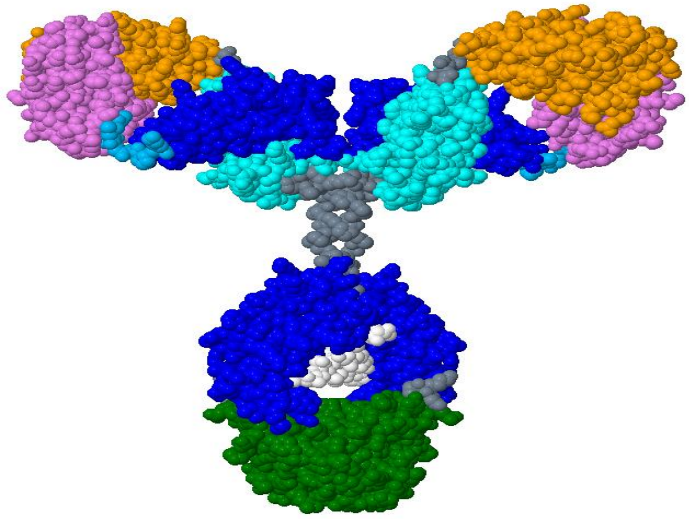
IgG
MW:
150,000

COMPARED TO SYNTHETIC, BIOLOGICS DRUGS ARE MORE SPECIFIC TO TARGET & THEREFORE LESS TOXIC

AMGEN
Pioneering science delivers vital medicines™

MONOCLONAL ANTIBODIES

- **Antibodies are a class of proteins known as immunoglobulins**
- **Found in blood or other bodily fluids of vertebrates**
- **Used by the immune system to identify and neutralize foreign organisms and molecules**
- **The huge diversity of antibodies allows the immune system to recognize an equally wide diversity of antigens**



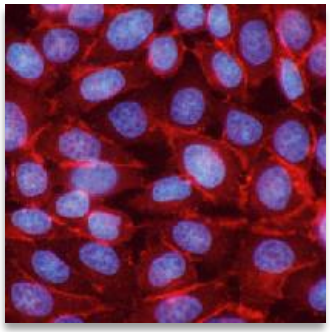
Large, complex molecule
Molecular Weight: 150 kDa

It has been estimated that humans generate about 10 billion different antibodies, each capable of binding a distinct antigen

MAMMALIAN VS MICROBIAL EXPRESSION SYSTEMS

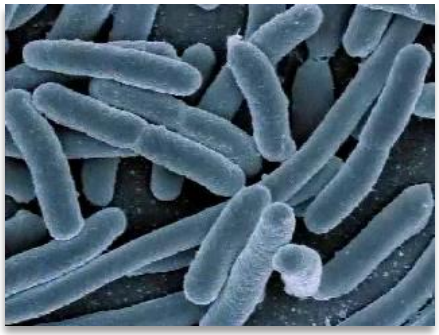
Mammalian Cells

- **Slower, more complicated cell growth**
- **Similar protein processing to humans (e.g. post translational modification)**
- **Less complicated recovery and purification**

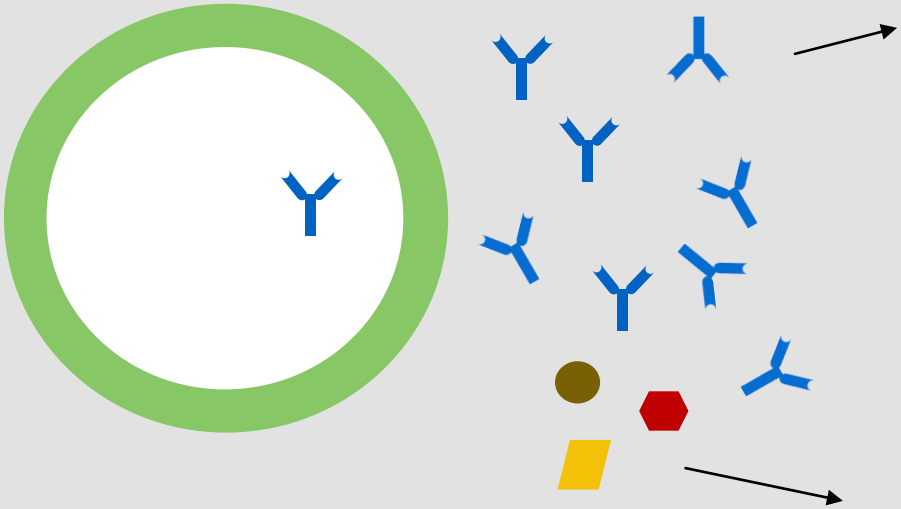


Microbial Cells

- **Faster, relatively straightforward fermentation**
- **High-yield inclusion bodies**
- **Difficult purification with lower yield**
- **Do not work for all protein therapeutics**



