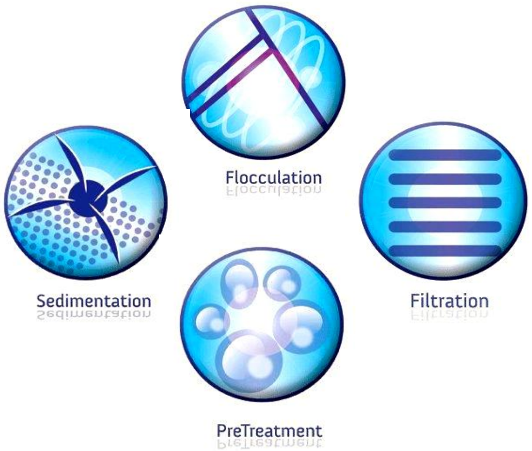
October 17th, 2017




# Development and Scale-up of Cell Culture Harvest Processes for Biopharmaceutical Production

BioProduction Conference 2017
Convention Centre Dublin, Ireland

**Markus Brakel**
Boehringer Ingelheim Pharma
Biopharmaceuticals Germany
BPAD Cell Culture CMB

# Boehringer Ingelheim **Bio**Xcellence™
## World-wide customer orientation

Boehringer Ingelheim

| | |
| --- | --- |
| 1963 | Research in chicken Interferon |
| 1983 | Registration biotech product Berofor (IFN-a) |
| 1984 | New GMP Biotech plants Biberach/Vienna |
| 1998 | Strategic decision for contract manufacturing business |
| 2003/5 | Additional new large scale plants Biberach/Vienna |
| 2010 | Establishment of global Biopharma Organisation with a global account management structure |
| 2011 | Acquisition of US biopharma plant (Fremont) |
| 2012 | Establishment of global brand: Boehringer Ingelheim **Bio**Xcellence™ |
| 2013 | Strategic alliance with Zhangjiang Base Co., to build a cGMP biopharmaceuticals facility in Shanghai |
| 2016 | BioBU (Biberach \| Vienna \| Fremont \| Shanghai \| Ingelheim) |

BIBERACH

VIENNA

FREMONT, CA

SHANGHAI

# Boehringer Ingelheim Biopharmaceuticals
## Production Network

## Microbial & Yeast

Mammalian…soon

### Vienna, Austria

Commercial capacity for microbials and yeast fermentation products

## Mammalian

### Biberach, Germany

Commercial capacity for cell culture products

### Fremont, USA

Commercial capacity for cell culture products

### Shanghai, China

Commercial capacity for cell culture products
Disposable Technology

### Vienna, Austria

Fermentation Capacity:
F1: 30 L,300 L, 6,000 L
F2: 30 L, 300 L, 6,000 L

### Biberach, Germany

Fermentation Capacity:
16x 15,000 L Bioreactors
12x 2,000 L Bioreactors

### Fremont, USA


Fermentation Capacity:
2 x 15,000 L Bioreactors
4 x 2,000 L Bioreactors

### Shanghai, China

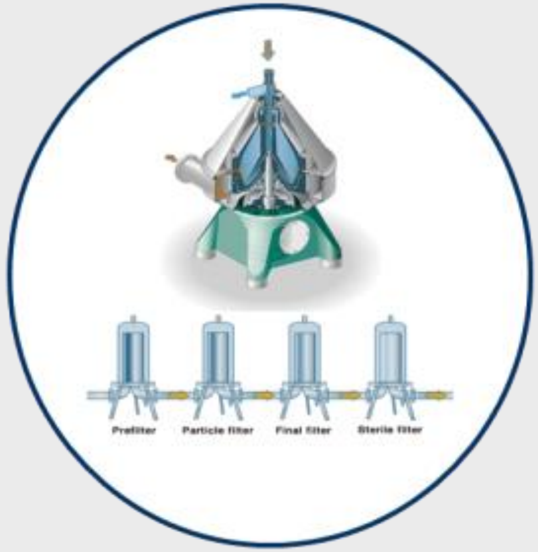
Fermentation Capacity:
1x 2,000 L SUB bioreactors
2x 500 L SUB bioreactors

# BI´s Cell Culture Harvest Processes

## Challenges for separation and filtration

**Cell culture process:**

- Biomass concentration
- Product titer
- Cell culture viability
- DNA/HCP impurities
- Particle freight




**Cell culture media:**

- Hydrolysates, peptones
- Trace elements
- Buffer system
- Amino acids
- High glucose

**Harvest technology:**

- Separator process
- Filter membranes
- Filter combination
- Particle size distribution
- Turbidity profile
- Filter capacity

# BI´s Cell Culture Harvest Processes

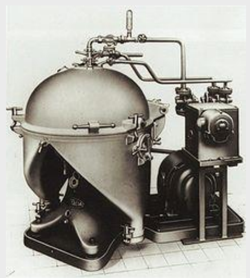
## History of centrifugation and filtration

Boehringer Ingelheim

**1893**


*

**1931**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**1985**


*

**1995…**

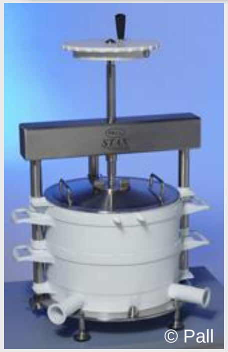

* GEA Westfalia

Milk - buttermilk – cream – butter
Food and beverage (beer, fruit juice)


