



FILTRODISC™ BIO SD

State of the art Midstream microfiltration e.g. for cell removal

MICROFILTRATION FOR VALUABLE LIQUIDS.

St. Gallen, March 1st, 2018, GMS

The FILTRON Group

The FILTRON Group is an international Group of Filtration Companies headquartered in St. Gallen, Switzerland

100 % privately owned

Founded 1938 as FILTRON Filter-Werke AG in the Sitter Valley, St. Gallen



2012 we were the first in the Industry who introduced body feed technology in single-use format for Midstream applications

Focused on depth filtration technologies in Pharma, Biotech as well as in Food & Beverage industries, single-use and not single-use





FILTROX WORLDWIDE



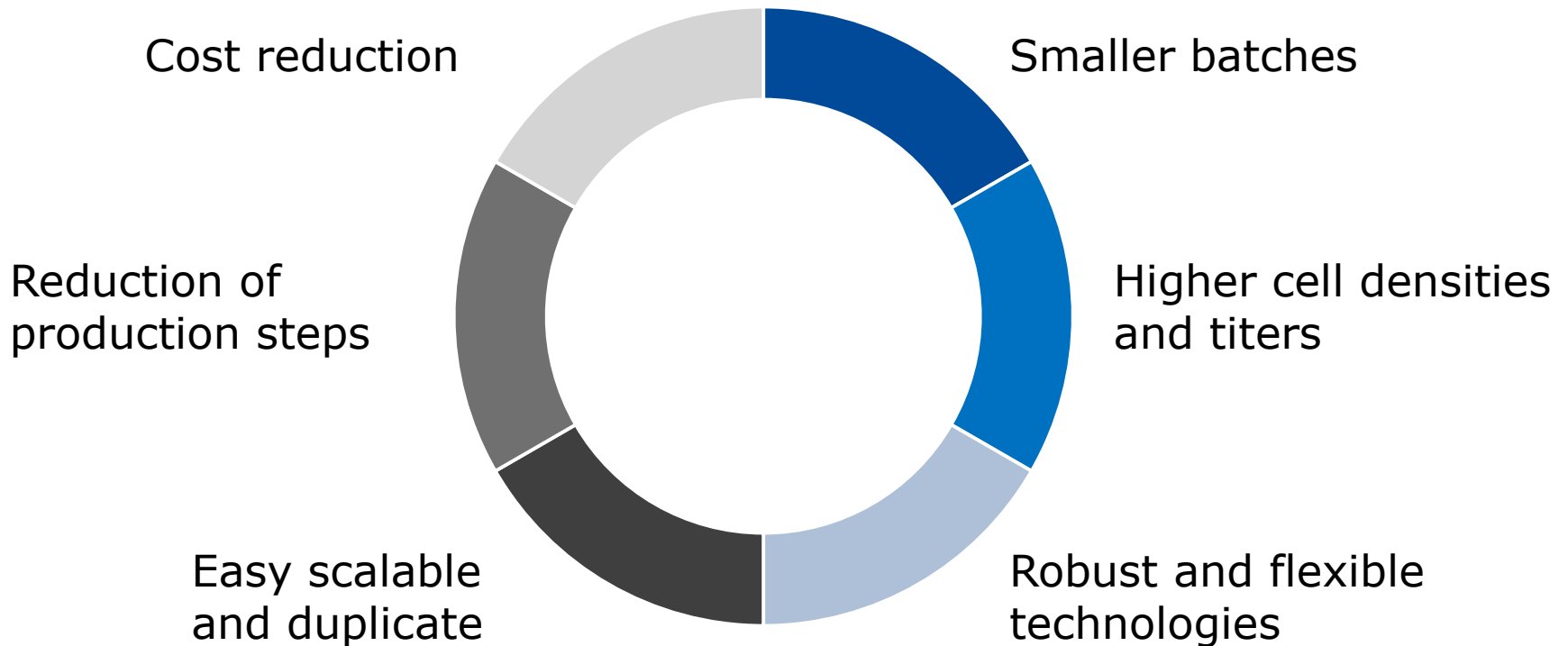
FILTRONDISC™ BIO SD

High performance
single-use microfiltration
system for Midstream
(e.g. cell removal) and
depth filtration applications



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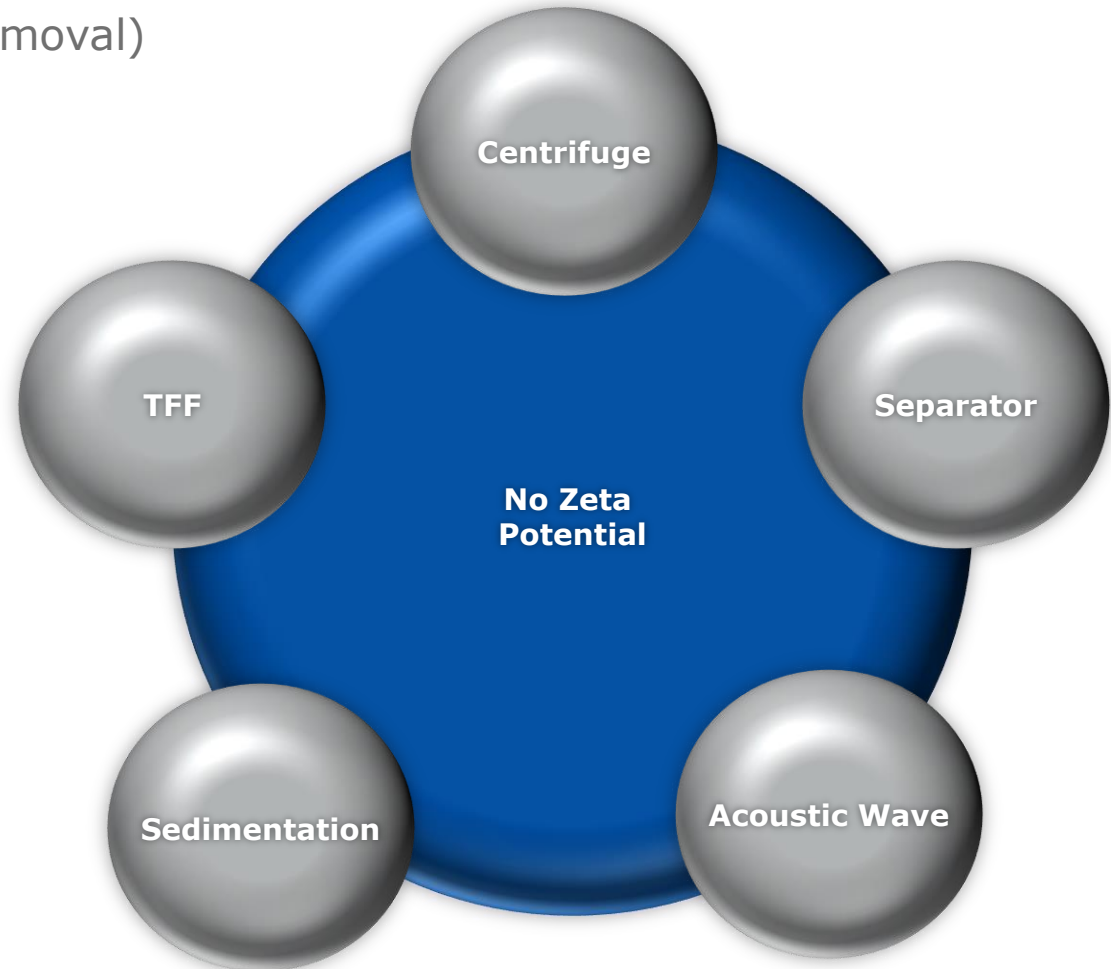
Development and challenges in Biomanufacturing



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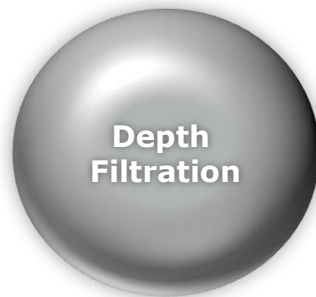
Solutions for Midstream (cell removal)

- High shear leads to additional cleaning (e.g. depth filtration)
- Difficult to develop and control
- Equipment and cleaning validation requirements
- Limited scalability
- High operation costs
- High investment costs
- High buffer and utility consumption
- Need time and in some cases additives (flocculants)
- So far only as lab scale
- Only for CHO cells



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Solutions for Midstream (cell removal)

