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Process intensification in biomanufacturing driven by advances in single use technologies

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Process Intensification in Biomanufacturing driven by Advances in Single use Technologies

Stefan Schmidt, Head of Operations/COO

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Theory and Practice of Process Intensification

Examples USP

Examples DSP

Future strategies

Summary

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BioAtrium AG: Company overview

- Joint venture between Lonza and Sanofi, established in February 2017
- New company with its own legal entity, manufacturing license and facility
- Co-located at Lonza's IBEX campus in Visp, Switzerland
- Shared investment/risk/capacity by Lonza and Sanofi

- Phase A: Building with 2x20kL mammalian Bioreactors, 1 DSP train
- Expected 1st Engineering batch : Q4 2020

- Phase B: Extension by 2x20kL mammalian Bioreactors, 1 DSP train
- Management by 4 persons, workforce (~380 FTE at full capacity) seconded by Lonza

BioAtrium: Construction site in Visp, Switzerland

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Different biotechnologically manufactured chemicals/products

Different targets for process intensification

Market demand and production scale

Selling price/manufacturing cost per kg

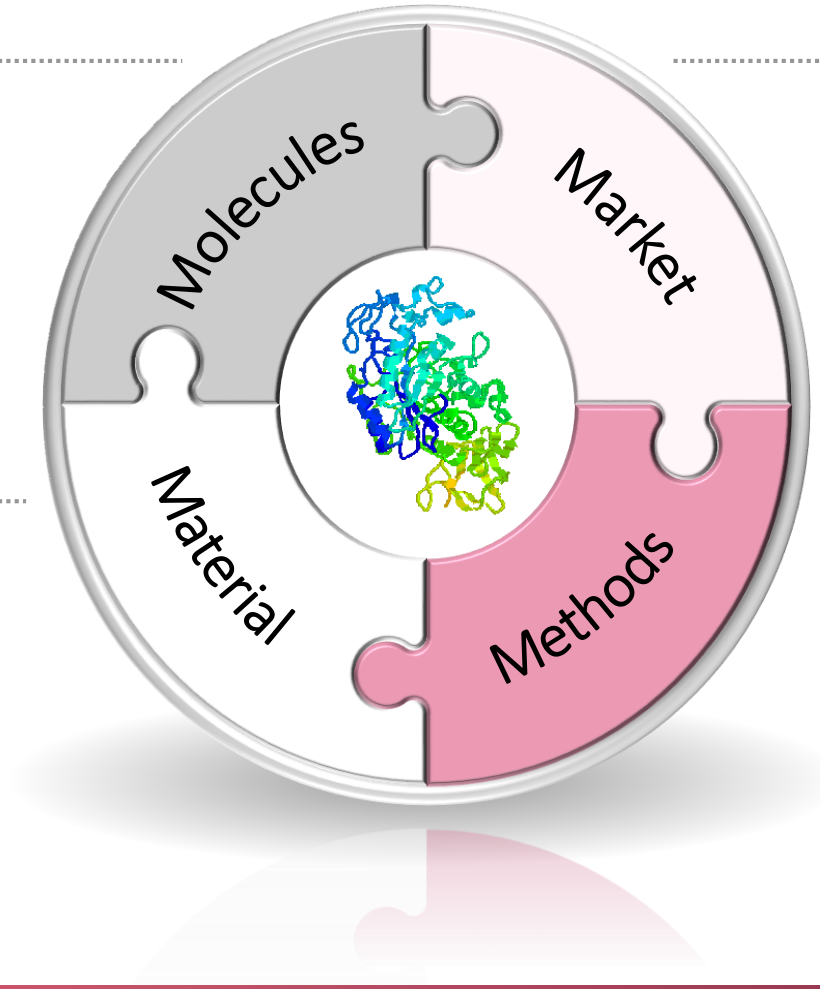
Bulk chemicals	Specialty chemicals/products	Fine chemicals/products	Pharmaceuticals
- citric acid - 1,3-propane-diol	- polymers (building blocks) - enzymes (e.g., lipases esterases)	- flavor and aroma compounds (e.g., menthol) - vitamins - nutrient additives	- antibiotics - therapeutic proteins (e.g., antibodies) - erythropoietin
Titer >100 g L^{-1} STY >10 g L h^{-1} Cost <1 € kg^{-1}	Titer 50-100 g L^{-1} STY 5-10 g L^{-1} h^{-1} Cost 1-10 € kg^{-1}	Titer 1-50 g L^{-1} STY 1 g L^{-1} h^{-1} Cost 10-100 € kg^{-1}	Titer max 5 g L^{-1} STY 0.1 g L^{-1} h^{-1} Cost > 100 € kg^{-1}

Regulatory requirements

Degree of required process intensification

The Manufacturing Landscape

- mAb
- Fusion protein
- Bispecific antibodies
- ADC



- Orphan diseases
- Biosimilars
- Development time
- Financing (IPO, M&A)

- Disposables
- Modular facilities

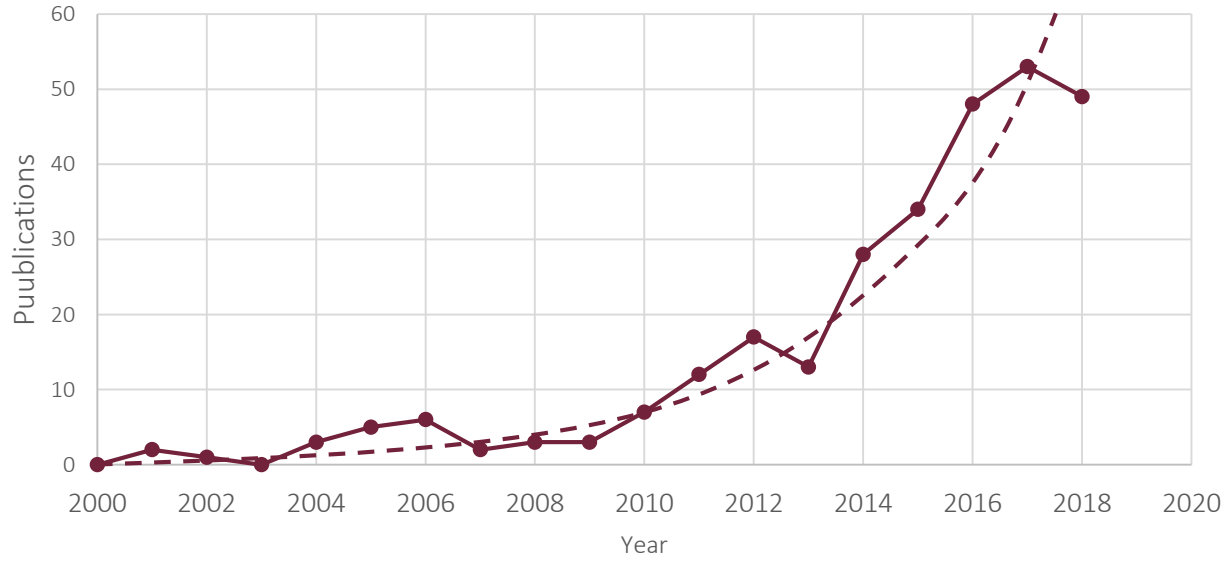
- High titer USP
- Continuous processing
- Platform processes
- Process intensification

What are the principles of process intensification?

- Combination and integration
  - Requires adaptation, potentially rearrangement
    - integration of operations
    - integration of functions
    - integration of phenomena
- Elimination
  - Biggest impact, hardest to achieve
- Simplification
  - Should always be possible
- Targeted enhancement of a phenomenon
  - Cell growth, protein stability
- Uncoupling and frontloading
  - Doing steps earlier and at different locations

Increased ↑	Decreased ↓
- Productivity - Capacity - Titer - Flexibility	- Complexity - Footprint - Byproducts - Energy usage - Waste - Investment - Cost

"Process intensification" in Pubmed



- Can single use equipment help in that context?

When did disposables start in DSP?

Labscale disposable membrane chromatography for R&D

- In 1991, six types of HiTrap columns were launched
- Today, there are over 130 different columns available
- Still focus on small scale and R&D purposes, not manufacturing
- All columns sold in 2010, would build a chain almost 5 km long

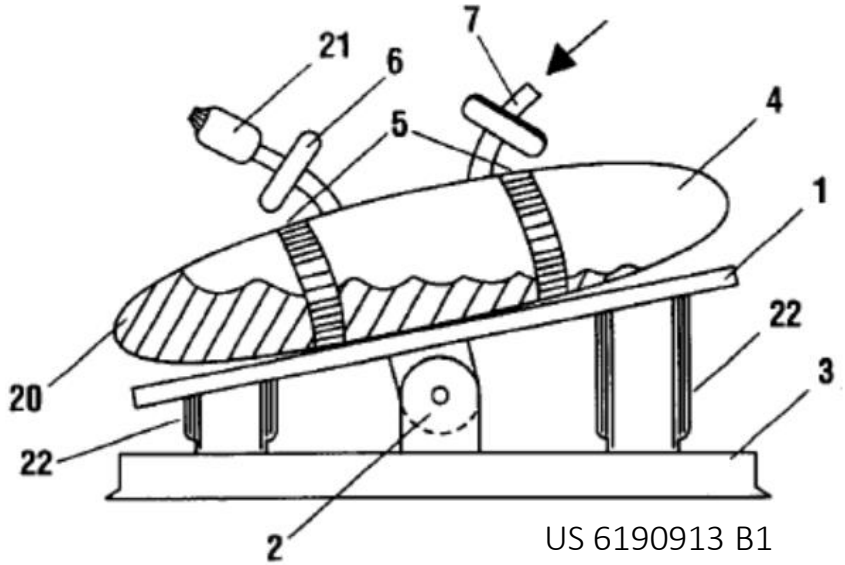
Labscale disposable membrane chromatography for R&D

- In the early 90‘s Millipore launched the ConSep LC-100 system
- 4 sizes of MemSep cartridges with 3 surface modifications
- First FDA approved product utilizing membrane chromatography in 2001 (Campath®)

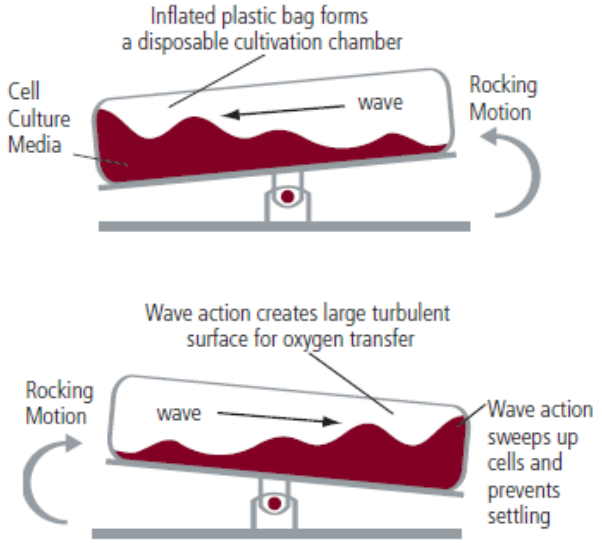
When did disposables start in USP?

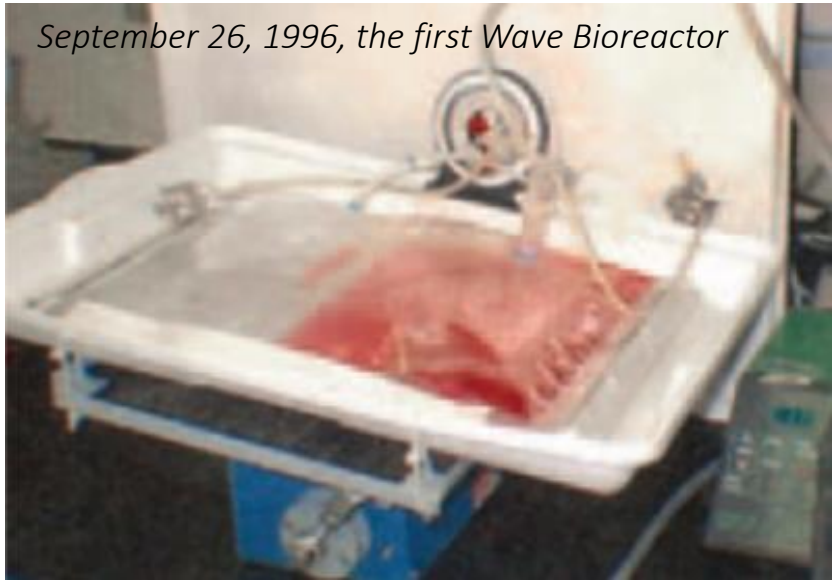
Wave Bioreactor

- First designed in 1996
- Smooth and gentle mixing
- High level of aeration
- Pre-sterilized and fully disposable



