DEPTH FILTRATION FOR VALUABLE LIQUIDS. SINCE 1938.

NEW APPROACH FOR SINGLE-USE CLARIFICATION OF BIOLOGICAL SOLUTIONS

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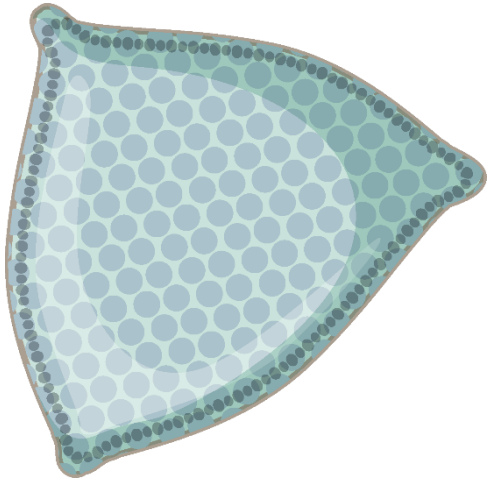
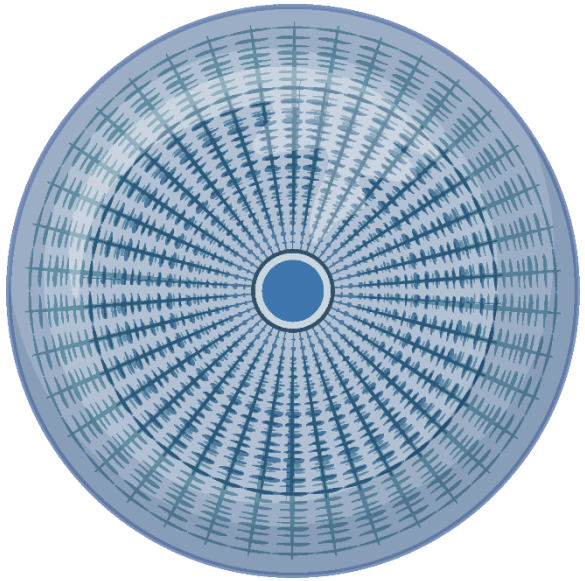
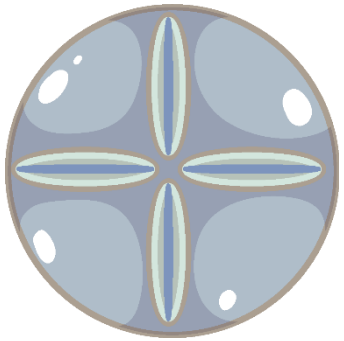
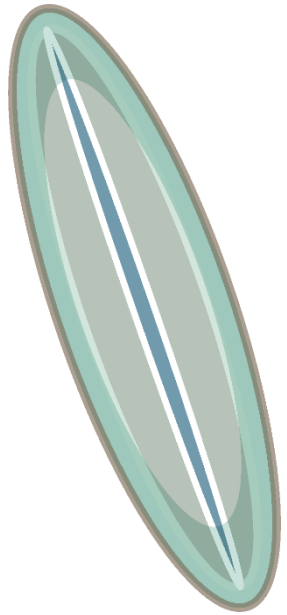
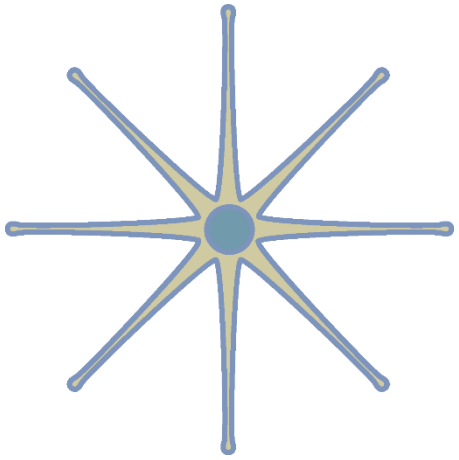
ONCE UPON A TIME…











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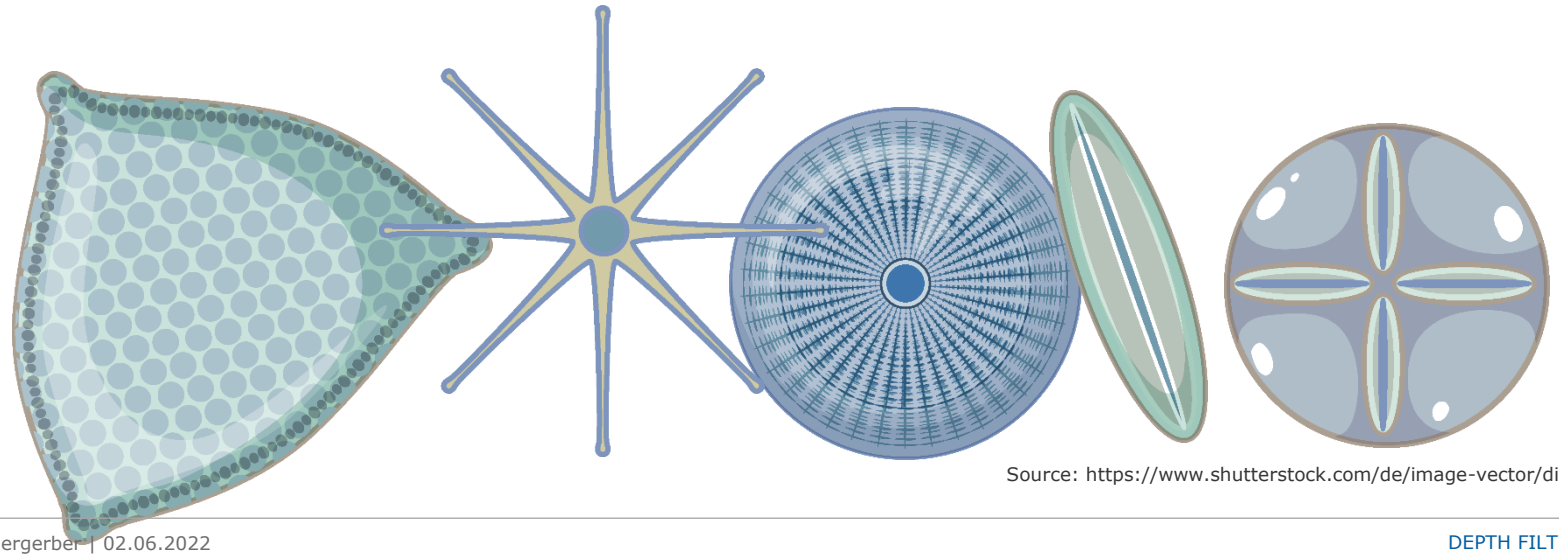
ONCE UPON A TIME…



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AGENDA

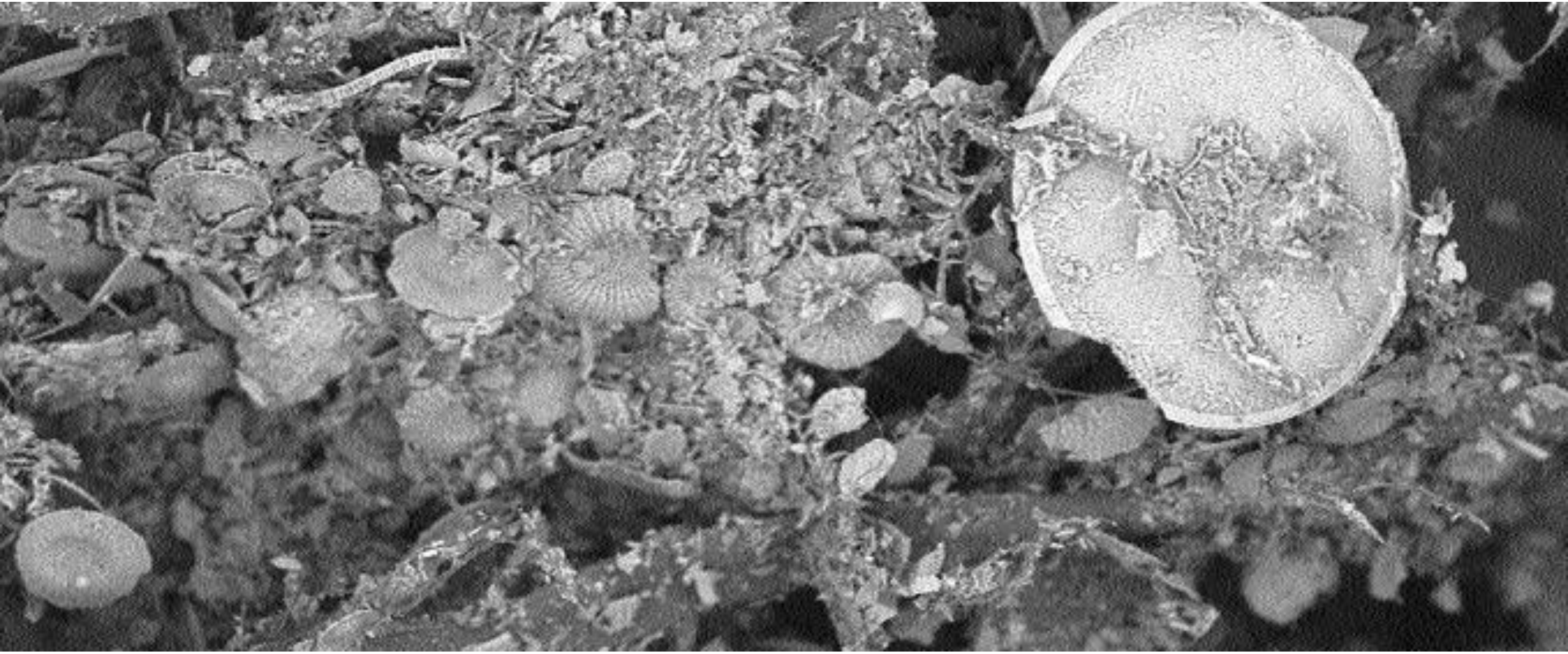
Basics of depth and alluvial filtration

- Trends & Technologies in biomanufacturing
- Sheet vs. Depth vs. Alluvial filtration