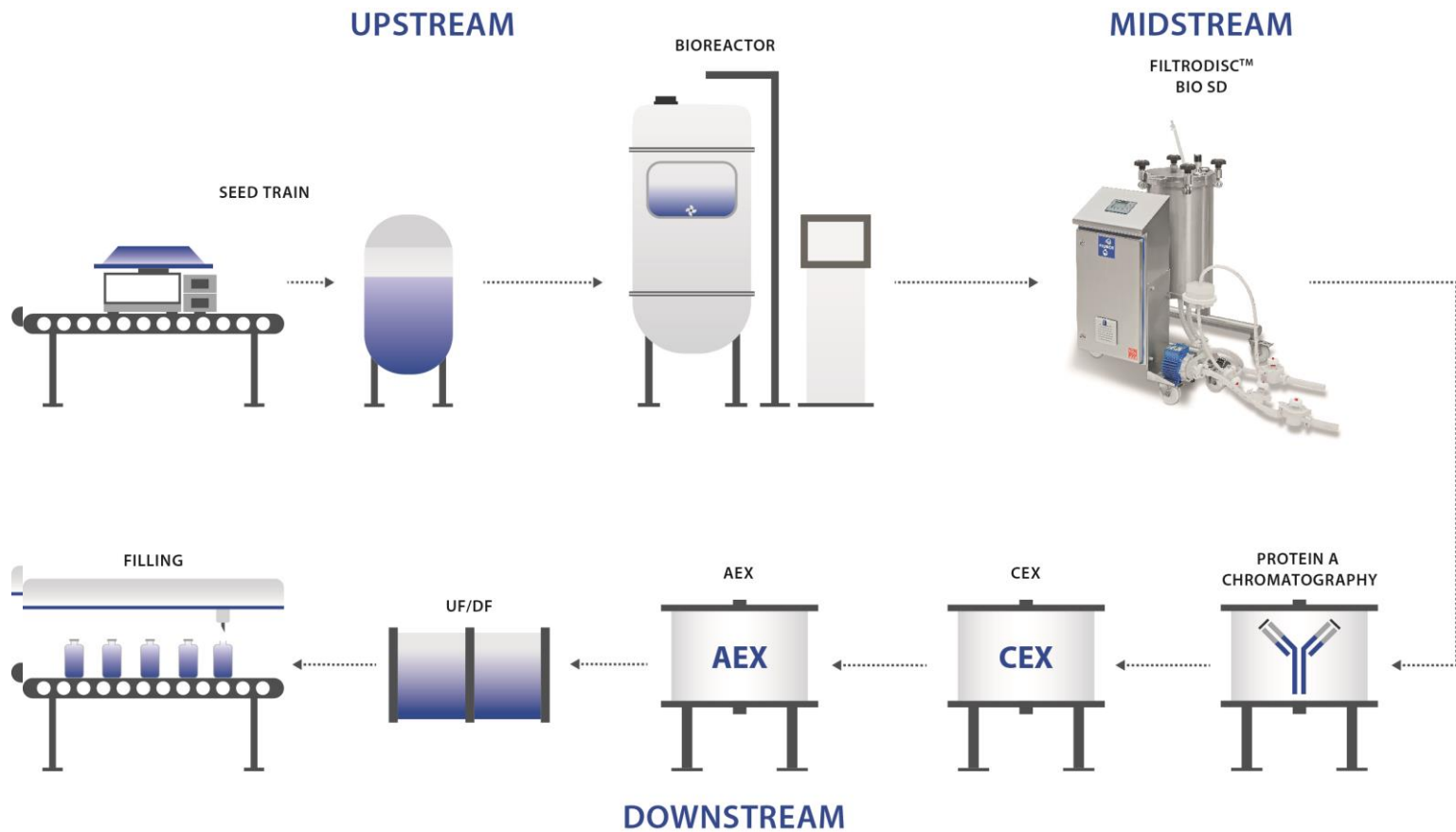


Depth filtration

- Capacity not high enough
- Easy to use and well known technology
- Easily and widely scalable
- Easy to develop and control
- No shear
- Single-use options available

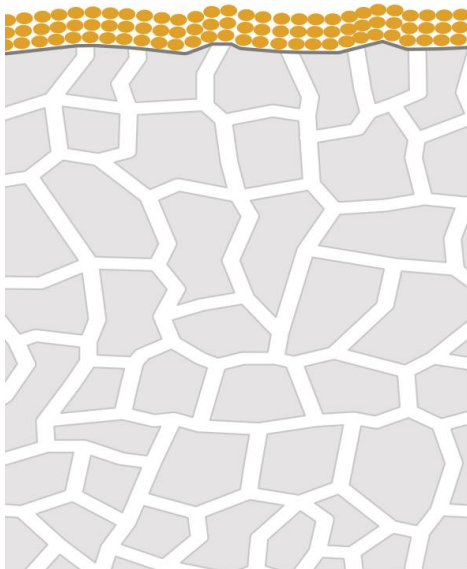
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Typical Bio-manufacturing Process



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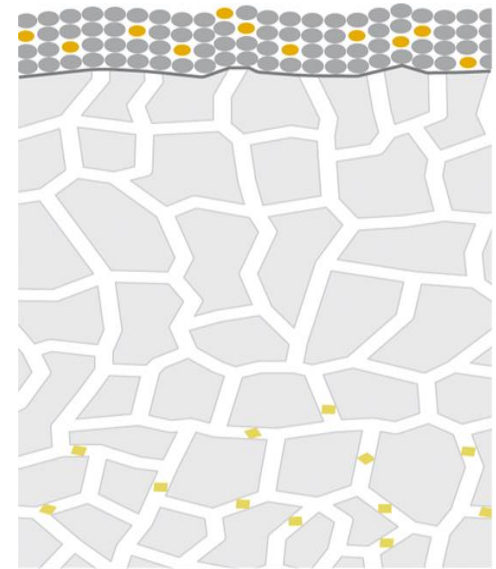
Depth Filtration Functional Principle



Surface filtration



Depth filtration

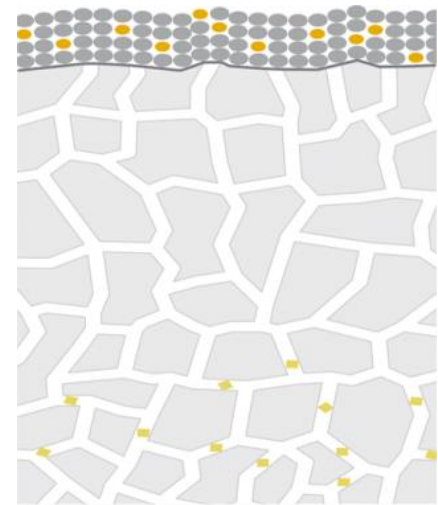


Alluvial filtration

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What means Alluvial Filtration?

- For decades an established technology in pharmaceutical filtration (e.g. plasma fractionation)
- Large capacity for high particle load (extend the filtration volume per area drastically)
- Long term experience
- Was not available as a single-use solution



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Filter aid / Diatomaceous Earth (e.g. Celpure® (Advanced Minerals Corporation))

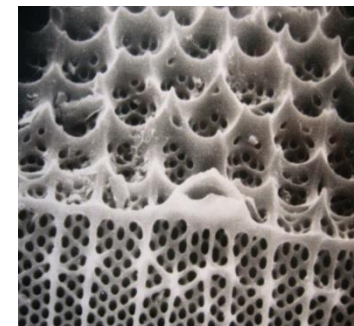


DE = Diatomaceous Earth = Kieselgur

- Inorganic filter aid
- Mineralized organisms (skeleton of diatoms)
- Pharma grade (Celpure®)
- Diatomaceous earth is like the Swiss army knife – the all-purpose tool for downstream processing (David Delvaile, Merck Serono France)



Grade	Permeability [mDarcy]	Solids removed* [µm]
C 65	40 – 80	0.3 – 0.45
C 100	70 – 140	0.3 – 0.45
C 300	150 – 300	0.45 – 0.6
C 1000	750 – 1250	> 1.0



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Filter aid / Cellulose (e.g. VIVAPUR® from JRS)



Cellulose

- Organic filter aid
- Microcrystalline Cellulose
- Pharma grade (e.g. VIVAPUR®)

Celpure® Grade	Cellulose grade
C 300	VIVAPUR 105
C 1000	VIVAPUR 101/102



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DE Function

- Clarification, already extensively used for highly turbid liquids (e.g. yeast removal in breweries) and well characterized in plasma fractionation
- Avoid clogging of depth filters
- Removal of impurities (HCP, DNA, Viruses, Endotoxin, ...)
- Can do all at the same time

Disadvantage

- Powdery material requiring closed system to avoid operator risk exposure (Powder transfer bags)



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BIO SD PTS / Powder Transfer System (EziDock™)