US 6190913 B1

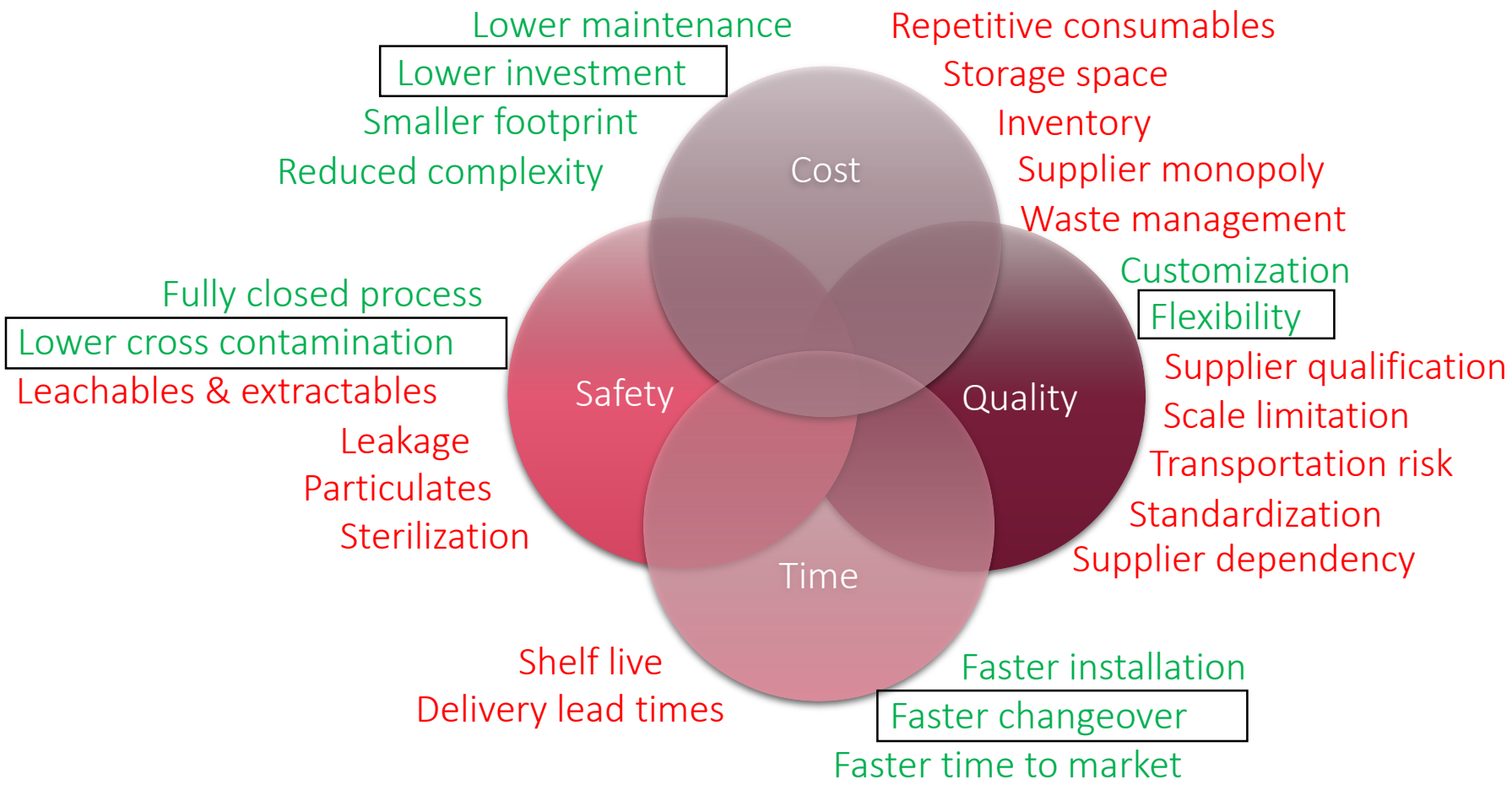




September 26, 1996, the first Wave Bioreactor

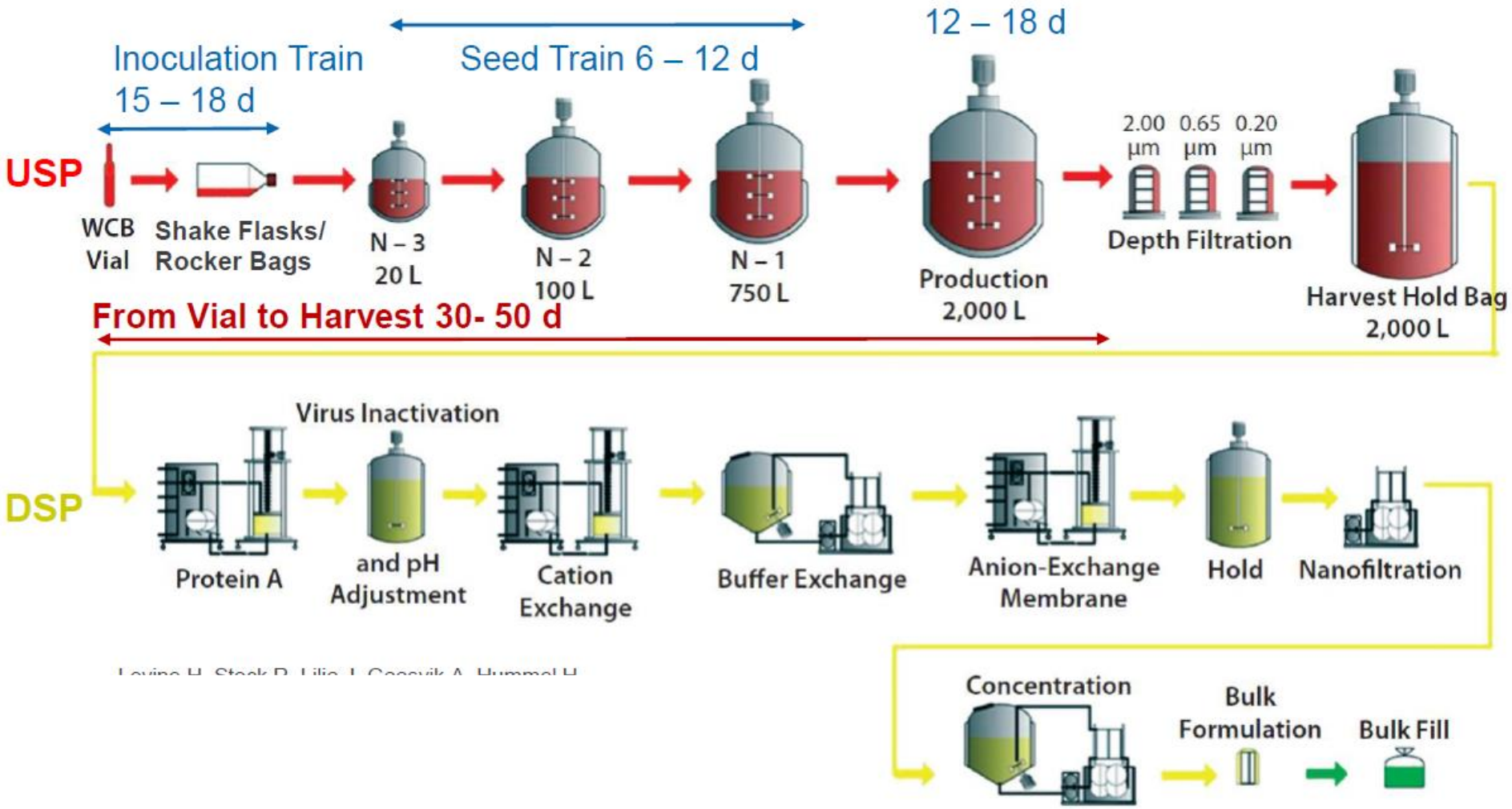
Singh, V. The Wave Bioreactor Story (2005)

Benefits and Limits of Disposables (CMO Perspective)



Schmidt, S. R. The Benefits and Limits of Disposable Technologies in Manufacturing Protein Therapeutics. *Am. Pharm. Rev.* 19, 60–62 (2016).

How can a biopharmaceutical process be intensified?



Levine, HL. et al. Single-Use Technology and Modular Construction. Enabling Biopharmaceutical Facilities of the Future. Bioprocess Int. 11(4), p40-45, (2013).

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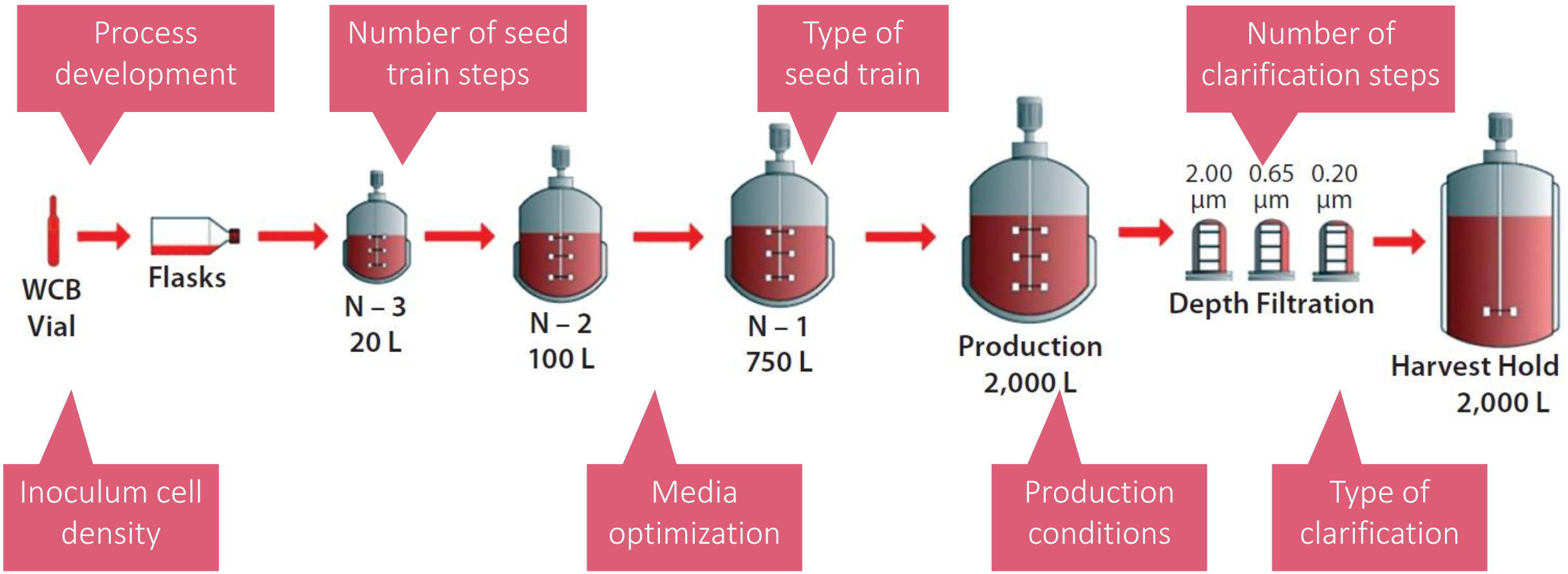
USP: Strategies for process intensification

Overview of approaches

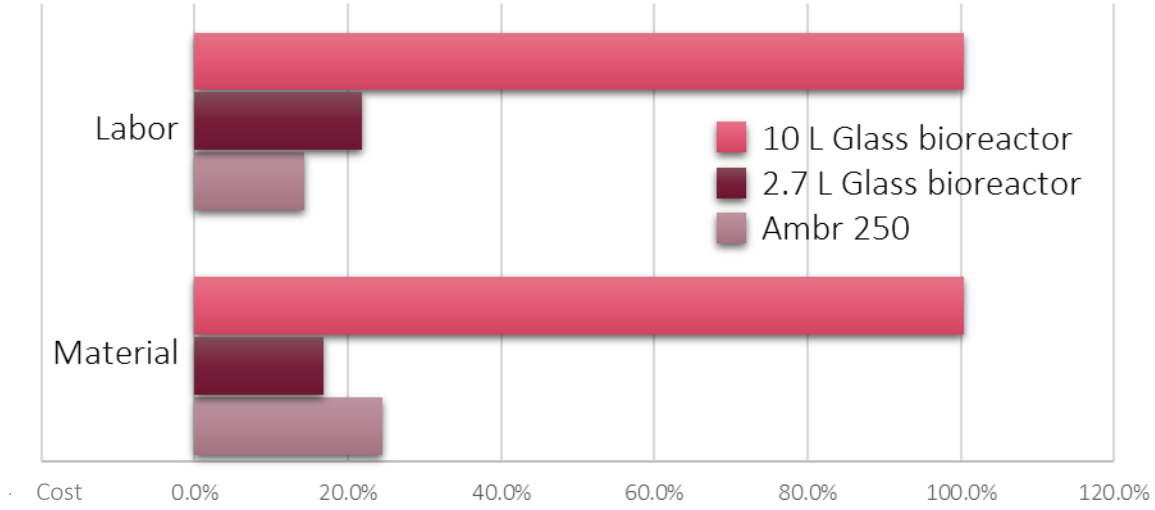


USP process development

Automation and miniaturization

- HTS-approach for process optimization including DoE capabilities
- Ambr® 250: good control and high capacity with short turnaround
- 24 individual bioreactors with automated sampling

- Process adjustment/optimization with **predictable scale-up**
- **Scale-down approaches**:
  - volumetric power input variation
  - gassing strategy
  - feeding volumes in triplicates
- Significant cost and labor reduction

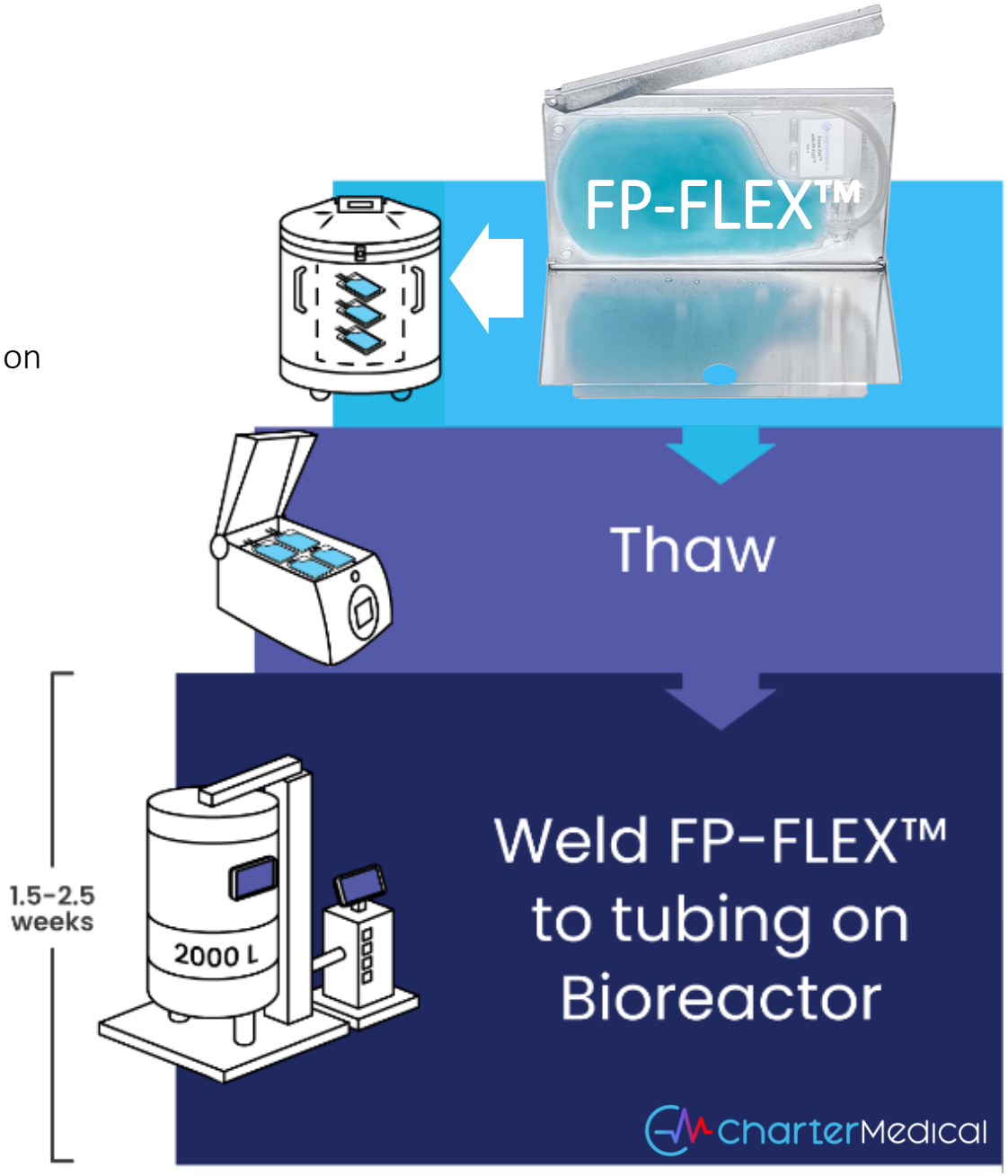


Inoculum cell density & volume

- Inoculum: 30-400 mL of cells at 20-50 M cells/mL in 7-10 days perfusion

- Tubing and containers
  - cryogenic storage and transport validated to -196°C
  - storage in metal freezing cassettes
  - compatible with sterile tube welder
- Closed process by aseptic transfer via tube-to-tube connection
- Reduces handling, contamination risk and seed train expansion steps

- DMSO concentration is critical
- 2-3 fold faster from inoculum to bioreactor



Sargent B., Direct inoculum of bioreactors with CHO cells from frozen seed bags to eliminate continual seed trains and improve facility utilization. Cell Culture Dish. January 24, (2017)