Hollow fibre
Filter discs

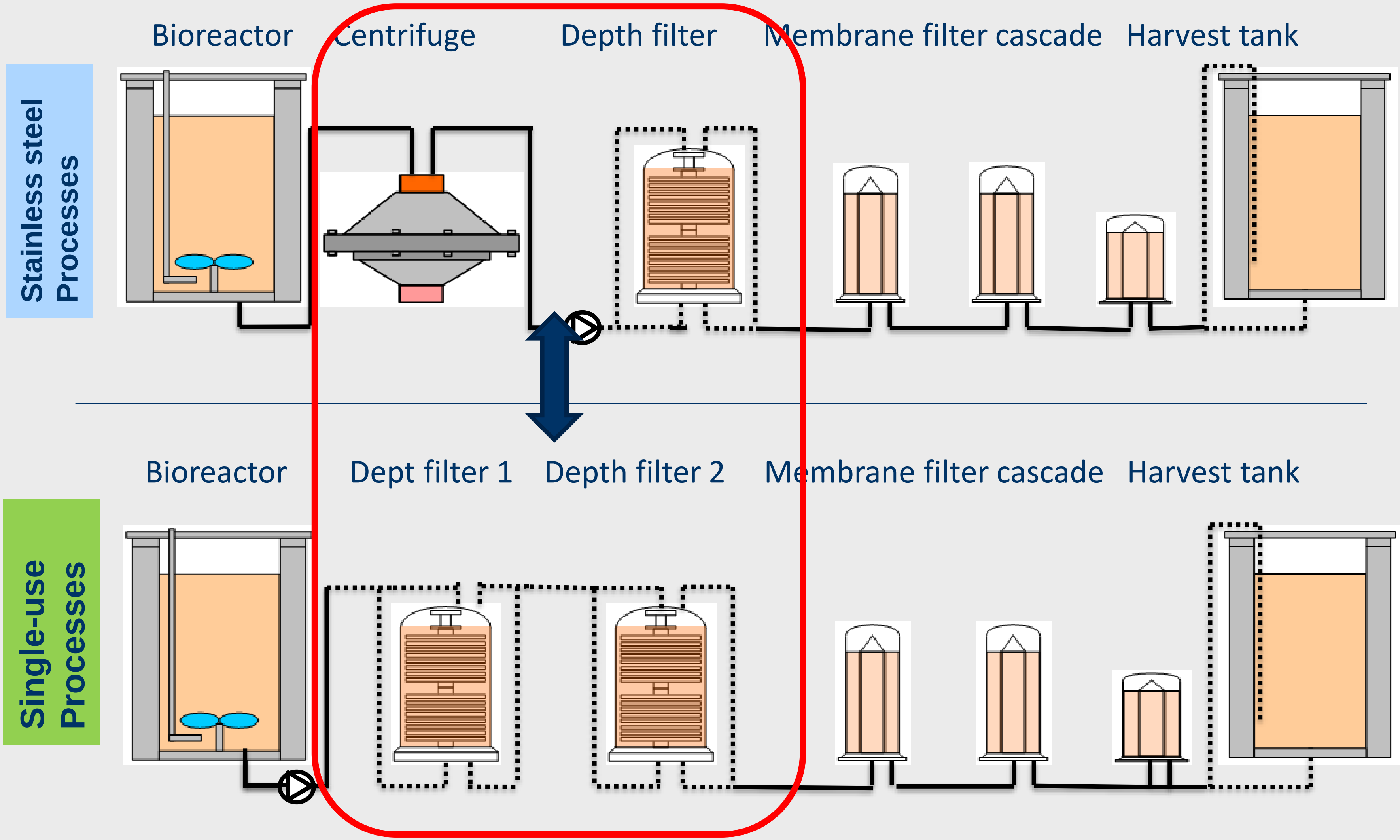

TFF Filters

**Centrifugation and filtration in modern Biotechnology**




# BI´s Cell Culture Harvest Processes

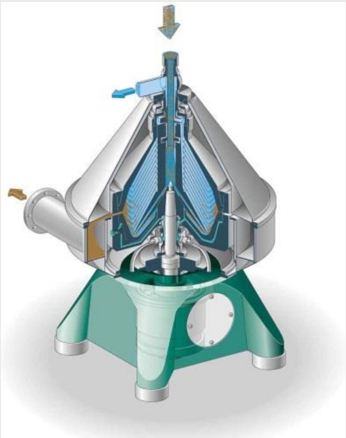
## Stainless steel vs. Single-use processes



# BI´s Cell Culture Harvest Processes

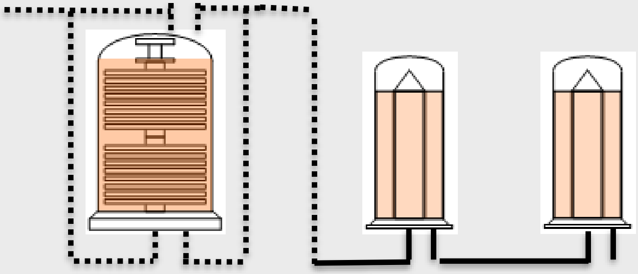
## Separation and filtration

Centrifugation / Separation



- Gravimetrical clarification of cell culture
- Reduction of biomass and turbidity
- Continuous desludging disc stack separator
- Key parameters: Biomass (PCV%)
  Feed flow rate Q
  Depletion time T
  Inlet/outlet pressure P
  Equivalent clearance area ∑
- Scale up criteria: Q/∑ = const.

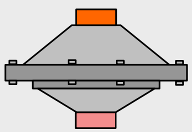
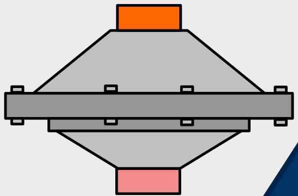
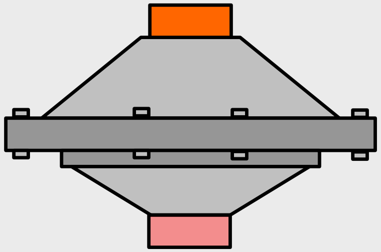
Depth- and Membrane filtration

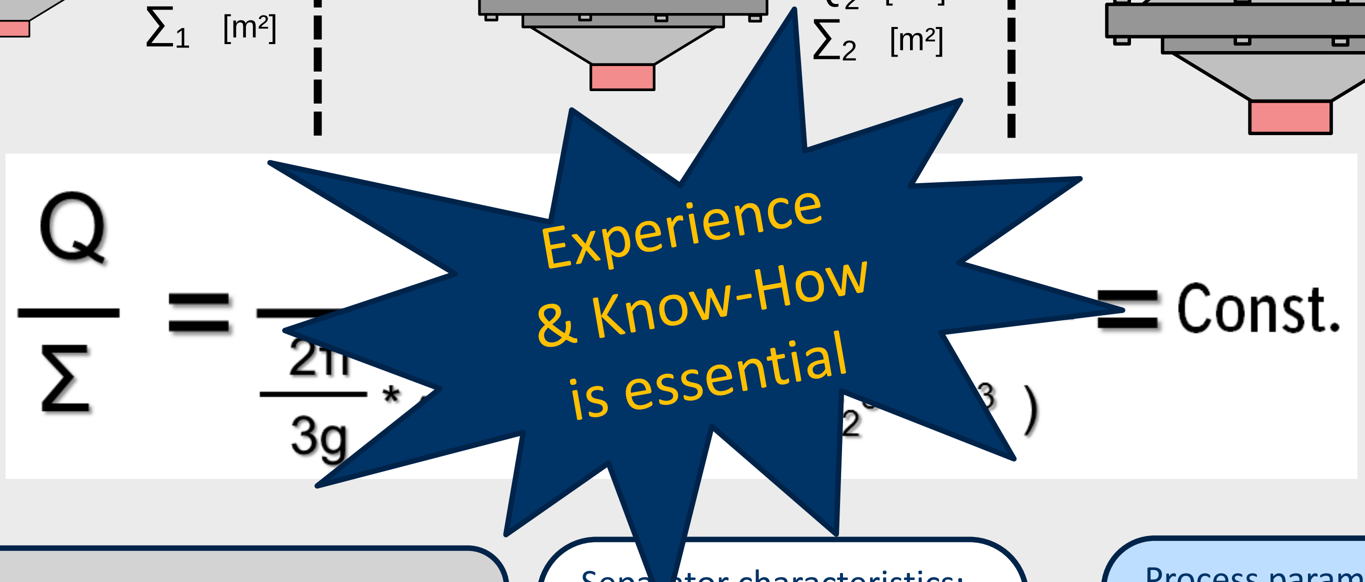


- Mechanical reduction of particles
- Reduction of PRI: DNA, HCP, cell debris, particles
- Key parameters: Filter types and combination
  Filter material characteristics
  Filtration flow rate and flux rate
  Total filter area
- Scale up criteria: Filter types and combin. = const.
  Flux rate = constant [L/m²h]
  Max. filter capacity = const. [L/m²]

# BI´s Cell Culture Harvest Processes

## Scale up of Separation processes

| ≤ 80 L Centrifuge | ≤ 2000 L Centrifuge | ≤ 12000 L Centrifuge |
|---|---|---|
|  |  |  |
| $Q_1$ [L/h] | $Q_2$ [L/h] | $Q_3$ [L/h] |
| $\Sigma_1$ [m²] | $\Sigma_2$ [m²] | $\Sigma_3$ [m²] |

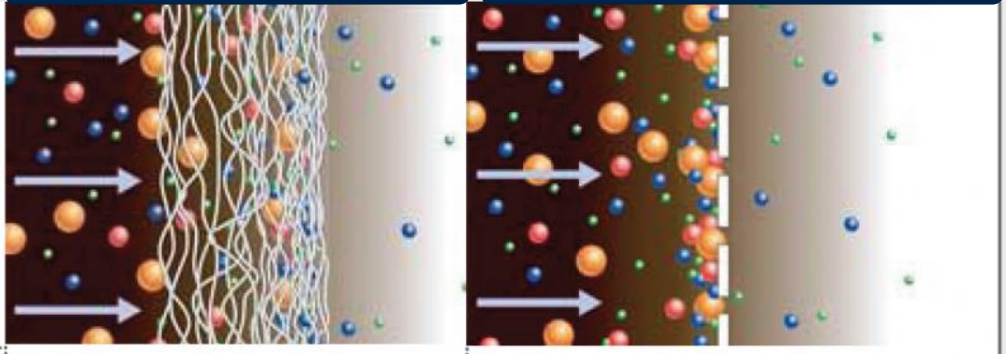



**Scale up factors:**
Q = feed flow rate of centrifuge [ L/h ]
Σ = equivalent clearance area [ m² ]
Q/Σ = const.

**Separator characteristics:**
ω = rotational velocity
N = number of discs
α = the conical half angel
$r_1$ = inner radius of disc
$r_2$ = outer radius of disc