Case Studies

1) Mammalian Cell removal
2) Plasmid DNA clarification
3) Depth Filtration vs. Alluvial filtration vs. pH adjustment
4) Influence of different filter sheet types
5) Scale up

BASICS OF DEPTH FILTRATION



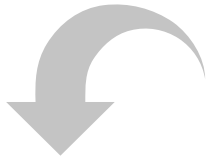
Source: FILTROX AG, 2008

TRENDS

Development and challenges in Biomanufacturing

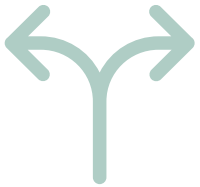
Reduction of process steps

Removal of impurities



Cost reduction

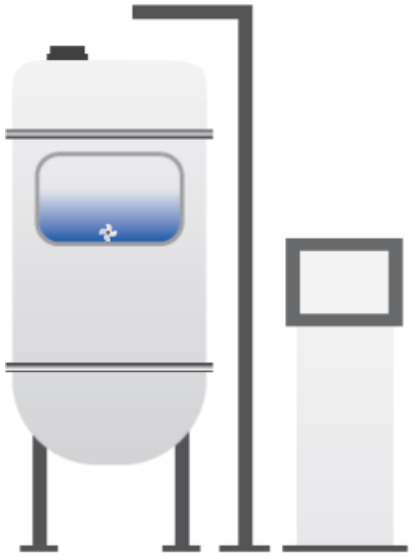
Fast and reliable



Robust and flexible technology

Easy scalable and duplicatable

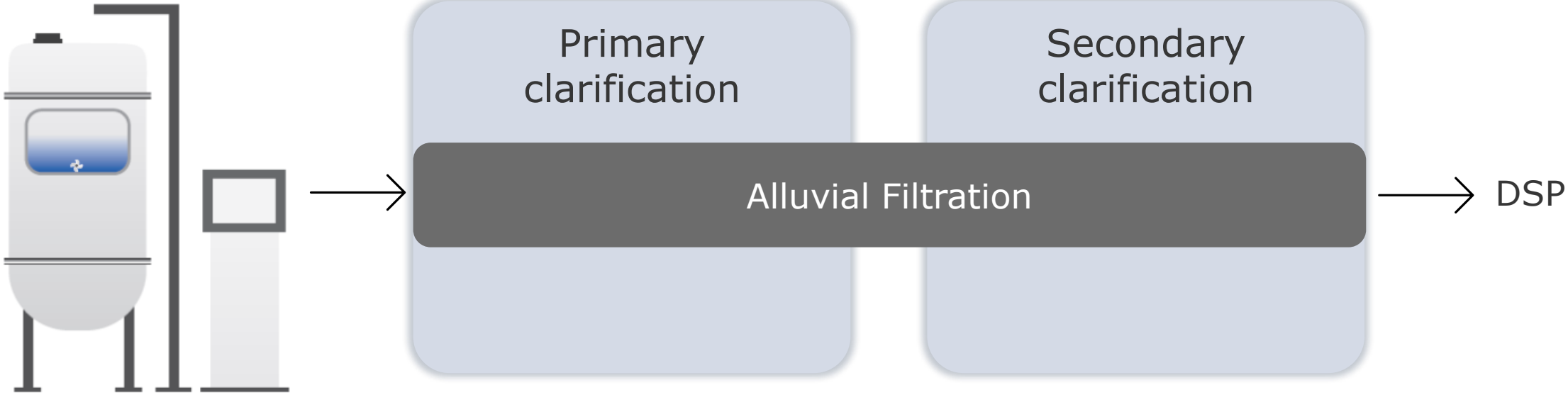
TECHNOLOGY COMPARISON



Primary clarification	Secondary clarification
Centrifugation	Depth Filtration
TFF	Depth Filtration
Depth Filtration	Depth Filtration
Accustic wave	Depth Filtration

DSP

TECHNOLOGY COMPARISON



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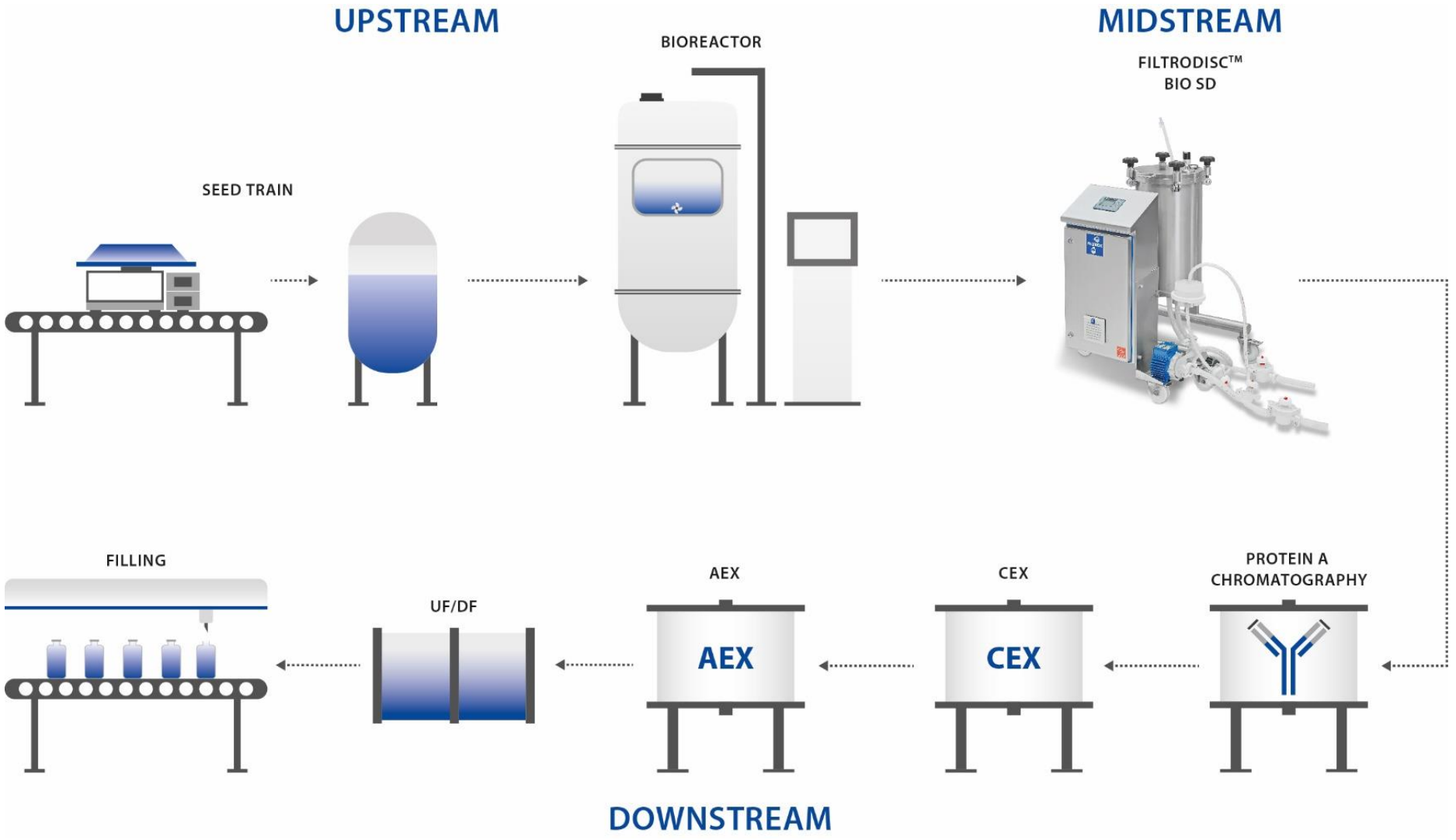
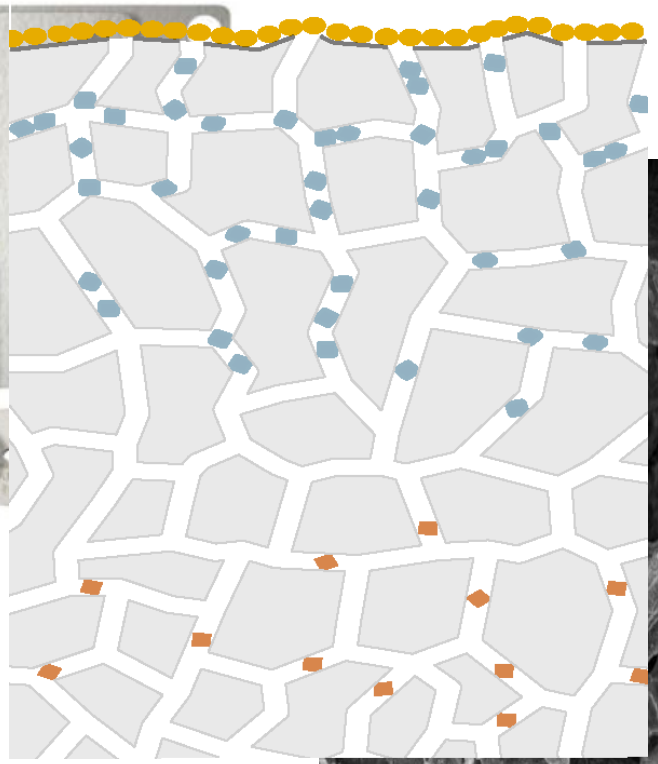
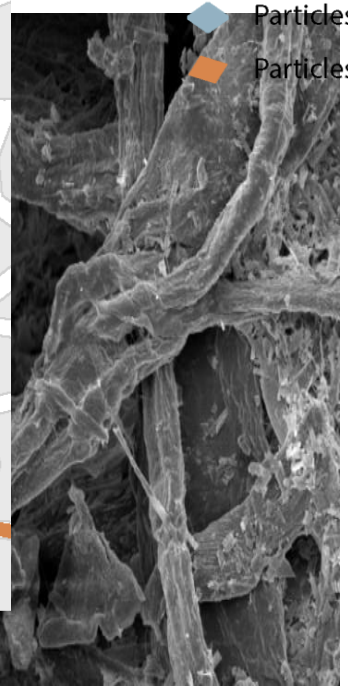