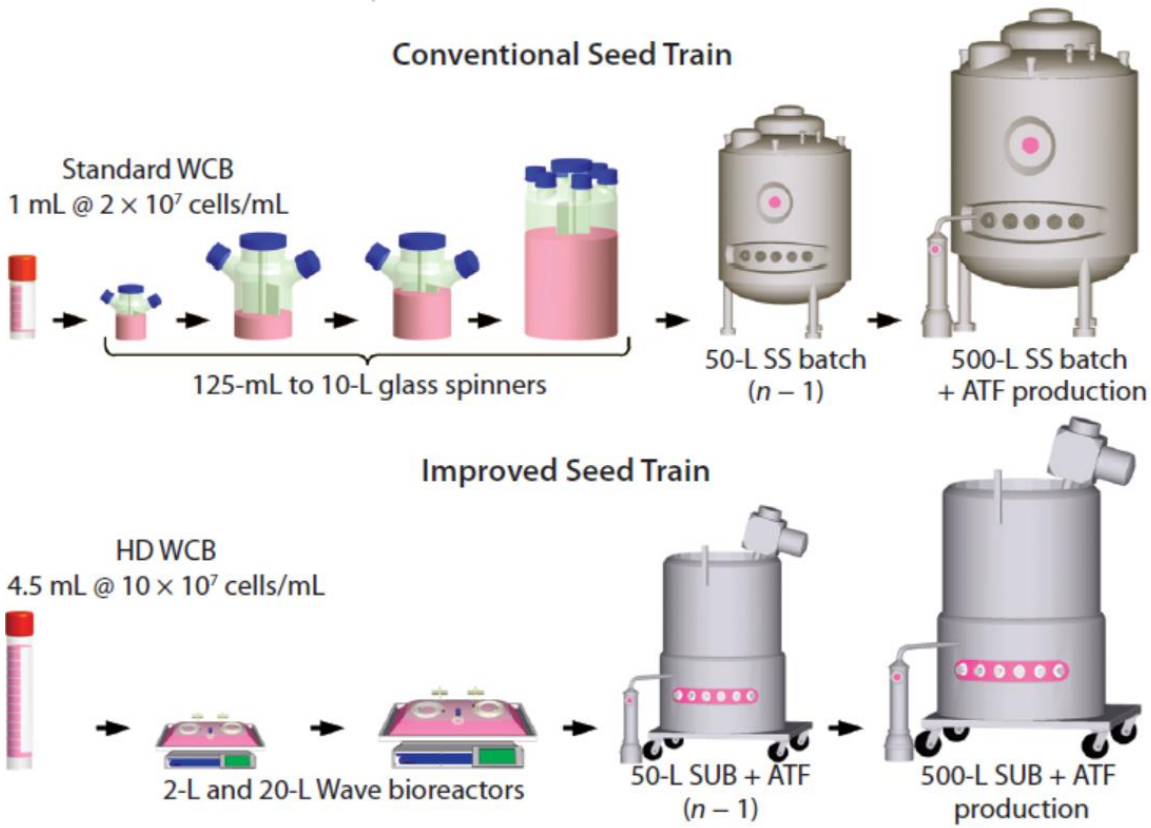
USP intensification

Seedtrain

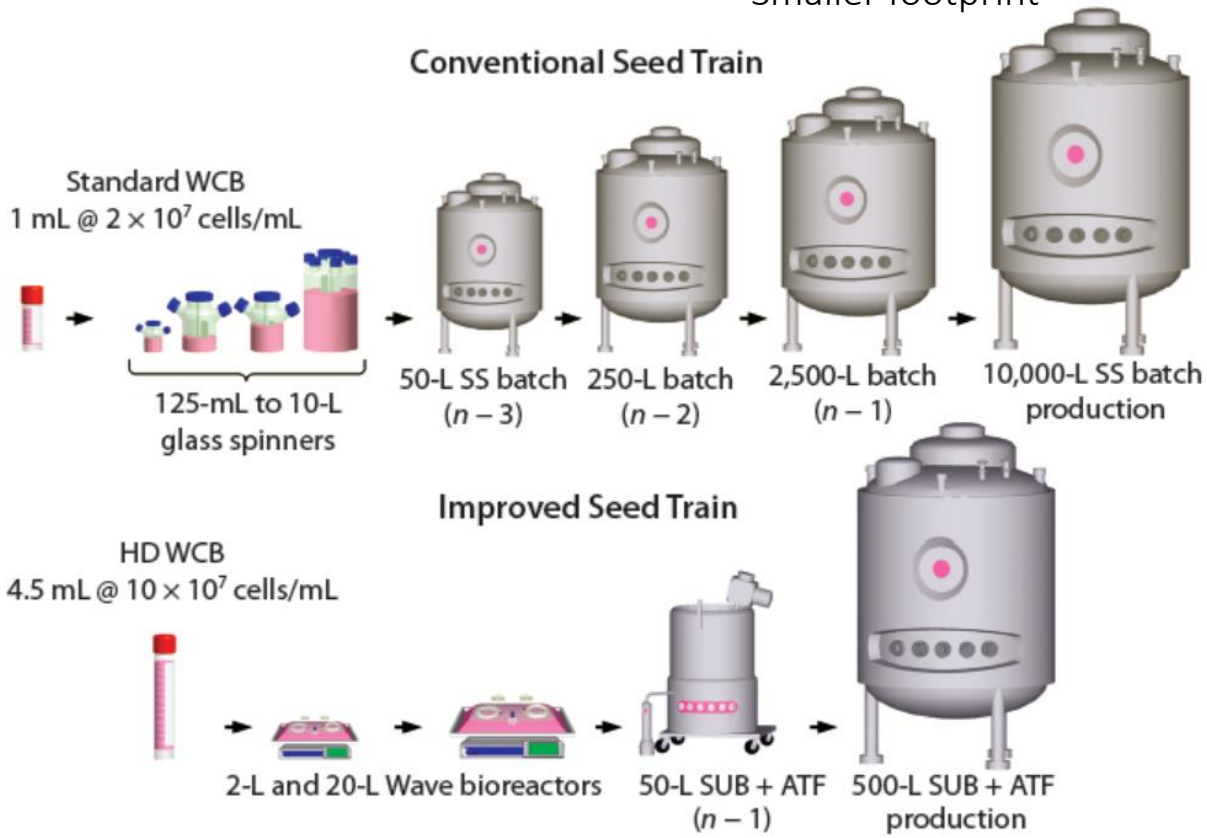
Benefits

- Process time savings
- Hands-on time reduction
- Process safety increase
- Less equipment
- Smaller footprint

Perfusion process

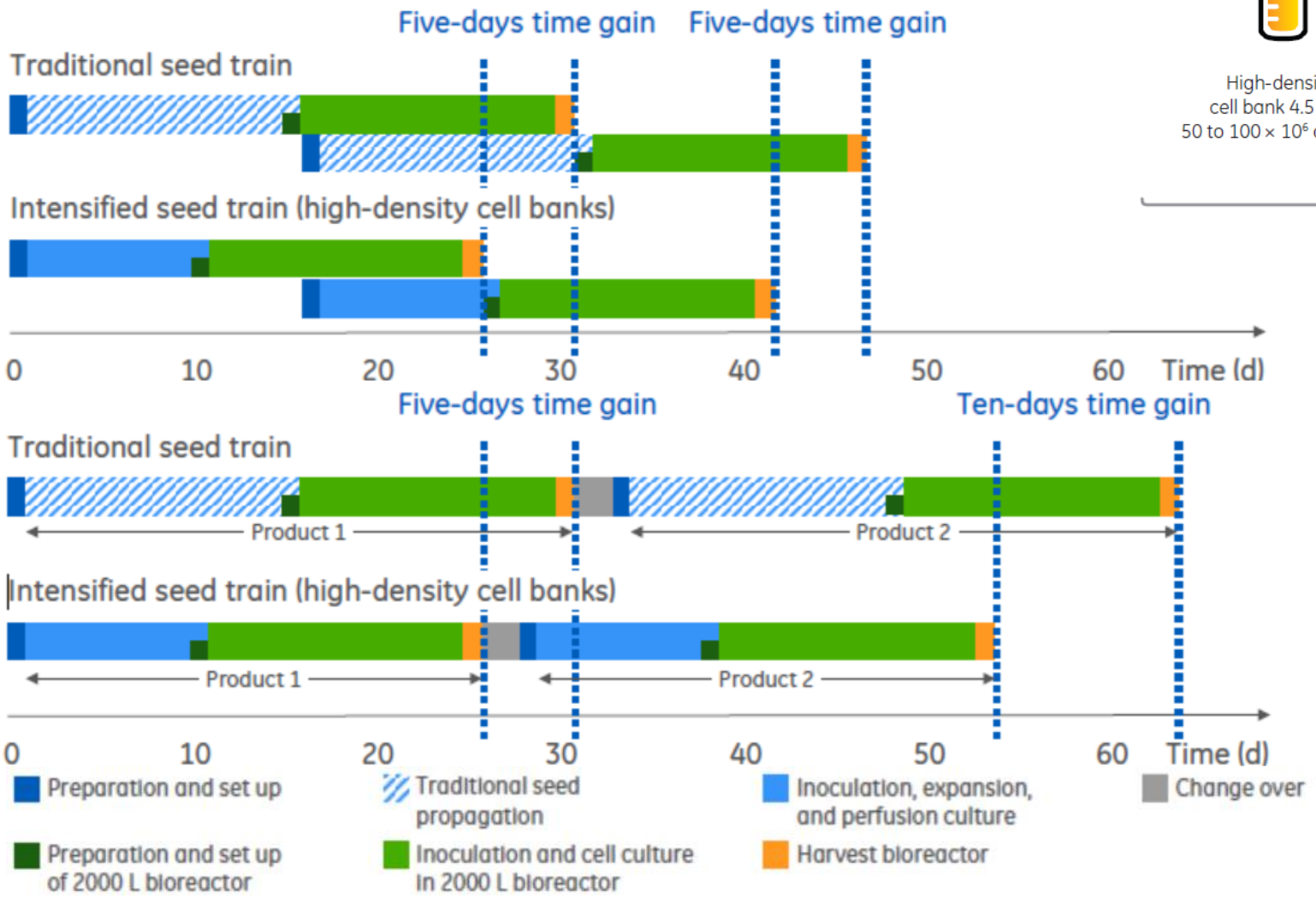


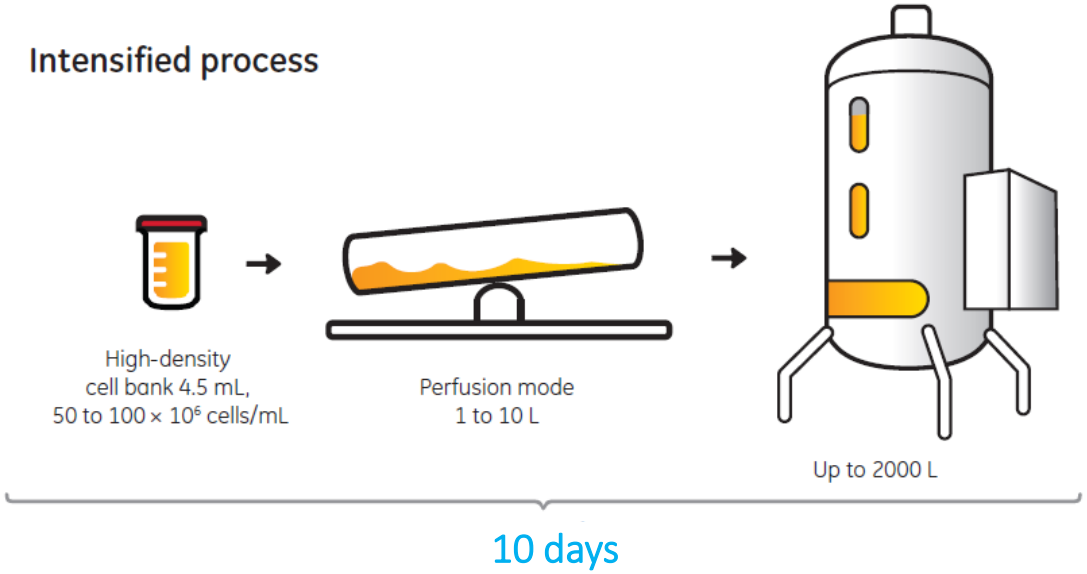
Fed-batch process



Wright, B. et al. A novel seed-train process: Using high-density cell banking, a disposable bioreactor, and perfusion technologies. Bioprocess Int. 13(3), p16-25, (2015).

Benefits from seed train intensification



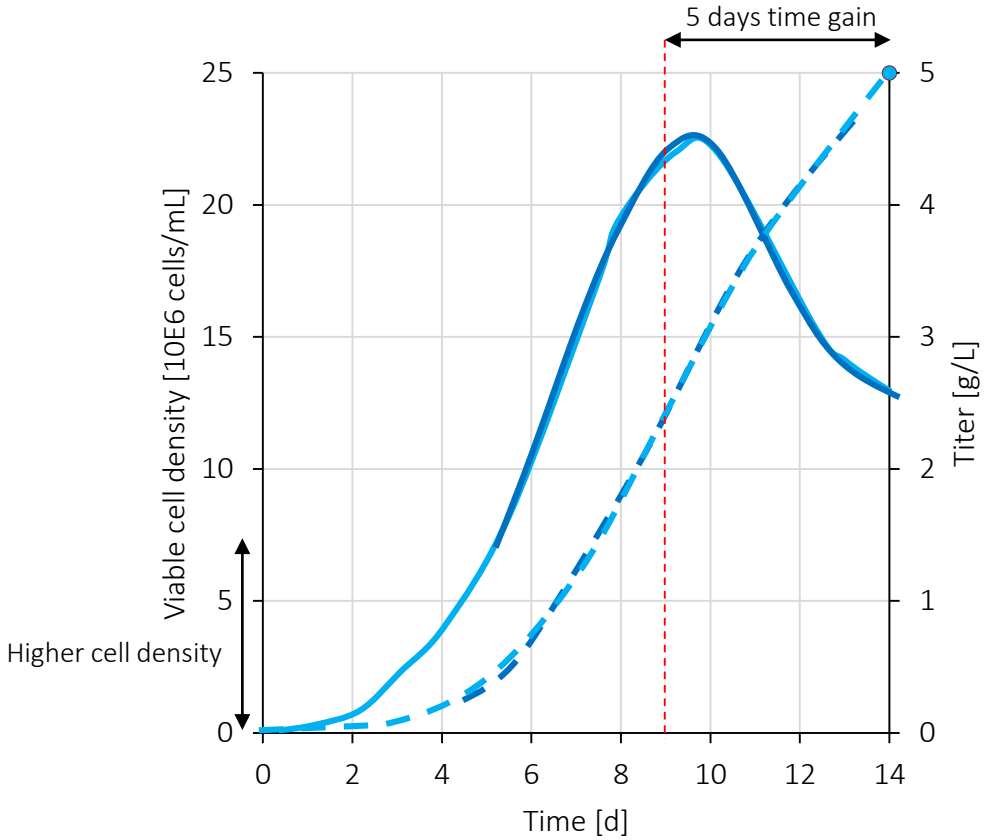


- Up to 2 batches more produced per year through higher plant utilization
- About 60 m^2 reduction in facility footprint with fewer bioreactors
- About 40% reduction of labor cost for seed preparation
- Between 10% and 20% reduction in upstream production cost
- Highest cost savings with small volume products with few batches per year

Time savings in main reactor

- Higher inoculation VCD through perfusion in N-1
- At end of N-1: density >40 × 10^6 cells/mL
- 30% increase in manufacturing capacity (5 days shorter process)
- Comparable product quality and yield

- Shorter cultivation time may even result in less degraded product
- Alternatively, if cells maintain high viability more product is generated at original cultivation time ➔ impacting DSP dimensions



Process simulation based on traditional N-1 step

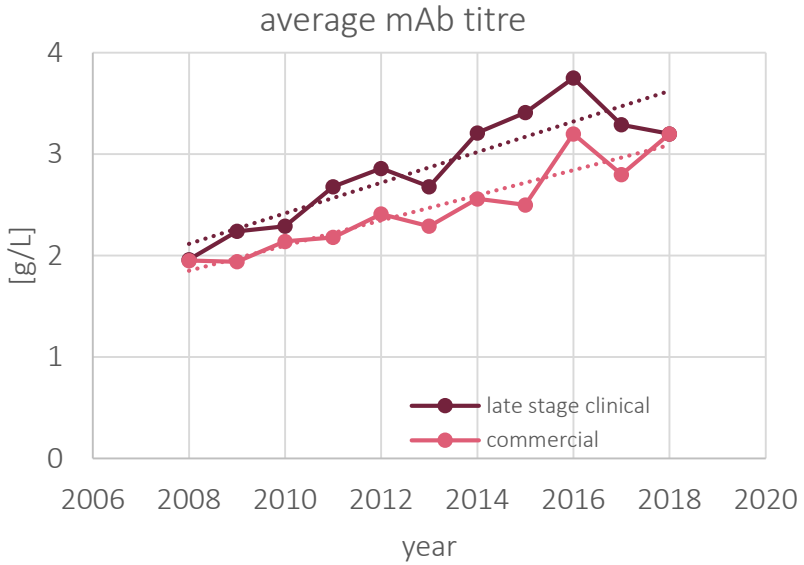
Process simulation based on perfusion in N-1 step

Yang, W. C. et al. Perfusion seed cultures improve biopharmaceutical fed-batch production capacity and product quality. Biotechnol. Prog. 30, 616–625 (2014).