MAMALLIAN CELL EXPRESSION SYSTEM

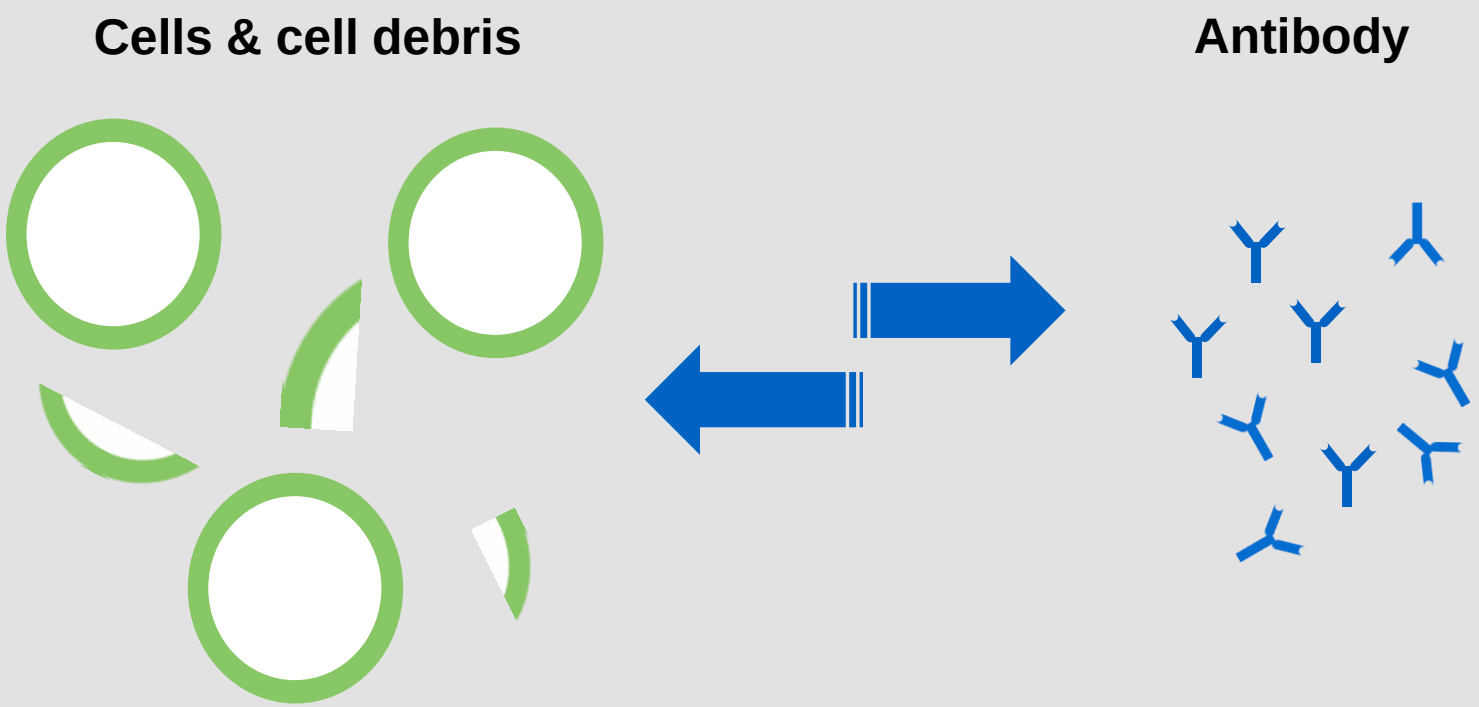


Antibody produced by mammalian cells are secreted extracellularly through the cell membrane

Small amount protein impurities

Antibody recovery process is relatively simpler with mammalian cell expression system and do not require cell disruption

HARVEST FOR MAMALLIAN CELL PROCESS



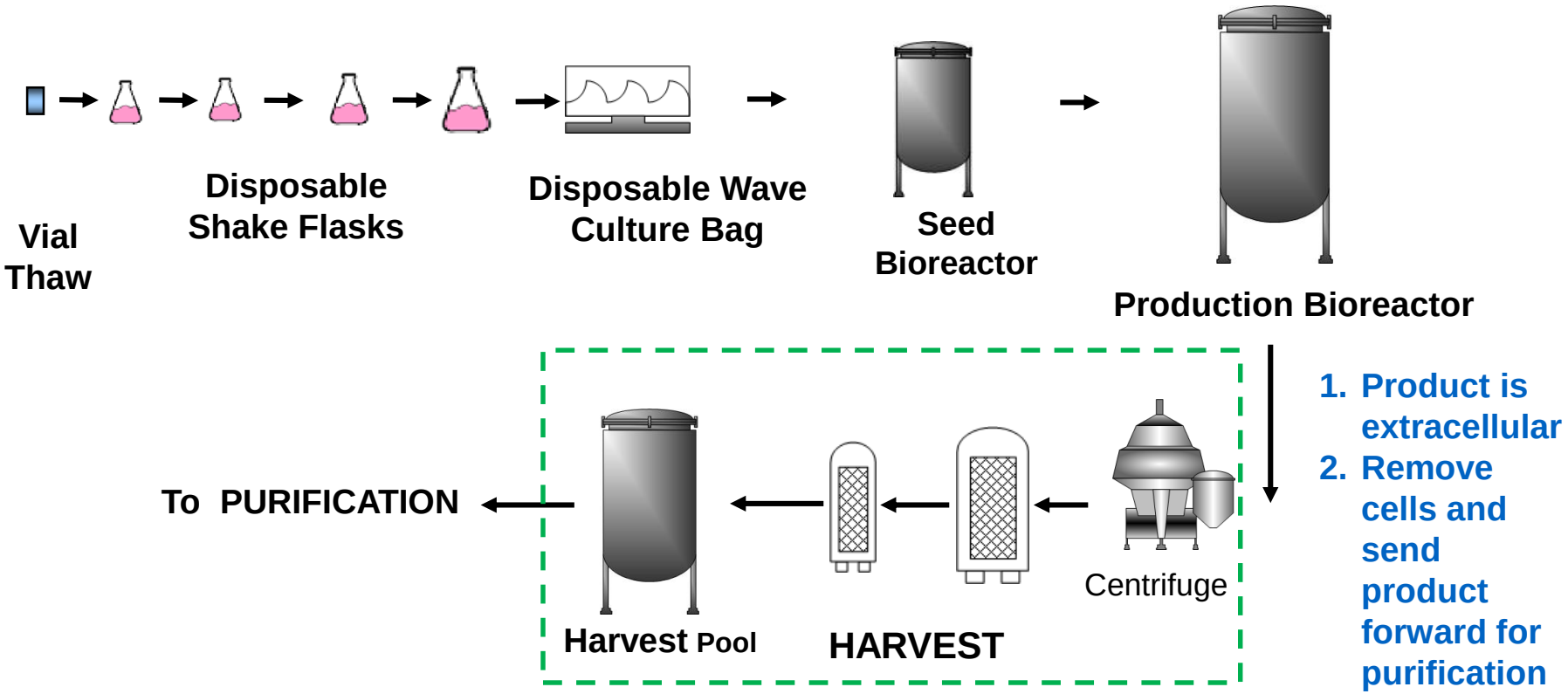
OBJECTIVE OF HARVEST PROCESS IS TO RECOVER THE ANTIBODY PRODUCT FROM CELLS & CELL DEBRIS