DEPTH VS. ALLUVIAL FILTRATION

Depth Filtration





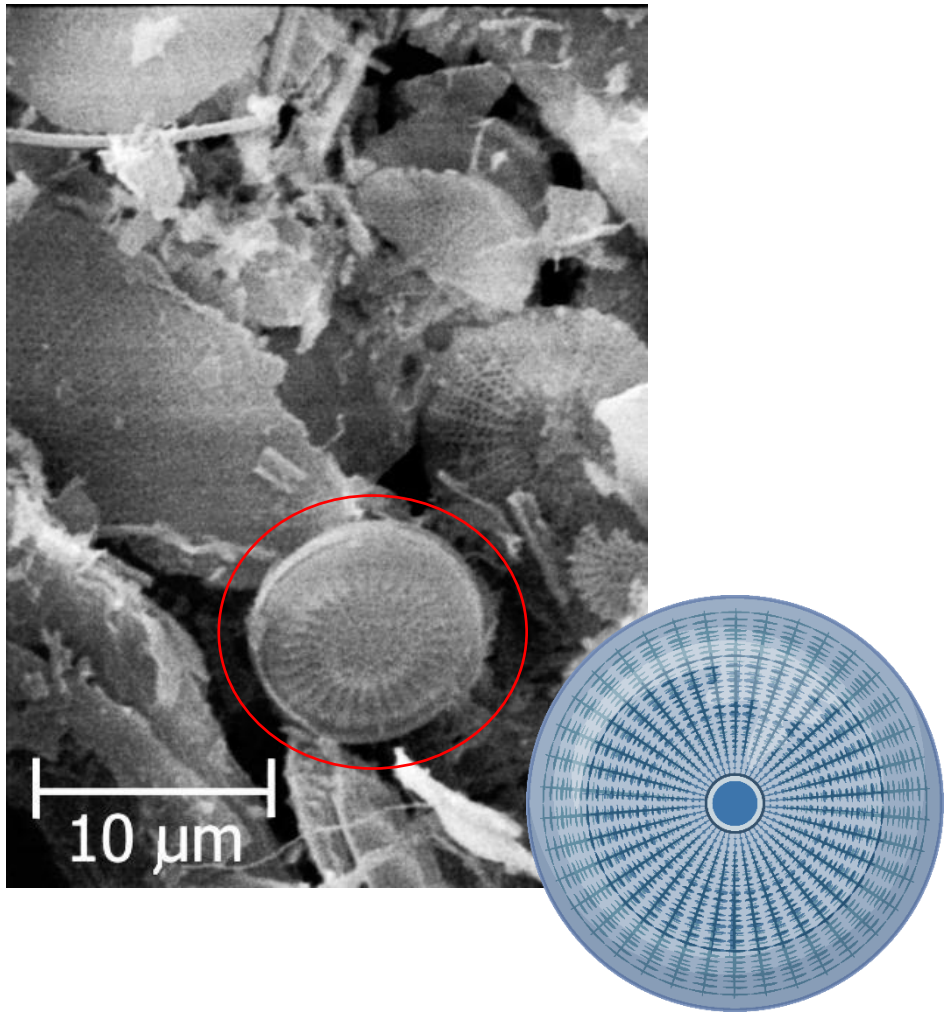
Particles > 5 µm
Filter aid > 10 µm
Particles < 5 µm
Particles < 1 µm

Alluvial Filtration

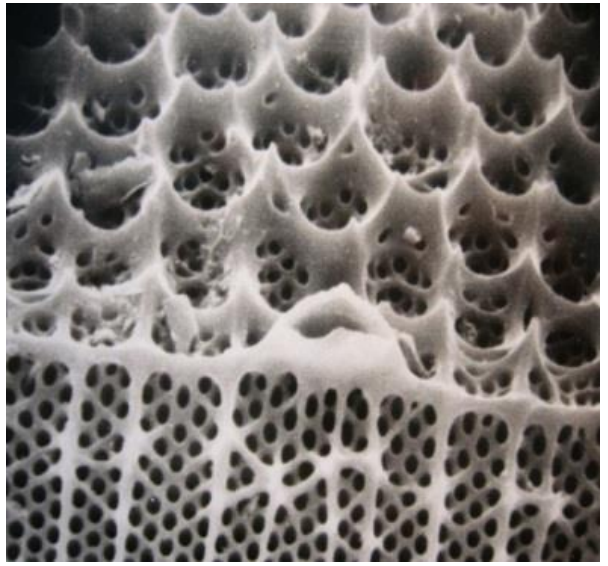
ALLUVIAL FILTRATION

- Large Capacity for high particle load
- An established technology in pharmaceutical filtration since decades (e. g. plasma fractionation)
- Long term experience (over 60 years in beer filtration)
- Filtration through 3D Network
- Economical

→ **Constantly renewed filter surface**



ALLUVIAL FILTRATION



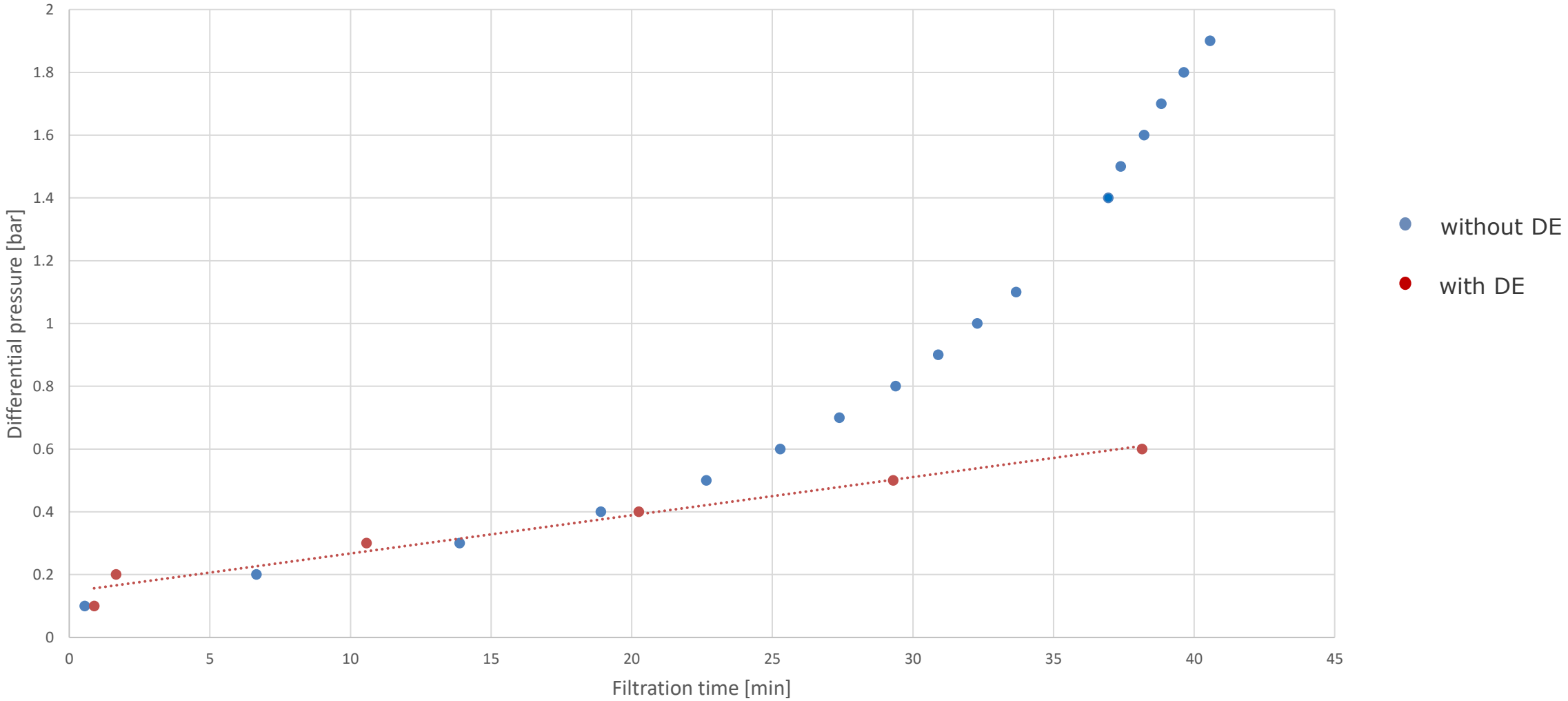
Diatomaceous earth = DE = Kieselguhr

- E. g. Celpure® (an imerys brand) → pharma grade
- Inorganic filter aid
- Mineralized organisms (skeleton of diatoms)

Grade	Permebaility [mDarcy]	Solids removed* [µm]
C65	40 – 80	0.3 – 0.45
C100	70 – 140	0.3 – 0.45
C300	150 – 300	0.45 – 0.6
C1000	750 – 1250	> 1.0

ALLUVIAL FILTRATION

ALLUVIAL FILTRATION



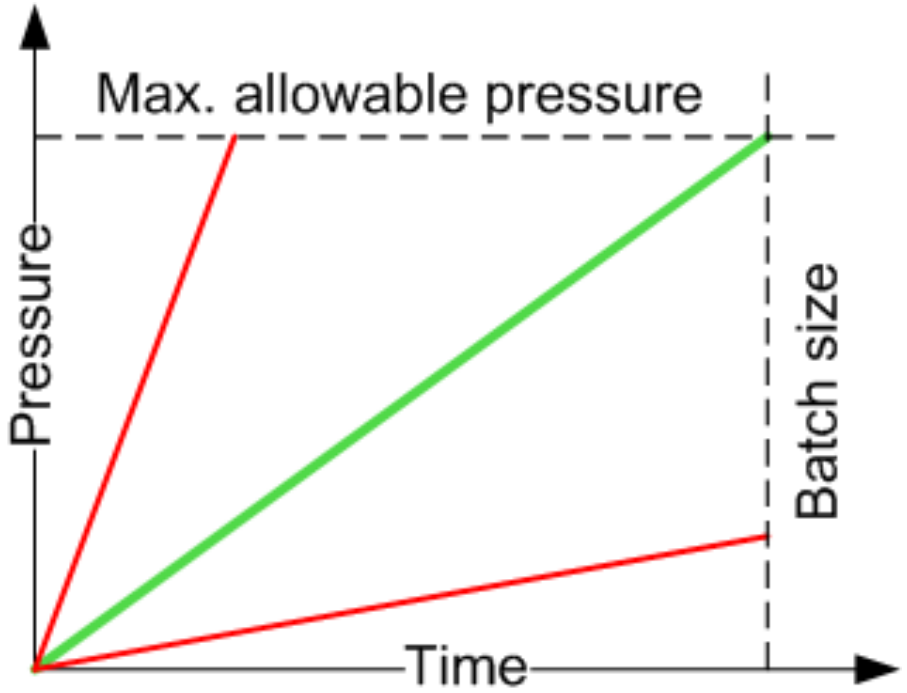
FILTRODISC™ BIO SD

Evaluation of the right DE grade

Low Turbidity

Optimization of DE dosage

High Efficiency



FILTRODISC™ BIO SD

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Basics of depth and alluvial filtration