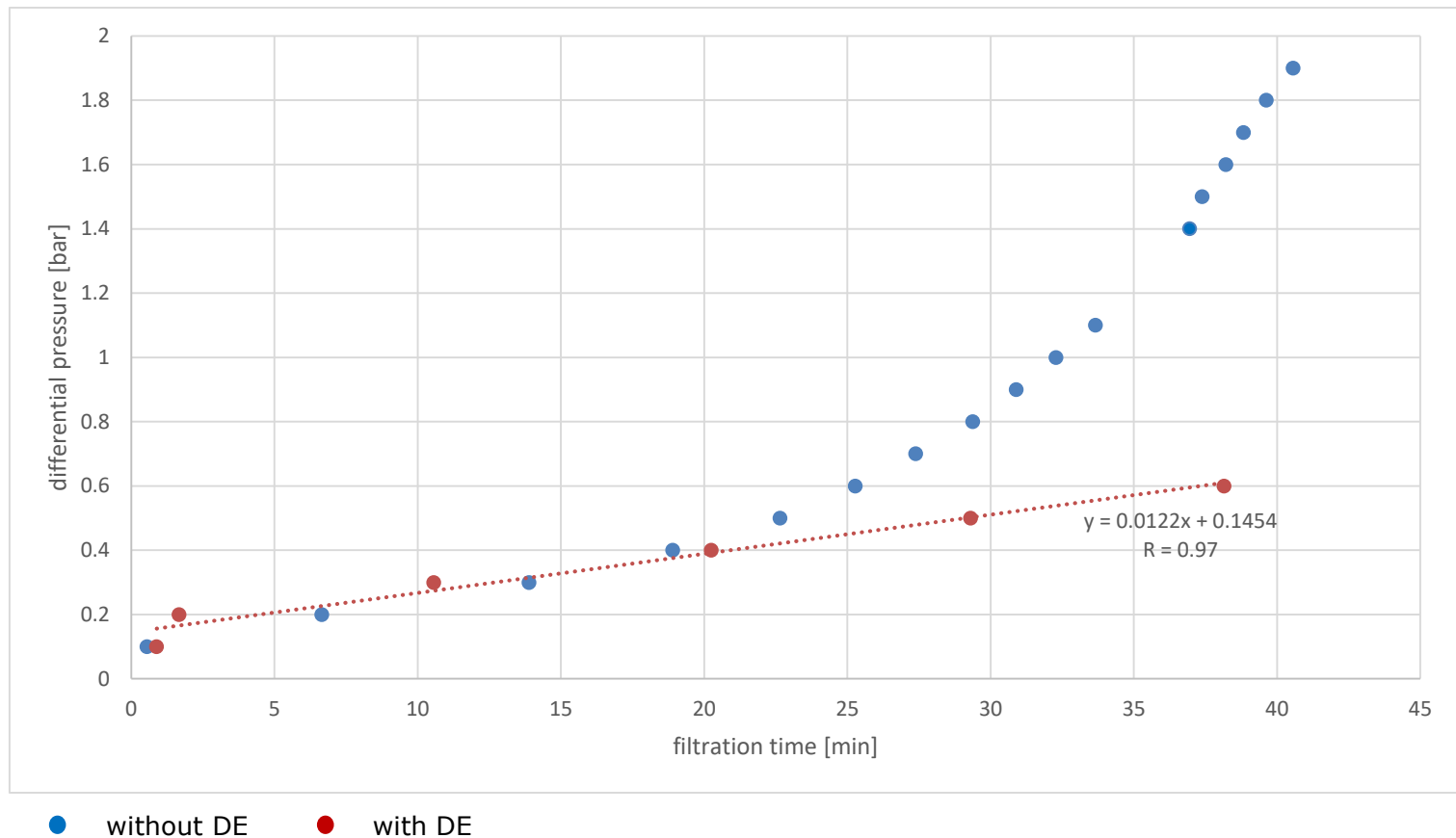
Capacity	Tri-Clamp Size
2L	2", 4" or 6"
5L	2", 4" or 6"
10L	2", 4" or 6"
15L	2", 4" or 6"
25L	2", 4" or 6"
40L	2", 4" or 6"



- <USP87> + <USP661> Compliant LDPE with Permanent Anti-Static Additive
- 2 Ply Body Construction
- 2/4/6/inch Tri-Clamp Ferrule - BS4825-3
- Individual Bag Label Showing Batch & Bag Number
- Available Gamma Sterilized
- Lower Transport & Storage Costs
- HDPE Blanking Caps available
- Handling aids available
- Comprehensive Range of Gaskets/Clamps available

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Depth Filtration vs. Alluvial Filtration / Clogging Curve



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Product Range / Disposable Polymer Housing with Single Disc

2" capsule



Batch range [l]	Up to 2
Cake volume [l]	0.06
Filter area [m ²]	0.002
Application	Proof of technology, evaluation of DE grade

5" capsule



Batch range [l]	Up to 10
Cake volume [l]	0.38
Filter area [m ²]	0.0127
Application	Evaluation of 2" results, Small pilot scale

Batch range and max. cake volume depends on amount of cells / particles and DE

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Product Range / Lab scale Test Kits



2" FILTRODISC™ BIO Kit

- Proof of technology
- Evaluation of DE grade
- Optimization work
- 10 capsules
- 4 Celpure® grades (C1000, C300, C100, C65)

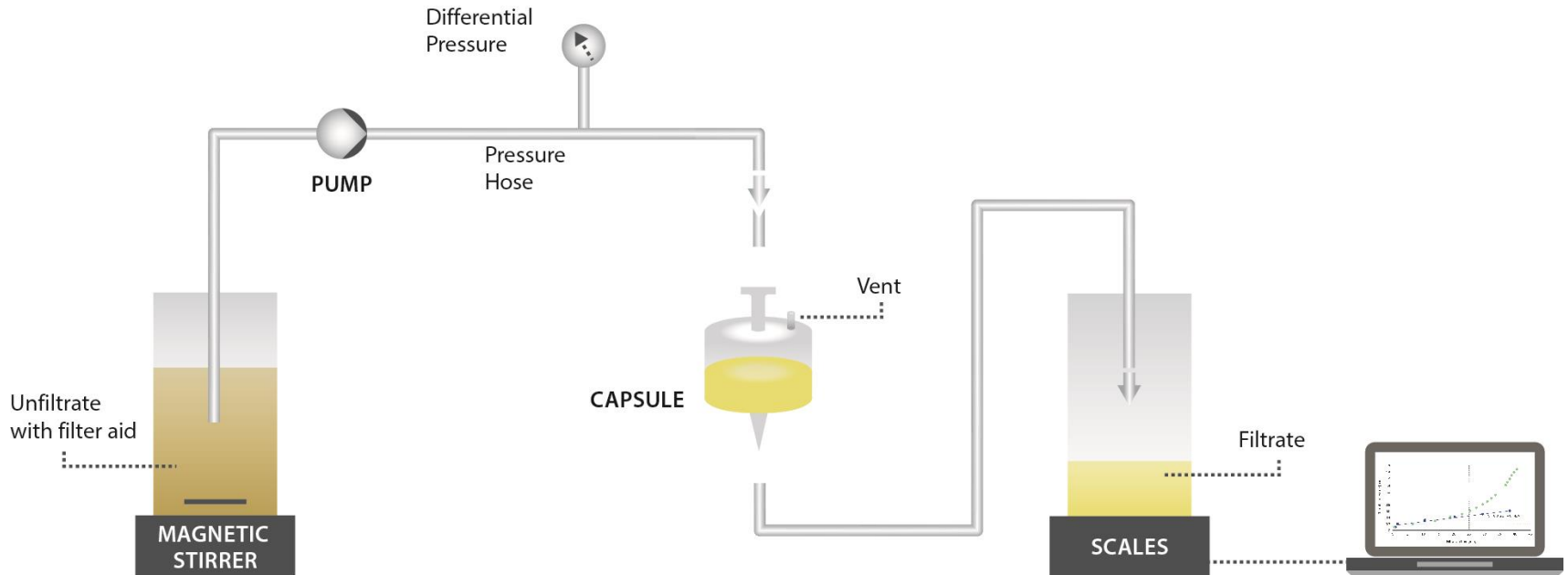


5" FILTRODISC™ BIO Kit

- Up to 10 l small pilot scale
- 3 capsules
- 1 Celpure® grade

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Product Range / Lab scale Test Kits



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Product Range / Lab scale Test Kits

Evaluation of
DE grade

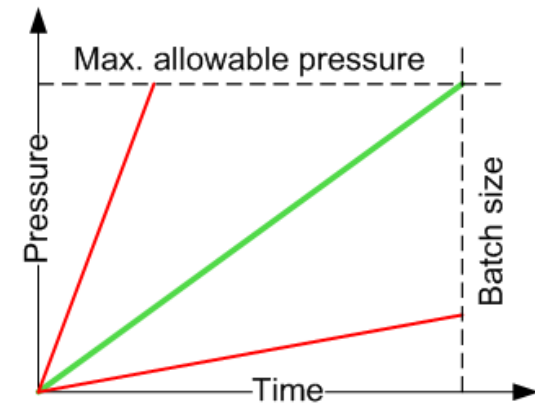


Low
Turbidity

Optimization
of dosage



High
Efficiency



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Product Range / Disposable modules in bag, in stainless support housings

12" short module



Batch range [l]	Up to 100
Max. cake volume [l]	4.8
Filter area [m ²]	0.33 (up to 0.675)
Application	Pilot scale, small production scale