PRINCIPLE IN CELL SEPARATION TECHNIQUES

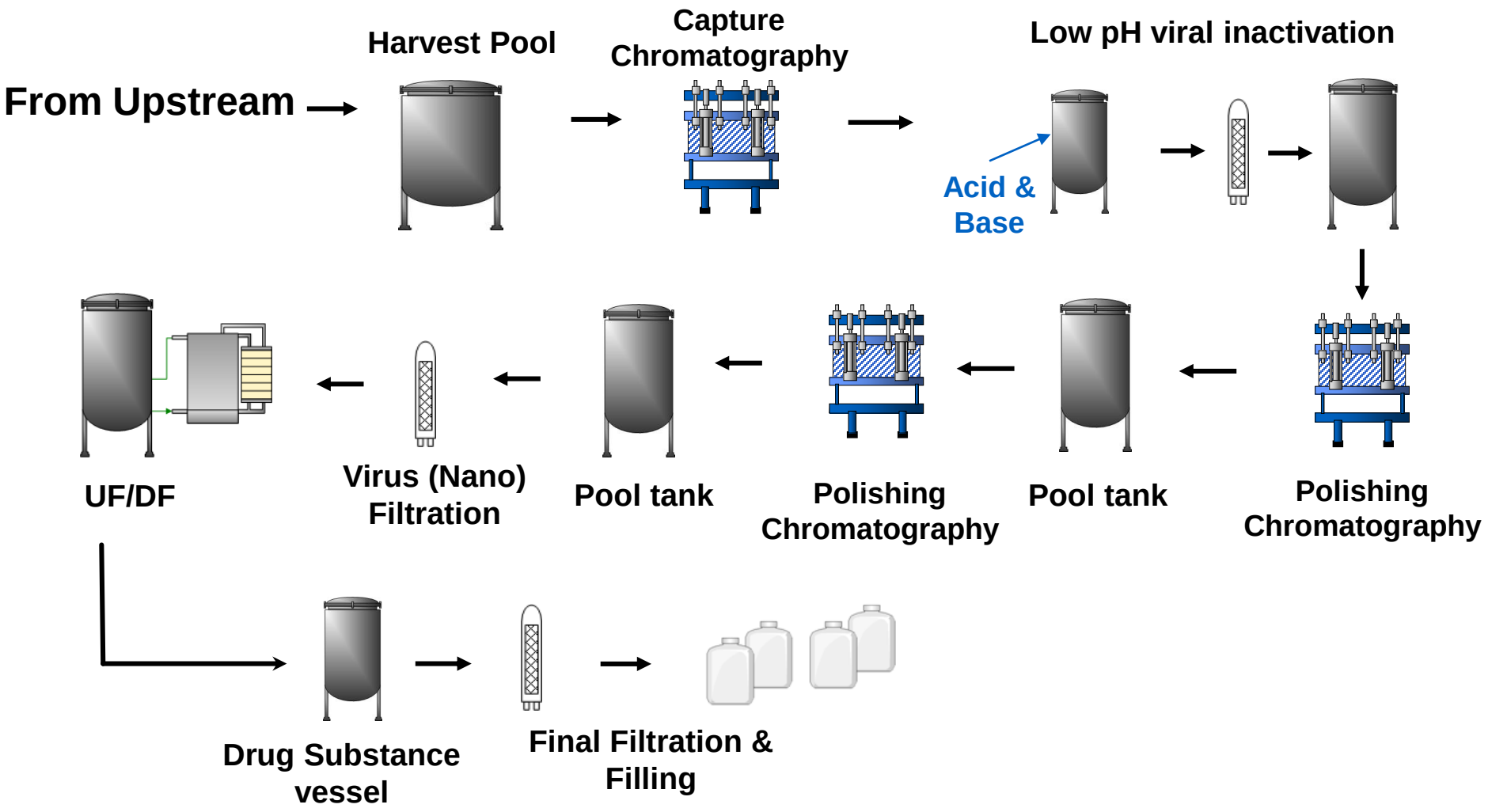
- **SIZE** (Cells are bigger than proteins)
  - Filtration
  - Retainment
- **DENSITY** (Cells are heavier than proteins)
  - Centrifugation
  - Sedimentation

CONVENTIONAL HARVEST PROCESS IN MAMALLIAN CELL CULTURE PROCESS

TYPICAL MAMALLIAN CELL CULTURE & HARVEST PROCESS FOR MONOCLONAL ANTIBODY PRODUCTION



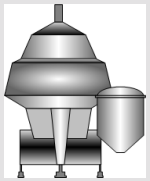
TYPICAL 3 COLUMN STEP DOWNSTREAM PROCESS