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Case Studies

1) Mammalian Cell removal
2) Plasmid DNA clarification
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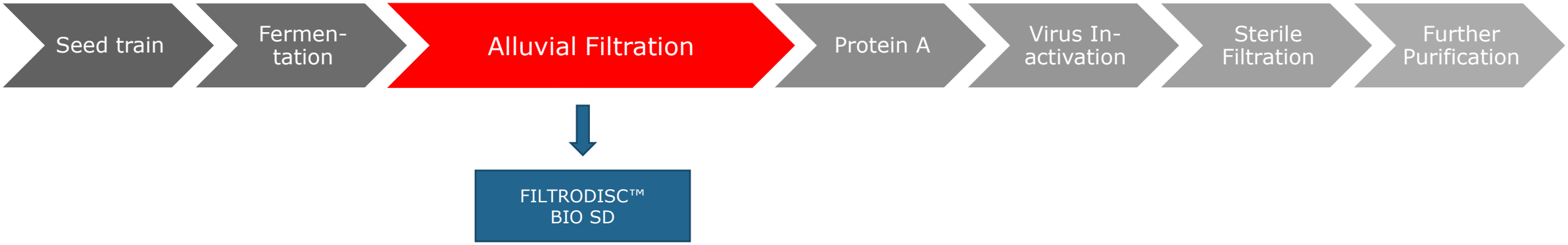
4) Influence of different filter sheet types
5) Scale up

CASE STUDIES

1. Mammalian cell removal
2. Plasmid DNA clarification
3. Depth filtration vs. DE filtration vs. pH adjustment
4. Influence of different filter sheet types
5. Scale up

1 CASE STUDY: MAMMALIAN CELL REMOVAL

Background: Typical clarification process for mammalian cells involves primary and secondary clarification step
→ Process step reduction with FILTRODISC™ BIO SD system and alluvial filtration technology



1

CASE STUDY: MAMMALIAN CELL REMOVAL

CHO cell culture producing IgG

- 2 days fed-batch cultivation
- 80 – 99 % viability
- 5 – 6 × 10^6 cells
- Harvest strategy:
  - FILTRODISC™ BIO SD 2"
  - PURAFIX® CH 09P
  - Comparison of Celpure® C100 and C300
  - Comparison of different Celpure® amounts
  - Filtration flux = 400 – 500 L/m²×h

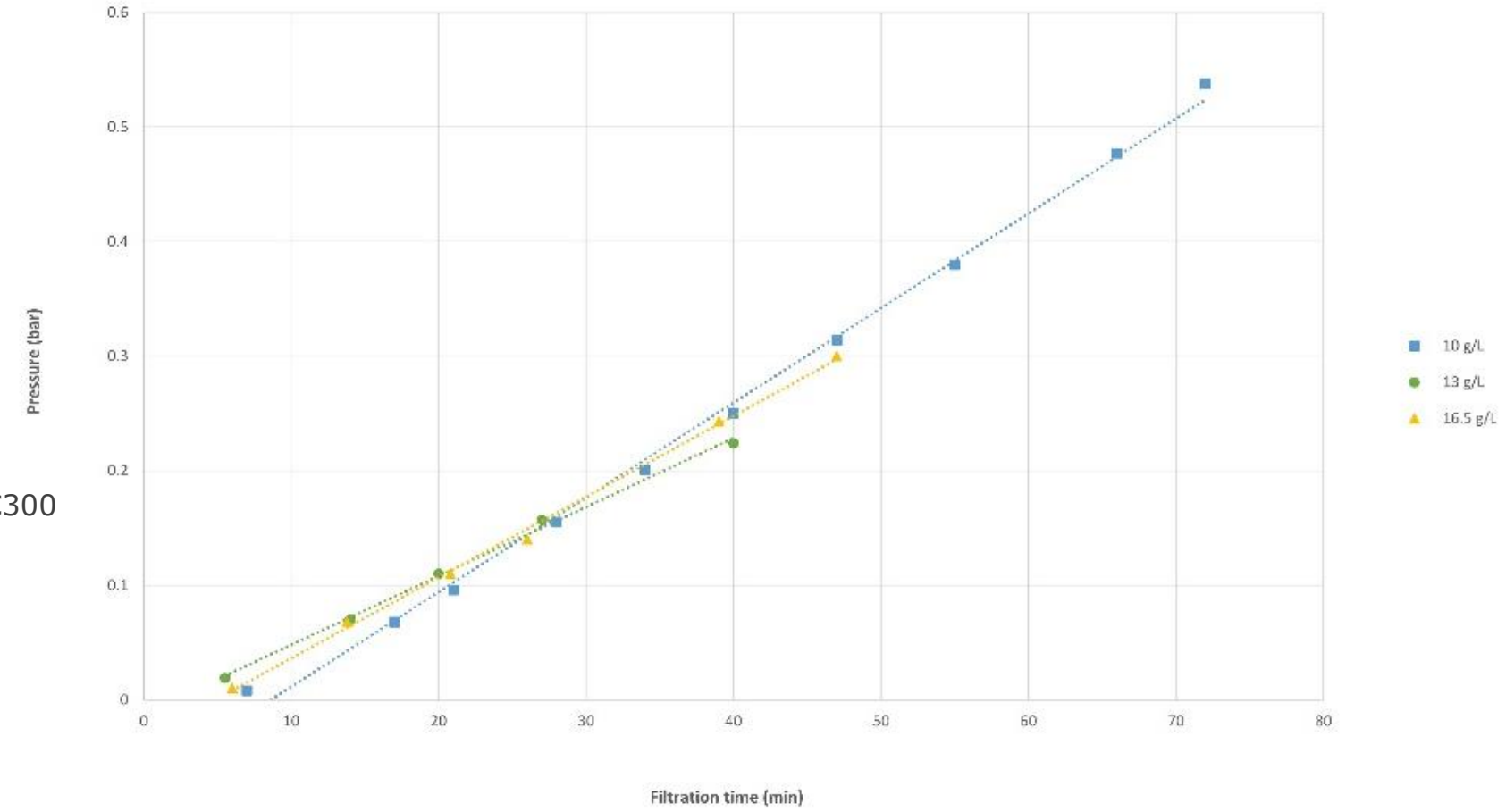


Figure: Pressure profiles as a function of throughput for different dosage of Celpure® 300

1

CASE STUDY: MAMMALIAN CELL REMOVAL

CHO cell culture producing IgG

→ Scale up from FILTRODISC™ BIO SD 2" to 5"

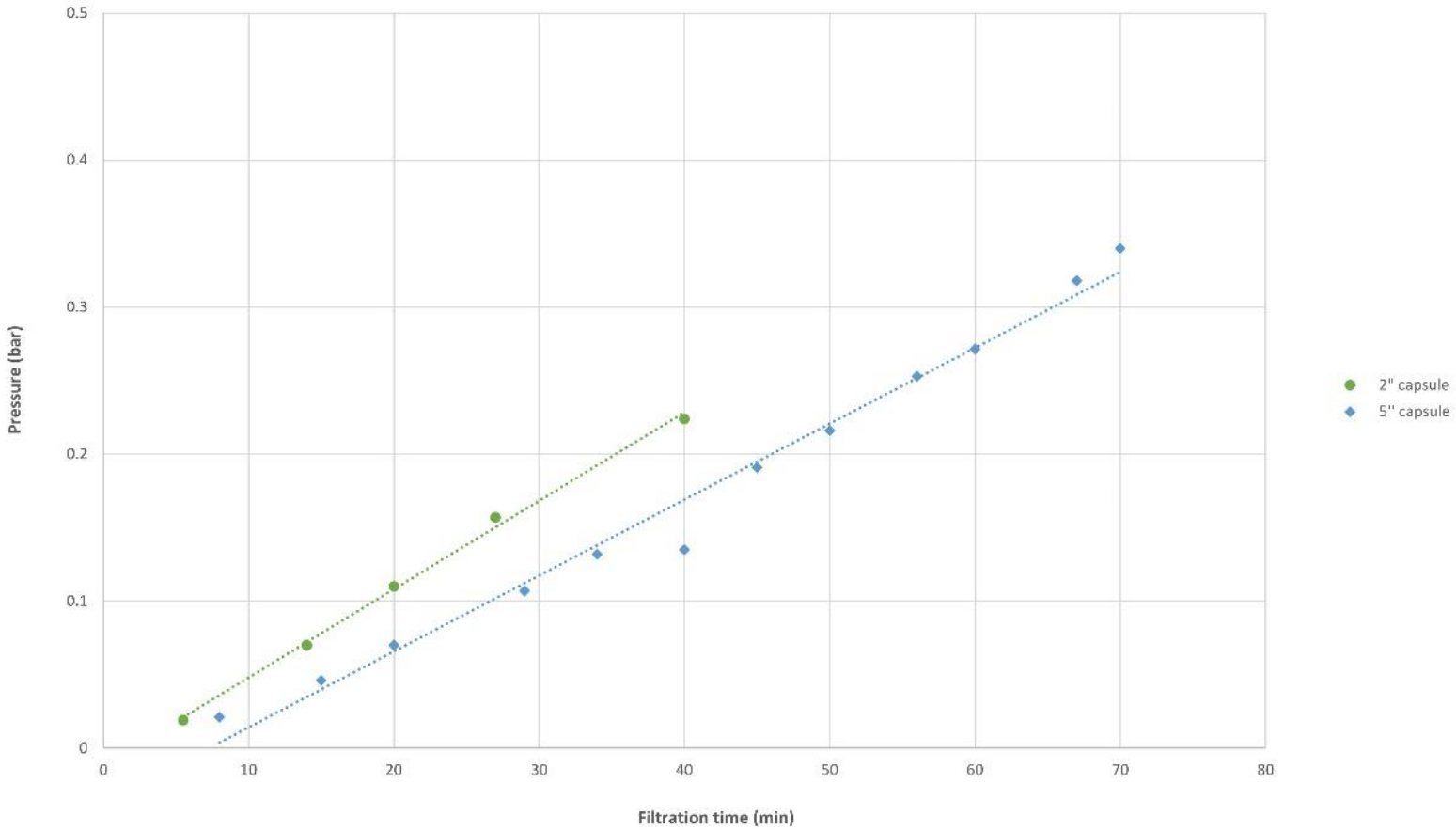


Figure: Pressure profiles (inlet) as a function of process time (throughput) for two different filtration FILTRODISC™ BIO SD 2" and 5" capsules and 13.5 g/L DE dose