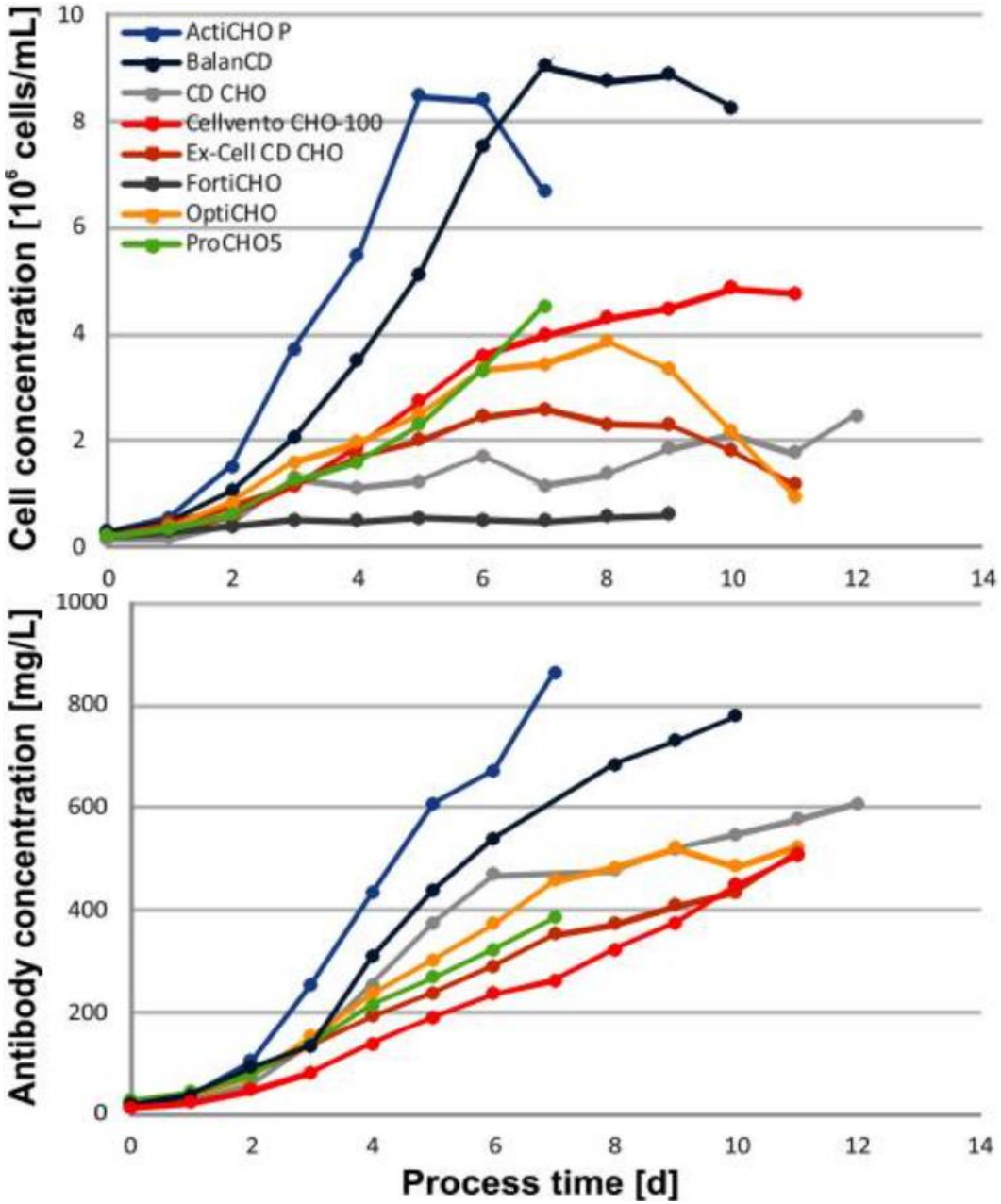
High titer processes and culture media

Drivers:

- Higher cell density
- Longer process time
- Genetic engineering
- Metabolic engineering
- Improved media

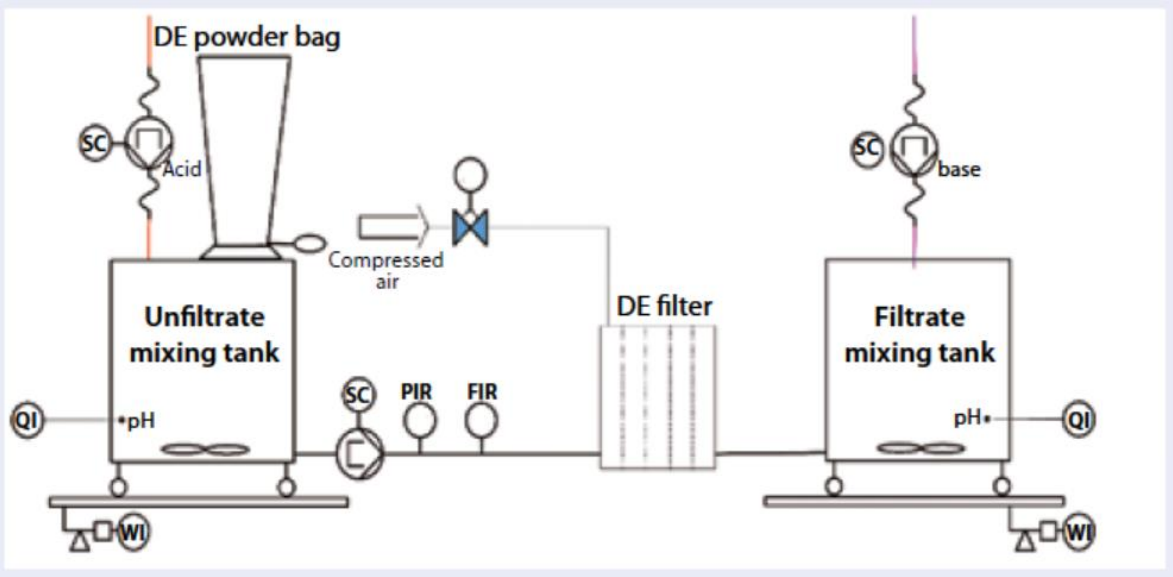


BioPlan 15th Annual Biomanufacturing Report, p 193, (2018)



Reinhart, D. et al. Benchmarking of commercially available CHO cell culture media for antibody production. Appl Microbiol Biotechnol. 99(11), p4645-4657, (2015)

Harvest clarification: Depth filtration with Diatomaceous Earth



pH 5.0 experiments with Celpure C300 with best performance:

- Filtration capacity (size of filter)
- Flux (duration of run)
- Impurity removal (HCP, DNA)

- Fast process: harvest of 600 L in 1 h with 7 DF modules
- Scalable to 3000L with 33 DF modules in same time

Minow, B. et al. High–Cell-Density Clarification By Single-Use Diatomaceous Earth Filtration. Bioprocess Int. 12(4), p16-46, (2014).

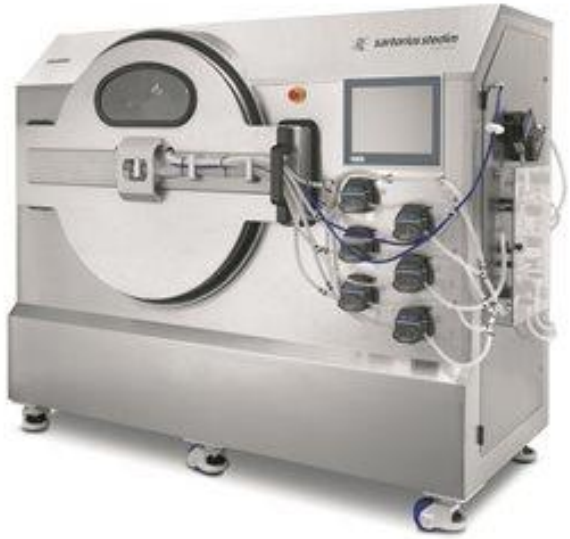
	Centrifugation	Tangential-Flow Filtration	Depth Filtration
Investment	High	Intermediate	Low
Free of particles	No	Yes	Yes
Maximum culture volume	6,000 L	2,000 L	"Unlimited"
Suitable for continuous processing?	Yes	Yes (ATF)	No
Scale-down model available?	No	Yes	Yes

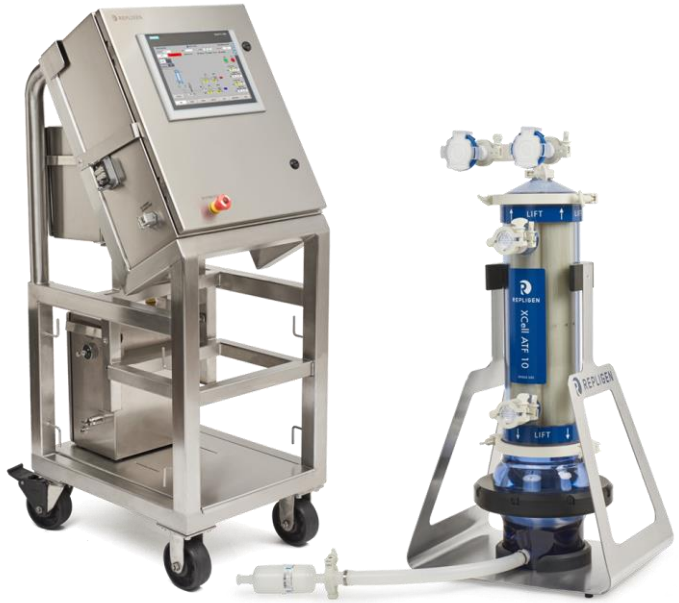
Schmidt, SR. et al. Single-Use Depth Filters Application in Clarifying Industrial Cell Cultures. Bioprocess Int. 14(1), p6-11, (2017).

Cell Removal/Retention with Disposable Systems

Scale and flow limitations vs time savings and lower contamination risk

- Single-use devices:
  - kSep-Systems (up to 2000x*g*, 720 L h^{-1})
  - ATF 10 single use (exchange rate up to 3600 L h^{-1})
  - Unifuge – Pneumatic Scale Angelus (up to 4000x*g*, 240 L h^{-1})





* Pictures are courtesy of Sartorius, Repligen and PSA

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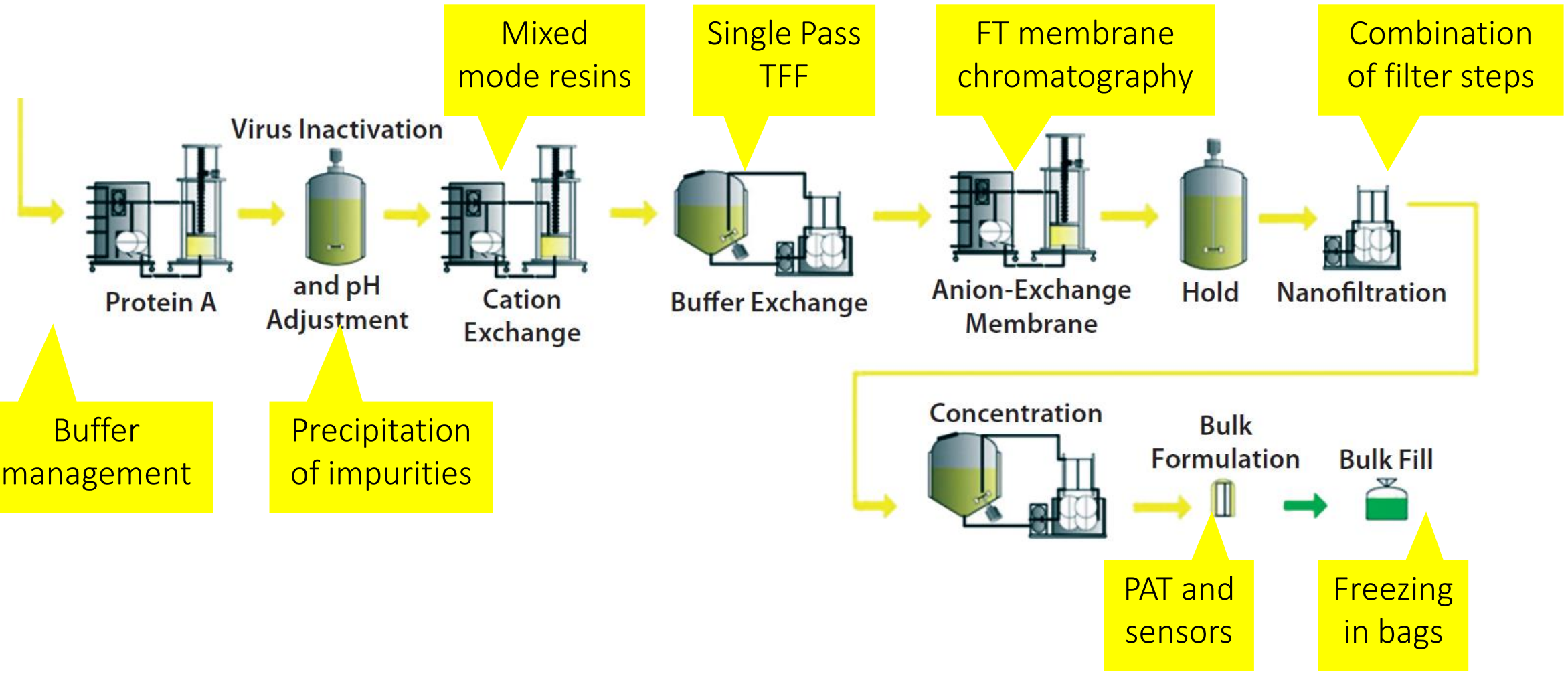
DSP: Strategies for process intensification

Overview of approaches



Buffer management

General strategies reducing cost, time and footprint

Generic buffers (recipes)

Reduce number of buffers and all subsequent efforts

Pre-dispensed **buffer substances in bags**

Ready to use, eliminate own preparation, closed system

Buffer prep in **disposable mixtainers**

Faster change-over, no cleaning

Robotic transfer of **filled bags**

Eliminate non- value adding work, enable traceability

Multi-bag concept

Optimal usage of buffer volumes

Inline blending/dilution based on (concentrated) stock solutions

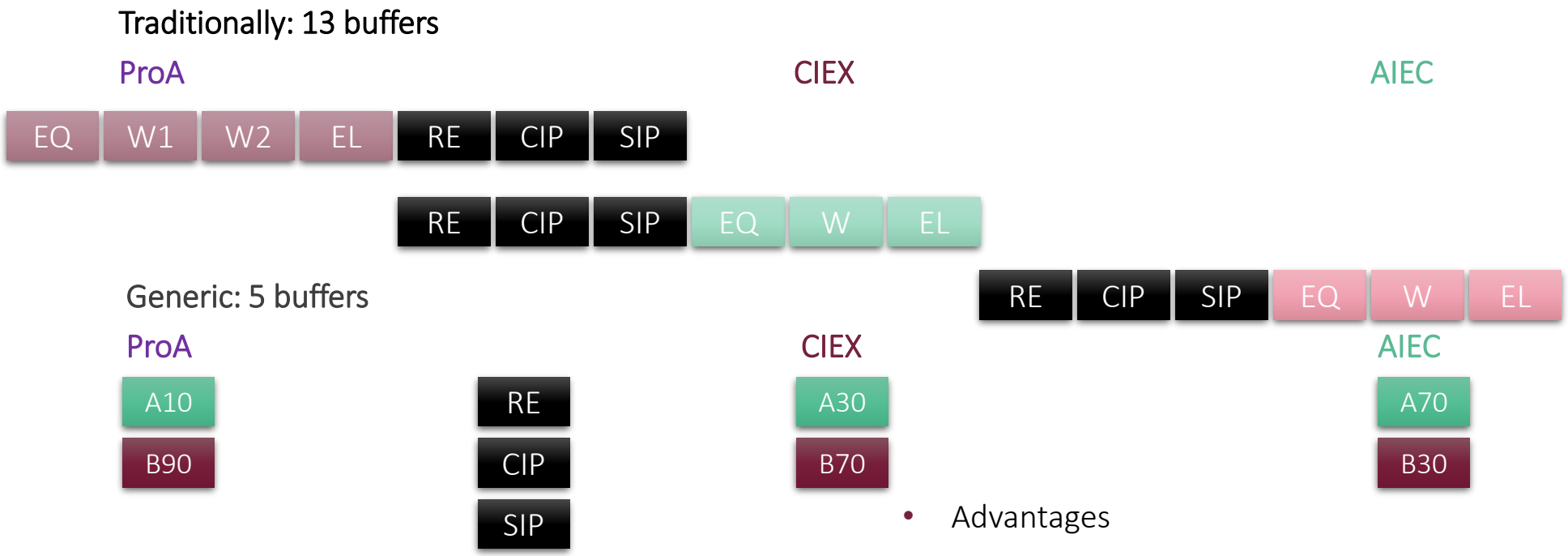
Optimal volume utilization


© ILC Dover


© Sartorius


© Argon medical devices

Generic buffers for DSP in a model mAb process



- Advantages
  - Less buffer definitions
  - Logistics: less transport and storage efforts
  - Costs: utilizable in more projects