CONVENTIONAL HARVEST PROCESS IN MAMALLIAN CELL CULTURE PROCESS

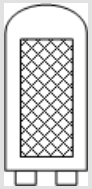
Bioreactor

Last day of production



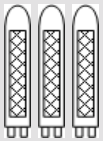
Centrifugation

Remove intact cells & large debris (micron size)



Depth filtration

Remove cell debris (Micron and sub-micron sizes)



Membrane filtration

Served as an aseptic boundary

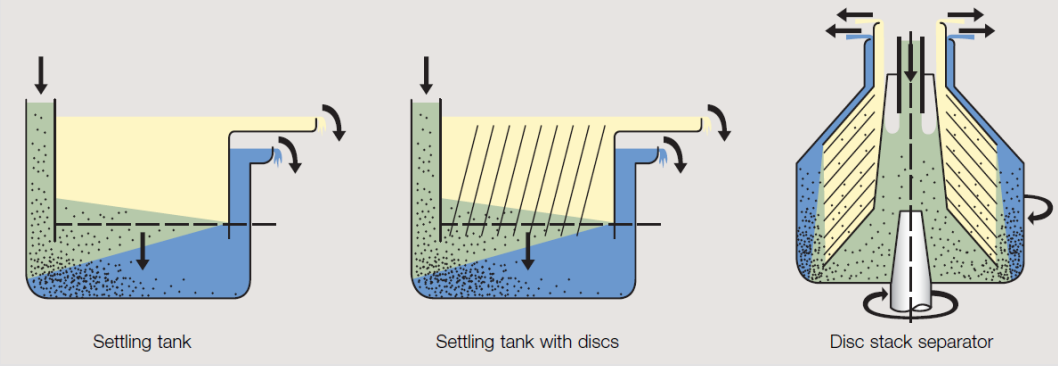
Harvest Vessel

Product solution, free of cell and debris, ready for purification

Harvest process with combination of centrifugation + filtration. Suitable for cell culture process with < ~10 million of cell density

DISC STACK CENTRIFUGE

Contain multi disc stacks for efficient separation



Mechanism of separation is similar to a settling decanter

Efficiency of separation depends on centrifuge rpm speed, residence time, size and no of disc, density difference between particle and solution

Pictures courtesy of Alfa Laval. Alfa laval is an example of centrifuge vendors

DISC STACK CENTRIFUGE

Design Parameters:

- Equivalent clarification surface area (Σ)
  - Centrifuge design (no of discs, dimension)
  - Bowl speed (rpm)
- Flow rate (Q)
- Feed material Cell culture viability
  - Cell density (% PCV -> pack cell volume)
  - Cell culture viability
- Solid discharge interval (volumetric, time, turbidity)
  - e.g. A centrifuge has a solid bowl capacity of 10L (target 80% fill). Cell culture feed of 2% PCV at 100LPM. Solid discharge interval/frequency can be set at 10L x 80% /(100LPM x 2%) =

4 min = 400L flowthrough

BioProcess International, NOV 2007, 38-50

$$\Sigma = \frac{2\pi}{3g} \times \omega^2 \times N \times \cot\alpha \times (r_o^3 - r_i^3)$$

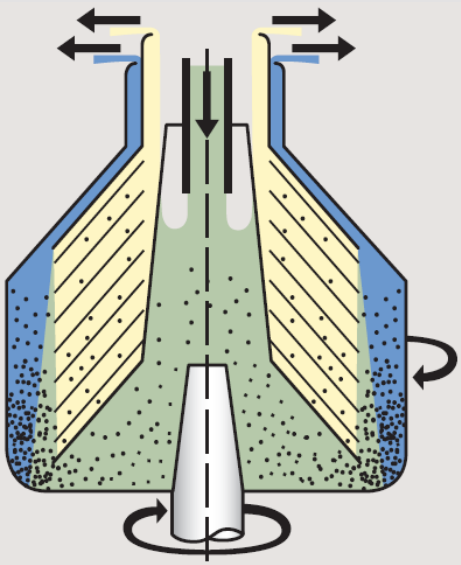
where

- Σ = equivalent clarification area of centrifuge (m^2)
- g = acceleration due to gravity (m/s^2)
- ω = angular bowl velocity (rad/s)
- N = number of discs in stack
- α = disc half-conical angle (°)
- r_o = outer radius of disc (m)
- r_i = inner radius of disc (m)

$$d_{min} = \sqrt{\frac{Q}{\Sigma}} \times \sqrt{\frac{18\eta}{\Delta\rho g}}$$

where

- d_{min} = diameter of minimum particle size that can be separated (m)
- Q = centrifuge feed rate (m^3/s)
- Σ = equivalent separation area of centrifuge (m^2)
- η = dynamic viscosity of culture medium ($kg/m \cdot s$)
- $\Delta\rho$ = density difference between cells and medium (kg/m^3)
- γ = acceleration due to gravity (m/s^2)