1

CASE STUDY: MAMMALIAN CELL REMOVAL

CHO cell culture producing IgG

→ Recovery Yield

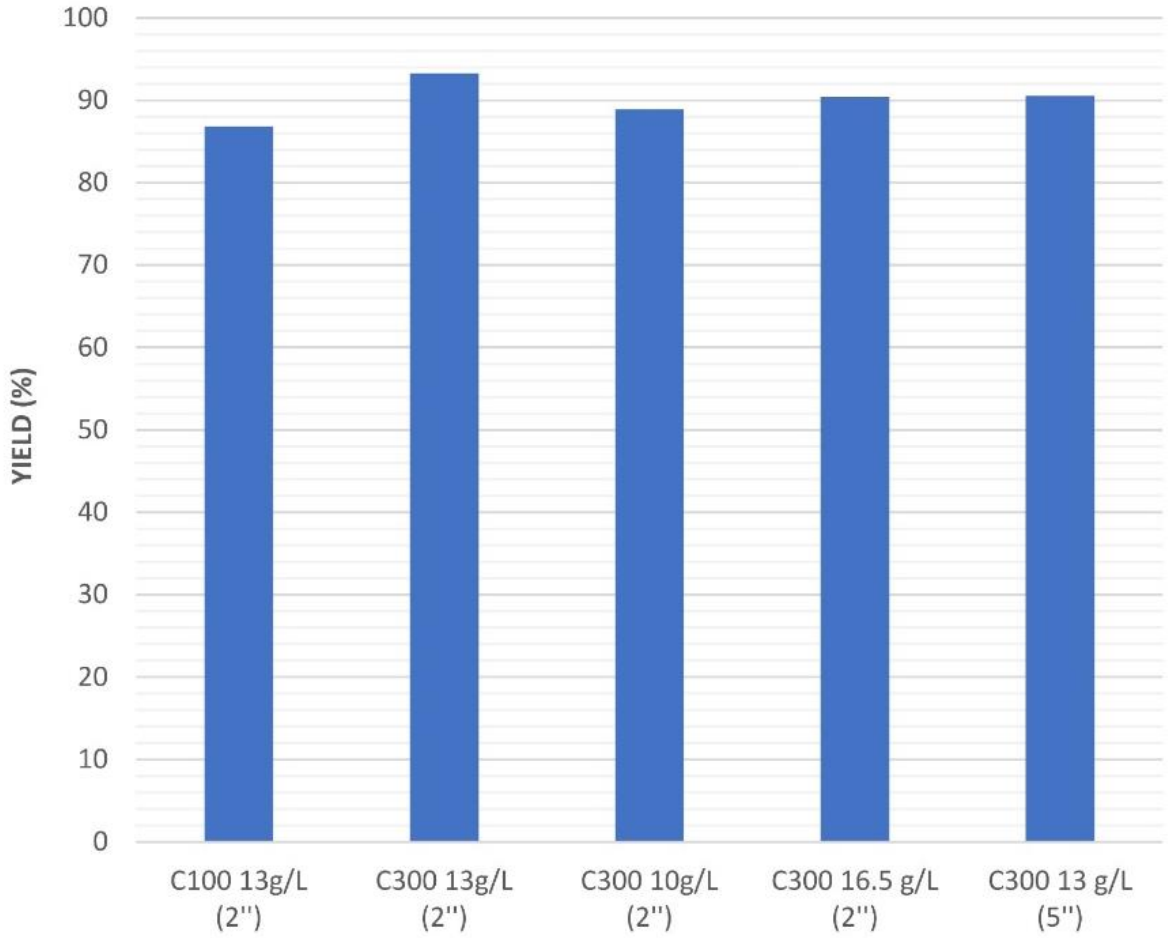


Figure: IgG recoveries in different filtration experiments

1

CASE STUDY: MAMMALIAN CELL REMOVAL

CHO cell culture producing IgG

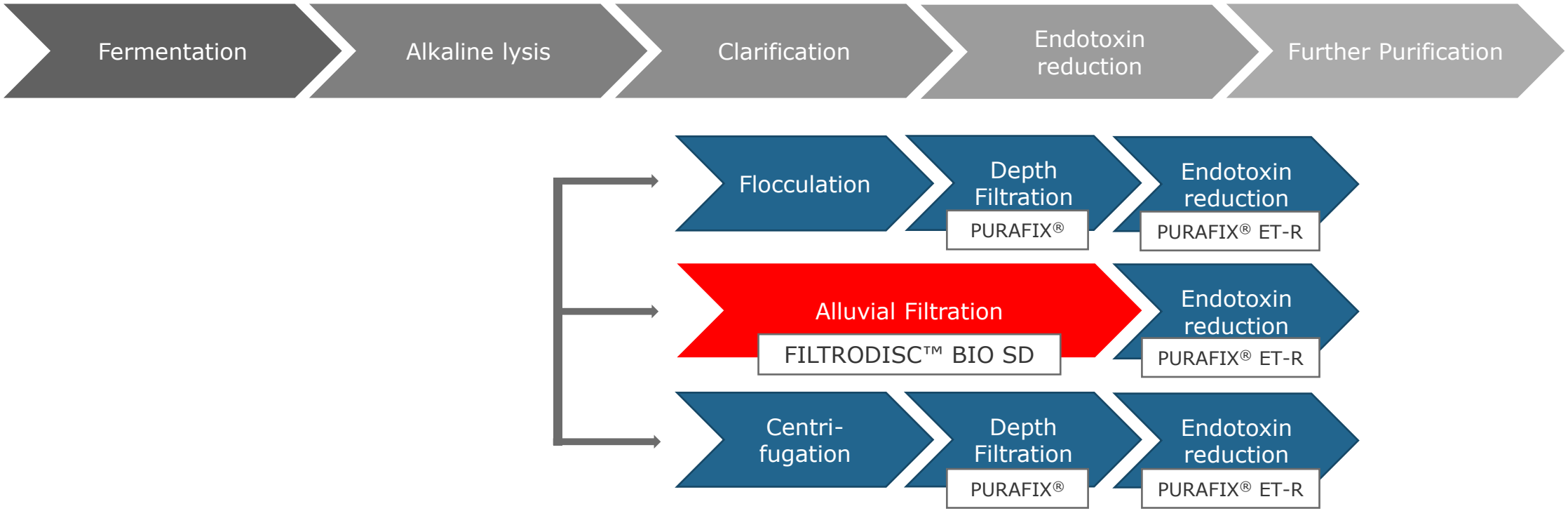
→Results

- Productive approach
  - High flux (450 L/m²×h)
  - High throughput (400 L/m² as standard)
- Consistent clarification performance
  - Filtration fluxes
  - Throughputs
  - Reduction of turbidity
- Simple scale up
- Superior performance in terms of capacity & productivity (compared to alternative technologies)
- Cost efficiency → important for processes that are associated with “acceptance criteria” in terms of manufacturing costs

2

CASE STUDY: PLASMID DNA CLARIFICATION

Background: Plasmid DNA is used in production of viral vectors.
→ Increasing demand for plasmid DNA leads to need for larger manufacturing capacities (due to increased clinical success and growing number of late-phase clinical studies)



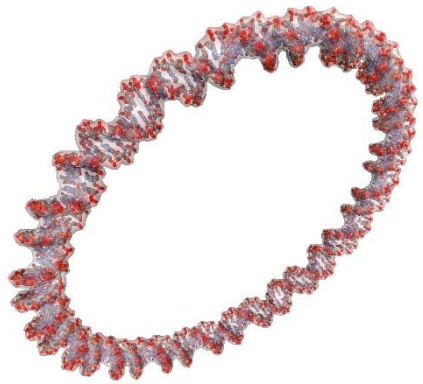
2

CASE STUDY: PLASMID DNA CLARIFICATION

Plasmid DNA production with FILTRODISC™ BIO SD and PURAFIX® ET-R

→Advantages

- Reduction of process steps (by elimination of centrifugation step)
- Process time reduction
- Cost effective reduction of impurities
- Flexible in terms of space for filter cake, retention rate, connectors etc.