Concept of multi-bag buffer supply

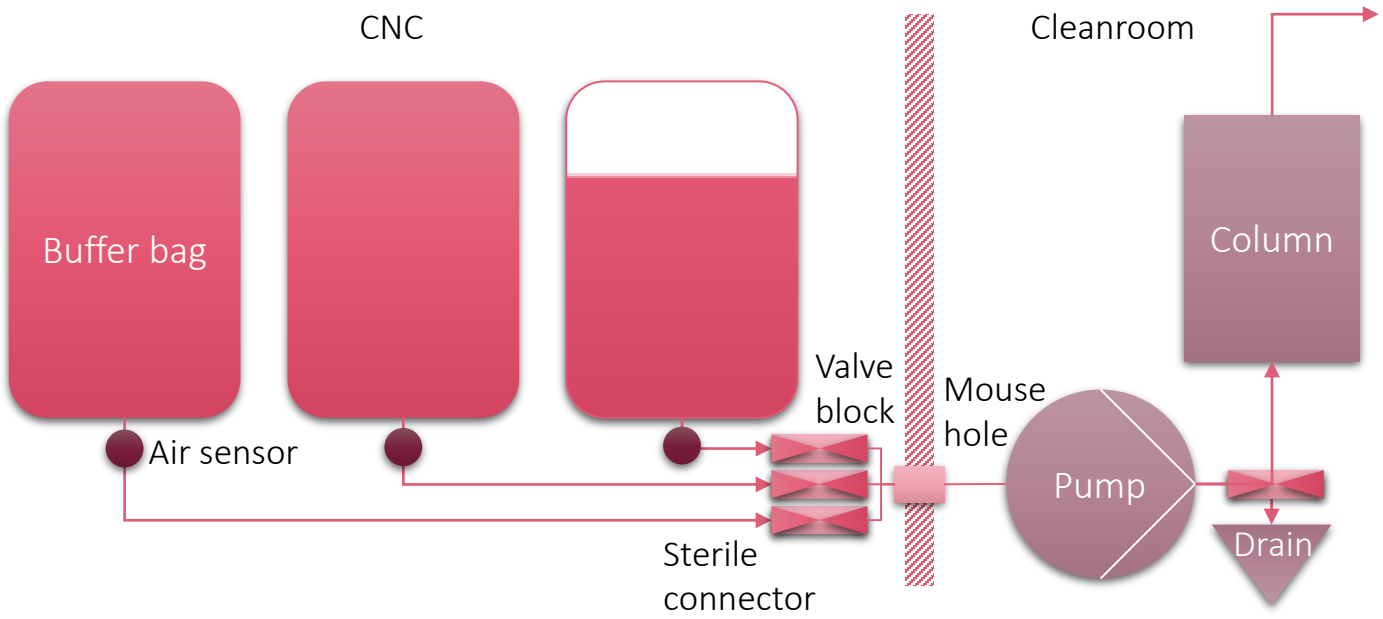
- No open handling, sterile connectors
- Buffer handling outside of cleanroom

- Tagged bags eliminating confusion
- Multi batch usage if hold times allow it

- Standardization of volumes enabled
- Reduced backup volume required
- Max. volume utilization (complete drain of bag)
- Eliminate drain need when discarding bags

- Automated switch when air passes
- Valve block to divert from different sources

- Tubing is washed and primed by skid-pump
- No CIP/SIP required

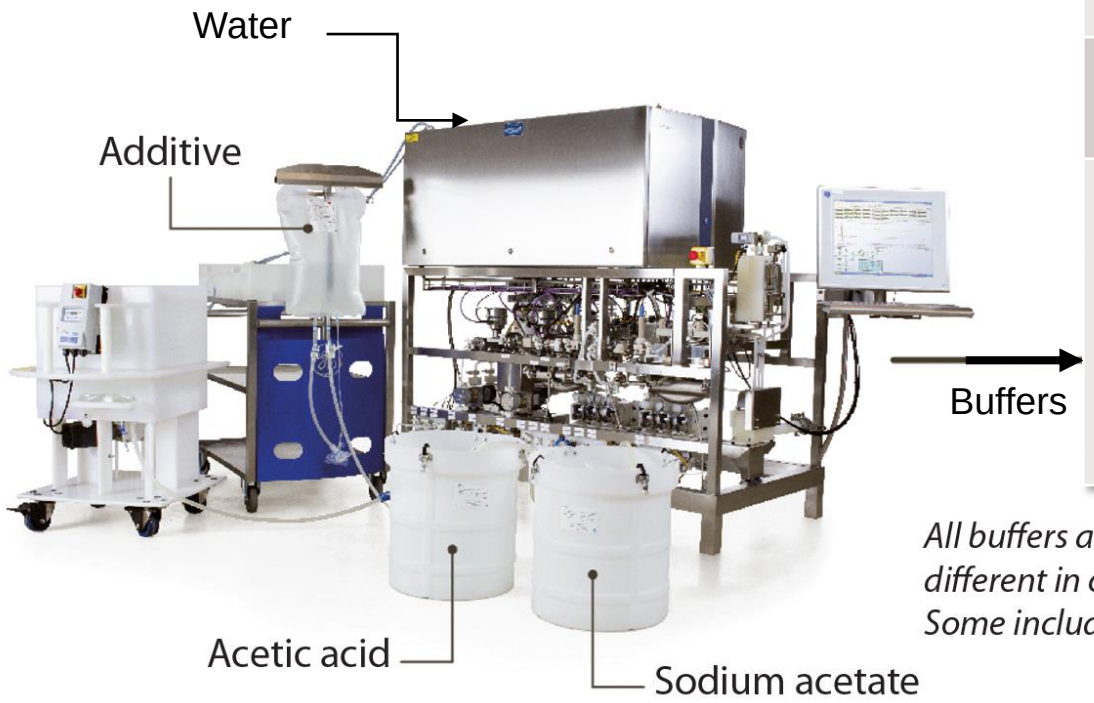


Buffer dilution and blending

Requires dynamic control of pH and conductivity

Increased complexity

Principle	Recipe and flow	pH and flow	pH and conductivity
Description	Buffer formulated using recipe	Buffer formulated based on target pH and concentration	Buffer formulated based on target pH and conductivity
Controlling probes	Flowmeters	pH and flowmeters	pH, conductivity, flowmeters
Monitoring probes	pH, conductivity, flowmeters	pH, conductivity, flowmeters	pH, conductivity, flowmeters
Benefits	Robust at constant T and accurate stock solutions	Delivers correct pH and concentration even if temperature varies	When variability in stock solution is expected, delivers correct pH and conductivity even if T varies



All buffers are acetate based and different in concentration and pH . Some include an additional additive.

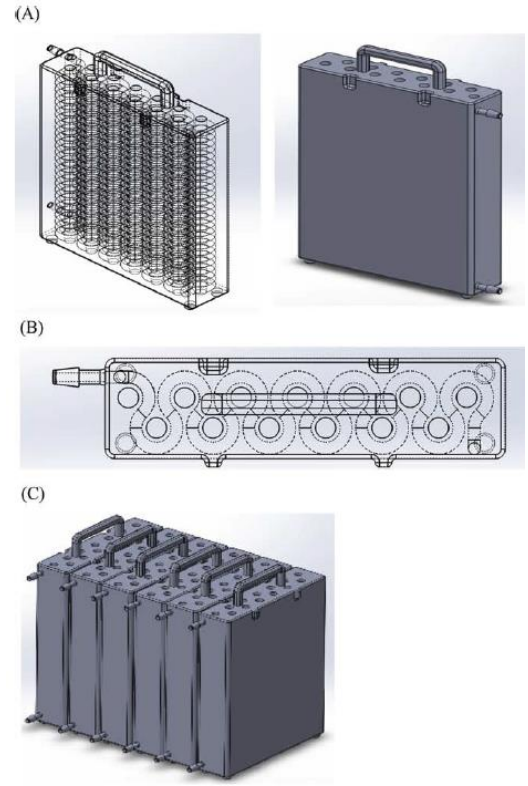
Fabbrini, D., et al. Addressing the Challenge of Complex Buffer Management. Bioprocess Int. 15, 43–46 (2017).

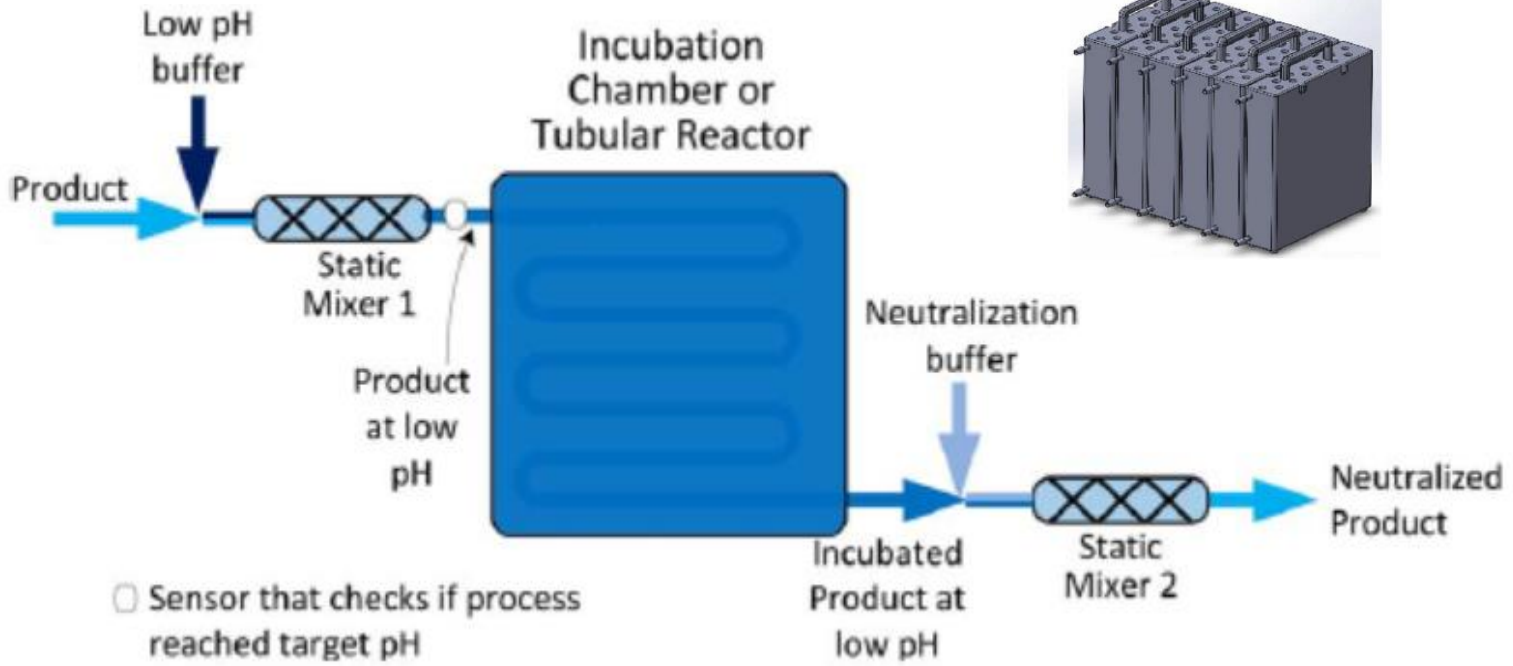
Continuous acidic pH virus inactivation

- Adjust flow to minimum residence time of 60 min.
- Titrating solution must be buffered to avoid pH fluctuations.
- Provide efficient radial and minimal axial mixing to ensure >99% liquid exits in <3 h.
- Enable validation for GMP use

Advantages:

- Easy scalable
- High reproducibility
- Sterilizable single-use material
- Inexpensive manufacture by molding or 3D printing.





Orozco, R. et al. Design, construction, and optimization of a novel, modular, and scalable incubation chamber for continuous viral inactivation. Biotechnol. Prog. 33, 954–965 (2017).

Pre-packed columns

Eliminating time for packing and qualification

Brand	Opus	RTP	Chromabolt	CIMmultus	EvolveD
Company	Repligen	GE-Lifesciences	Merck/Millipore	Bia Separations	Prometic
Diameter [cm]	10-80	8-45	10-32		7/10/20
Bedheight [cm]	5-40	20	20		10 or 20
Volume [L]	0.5 - 150	0.8 - 32	1.6 - 16.1	0.001 - 8	0.38 - 6
Pressure [bar]	3	4	3.5	14	4
Resins	all	own	own	own (monolithic)	own
Design					

Mixed mode chromatography

Combination of multiple separation methods in one step

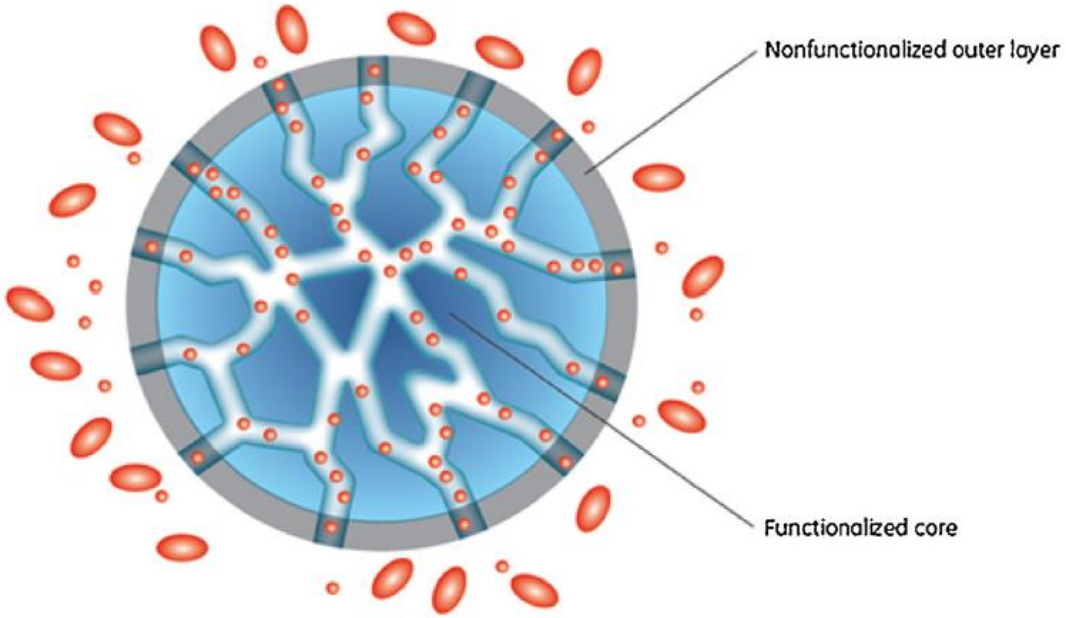
Capto Core 700: both size exclusion and IEX/HIC binding properties

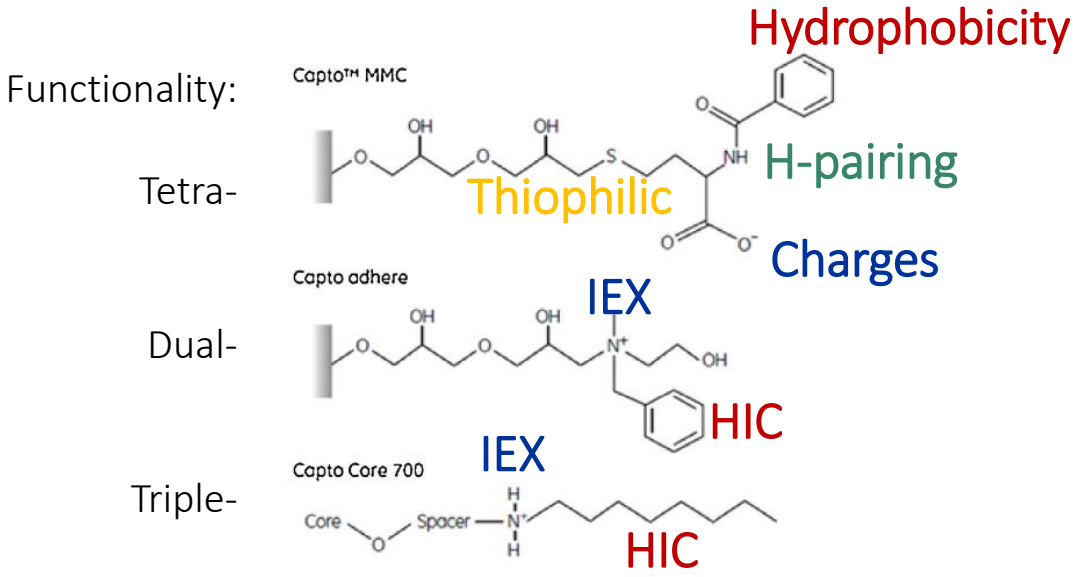
- Efficient capture of contaminants, target molecules in the flow through
- Improved productivity and higher flow rates (compared SEC)
- Easy optimization due to flow through chromatography and robust performance

Capto MMC: fourfold functionality

- Bind elute with conductivity, pH, hydrophobicity
- Potential of FT chromatography

- Simultaneous enrichment of product while removal of impurities

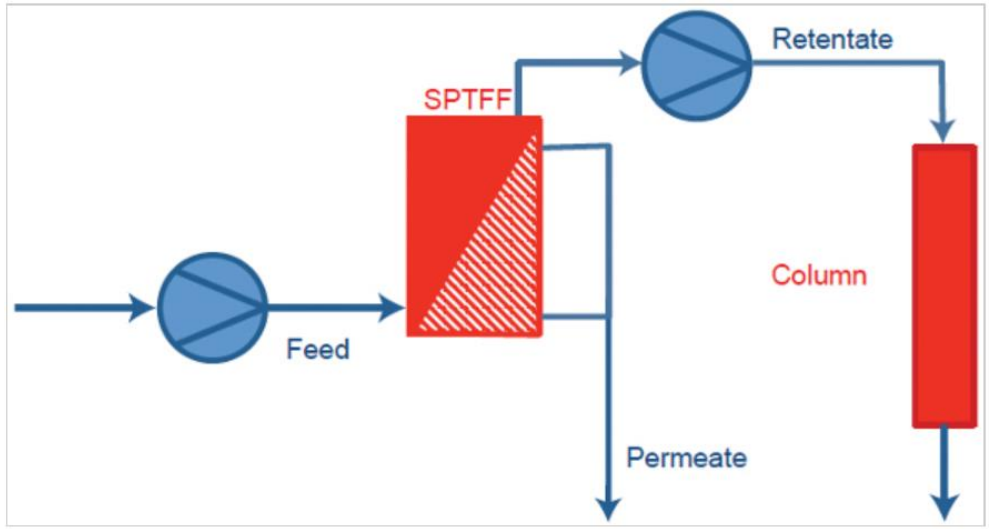




Zhang, K. et al. Mixed-mode chromatography in pharmaceutical and biopharmaceutical applications. J Pharm and Biomed Anal. 128, p73-88, (2016).