Picture from 'Alfa Laval – disc stack separator technology' brochure

Q / Σ factor is maintained constant during scale up/down

DEPTH FILTRATION

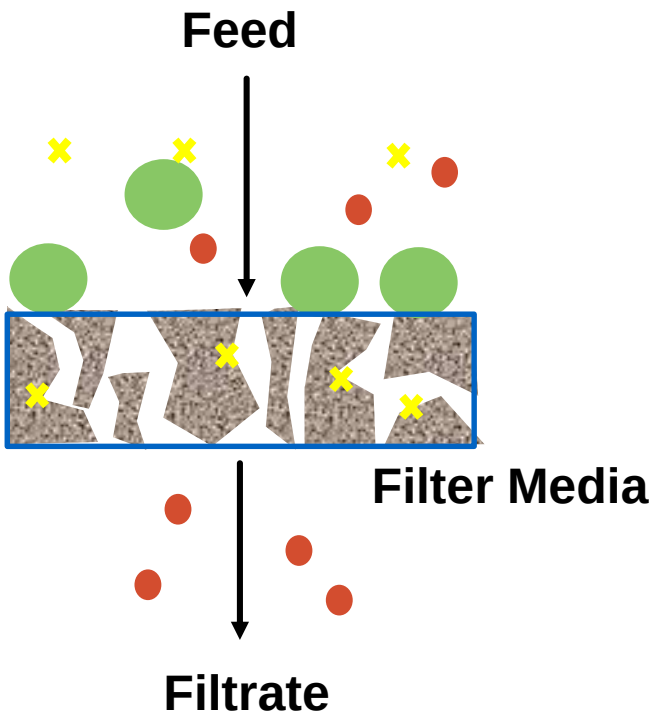


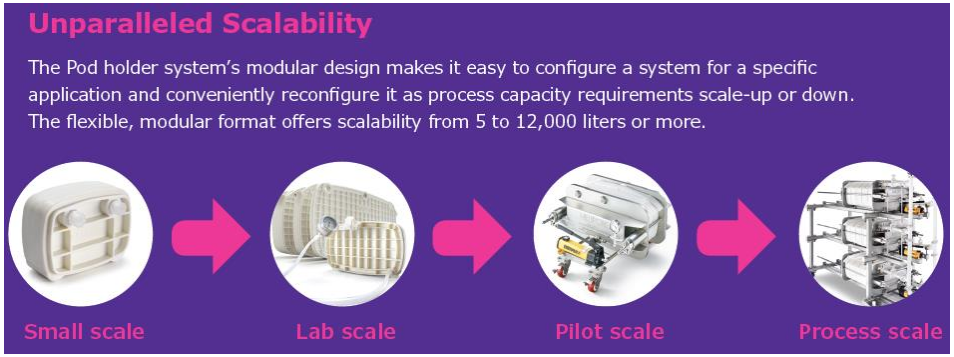
FIGURE 1. Main components of depth filters: **(A)** cellulose fibers; **(B)** diatomaceous earth; and **(C)** polypropylene fibers.

- Depth filters are typically composed of cellulose fibers impregnated with diatomaceous earth and polymeric binding additives
- Mechanism of separation : sieving & adsorption
- Filter media may contain charge species (typically positive charge) which can bind Host Cell Protein (HCP) and DNA
- Pre-use water flush is required to remove extractables & leachables

Sarah Le Merdy, Bioprocessing journal, Oct, 2015

DEPTH FILTRATION

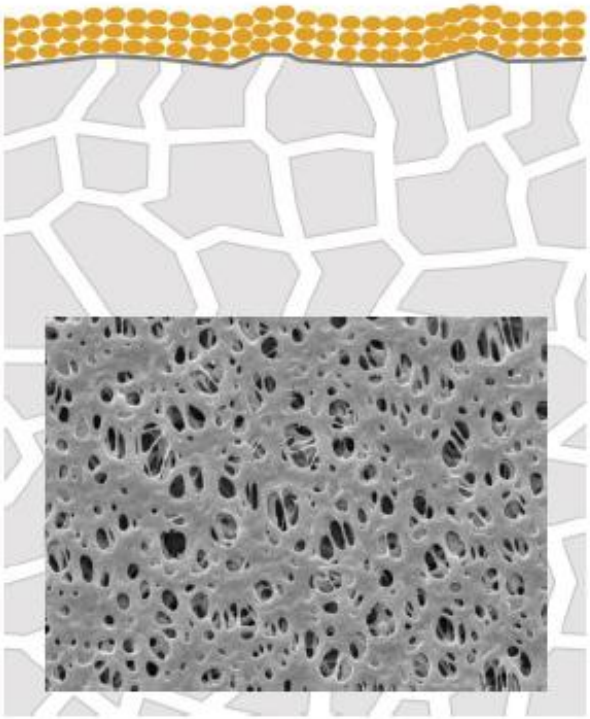
POD disposable depth filter system, EXAMPLE from MerckMillipore



Lenticular depth filter system, example from 3M (available in disposable or SS housing format)

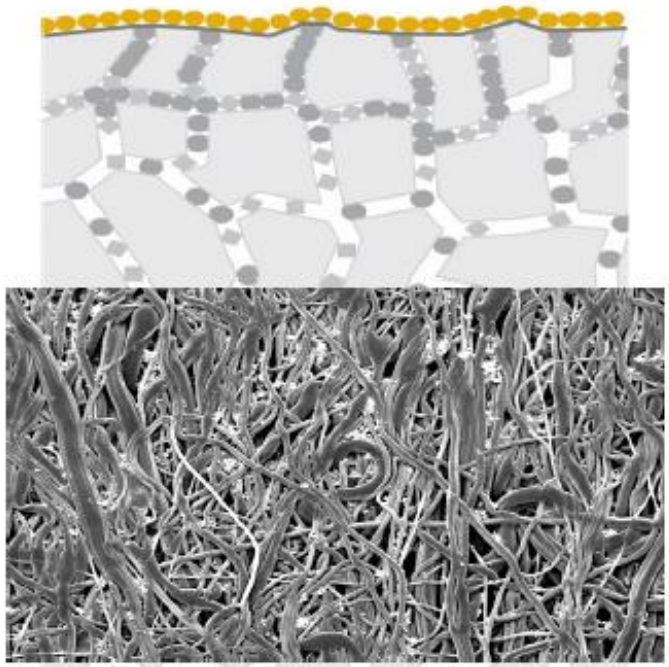
Zeta Plus™ Cartridges and Capsule Family