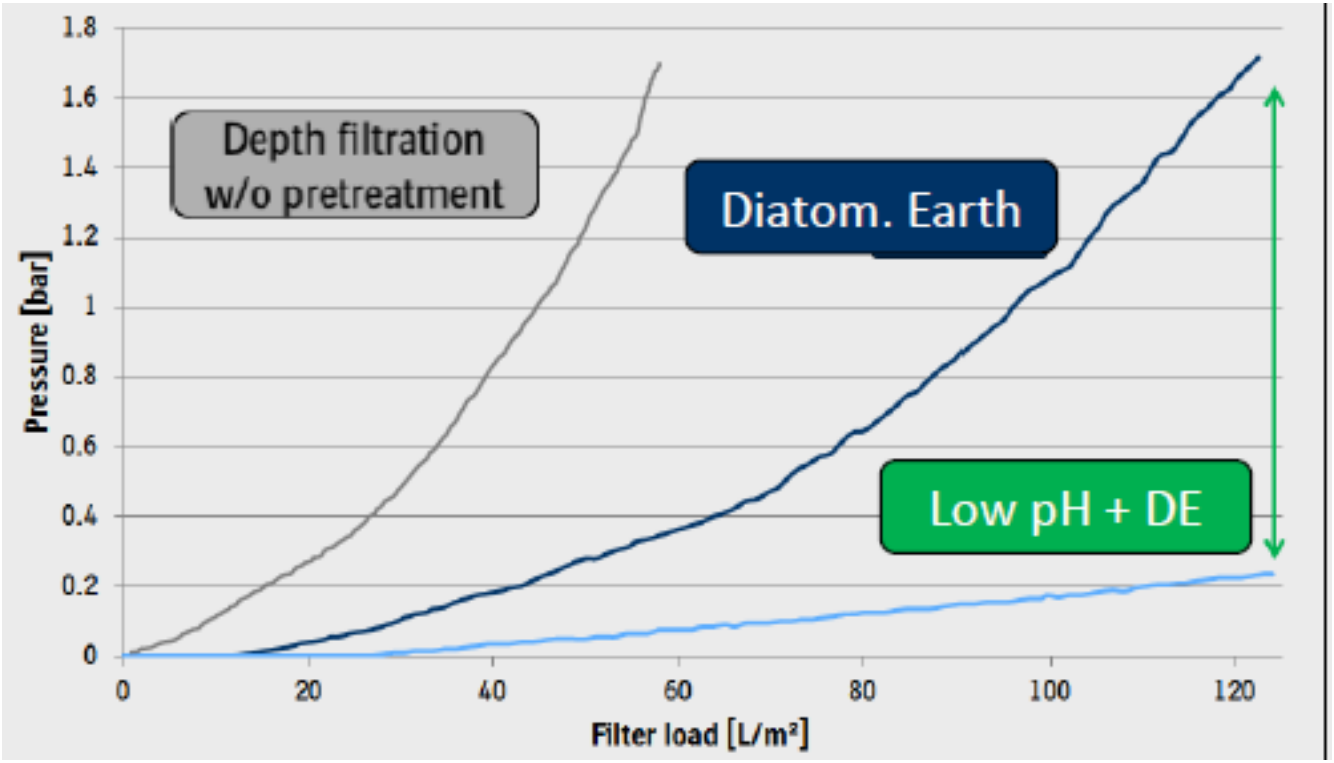


3

CASE STUDY: DEPTH FILTRATION VS. DE FILTRATION VS. ACID PRECIPITATION

Acid treatment / low pH:

- Leads to bigger particles
- Easier to filter
- Influence on target molecule?
- Afterwards pH needs to be adjusted again



Data published by Dr. Markus Brakel, Boehringer Ingelheim, BioProduction 2017 Dublin

4

CASE STUDY: INFLUENCE OF DIFFERENT DEPTH FILTER SHEET TYPES

Background: The use of filter sheets with different characteristics (retention rate) have an influence on the turbidity

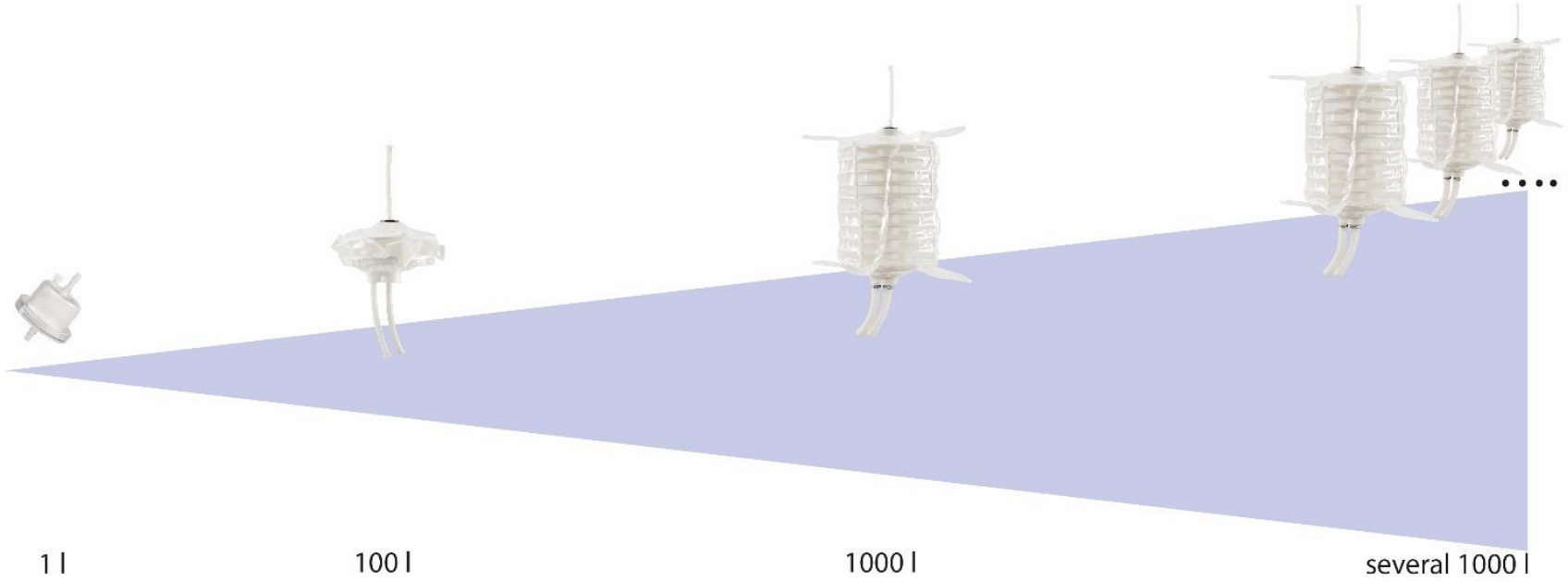
Conditions:

- 6 × 10^6 cells / mL
- Viability: 90 %
- Turbidity: 295 NTU
- DE grade: Celpure® C1000
- DE dosage: 25 g/L

	Trial 1	Trial 2
Filter sheet grade	PURAFIX® CH 9P	PURAFIX® CH ST 130P
Renteion rate [µm]	30 – 10	0.6 – 0.4
Average flux [L/m²×h]	648	420
Turbidity after filtration [NTU]	3.8	1.5

5

CASE STUDY: SCALE UP



$$C_P = \frac{V_P \times C_L}{V_L}$$

$$C = h \times A$$

C: cake volume [m³]
V: filtered volume [L]
L: lab scale
P: production scale
h: cake height [m]
A: filter area [m²]

5 CASE STUDY: SCALE UP

Background: Laboratory scale filtration of a CHO cell culture

Conditions:

- FILTRODISC™ BIO SD 2" capsule (21 cm²)
- Filtered volume = 380 mL
- Final ΔP = 2.3 bar
- Filter aid type = Celpure® C300
- Filter aid dosage = 50 g/L
- Cake height = 3.0 cm
- Batch size = 100 L

Scale up:

$$A_{prod.}[m^2] = \frac{V_{prod.}[L] \times A_{test}[m^2]}{V_{test}[L]} = \frac{100\ L \times 0.0021\ m^2}{0.38\ L} = 0.55 = 0.6\ m^2$$

$$c_{prod.}[m^3] = \frac{V_{prod.}[L] \times c_{test}[m^3]}{V_{test}[L]} = \frac{100\ L \times 0.000063\ m^3}{0.38\ L} = 0.0166\ m^3 = 16.6\ L$$

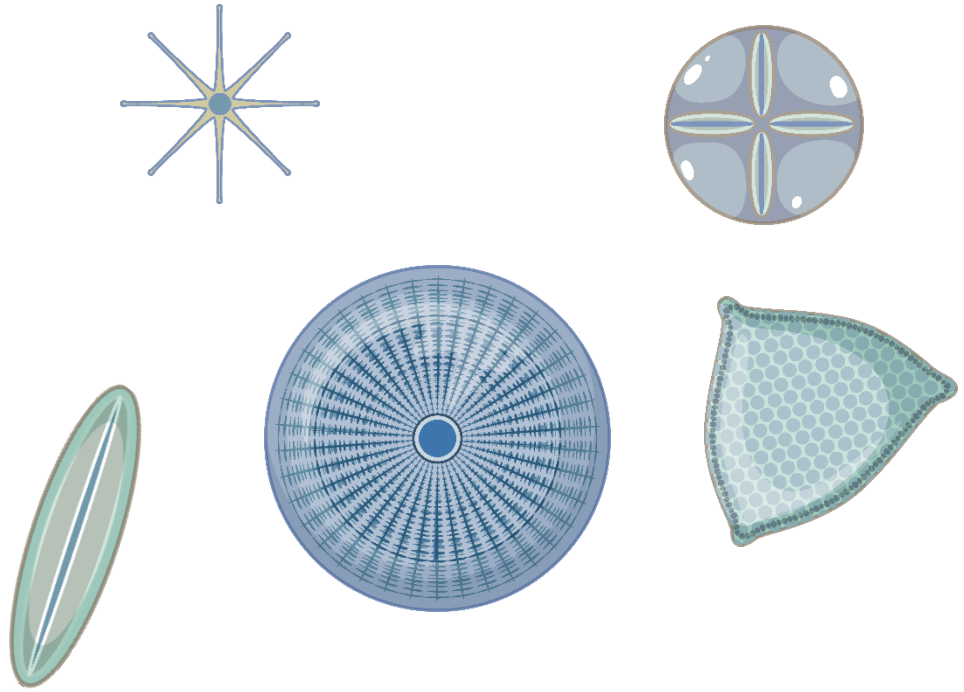
→ FILTRODISC™ BIO SD 12" double module

- 6 lenses per module (overall 12) = 1.32 m²
- Max holding volume = 17.8 L

TAKE-HOME MESSAGE

Advantages of Alluvial Filtration technology

- Well know and accepted technology (e. g. plasma fractionation)
- Removes cells and impurities in one step
- No need for pH changes or flocculants
- Reduce process time
- Different DE types available



DEPTH FILTRATION FOR VALUABLE LIQUIDS. SINCE 1938.