12" single module

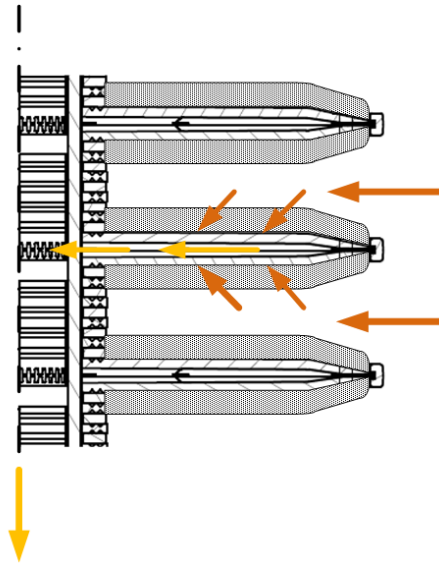


Batch range [l]	Up to 200
Max. cake volume [l]	11.2
Filter area [m ²]	0.55 (up to 1.8)
Application	Bio process scale

Batch range and max. cake volume depends on amount of cells / particles and DE

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Product Range / Pilot scale – 12" short module

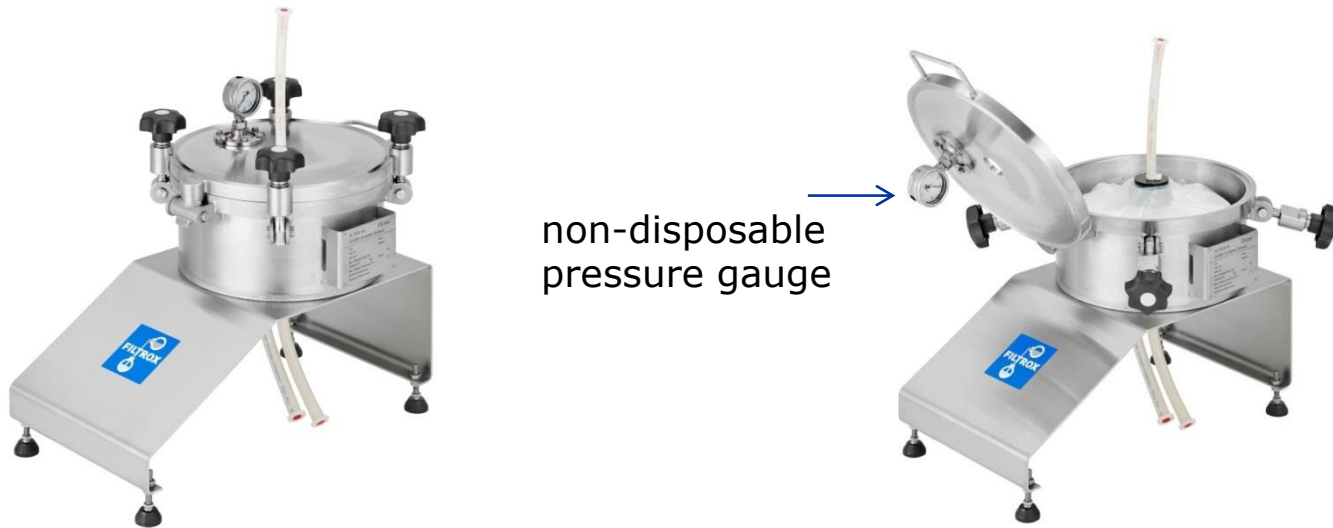


Module with 2 or 3 lenses (up to 6 possible)

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Product Range /

Pilot scale – 12" short module stainless steel support vessel



Designed according to PED 97/23/CE; Max. operating pressure 2.5 bar
Test pressure 8.3 bar → high safety factor

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Product Range / Disposable modules in bag, in stainless support housings

12" double module



Batch range [l]	Up to 500
Max. cake volume [l]	22.4
Filter area [m ²]	1.1 (up to 3.6)
Application	Bio process scale

16" double module



Batch range [l]	Up to 1000
Max. cake volume [l]	46.1
Filter area [m ²]	2.26 (up to 7.2)
Application	Bio process scale

Batch range and max. cake volume depends on amount of cells / particles and DE

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Product Range / Bio Process scale – 12" and 16" double module

Module with 8, 10 or 12 lenses (up to 16 possible)



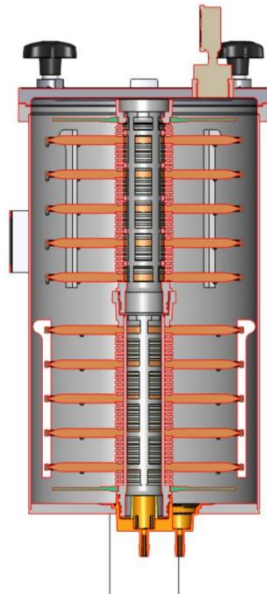
Multilayer plastic film with:

- PE contact layer to minimize extractables
- EVA layer as gas barrier
- PA layer for high mechanical performance

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Product Range /

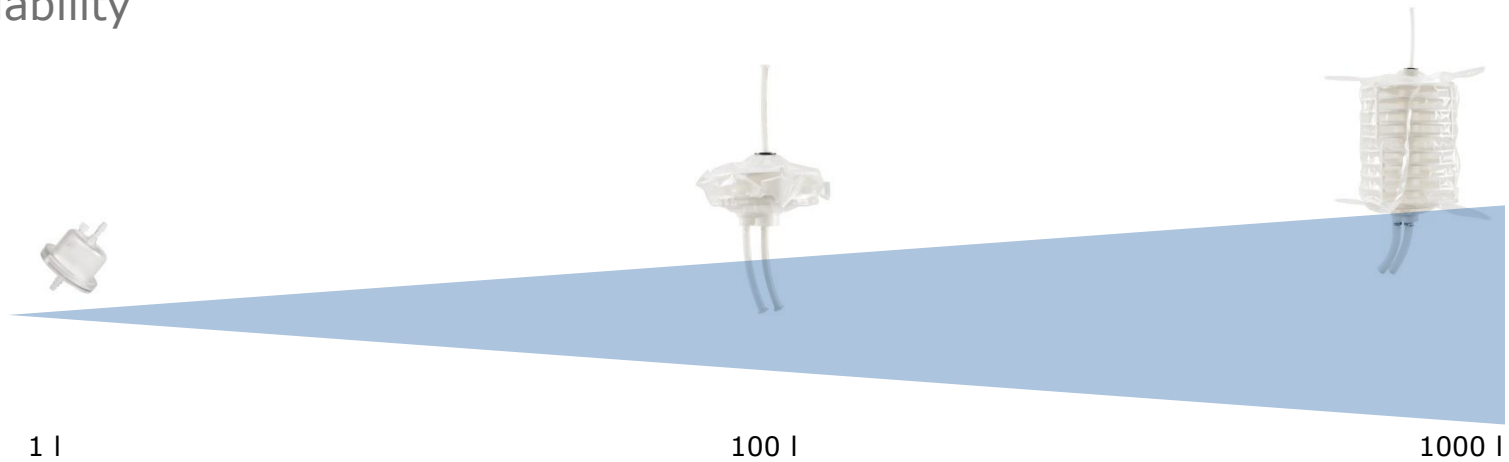
Bio Process scale – 12" and 16" double module stainless steel support vessel