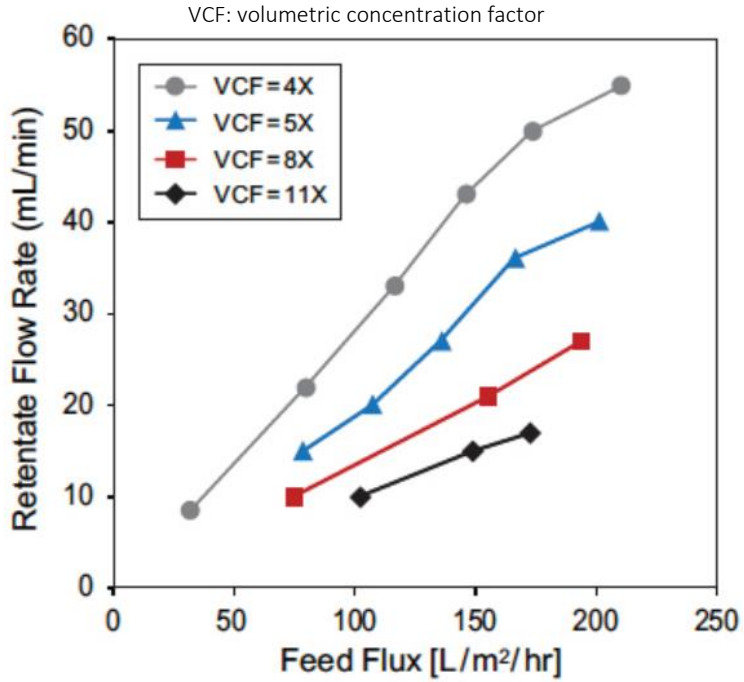
Single pass TFF (SPTFF)

Concentration

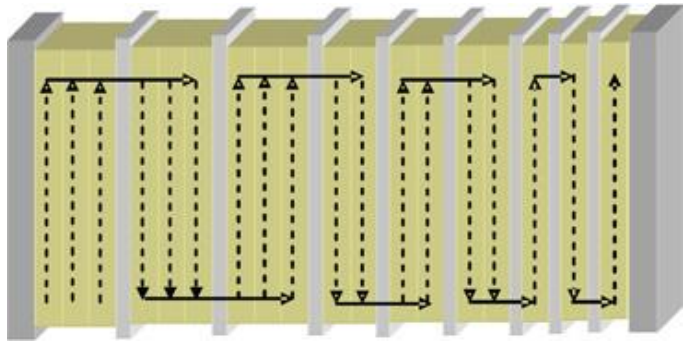


Development parameter

- Cross flow rate
- Number of sections (serial)
- Membrane area per section
- Serial vs parallel



- SPTFF eliminates the conventional TFF recirculation loop
- Concentrated retentate of the TFF device loads directly onto the column.
- The lower volumes and higher titers decrease loading time, reducing tubing and equipment sizes
- Reduced volumes also enable the utilization of disposable, single-use technologies
- Concentration factors of 2 to 30X



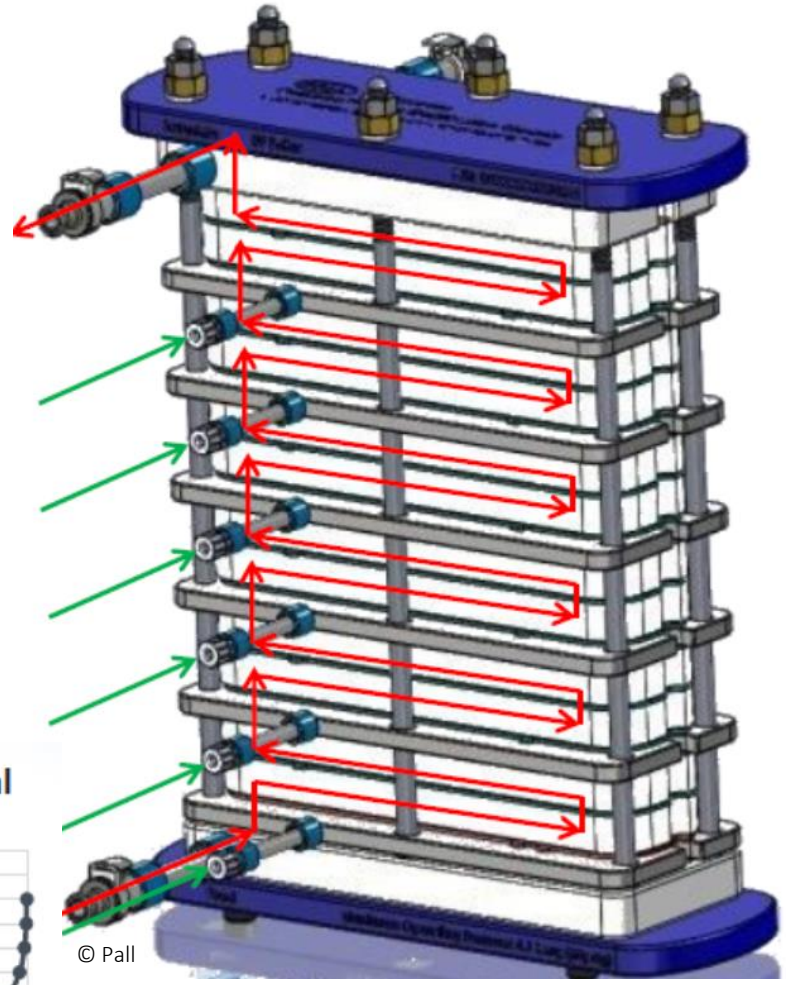
© Pall

Casey, C., et al. Cadence™ Single-pass TFF Coupled with Chromatography Steps Enables Continuous Bioprocessing while Reducing Processing Times and Volumes. American Pharm. Rev. (2016)

SPTFF or ILDF (Inline diafiltration)

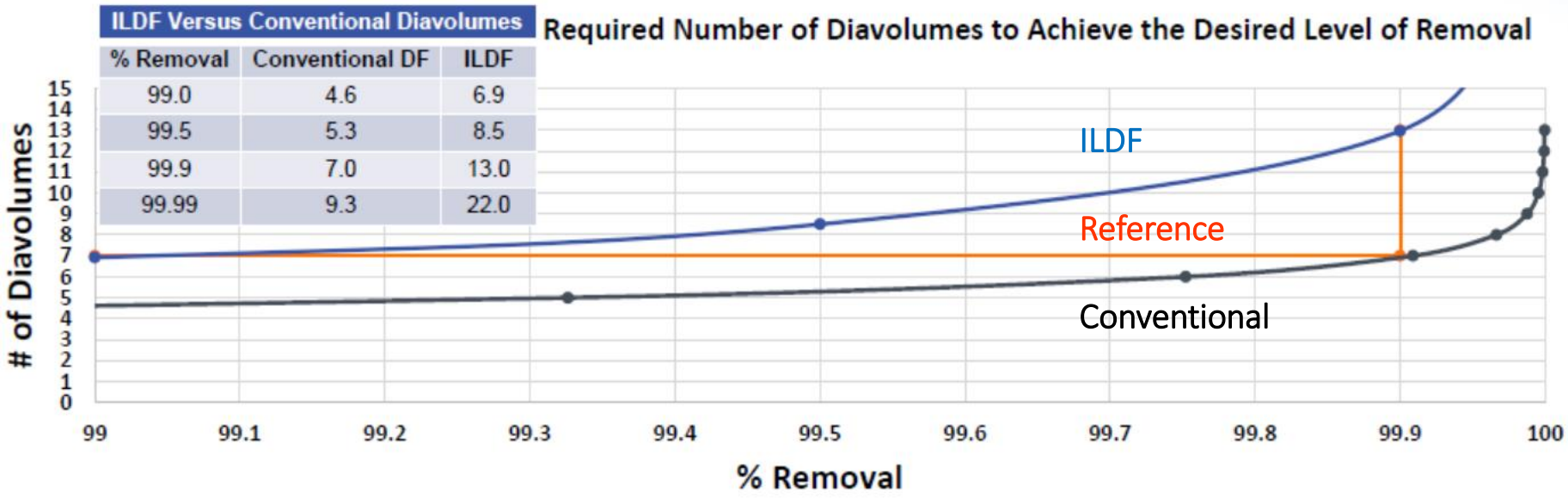
Diafiltration

- Repeated dilution and concentration without recirculation
- Product concentration impacts feed pressure profile
- 3 log removal (99.9 %) requires 13 DF in SPTFF versus 7 DF conventionally

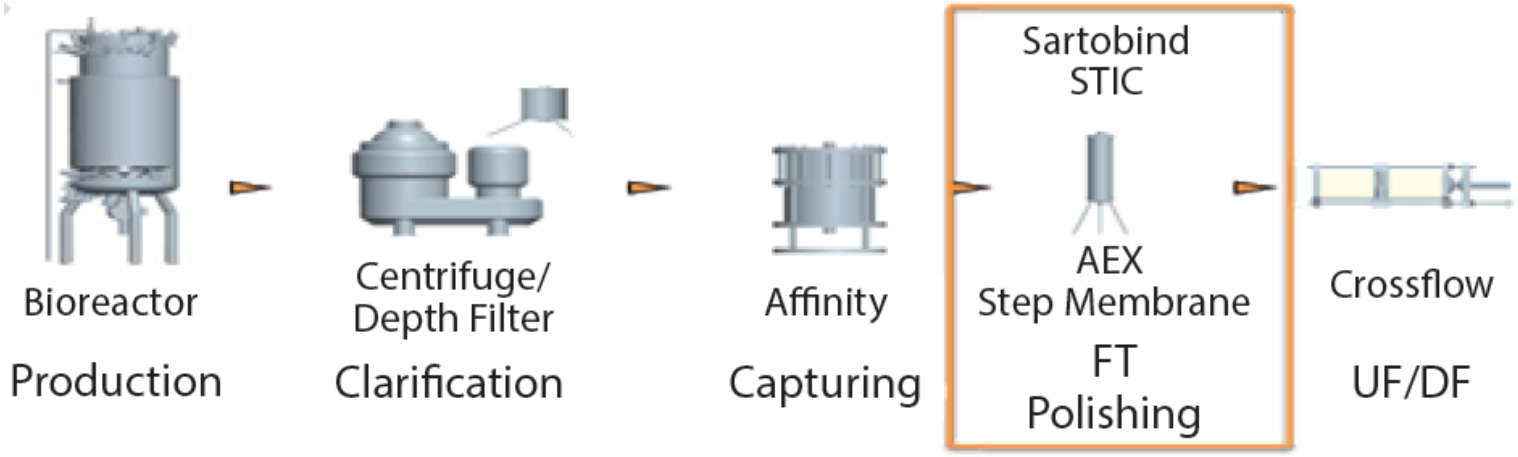


ILDF Versus Conventional Diavolumes

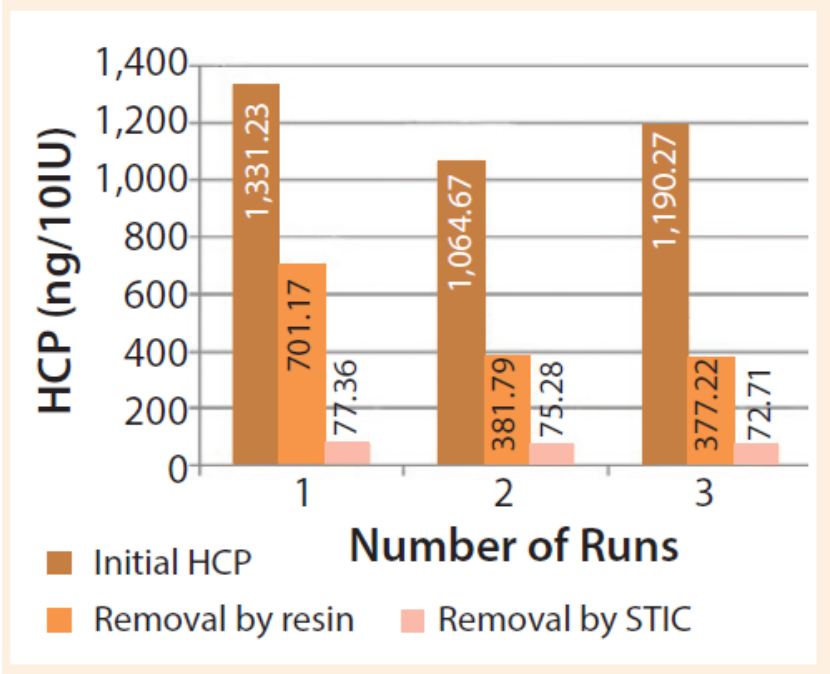
% Removal	Conventional DF	ILDF
99.0	4.6	6.9
99.5	5.3	8.5
99.9	7.0	13.0
99.99	9.3	22.0



Membrane chromatography in FT mode



- Larger pores, negligible diffusion time, higher flow rates
- Available as disposable module
- Sufficient capacity in FT mode, only binding of impurities (HCP, DNA)

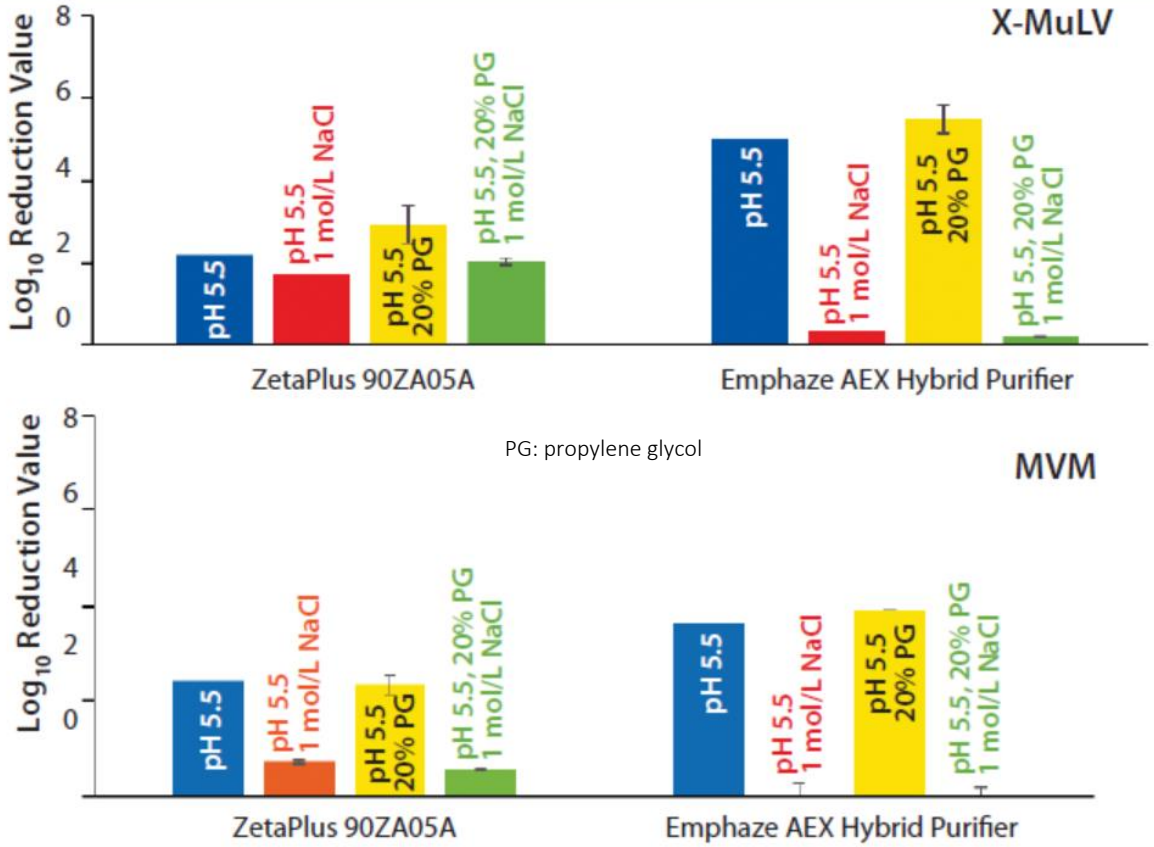


Gupte, P., et al. Establishing Effective High-Throughput Contaminant Removal with Membrane Chromatography. Bioprocess Int. 16(1-2), p60-63, (2018).