**Process parameters:**
Inlet/outlet pressure
Depletion strategy
Feed flow rate
Rotation speed [rpm]
G-force [m/s²]

# BI´s Cell Culture Harvest Processes
## Filtration development & scale up



| | Depth filtration | Membrane filtration |
|---|---|---|
| Principle | Particles trapped in filter depth | Particles stopped on filter surface |
| Filtration media | Deep filtration media | Thin filtration media |
| Applications | - Filtration of larger suspended solids<br>- Large or undefined particle sizes<br>- Prefiltration | - Filtration of finer suspended solids<br>- Narrow or defined particle sizes<br>- Prefiltration and final filtration |
| Cost | Low (disposable after use) | High (cleanable with hot water or chemicals) |
| Type of cartridges | Wound and meltblown cartridges | Pleated cartridges |

www.pentairaquaeurope.com

**Filter characteristics:**

- Filter materials: PES, PTFE, Polypropylene, Polyethylene, Cellulose
- Hydrophilic / hydrophobic / charged / coated
- Filter design and „architecture“: hollow fibre, sheet, plate, column
- Porosity, Pore size distribution, pore symmetry
- Filter size and total membrane area

# BI´s Cell Culture Harvest Processes
## Filtration development and scale-up

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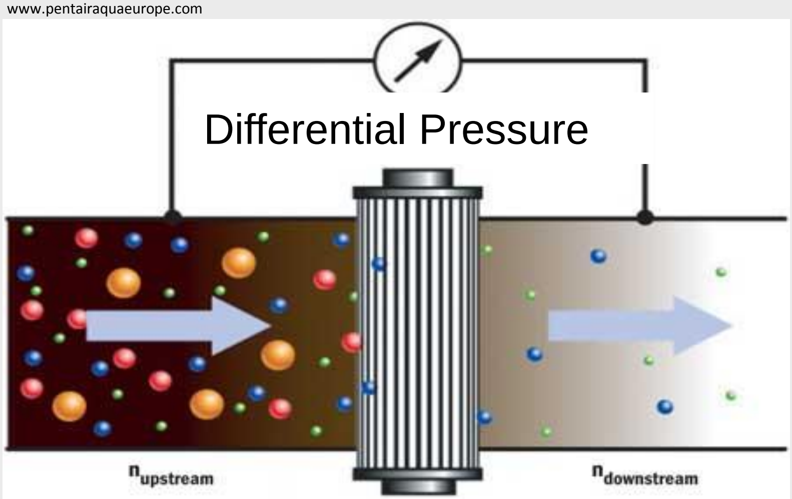
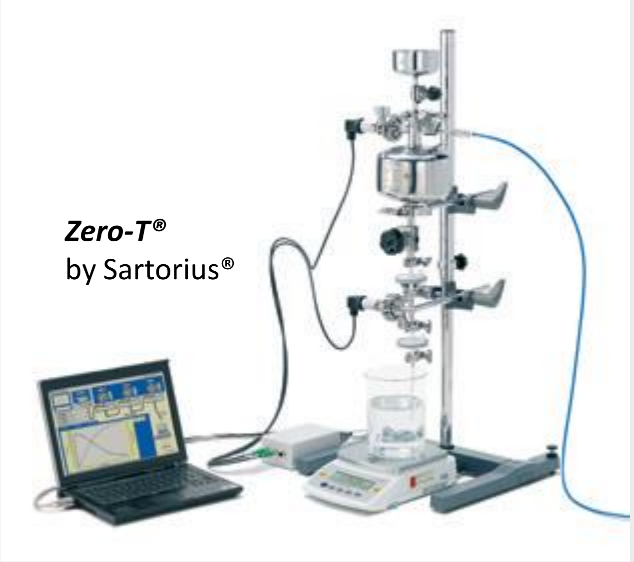



- Linear filter scale-down 12kL ➔ 80L
- $P_{max}$ studies at constant flow rates
- Differential pressure profile analysis ($P_{max}$)
- Maximum filter capacity analysis ($C_{max}$)
- Particle-size-distribution analysis
- Turbidity trending analysis
- Calculation of product step yields
- Impurity reduction analysis (DNA, HCP)
- Small scale filtration studies and SDM dev.

**Scale up strategy:**

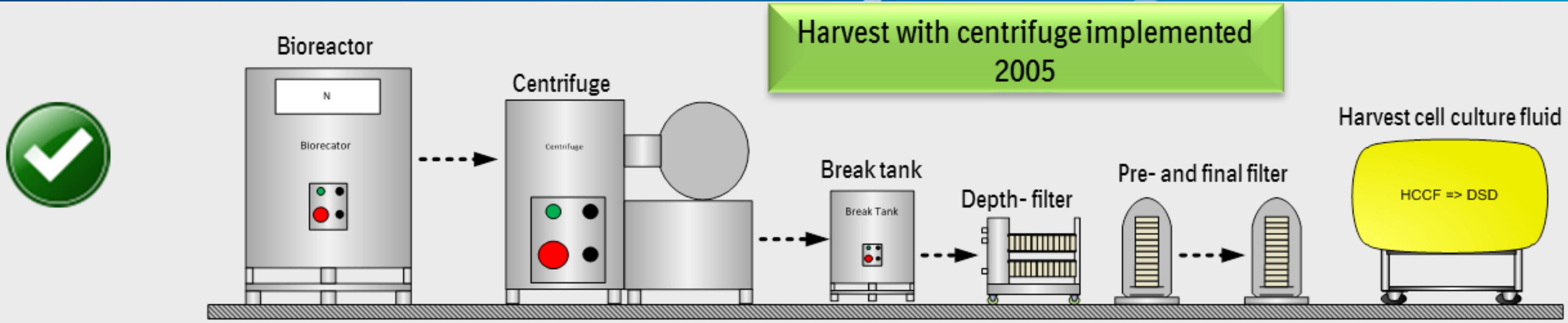
- Identical filter types
- Filter flux LMH [L/m²h] = const.
- $C_{max}$ at $P_{max}$
- Linear scale up 80L ➔ 2kL ➔ 12kL