Tilting housing with trolley for easy discharge

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Scalability



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

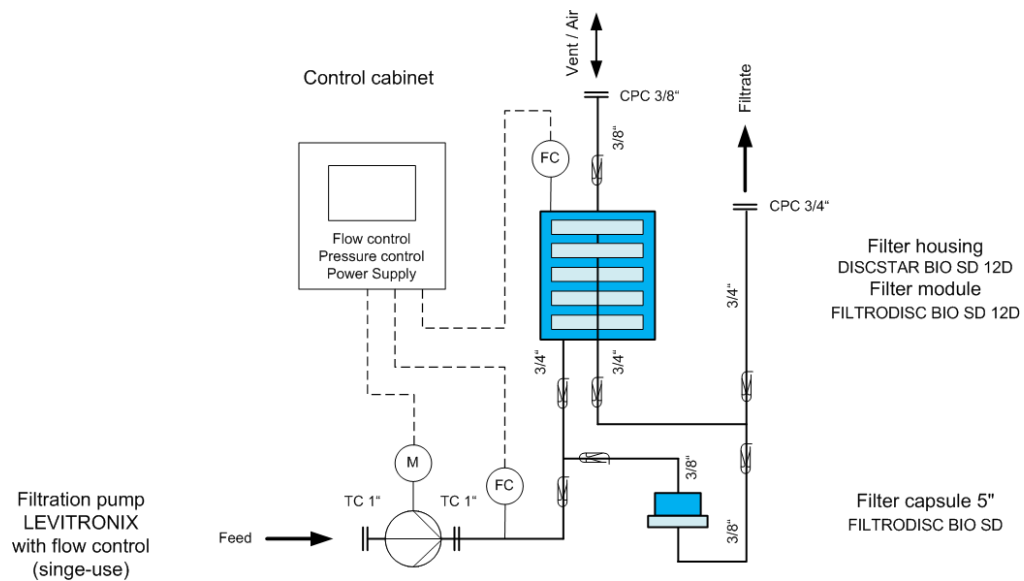
$$C = h \times A$$

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

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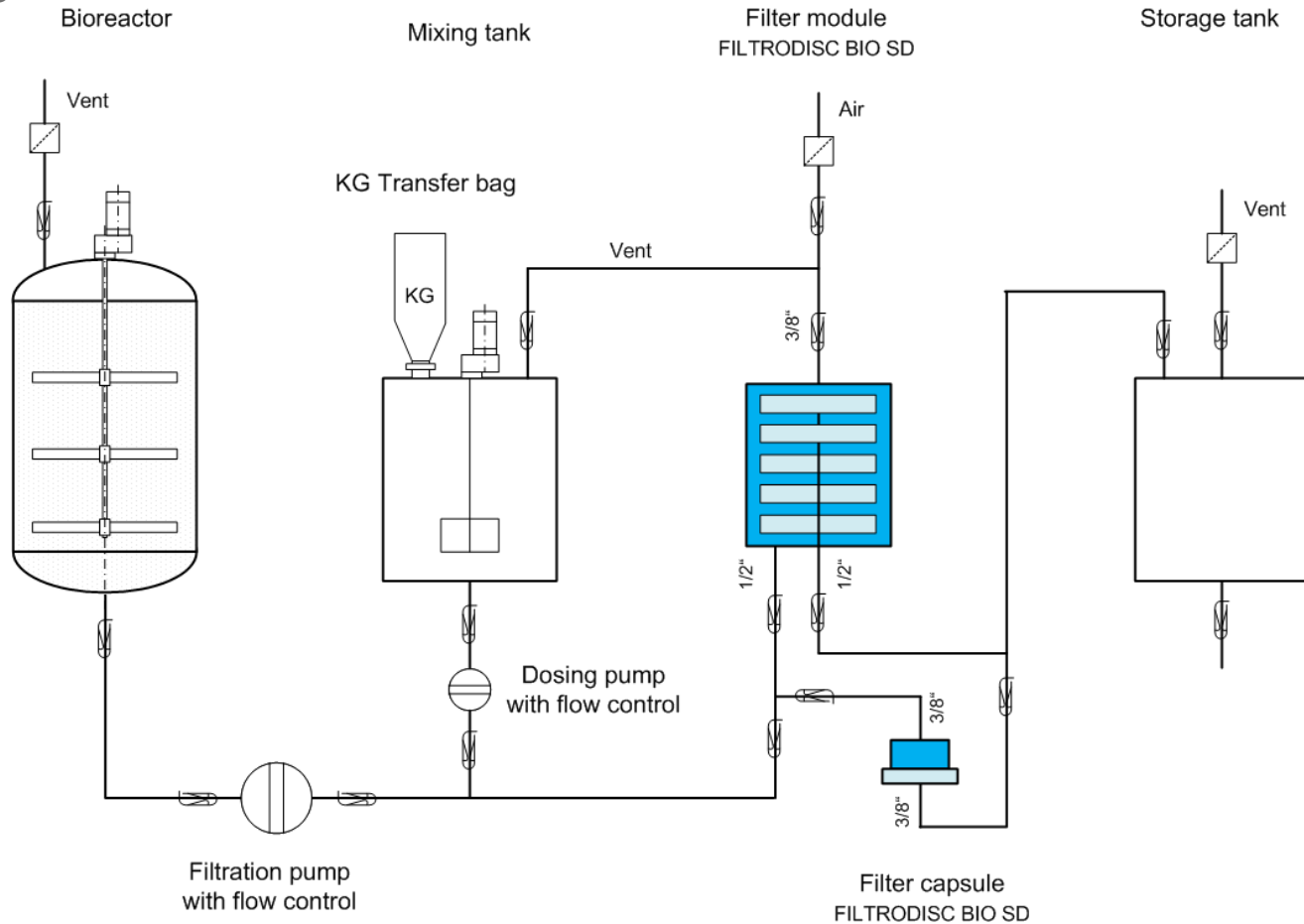
Product Range / Bio Process Skid

Bio Process scale – 12" double module stainless steel support vessel incl. pump and control unit



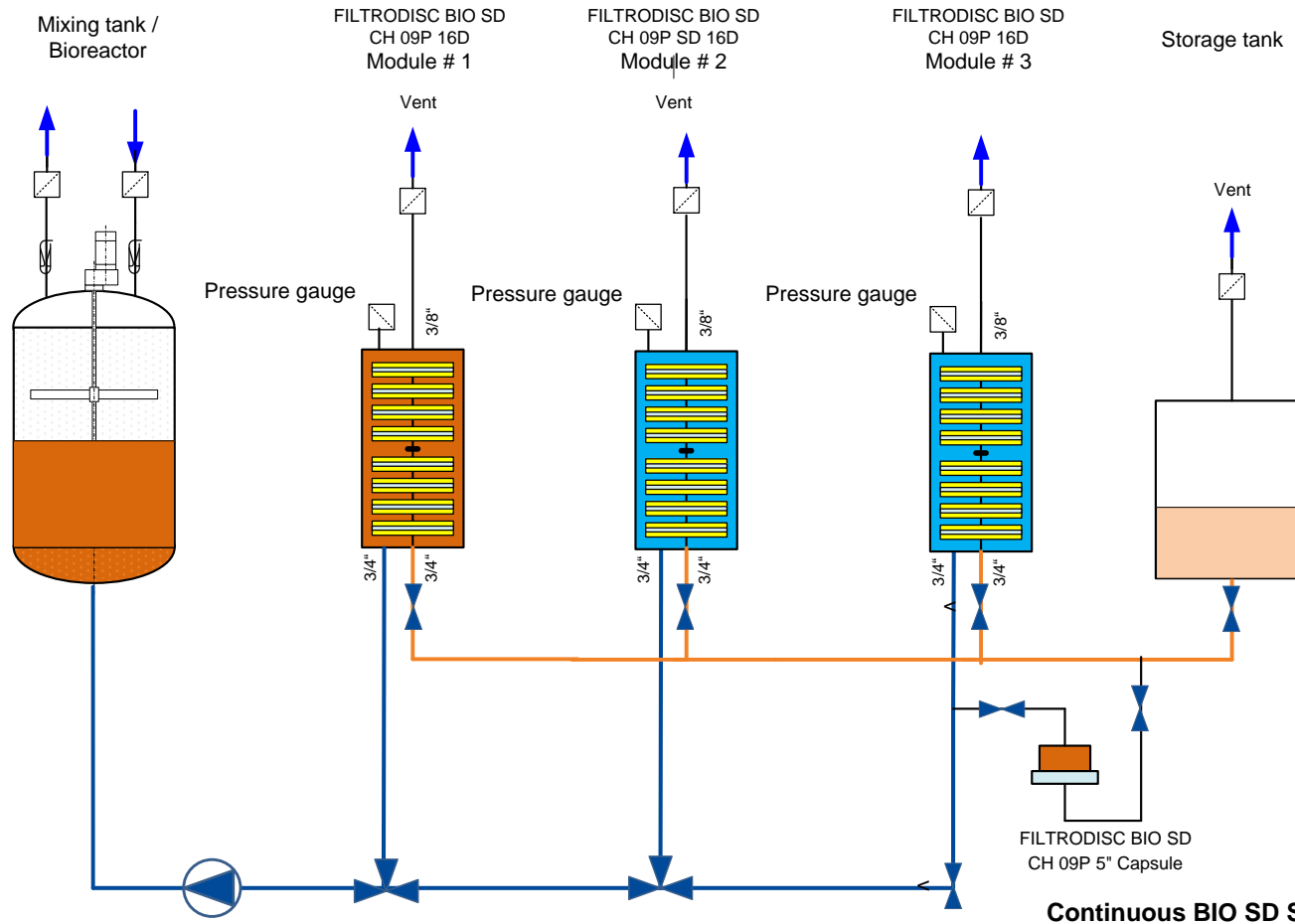
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Plant Configuration