MEMBRANE FILTER VS DEPTH FILTER



Surface filtration

Membrane Filters



Depth filtration

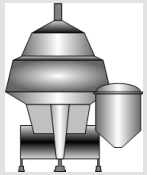
Depth Filter Sheets / Modules

Pictures courtesy of Filtrox

COMMON PROCESS MONITORING IN CONVENTIONAL HARVEST PROCESS

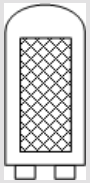
Bioreactor

- Cell culture viability
- Pack cell volume
- Temperature



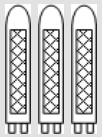
Centrifugation

- Turbidity (outlet)
- Feed flow rate
- Discharge interval



Depth filtration

- Pre-use water flush (volume, pressure, flow rate)
- Pressure drop



Membrane filtration

- Pressure drop
- Integrity test

Harvest Vessel

- In process testing (e.g. Titer, Bioburden, endotoxin, LDH assay, TEM)
- Yield

NEW HARVEST TECHNOLOGIES FOR HIGH CELL DENSITY CELL CULTURE PROCESS

HARVEST TECHNOLOGIES FOR HIGH DENSITY CELL CULTURE PROCESS

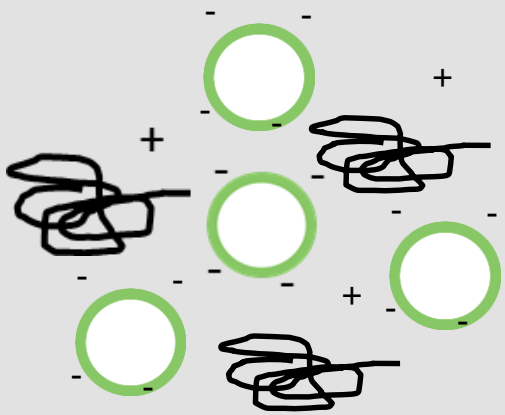
- **Latest trend of cell culture processes with cell density of >10 million cells and solid content of >15%**
- **Conventional platform process is limited by centrifuge bowl capacity and depth filter surface area, which impact on harvest yield and production cost**
- **Alternative technologies:**
  - **Accelerated sedimentation (Flocculation)**
  - **Tangential Flow (TF)- Microfiltration (MF)**
  - **Alluvial filtration**
  - **Acoustic Wave Separator**

INDUSTRIAL TREND IS MOVING TOWARDS HIGH DENSITY CELL CULTURE PROCESS

ACCELERATED SEDIMENTATION (FLOCCULATION)

- **Particulates in CHO culture process are typically <10 µm, and can be induced to form flocs or precipitates sizing between 20 and 100 µm.**
- **Methods:**
  - **Cationic polymers (pDADMAC, PEI, Chitosan)**
  - **Non ionic polymers (PEG, Dextran)**
  - **Acid titration (to pH ~5)**
  - **Salt addition, for e.g $(NH4)_2SO_4$, K_2SO_4, KH_2PO_4**

Crosslinking of negatively charged Cells and cationic polymers through ionic interaction

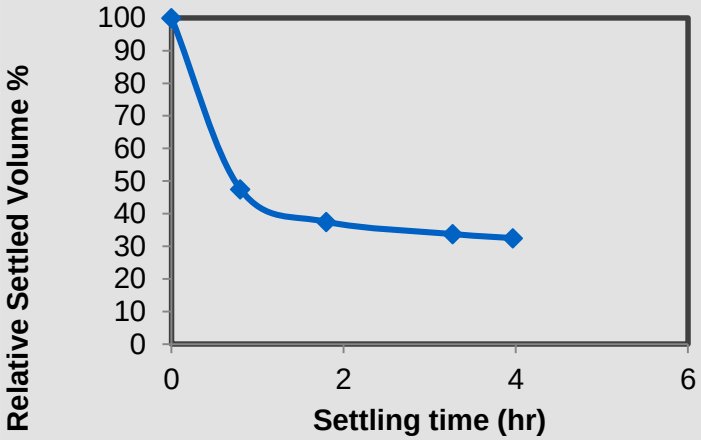


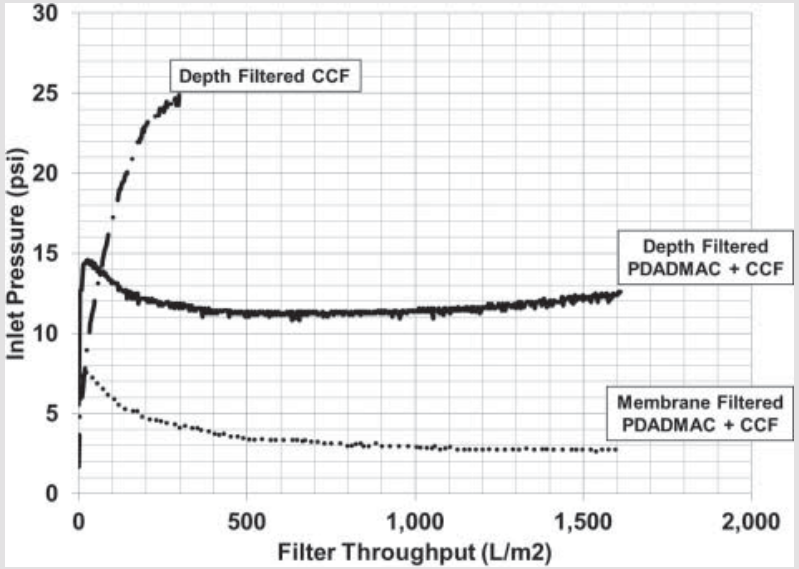
Sources:
Sarah Le Merdy, Bioprocessing journal, Oct, 2015
J Biotechnol, 2007; 128(4): 813–23.
Biotechnol Bioeng, 2013; 110(11): 2928–37.
mAbs, 2015; 7(2): 413–27
Biotechnol Bioeng, 2011; 108(1): 50–8.