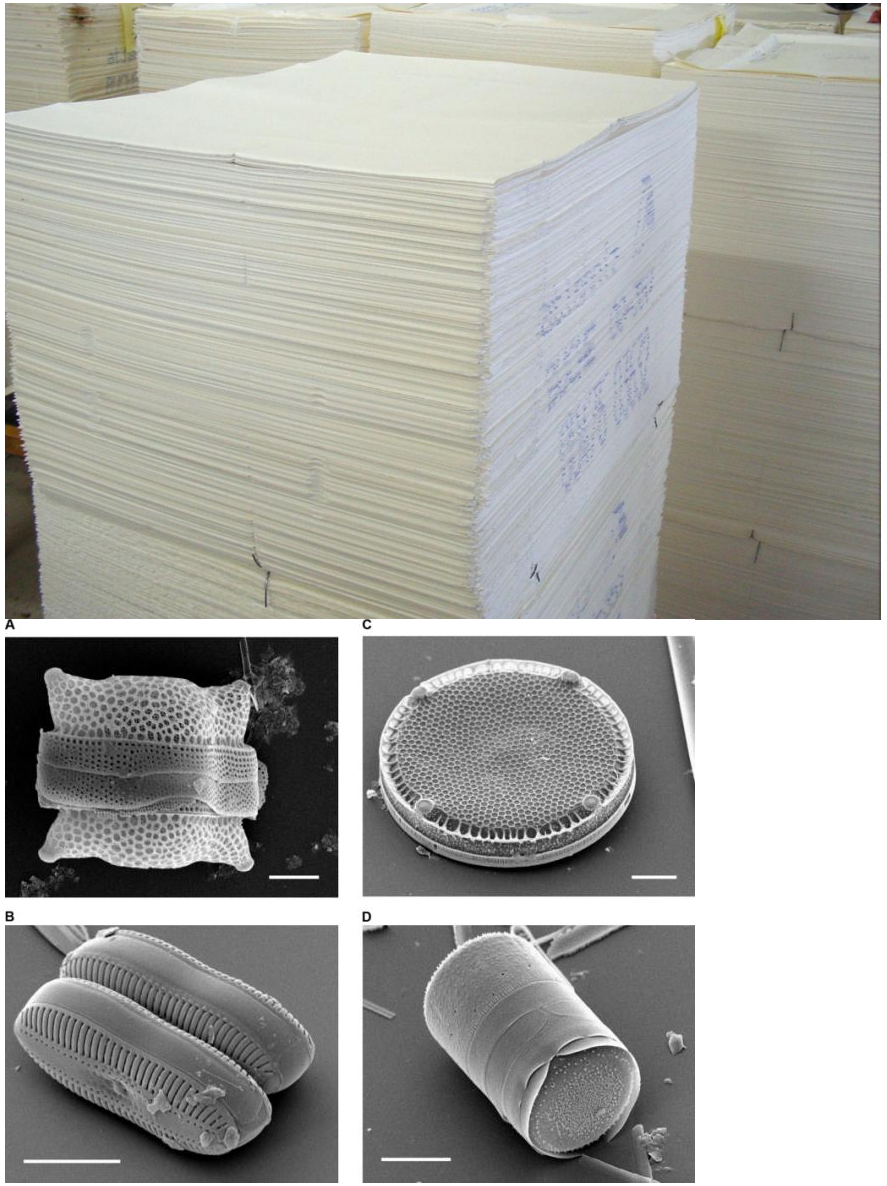
Let us solve your filtration task!

APPENDIX

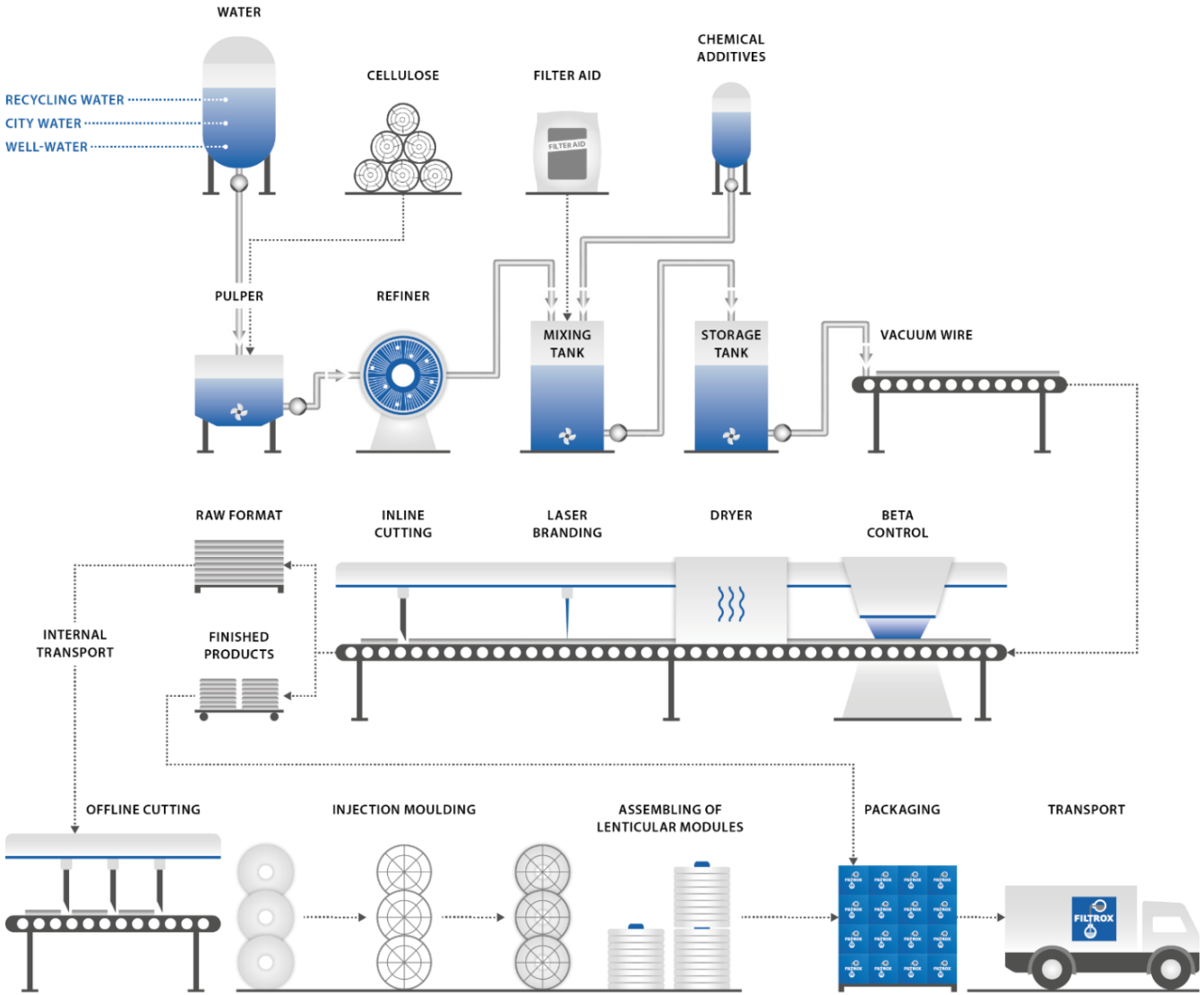
- Filter sheet composition
- Filter sheet production process
- Validation
- FILTRODISC™ BIO SD series
- FILTRODISC™ BIO SD: Filtration principle
- Laboratory scale set-up
- Powder Transfer
- Case Study: Viral Vector Clarification

FILTER SHEET COMPOSITION

- Cellulose fibers
  - Ground cellulose fibers form a 3D matrix
- Filter aid (e. g. Kieselguhr, Perlite)
  - Enlarge the inner surface
  - Improve the filtration effects
- Wet strength agent
  - Increase of wet tensile strength
  - Positive charge for the adsorption of negatively charged suspended solids