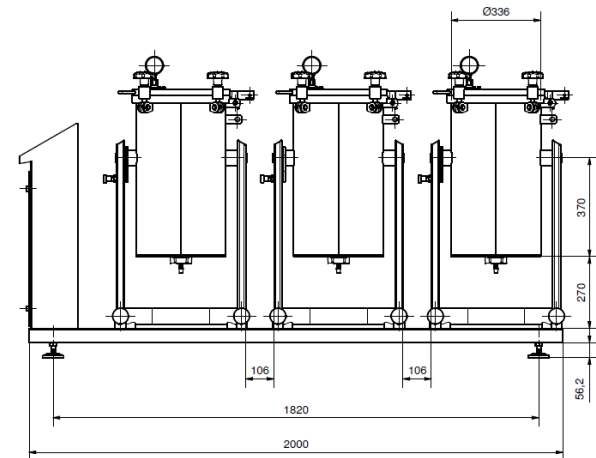
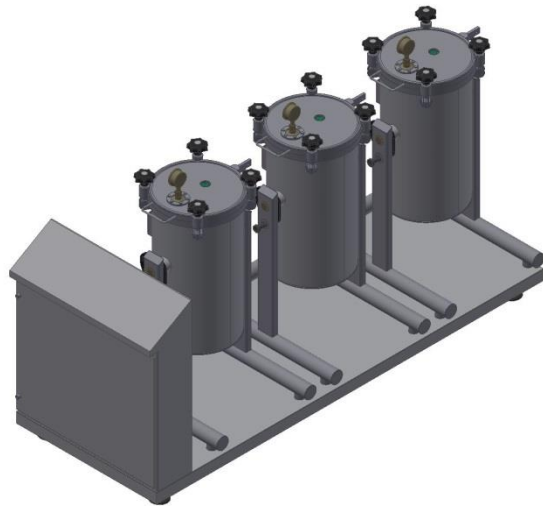
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Continuous solution for bigger production scales



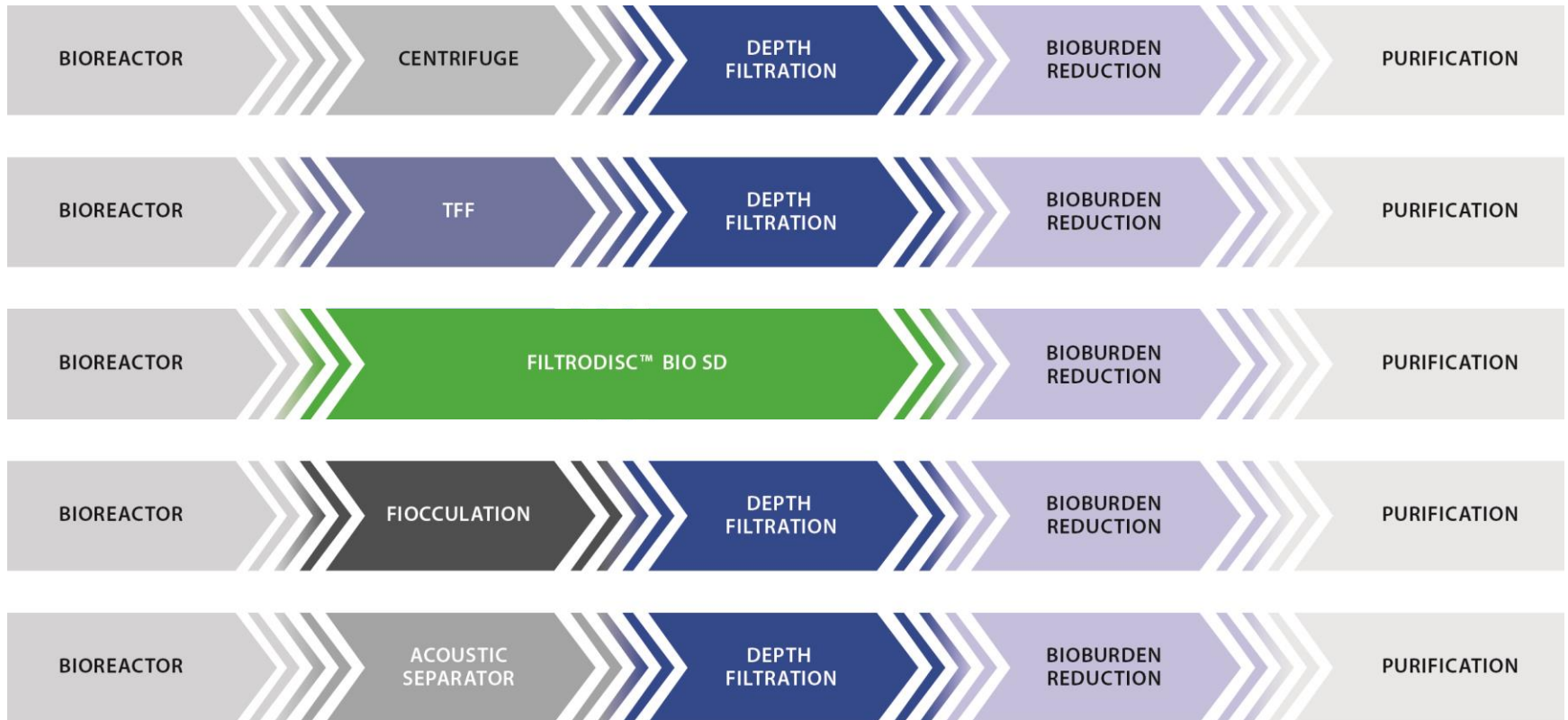
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Continuous solution for bigger production scales



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Typical set up Mammalian cell culture Midstream clarification



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Powder Transfer / Handling

Capacity	Tri-Clamp Size
5L	2"
5L	4"
15L	2"
15L	4"
30L	2"
30L	4"
50L	4"
50L	6"
100L	4"
100L	6"



50 L bag
with 10 kg DE



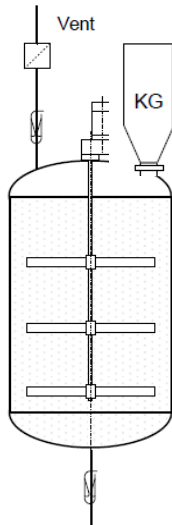
Dust free powder transfer !

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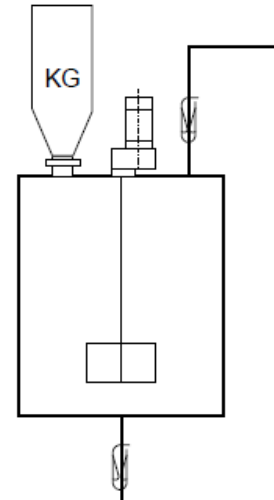
Powder Transfer into the Process

Direct mixing with fermentation broth / cell homogenate

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

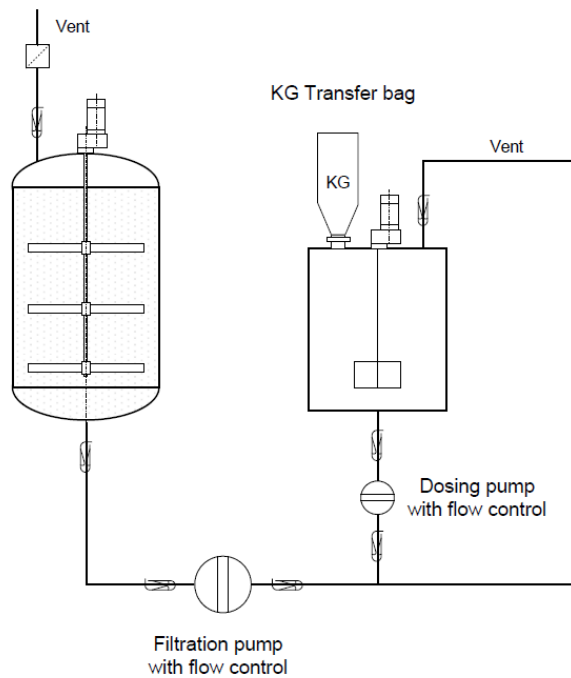


2. Feeding mixing unit with filter aid
and fermentation broth



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Powder Transfer into the Process Body Feed



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

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Material Validation Aspect

All materials involved are already part of the process:.

- Supporting sheets → PURAFIX® low ion and low pyrogen containing filter sheets (Validation guide for sheets and modules available)
- Inorganic filter aid → Celpure®; filter aid is already included in the filter sheet (depending on the grade) (Validation guide available)
- Bag material → extensively used for single-use storage tanks or bioreactors; very well known supplier (Validation guide available)
- Plastic parts → all disposable material USP class VI (e.g. core body of the modules)

→ No material validation risk!