FLOCCULATION USING CATIONIC POLYMER PDADMAC

McNerney et al., (2015) PDADMAC flocculation of Chinese hamster ovary cells:, mAbs, 7:2, 413-427

Time Based Settling



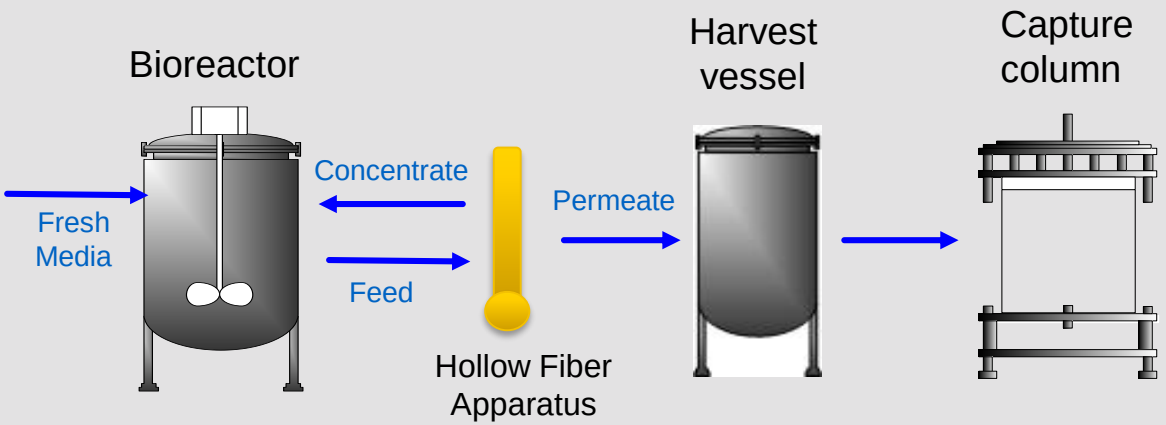


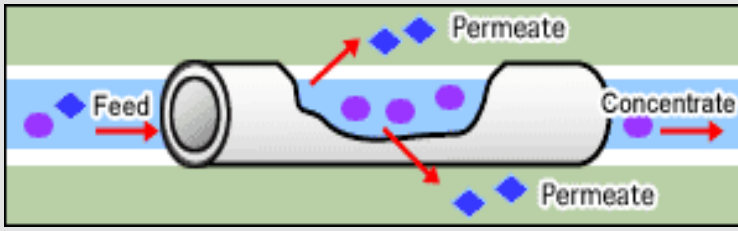
The clarity of the flocculated supernatant is comparable to centrifugation followed by depth filtration and membrane filtration

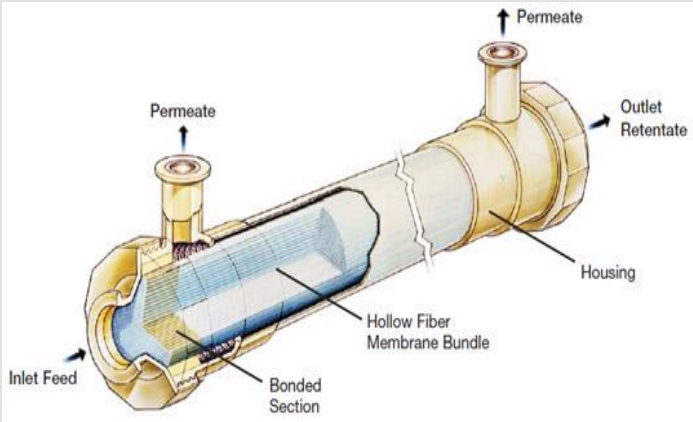
KEY CONSIDERATION FOR FLOCCULATION METHOD

- **Evaluating impact of additives on product quality attributes**
- **Impact on downstream processing**
- **Demonstrating the downstream process clearance for additives**

TANGENTIAL FLOW (TF)-MICROFILTRATION (MF) HARVEST SYSTEM







Sieving Coefficient

$$(S_o)=\frac{\textit{Product Concentration in Permeate}}{\textit{Product Concentration in Feed}}$$

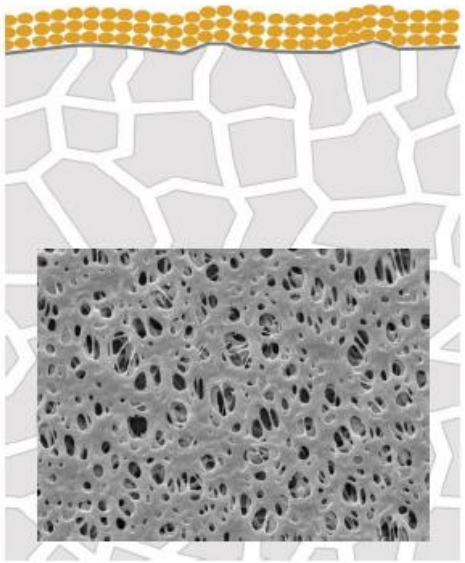
Product is small to permeate through the membrane, while cell and larger debris are retained in the concentrate line returning to bioreactor

KEY CONSIDERATION FOR TF-MF SYSTEM

- **Filter dimension & surface area**
- **Filter pore size**
- **Cross flow rate → cell damage (shear), sweeping action**
- **Transmembrane pressure**
- **Permeate flux**