Combination of impurity elimination and virus removal in depth filtration

Functionalized (positively charged) filters

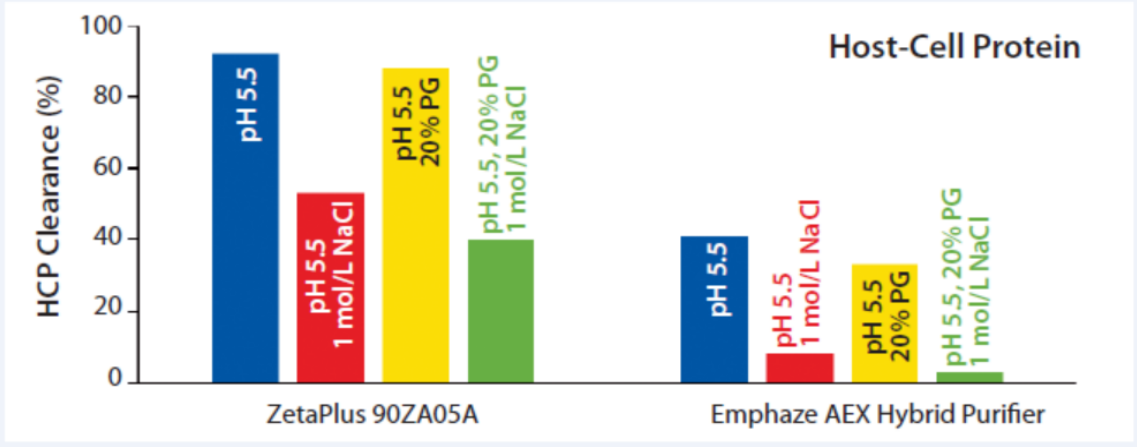
Figure 2: Log_{10} reduction values (LRVs) for (TOP) X-MuLV and (BOTTOM) MVM at low-salt, high-salt, high-PG, and high-salt + high-PG conditions with two filter devices



PG: propylene glycol

Trapp, A. et al. Evaluating adsorptive filtration as a unit operation for virus removal. Bioprocess Int. 16, p58–62 (2018).

Figure 4: Host cell protein (HCP) clearance at low-salt, high-salt, high-PG, and high salt + high PG conditions for MAb 2 (CHO HCP Kit #F550, third generation, from Cygnus Technologies)



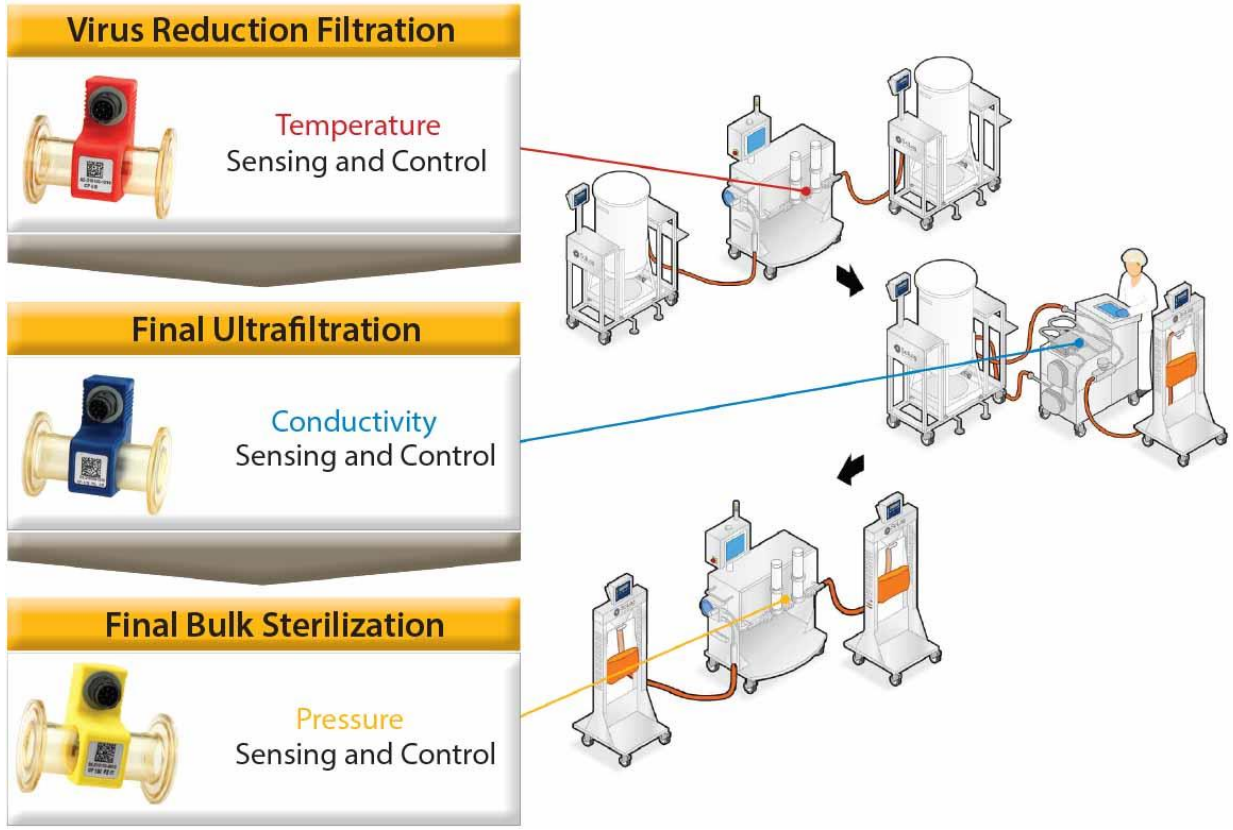
Advantages

- Higher flow rate
- Reduced process time
- Lower buffer consumption
- Disposable membrane
- Integration of virus and HCP reduction

Disposable sensors in critical unit operations

Increasing safety and process performance

- Virus-Reduction Filtration: variations in **temperature can affect the polymeric structure** of the filter or the mechanism of virus retention.
- Final Ultrafiltration: this operation often is the **final conductivity** measurement of a drug product before it enters a vial.
- Sterilization: the validated cross-filter pressure drop must not be exceeded to **prevent breakthrough**
- Ideally all sensors used for monitoring can also actively regulate the measured parameter by automation:
  - Flowrate reduction when pressure builds up
  - Extending dia-filtration time and volume if conductivity is not reached

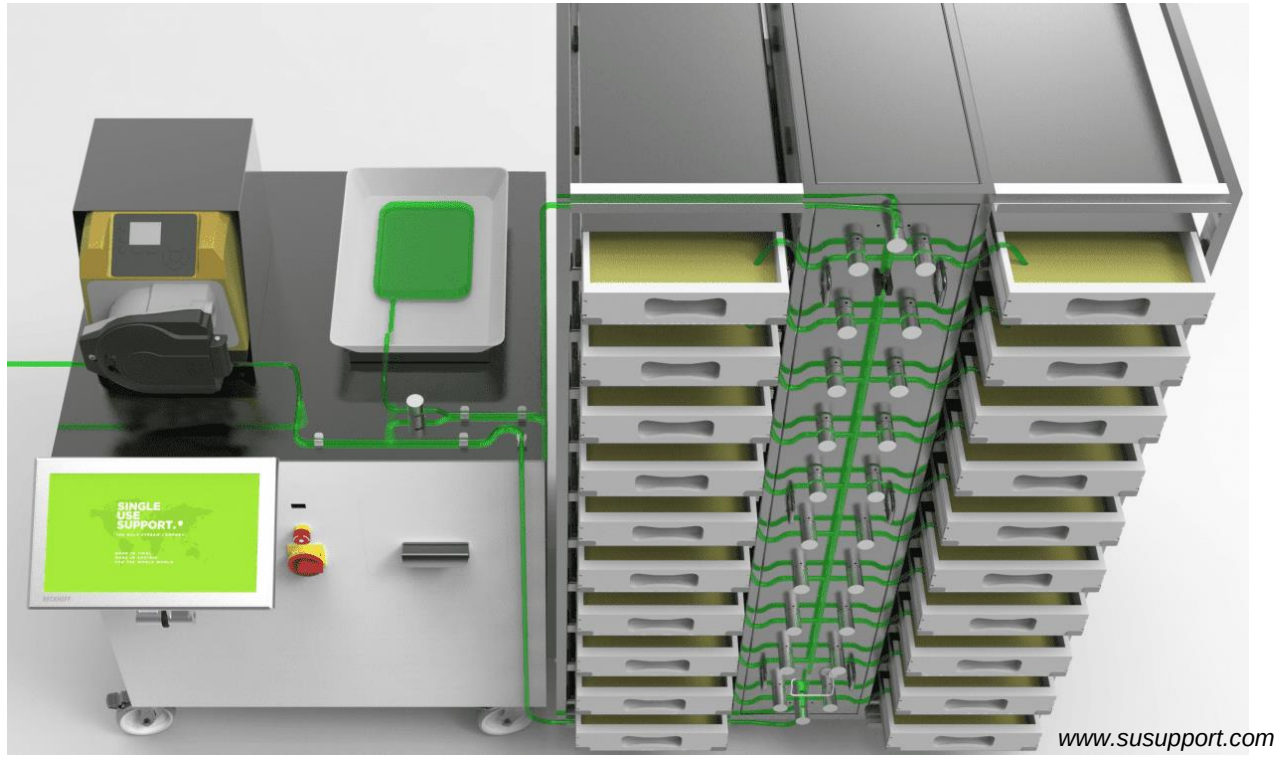


Hutchinson, N. Understanding and Controlling Sources of Process Variation. *Bioprocess Int.* **12,** 24–29 (2014)

Automated final fill of bulk drug substance

Faster, less effort, better control, lower risk, smaller footprint

- Fully automated & closed fill/drain system of single-use bags
- Filling up to 200 L bulk drug substance in < 1 hour
- Accuracy +/-10 mL for 10 L with single weight cells



www.susupport.com

- Automated fill in bottles, fully disposable closed flow path
- Standardization of operations, less variability and manual interference

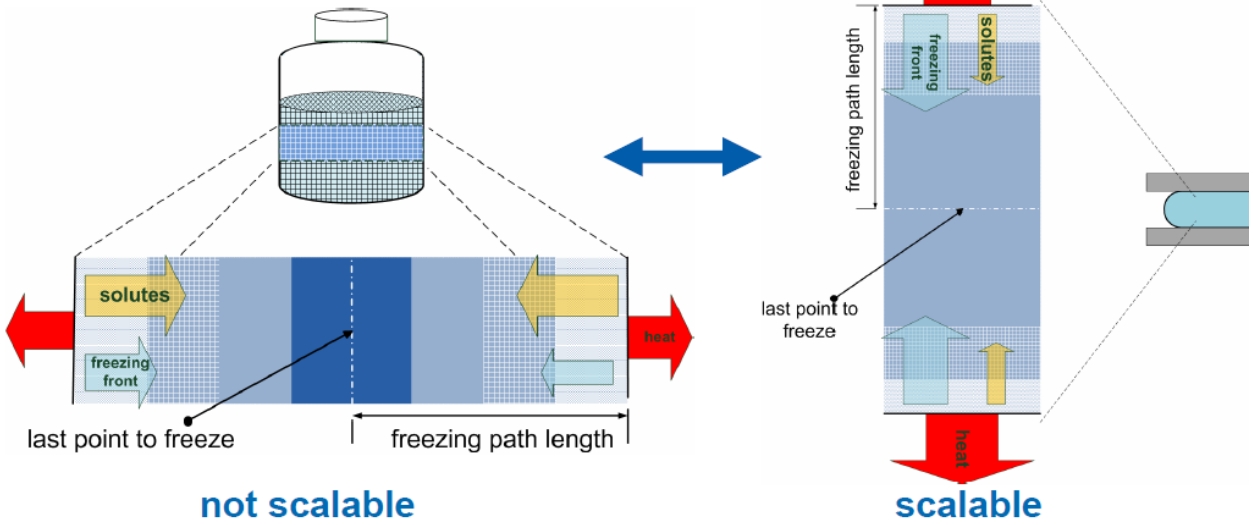
Hutchinson, N. Understanding and Controlling Sources of Process Variation. *Bioprocess Int.* **12,** 24–29 (2014)

Freezing in bags

- Isolating air layer in head space of the bottle and to the freezer
- Large layer thickness in the bottles
- Freeze/thaw time approx. 20h.

- Large contact area to plate freezer
- Small layer thickness ➔ fast and gentle
- Freeze & thaw time approx. 3h.
- Critical zone in bags where different plastics come together

- Plate-based freeze-thaw unit
- 1 L- 10 L nominal filling volume, maximum capacity 240 L
- Shell required for transport and storage
- Any volume possible



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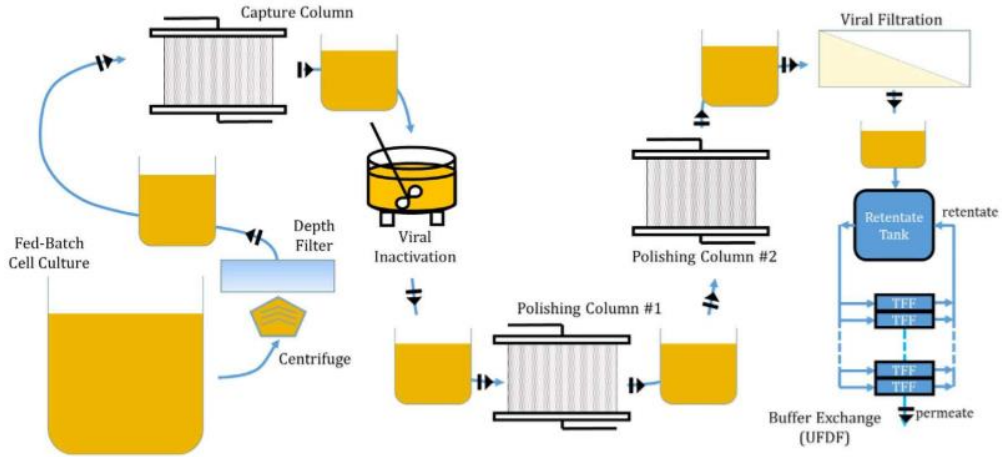
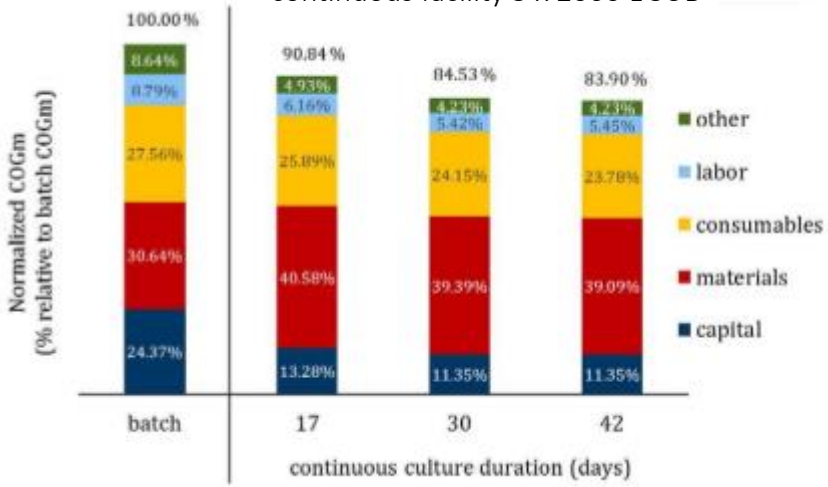
Process intensification and continuous processing