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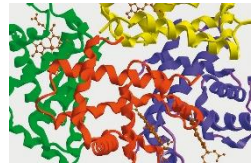
Applications



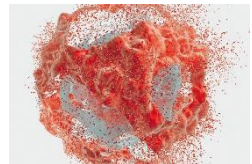
Plasmid-DNA



mAB`s

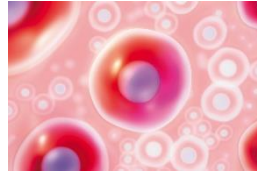


Proteins



Cell debris

Removed Organisms



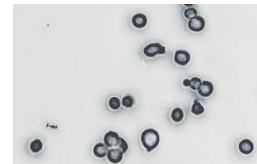
Mammalian cells (CHO)



Bacteria (E.coli)



Yeast (Pichia)



Insect cells

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Clarification Experiments with CHO cells optimized for HCP removal
 Experimental conditions

Molecule	Antibody	FC-Fusion
Cells	CHO cells	
Max. cell density	3-5 x 10 ⁶ cells/ml	8-10 x 10 ⁶ cells/ml
Clarification	Depth filter without and with DE alluvial filtration	
Process	<ol style="list-style-type: none"> 1. Filterability and optimization at 2" scale 2. Upscale and 2" result verification at 50L bioreactor scale 	

(Trails at: MerckSerono, David Delvaille 2010)

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Filterability data at 2" scale

Produced molecule: Antibody

Condition	Filterability [L/m ²]	HCP removal
Harvest with optimized depth filtration	376	8%
Harvest with depth filtration DE filter aid	500	12%
Harvest with depth filtration and optimized DE conditions (amount and grade)	676 (+80%)	33%

Product yield after filtration: 98%

Impurity removal: up to 50%

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Filterability data at 2" scale

Produced molecule: FC-Fusion protein

Condition	Filterability [L/m ²]	HCP removal
Harvest with optimized depth filtration	192	0%
Harvest with depth filtration and DE filter aid	313	0%
Harvest with depth filtration and optimized DE conditions (amount and grade)	556	39%
Harvest with depth filtration and optimized DE grade mixture conditions (amount and grade)	455	42%

Product yield after filtration: 98%

Impurity removal: up to 50%

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Clarification Conclusion

DE is efficient enough to clarify in a single step high cell density fermentation broth

- Filterability achieved using 2" scale experiments
- Scale up successfully done at 50 L scale

Reduced filter area for clarification

- The filter sheet is only used to retain the DE, the cells removed by the DE
- Efficient for 10 µm and/or 0.2 µm membrane filtration afterwards
- Reduces cost for clarification: less surface needed, eliminate one step (centrifugation) and raw material is cost effective (50 € / kg)

Can remove up to 50% of the impurities during clarification

- HCP removed 33% up to 42%
- DNA removal is also possible (no data)

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Scalability Experiments

Initial situation:

- Cell culture:
 - TCD: 4.4×10^6 cells
 - NTU: 690
- 2" FILTRODISC™ BIO SD CH 9P capsules (0.0020m²)
- Filter aid: Celpure®
- Min. specific flow rate: min. 300 -350 l/m²*h (process filter)
- Min. effective flow rate: min. 10 – 11.7 ml/min (2" capsule)



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Scalability Experiments

Scale up trial:

- Batch size: 600 l
- Filter aid: Celpure® C1000
- Dosage: 8 g/l
- Wet density of DE: 3.9 l/kg

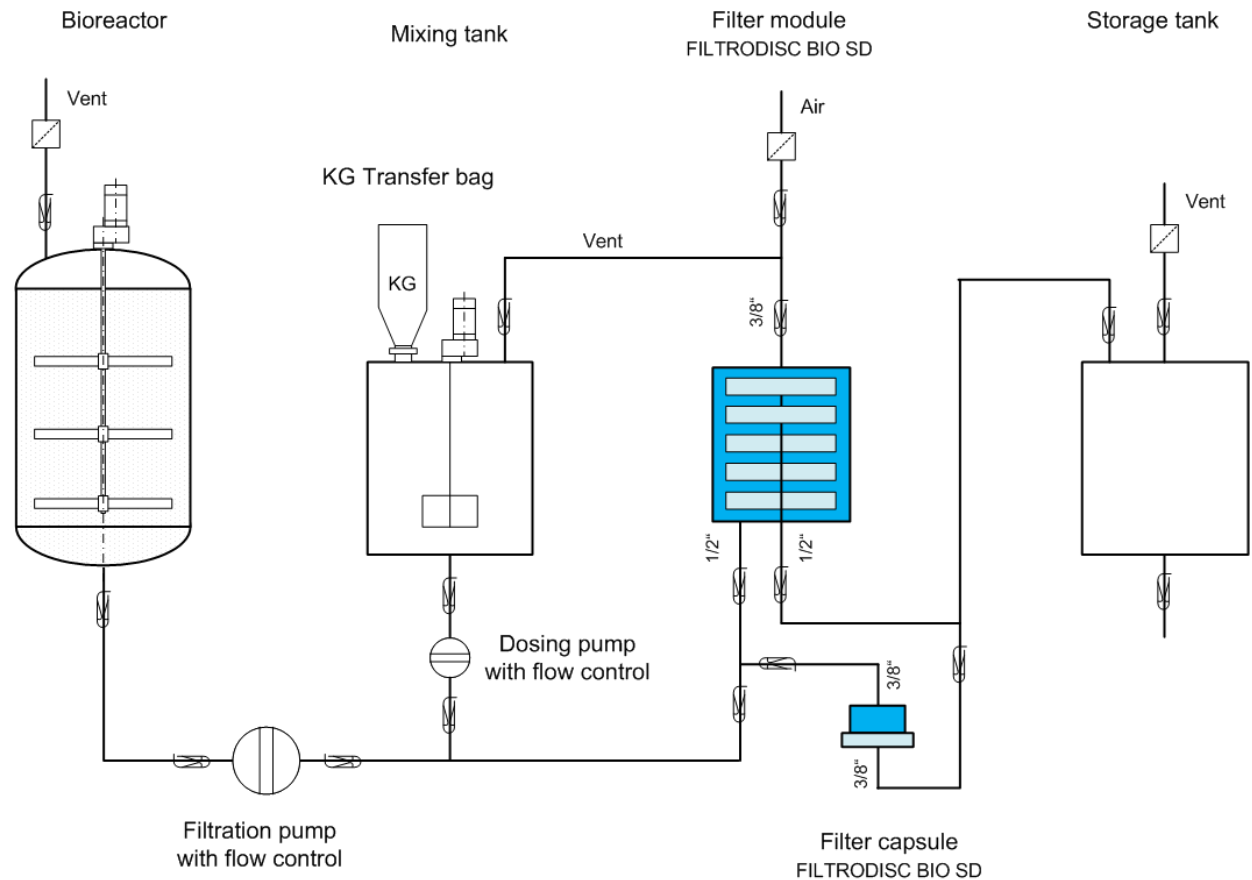
Due to this data a FILTRODISC™ BIO SD 12" double module was chosen:

- 5 lenses per module (overall 10)
- Max. holding volume: 19.8 l
- Max. amount of DE: 5.1 kg



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Scalability Experiments Pilot scale set up



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Scalability Experiments
Pilot scale set up

Experimental set-up

- Bioreactor
- Pump
- Mixer
- Pump
- Filter



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Scalability Experiments

Procedure:

- Flush the filter with water / buffer at 1 bar, afterwards keep the liquid in the filter
- Meanwhile transfer the fermentation broth into a mixer
- Add 4.8 kg Celpure® C 1000 (= 8 g/l)
- Mixing approx. 10 min, keep in motion afterwards
- Remove liquid from filter
- Start filtration with a min. flow rate of 300 - 350 l/m²*h
- Remove rest liquid with air or gas after filtration



Result:

- NTU reduction from 690 down to 3.5

Scale up works perfectly



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Influence of filter sheet type

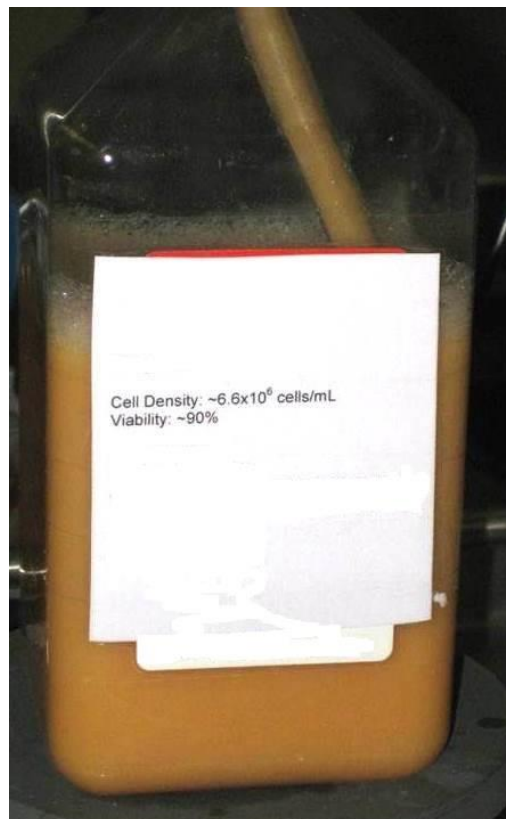
Conditions			
TCD: 6×10^6 cells/ml			
Viability: 90%			
Turbidity: 295 NTU			
DE grade: Celpure [®] C1000			
Dosage: 25 g/l			
	Trial 1	Trial 2	
Filter sheet grade	CH 09P	CH 133P	
Average flux [$l/m^2 \cdot h$]	648	420	
Turbidity after filtration	3.8 NTU	1.5 NTU	

A = coarse filter sheet (CH 9P, 30 – 10 μ)

B = fine filter sheet (CH 133P, 0.6 – 0.4 μ)