FILTER SHEET PRODUCTION PROCESS



PRIMARY PRODUCTION

Pulper

Wet sieve section

Drying Oven

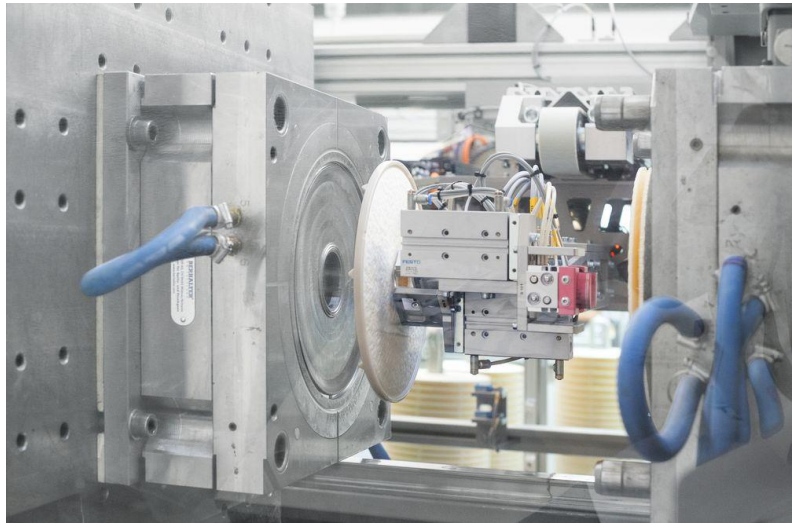
Laser marking

SECONDARY PRODUCTION

Stamping

Water jet

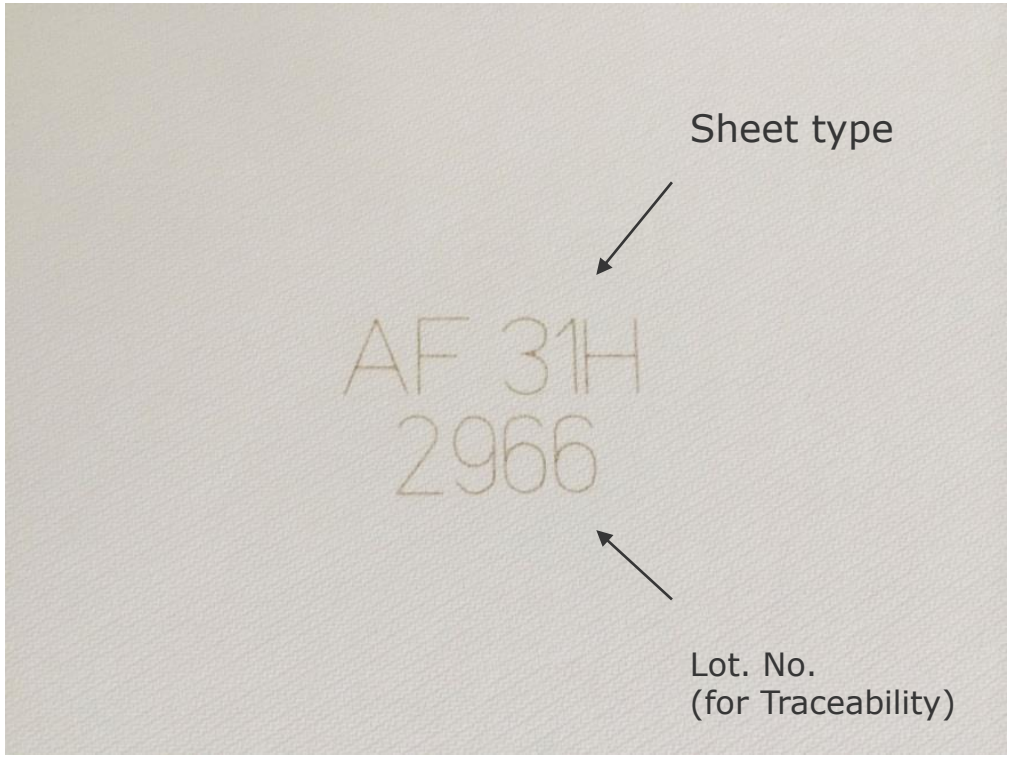
Modul-
robot



Packaging

PRODUCTION PROCESS

Unfiltrat (coarse surface)



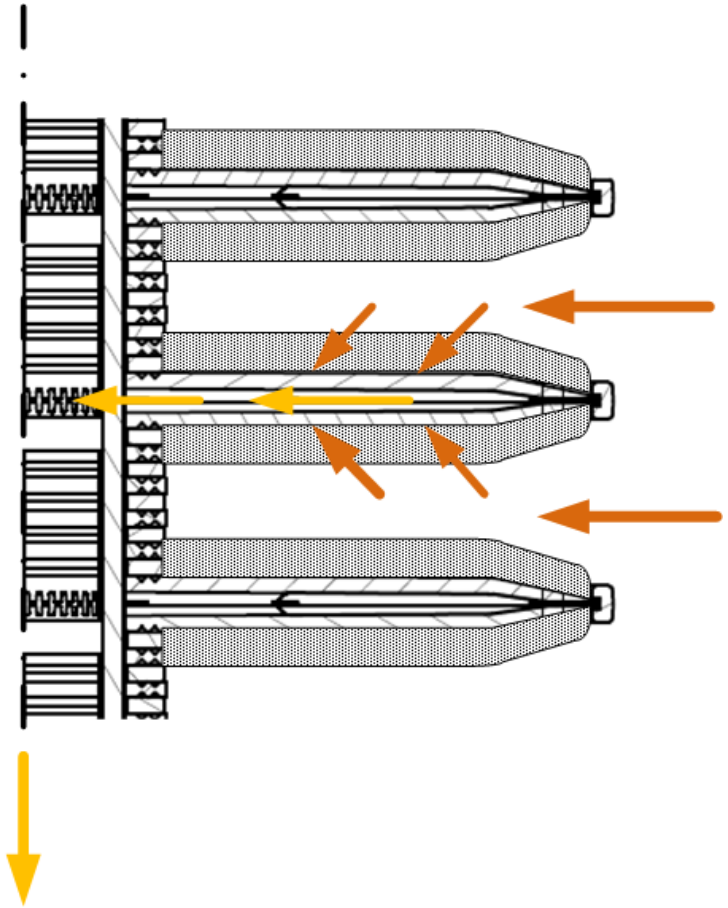
Filtrat (fine surface with laser marking)

VALIDATION

Material of Construction

Supporting filter sheets:	PURAFIX® low ion and low pyrogen containing filter sheets
Filter aid:	Pharma grade Diatomaceous earth Celpure®; DE is already part of some sheet types
Bag material:	Double layer PE material, already used for other single-use applications (e. g. Bioreactors)
Tube:	APSH - Platinum Cured Silicone
Other plastic parts:	USP class VI material (e. g. core body and drainage nets of the modules)
Powder transfer bags:	LDPE with Permanent Anti-Static Additive

FILTRODISC™ BIO SD: FILTRATION PRINCIPLE



FILTRODISC™ BIO SD SERIES

2"

5"

10"

12"

16"

LABORATORY SCALE SET-UP

Capsule test setup

Some important test parameters:

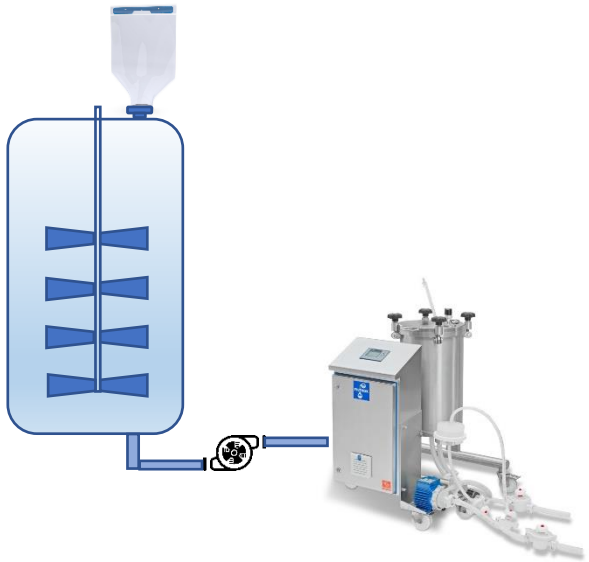
- Pump need to keep the flow even with increasing pressure
- Tube connection as short as possible
- Follow the instruction in the handbook or in the playbook alluvial filtration

POWDER TRANSFER

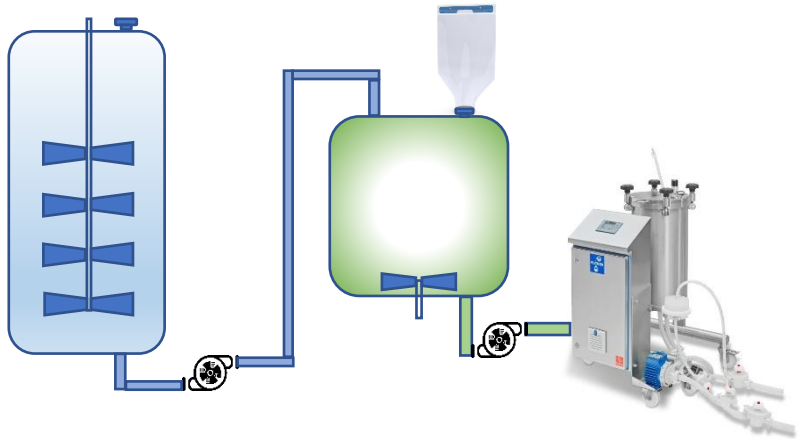
Powder Transfer into the Process

Direct mixing with fermentation broth / cell homogenate

1. Feeding filter aid into bioreactor
   (only if single-use bioreactor)



2. Transfer fermentation broth into mixing unit and feeding filter aid

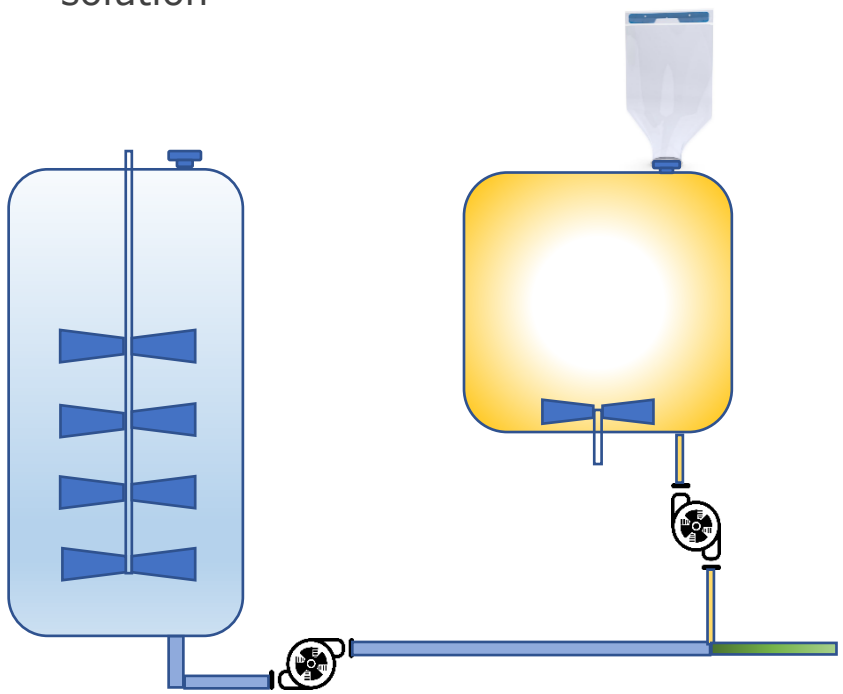


POWDER TRANSFER

Powder Transfer into the Process

Direct mixing with fermentation broth / cell homogenate

3. Body feed with a pre-prepared slurry solution



Mixing buffer with filter aid in a single-use mixer and add the solution into the line to the filter

CASE STUDY: VIRAL VECTOR CLARIFICATION

Background: Adeno-associated viruses (AAV) and lentiviruses are currently being developed for numerous indications in field of gene therapy.
→ First step in both direct and cell-based gene therapy = packaging a therapeutic transgene into a delivery vehicle (e. g. a viral vector), followed by expansion of host cell lines to produce high-enough vector concentrations