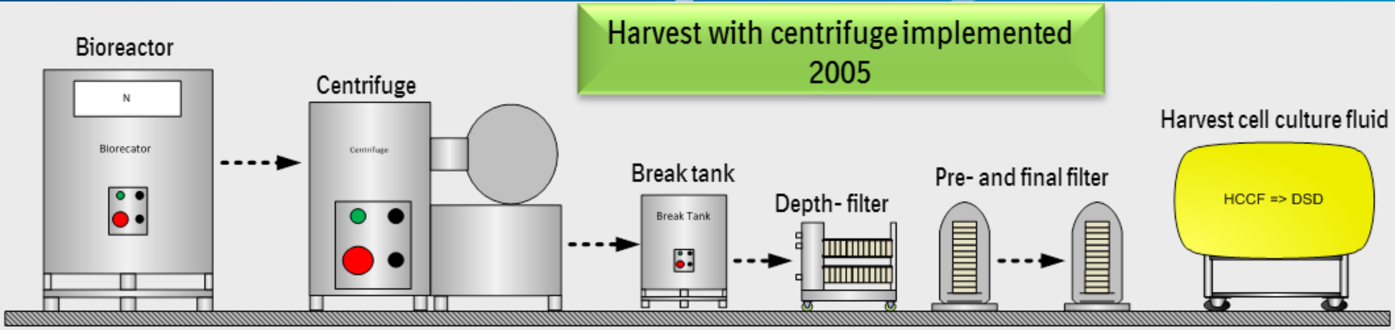

www.sartorius.de

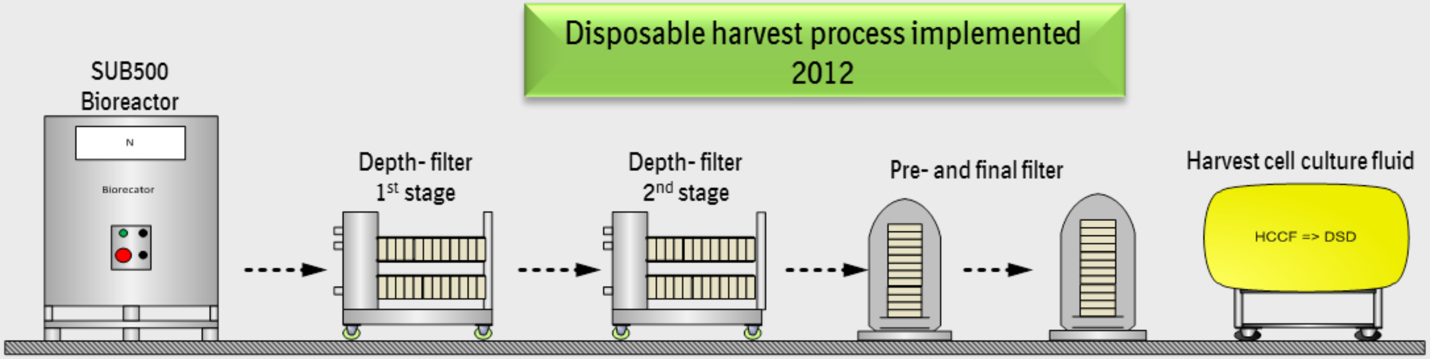
# BI´s Cell Culture Harvest Processes
## Current Technology Status

Boehringer Ingelheim

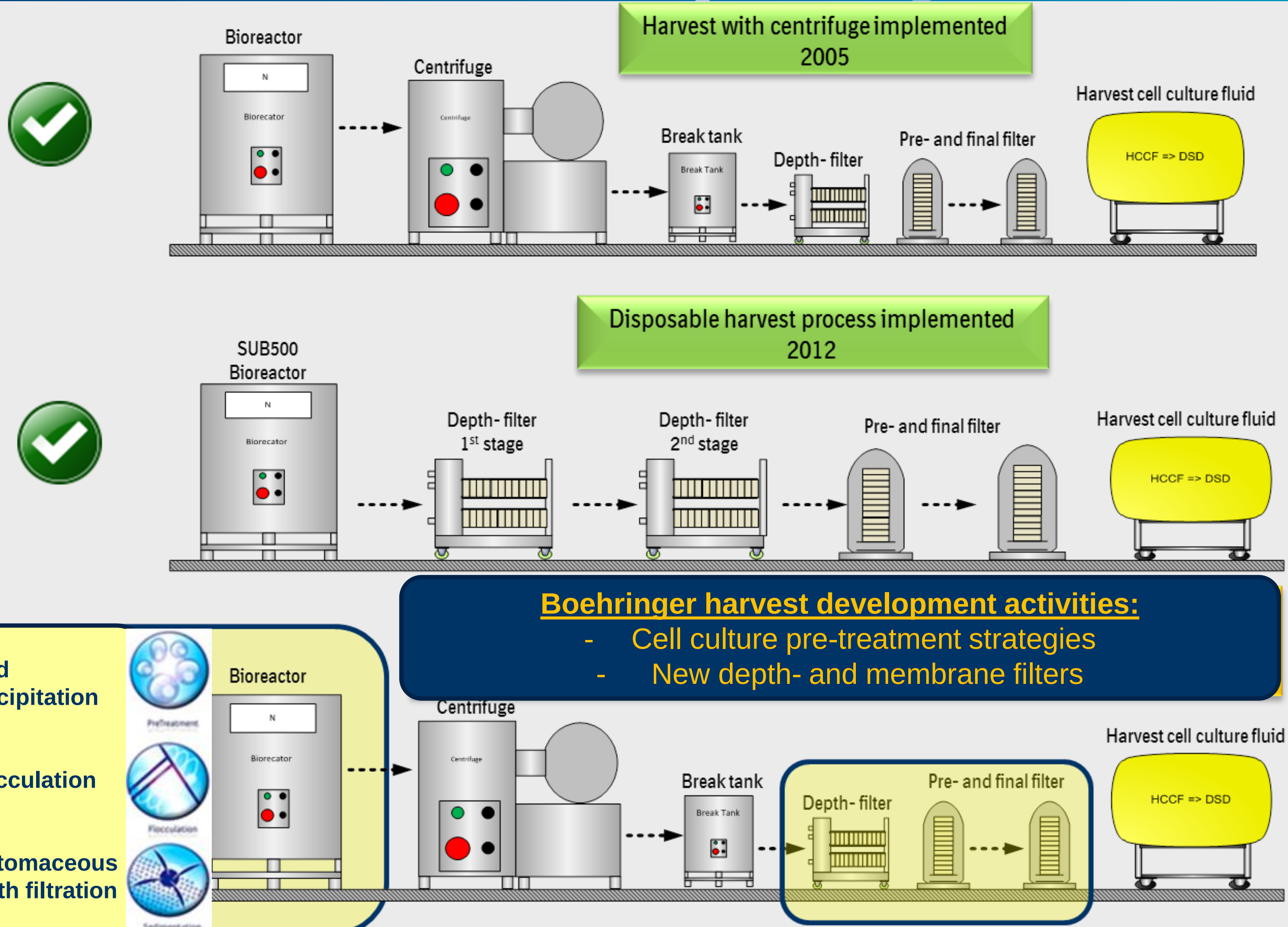


# BI´s Cell Culture Harvest Processes
## Current Technology Status



# BI´s Cell Culture Harvest Processes
## Current Technology Status



# Case studies…

# BI´s Cell Culture Harvest Processes

## Case study I: Process scale up & optimization in 12kL

**Background:**

- Cell Culture Process development and successful consolidation in 80L stainless steel at BI.
- Process transfer from 80L Development scale to 12.000L production scale.
- Standard transfer procedures and scale up criteria were used.

**Situation:**

- First 12.000L large scale run showed a significant pressure increase on depth filters.
- Data analysis revealed an uncommon pattern of online turbidity profile in the centrifuge.
- Consultation of experts from development, operations and the supplier of the centrifuge.
- The inlet and outlet pressure in the separator was identified a key process parameter.
- The second process was performed using different pressure levels in the centrifuge.

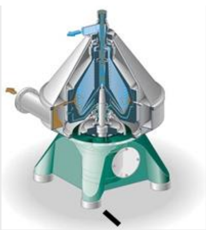
**Conclusion:**

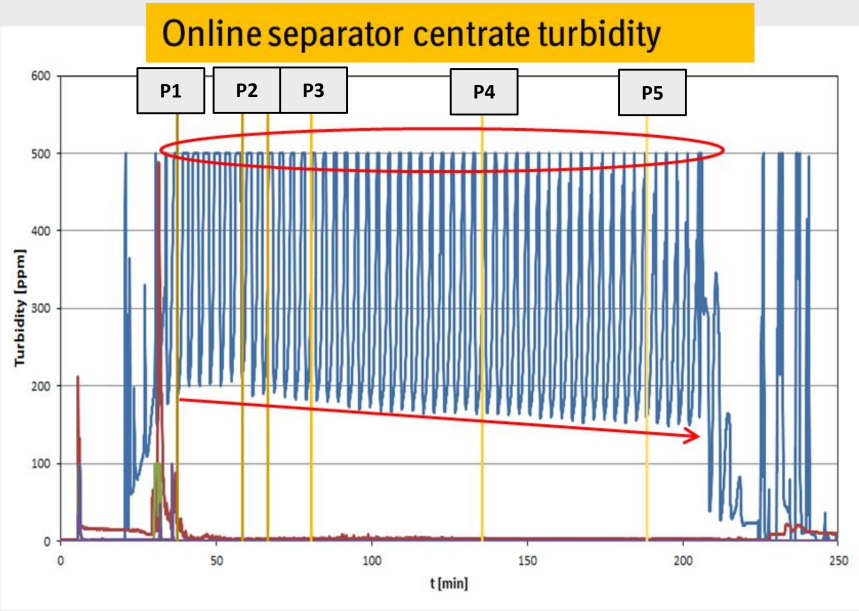
- By the optimization of the inlet and outlet pressure of the centrifuge the clarification of biomass could be improved significantly .
- Finally, the process performance was very comparable to the 80L scale consolidation run.
- Successful process transfer and optimization by collaboration of technial experts from different departments.