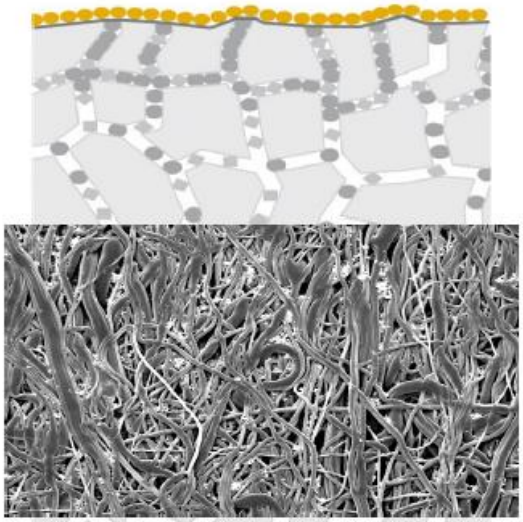
ALLUVIAL FILTRATION

Different Filtration Functional Principles



Surface filtration

Membrane Filters



Depth filtration

Depth Filter Sheets / Modules

Alluvial filtration

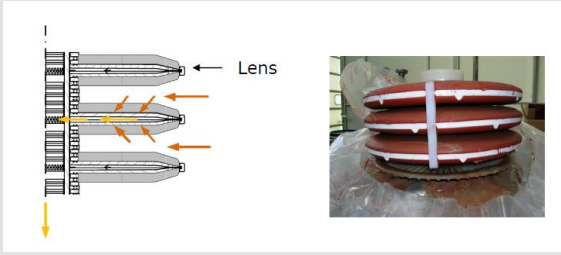
Body Feed / Pre-Coat Filtration

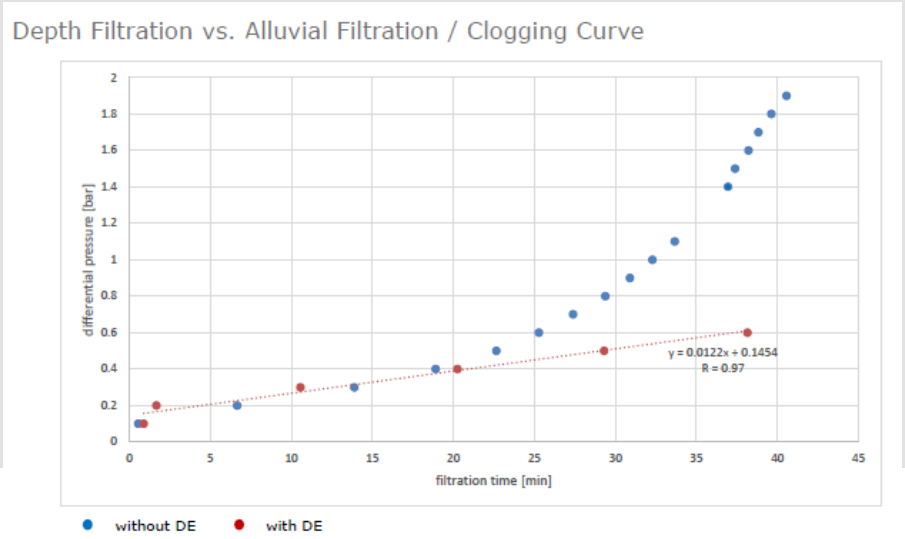
Pictures courtesy of Filtrox

ALLUVIAL FILTRATION

Mix the cell culture solution with filter aid material (DE)

Filtration through a specialized design filter

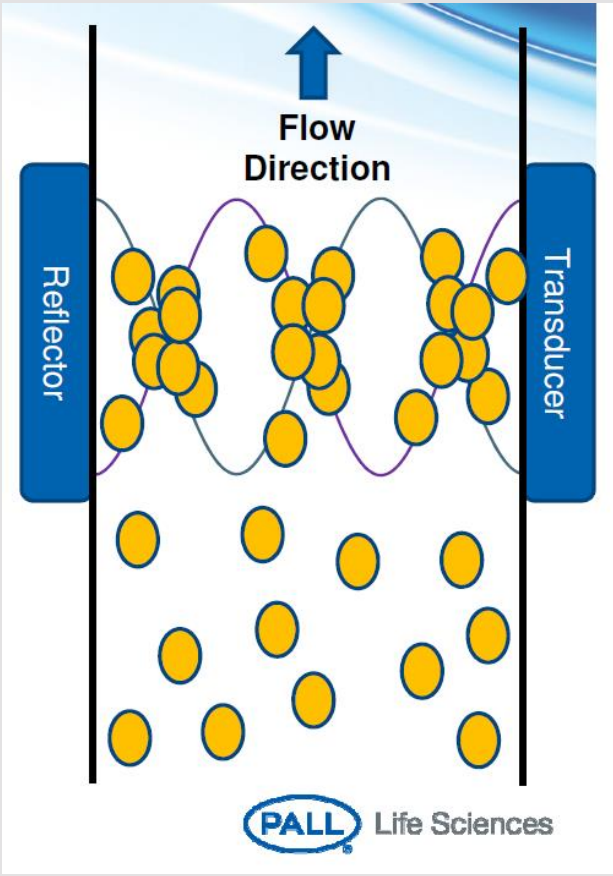




Pictures courtesy of Filtrox

CADENCE® ACOUSTIC SEPARATION (CAS®)

- Acoustic wave separation (AWS) technology involves the use of low frequency acoustic forces to generate a 3-dimensional (3D) standing wave across a flow channel.
- Cells are trapped by the acoustic forces, while small proteins will flow through.



ACKNOWLEDGMENT

Mahsa Rohani

Amgen Pre-Pivotal & Pivotal Process Development