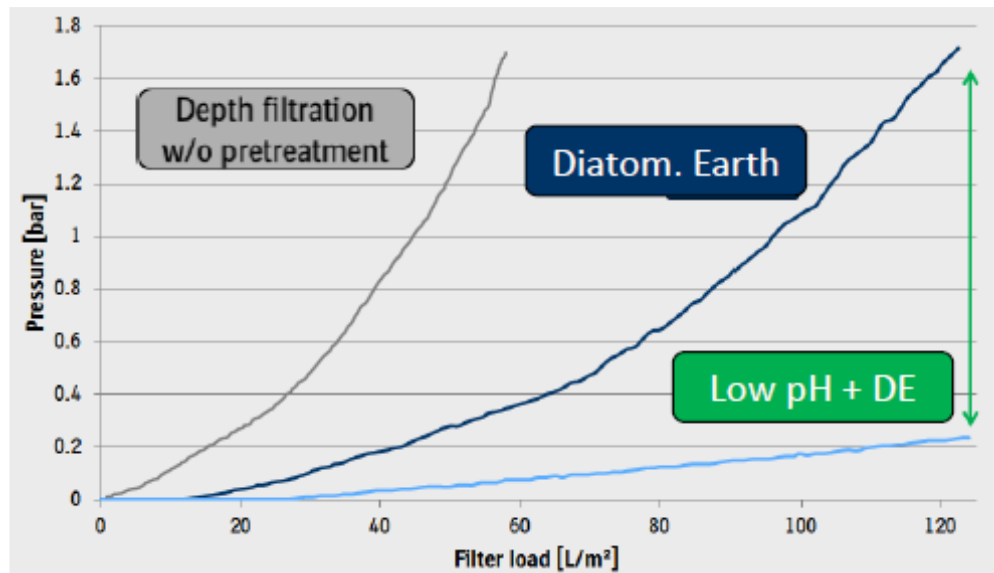
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Before and after filtration



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Comparison DE vs. DE and acid precipitation



Data provided by Markus Brakel, Boehringer Ingelheim, BioProduction 2017 Dublin

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Test set up



Pichia yeast trials

Starting conditions:

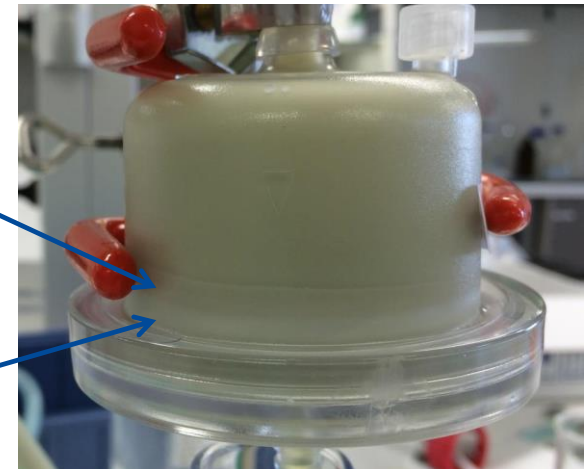
- NTU: ~40.000
- Cell amount: ~1.25 x 10⁹
- Celpure® grade: C300 and C1000

Process conditions:

- DE amount: 45 g/l
- Precoat: 10g/l – 20g/l
- Flow: 3 – 5 ml/min

Filter cake

Precoat



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Pichia yeast trials

Result:

- **Reducing NTU from ~40.000 to ~20 in just one step**
- Overall product lost was 10%
- C300 will reduce the yield more than C1000

Conclusion:

- Still need work on flow (in this case it was not so important because the production scale is small enough to work with a 5" capsule)
- The FILTRODISC™ BIO SD system works with Pichia yeast but it needs more optimization work than e.g. CHO cells or bacteria

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Cell debris removal

Starting conditions:

- Recombinant E.coli, producing cytochrome P450 enzyme
- Efficiency of rupture: $\geq 99\%$
- Concentration of debris after rupture: ~ 25 g/l (wet biomass)

Process conditions:

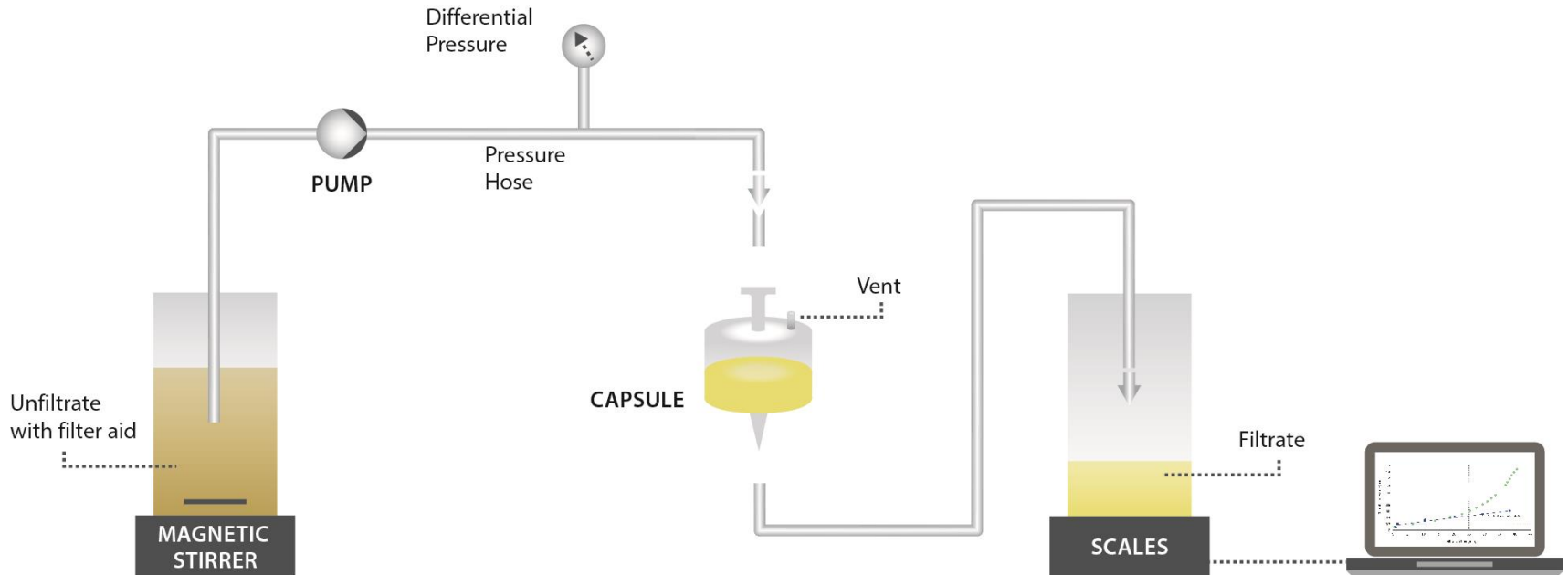
- Volume flow: min. $300 - 350$ l/m² * h (= 10 – 12 ml in 2" capsule)
- Pressure max.: 2.5 bar
- Turbidity measurement: OD₆₀₀

Optimization work on:

- Volume flow
- Filter sheet and filter aid grade
- Concentration of filter aid
- Precoat or standard alluvium filtration

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Cell debris removal
Test set-up



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Cell debris removal

Result overview:

- Selection of filter aid based on OD_{600} of the filtrate:
 - PURAFIX® CH 73P, Celpure® C65 = OD_{600} 0.056
- Selection of filter sheet grade based on OD_{600} of the filtrate:
 - PURAFIX® CH 103P, Celpure® C65 = OD_{600} 0.183
- Influence of precoat filtration:
 - PURAFIX® CH 103P, Celpure® C65 20 g/l + 20 g/l in the cell debris suspension =

$$OD_{600} = 0.004$$

FILTRODISC™ BIO SD

Advantages of FILTRODISC™ BIO SD

- Flexibility
 - Space for filter cake / Filter area
 - Connectors (fits to all single-use systems)
 - Retention rate
- Very small dead volumes
- Small footprint (1 meter square)
- High supply safety (three production plants)
- Short lead-times (normally 6 – 8 weeks)
- Widely scalable (process development to production also non single-use systems)
- Well know and accepted technology (no validation risks)
- Removes cells and impurities in one step
- No need for pH changes nor flocculants

FILTRODISC™ BIO SD

Advantages of FILTRODISC™ BIO SD

- Variable operation possibilities (alluvial filtration, sheet filtration)
- Reduce overall costs and cost of goods
- Reduce cross contamination / Eliminates cleaning validation
- Reduce downtime
- Available as a skid version (incl. pump and control unit)

- **First in the life science industry to introduce body feed filtration for Midstream applications in a single-use format**

FILTRODISC™ BIO SD



FILTRODISC™ BIO SD

Diatomaceous earth is like the Swiss army knife –
the all-purpose tool for downstream processing

(David Delvaille, Merck Serono France)

It is like: **Build your own depth filter**

(comment from a famous US Biotech company)



SWISS QUALITY.
SINCE 1938.



Let us solve your filtration task!

MICROFILTRATION FOR VALUABLE LIQUIDS.