# BI´s Cell Culture Harvest Processes

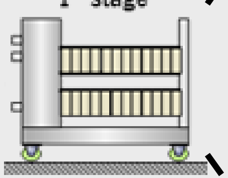
## Case study I: Process scale up & optimization in 12kL

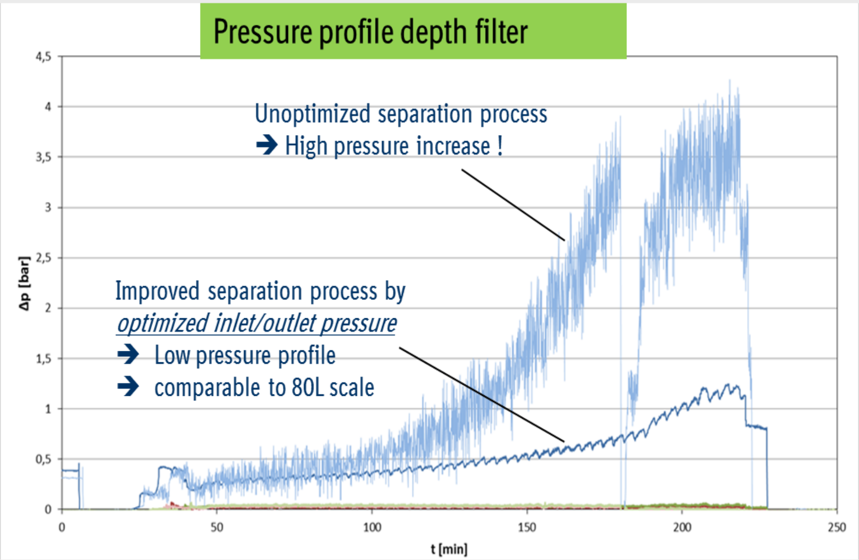
Centrifugation






Depth- filter
1st stage






- Inlet and outlet pressure in centrifuge optimized at scale
- Inlet/outlet pressure influences centrate turbidity after centrifuge
- Direct impact on clarification efficiency in separator
- Higher clarification efficiency improves particle freight on depth filters
- Lower pressure profile and higher filter capacities!
- Comparable performance 12.000L and 80L scale

# Cell Culture Harvest Processes Development

## Case Study II: Bringing Know-how together !

**Background:**

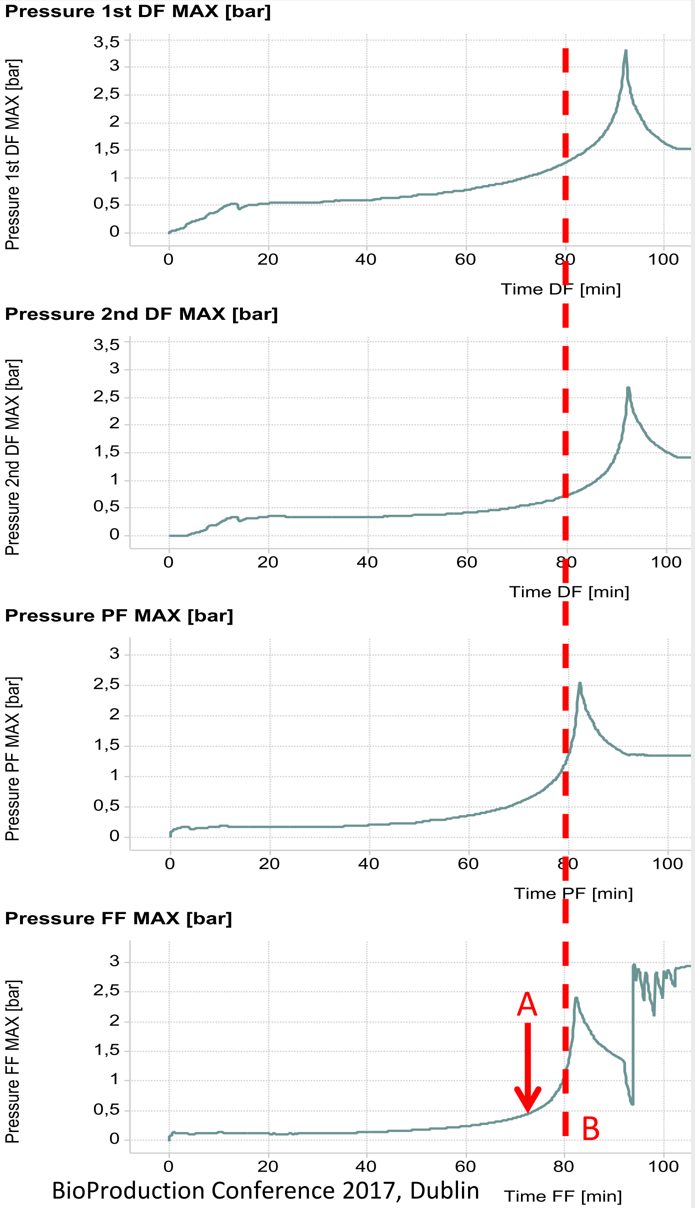
- Direct adaptation of 80L stainless steel into 500L SUB process in first run at BI
- Cell culture process with a high cell mass and a very high demand for glucose
- Initial harvest process using a two-stage depth filtration showed filter blocking
- Root cause: Cells show a high tendency for settling
- Filter blocking ➔ very low product yield ➔ optimization necessary ! FAST !

**Joint Harvest development:**

- Collaboration with filter supplier for development of new depth filtration strategy
- BI: Introduction of a separate mixing tank in order to ensure good suspension
- Lab scale experiments in 2L - 80L scale by filter supplier at BI labs for testing filter options
- Successful implementation and scale up of new harvest strategy in 500L scale

# Cell Culture Harvest Processes Development

## Case Study II: Bringing Know-how together !




**A : Fast pressure increase**

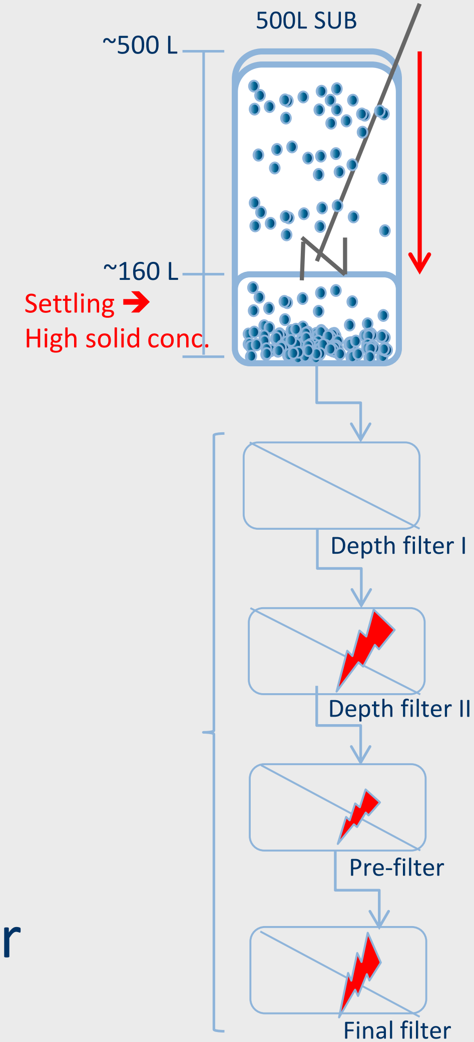
➔ fast settling of solids (cells, debris etc.) to the bottom of the bioreactor!

➔ Fast increase of solid particle concentration in filtration feed stream!

**B : Filter blockage**

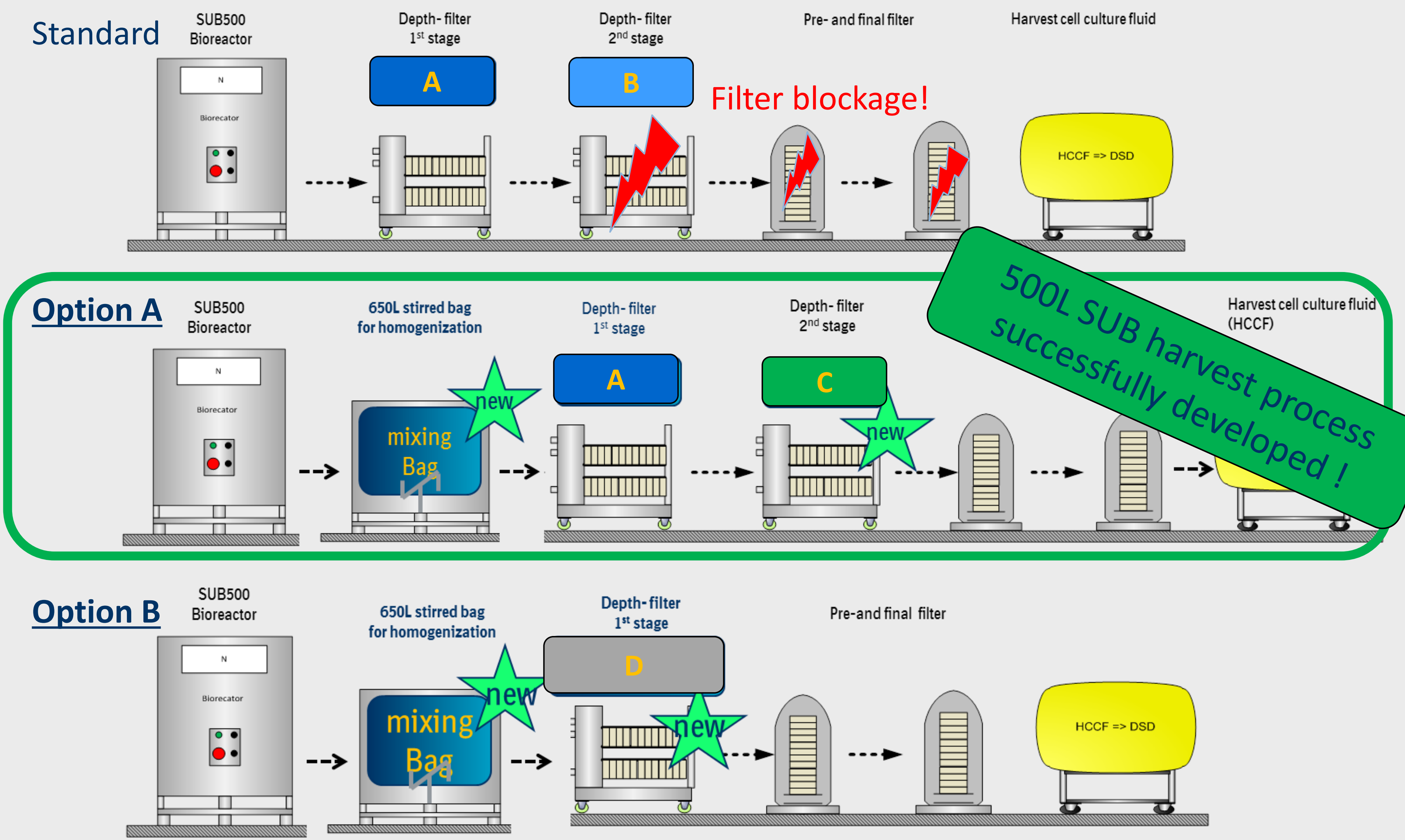
➔ High concentration of solids at bottom
➔ Rapid pressure increase in all filters
➔ Insufficient particle clarification
➔ Bottleneck in depth filter capacity!

➔ Alternative depth filter needed!



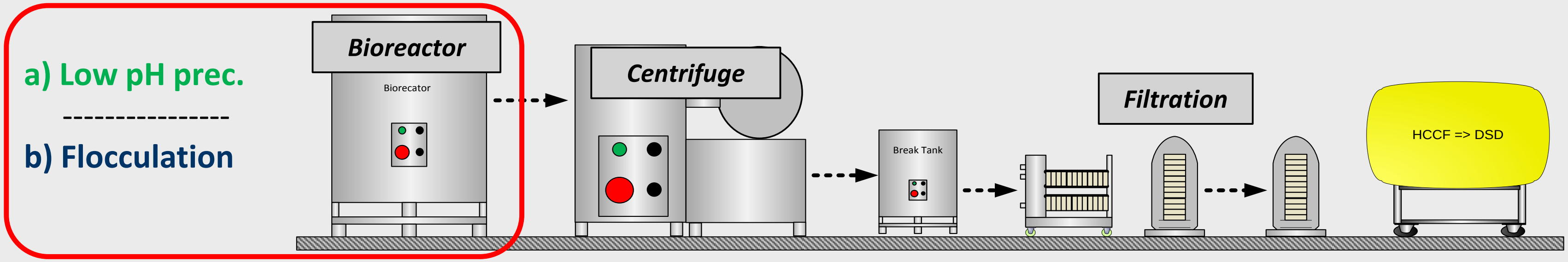
# Cell Culture Harvest Processes Development

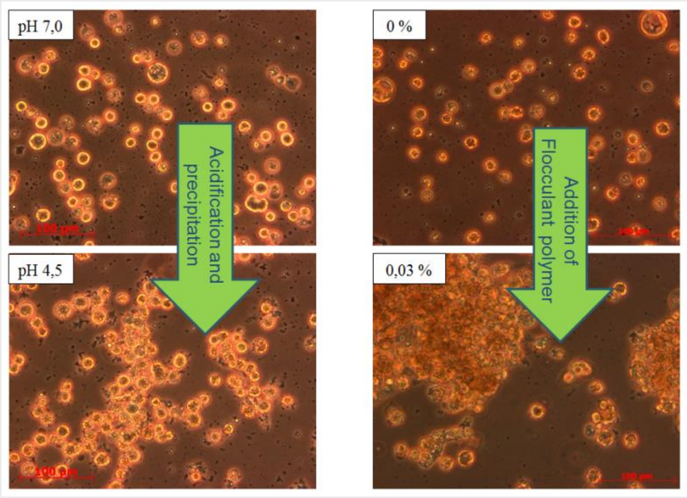
## Case Study II: Bringing Know-how together !

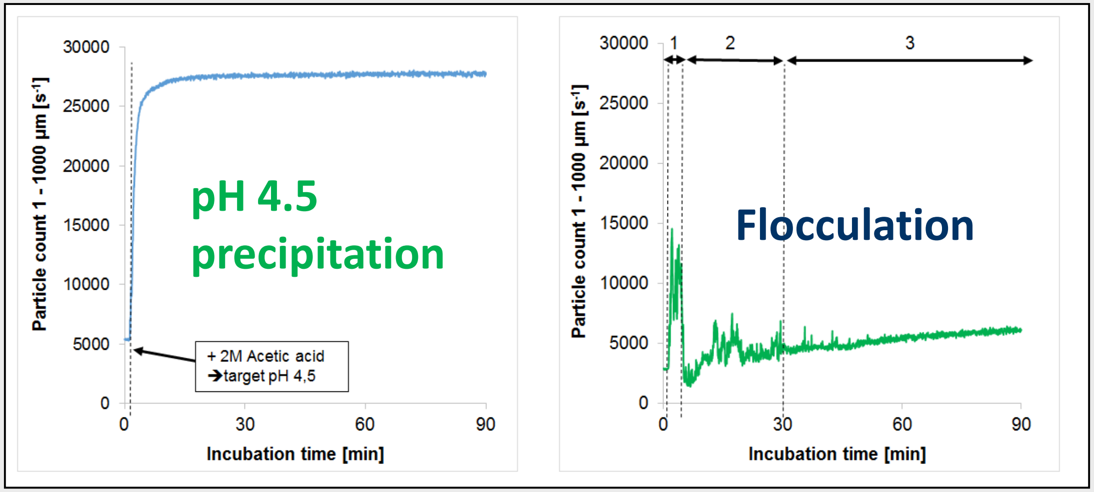


# BI´s Cell Culture Harvest Processes

## Case Study III: Pretreatment by precipitation vs. flocculation




### Microscopic analysis:

➔ A direct low pH precipitation and flocculation in the cell culture have a different impact on particle formation and cell aggregation / cell lysis

### Online Particle size distribution analysis:

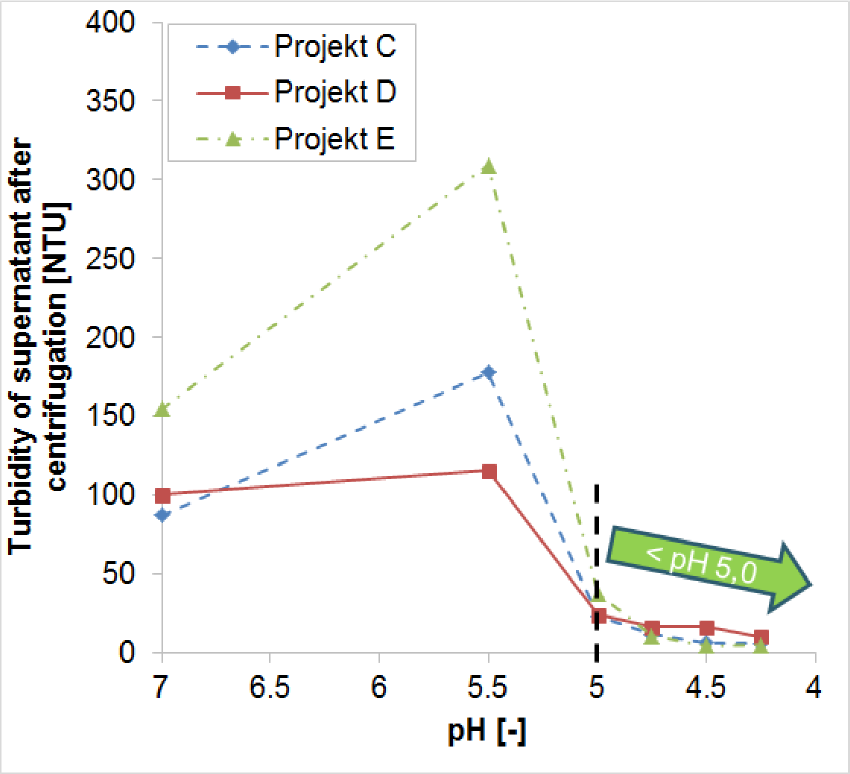
➔ Online FBRM probe (Mettler Toledo®)

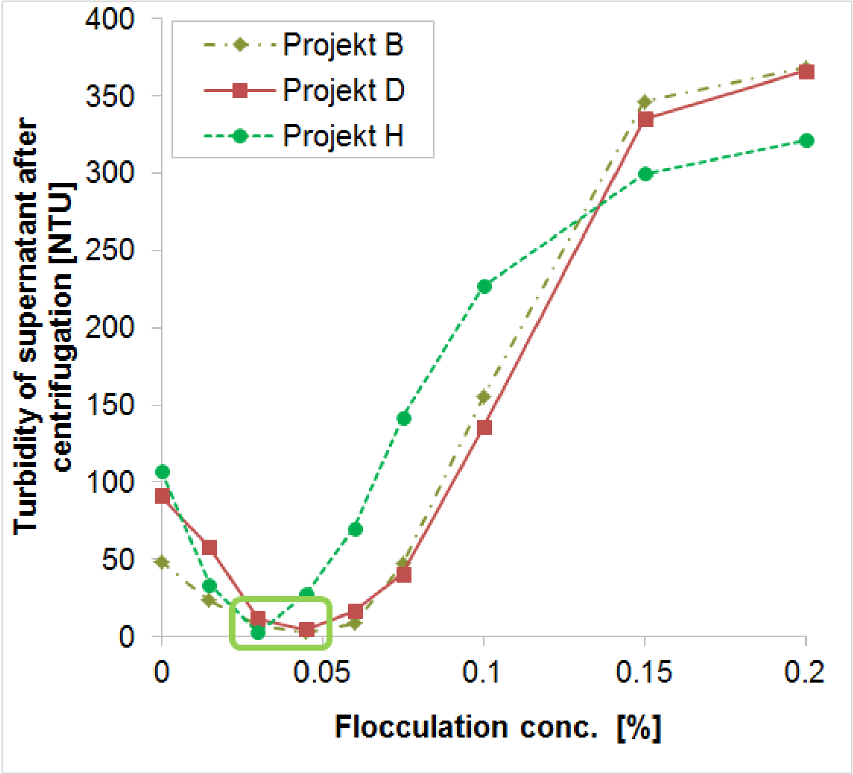
➔ Precipitation/flocculation kinetics

➔ Low pH precipitation and flocculation have different particle formation kinetic

# BI´s Cell Culture Harvest Processes

## Case Study III: Pretreatment by precipitation vs. flocculation

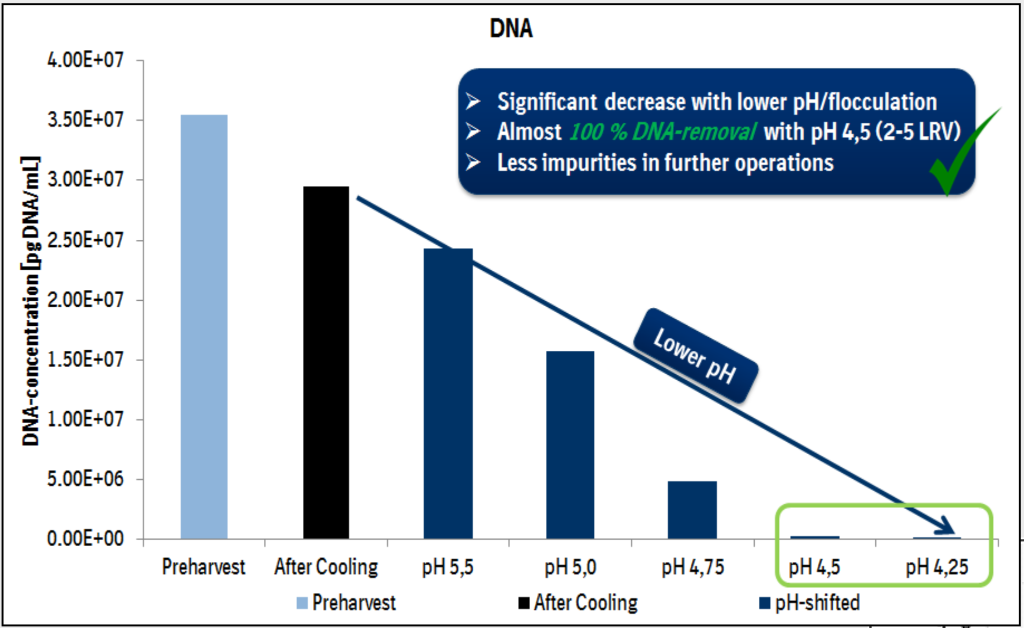


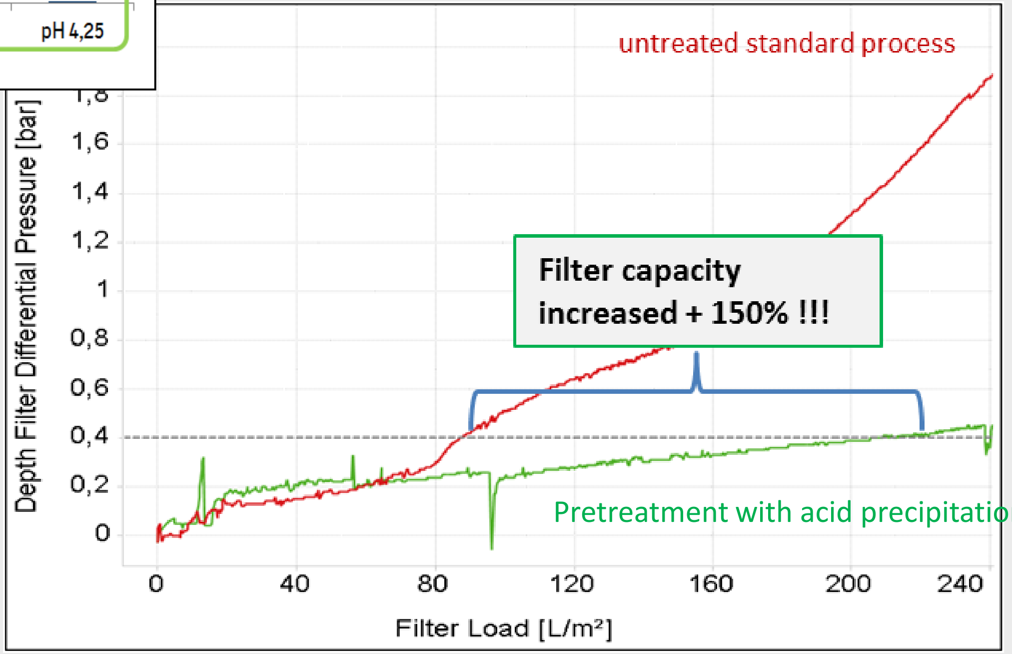

- Flocculation process with a small operational window (conc. range)
- Best precipitation results with low pH < 5.0 ➔ Optimum: pH 4.5
- Flocculation process more challenging: polymer choice, concentration, incubation, analytics, removal of polymer during purification process

# BI´s Cell Culture Harvest Processes

## Case Study III: Pretreatment by precipitation vs. flocculation




- > 99% DNA removal by low pH precipitation
- Optimal precipitation at pH 4.5
- Easy implementation in standard process



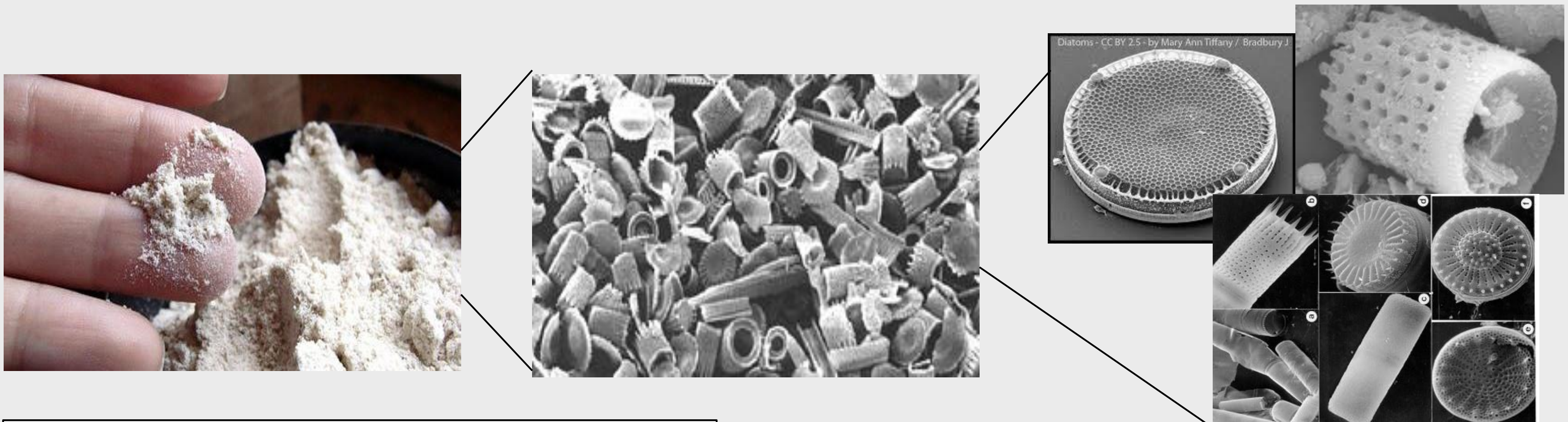

- Significant filter capacity increase by low pH precipitation processes
- Reduction of COGS for filtration

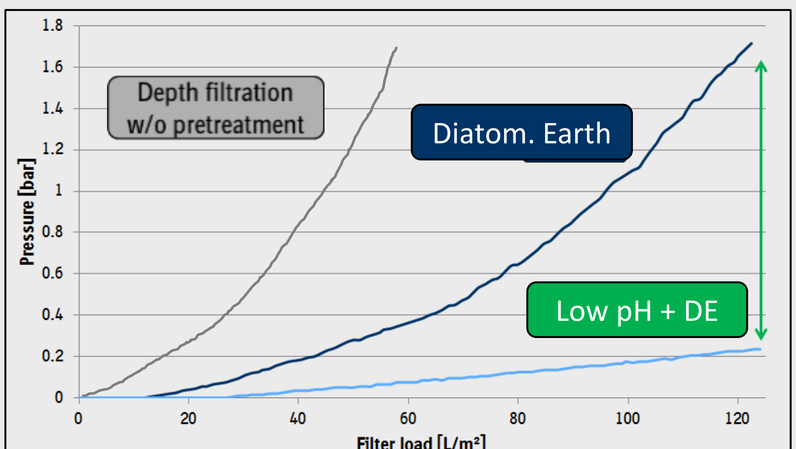
# BI´s Cell Culture Harvest Processes

## Case Study III: Tech evaluation - Diatomaceous Earth Filtration

Diatomaceous Earth Filtration:

- Established technology in food & beverage and pharmaceutical industry since decades
- Diatomaceous earth = inorganic, incompressible, inert and higly porous filter aid




- D.E.-Filtration: Proof-of-Concept studies in 2016 /17
- Lab scale experiments performed up to 80L scale
- Easy handling, good scalability
- High performance in combination with acid precipitation
- A real alternative for future  SUB harvest processes !

# Cell Culture Harvest Development at BI
## Final Facts

Boehringer Ingelheim

- Boehringer Ingelheim BioXcellence® offers a high level of expertise in cell culture and harvest process development, scale up and process transfer of clinical trial or manufacturing processes for customers world-wide.
- BI´s large network with global suppliers for separation and filtration technologies ensures the usage of state-of-the-art equipment and technology.
- Boehringer Ingelheim is continuously working on the evaluation of new cell culture harvest strategies like low pH precipitation, flocculation or diatomaceous earth filtration.
- Boehringer Ingelheim BioXcellence® offers a high degree of know how and expertise to develop processes from 500L – 2.000L SUB and up to 12.000L stainless steel production.

# Thank you for your attention.