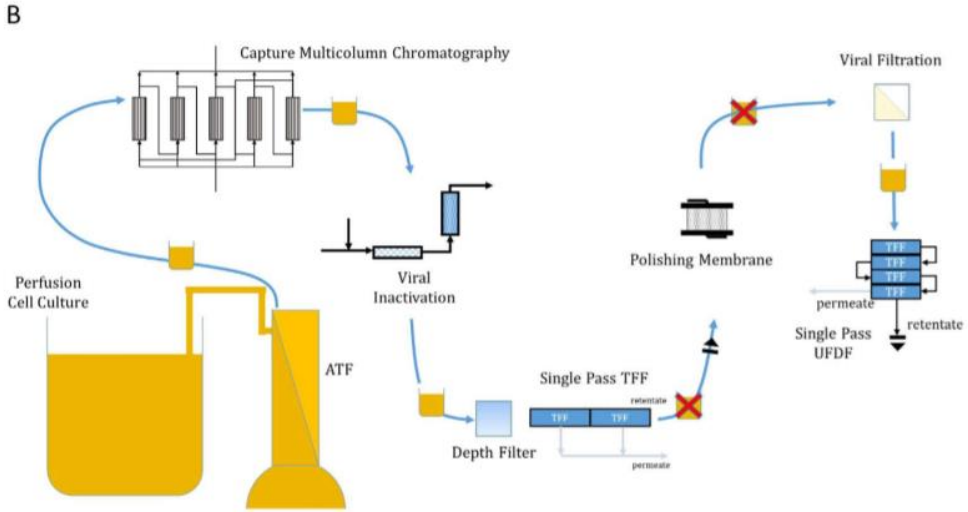
A cost of goods perspective



fed-batch with 4 x 12 500 L SS bioreactors

continuous facility 5 x 2000 L SUB





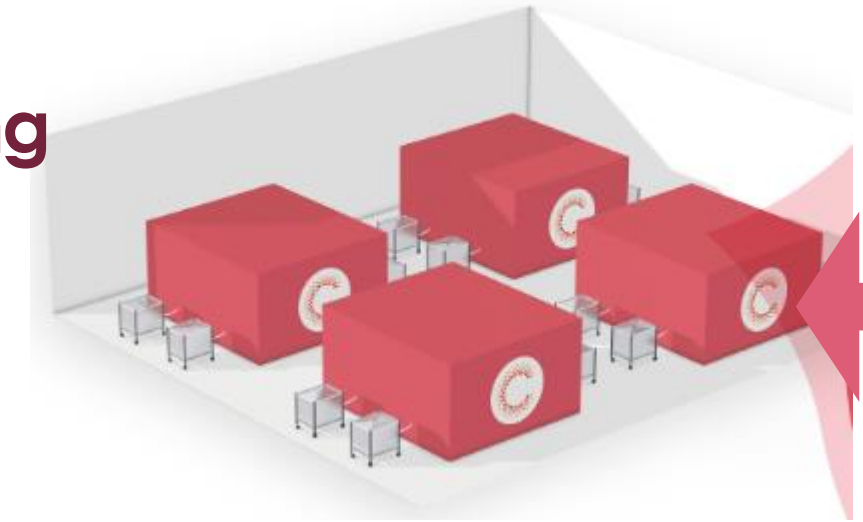
- 1 kg of purified mAb every 4 days (in a 30 days campaign) from a 200L SUB
- 15% cost reduction at 30 day campaign
- Approximately 50% lower capital investment

- Small columns 10–11 cycles/d, lifetime reached in 3 weeks
- Small surge tanks only used for pressure relief, no need of large pool tanks

Arnold, L., et al. Implementation of Fully Integrated Continuous Antibody Processing: Effects on Productivity and COGm. Biotech J., (2018)

Cost driven integration of manufacturing

Univercells modular concept



Concept

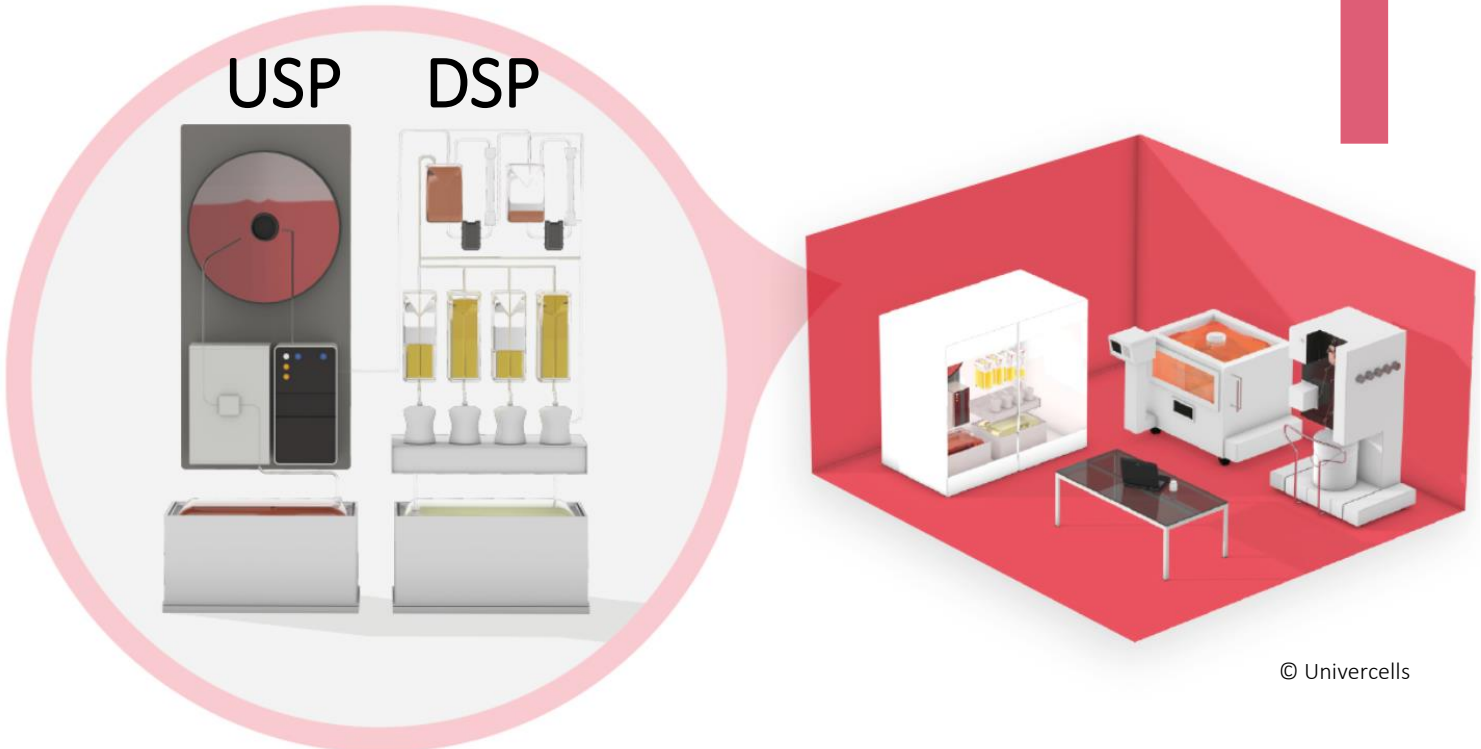
- Increased integration ➔ footprint reduction
- Minimal infrastructure due to single use
- Cost-effective modular production of biologics
- Small to medium-size markets

Upstream

- Suspension or fixed-bed single-use bioreactors
- Fed-batch or perfusion mode

Downstream

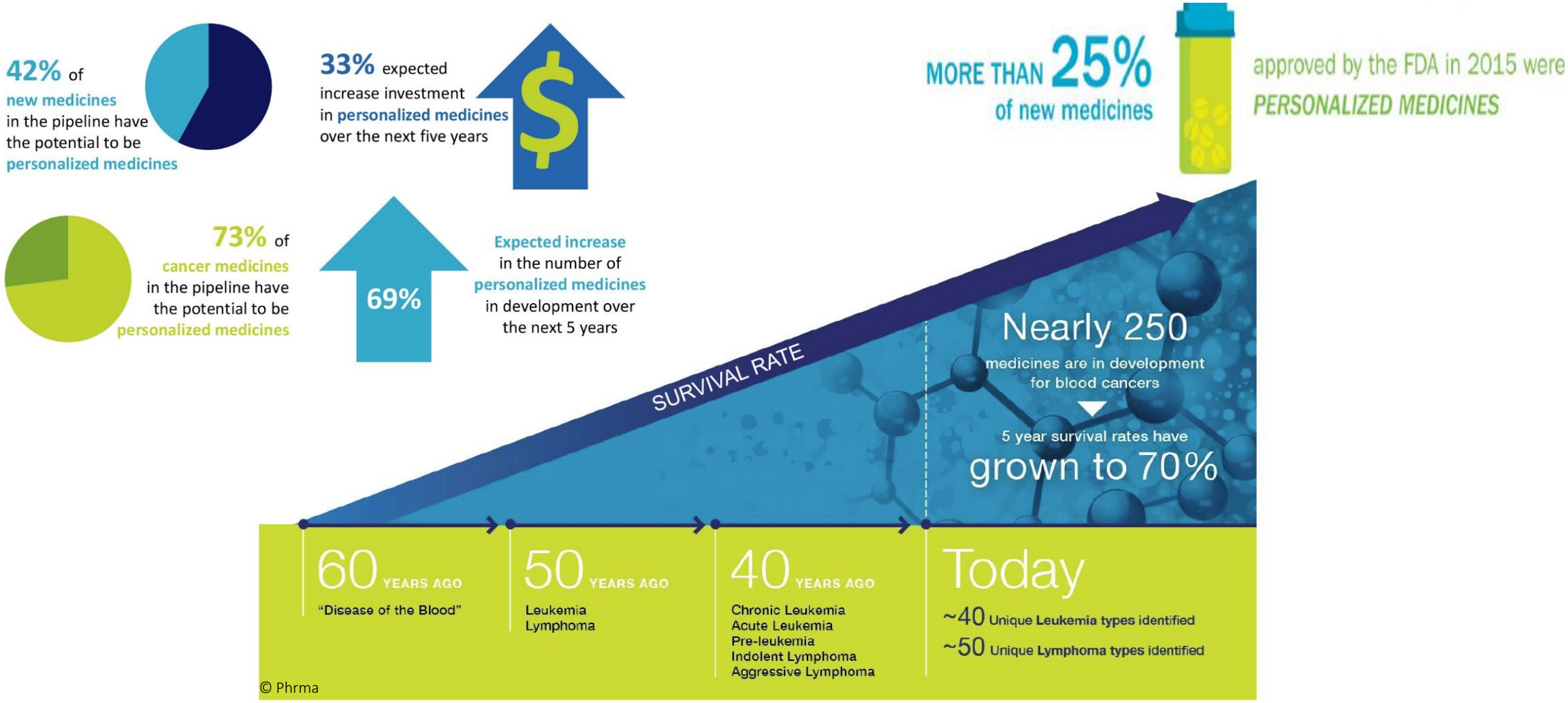
- Proprietary innovative clarification technology
- Integrated SMB purification



Jacquemart, R. et al. A Single-use Strategy to Enable Manufacturing of Affordable Biologics. Comput. Struct. Biotechnol. J. 14, 309–318 (2016).

Megatrend personalized medicine

Specific demand for cell therapy manufacturing in close proximity to hospitals



Trend towards personalized medicine I

Integrated systems

- Cell therapies as next generation treatments
- Autologous ⇔ allogeneic
- Lot size 1 (patient), small volume
- Proximity to patients in hospitals
- No need for re-use
- No DSP processing required

Cocoon™:

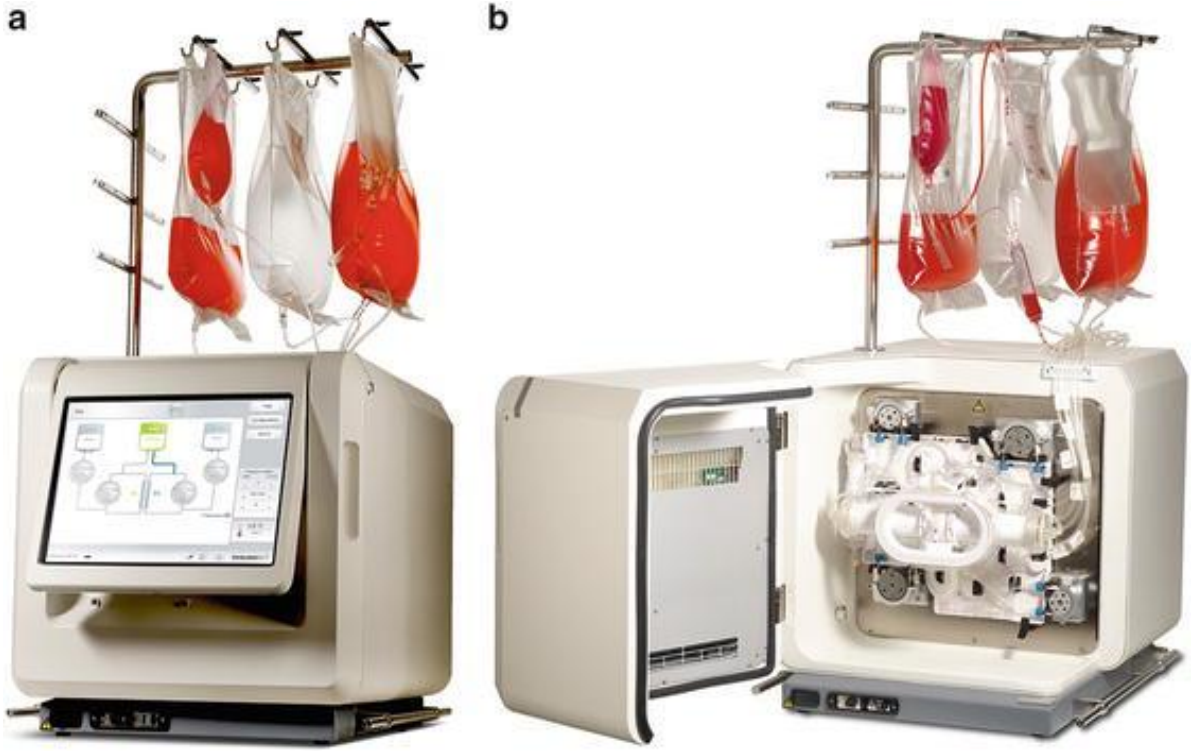
- High level of system integration, fully encapsulated
- Cell isolation, washing, expansion and formulation
- Reduced need for GMP facility environment
- Cells are refunded back into patient

Octane Cocoon™ cell production platform for personalized cell therapy manufacturing

Trend towards personalized medicine II

Quantum®

- Single use bioreactor (11,500 hollow fibers with a surface area of 2.1 m^2)
- Expands adherent cells (e.g. mesenchymal stem cells)
- Fully closed, automated, GMP compliant
- Continuous temperature control, cell feeding and waste removal
- Small footprint, direct installation in hospital possible



Quantum® automated adherent cell expansion system by TerumoBCT

Hanley, P. J. et al. Efficient manufacturing of therapeutic mesenchymal stromal cells with the use of the Quantum Cell Expansion System. Cytotherapy 16, 1048–1058 (2014).

Autologous Cell therapy

Technology combining multiple operations into one automated platform

Counterflow Centrifuge Device (CFC): Invetec & Caladrius Biosciences

- Concentration/volume reduction
- Cell washing
- Media exchange
- Particle depletion
- Short-term incubation

System components

- Instrumentation platform,
- Novel disposable flow path
- Operating and application software for automated execution of user-selected protocols

Human umbilical tissue-derived cells (hUTCs) therapies

Manufacturing automation equipment platforms for allogeneic cell therapy development

System components include:

- Four, 100-layer cell culture containers
- Cell expansion platform to handle expansion containers and medium fluidics
- Formulation platform for automated, closed, accurate and rapid formulation of concentrated cell suspension with cryo-preservative
- WCB dispensing platform, enabling automated, closed, cryo-bag filling for up to 100 bags
- Large populations of adherent cells in multiple parallel containers as a single batch

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Technical approaches with disposables

Summary

- Cell banking in bags
- Seed train in SUB or Wave
- Perfusion at N-1 step using ATF
- Continuous virus inactivation
- Single use based buffer management
- Prepacked columns of different variations
- Concentration by SPTFF
- Flow-through steps and membrane cartridges
- Mixed mode single use virus filter
- Online monitoring with single use sensors
- Final fill in bags

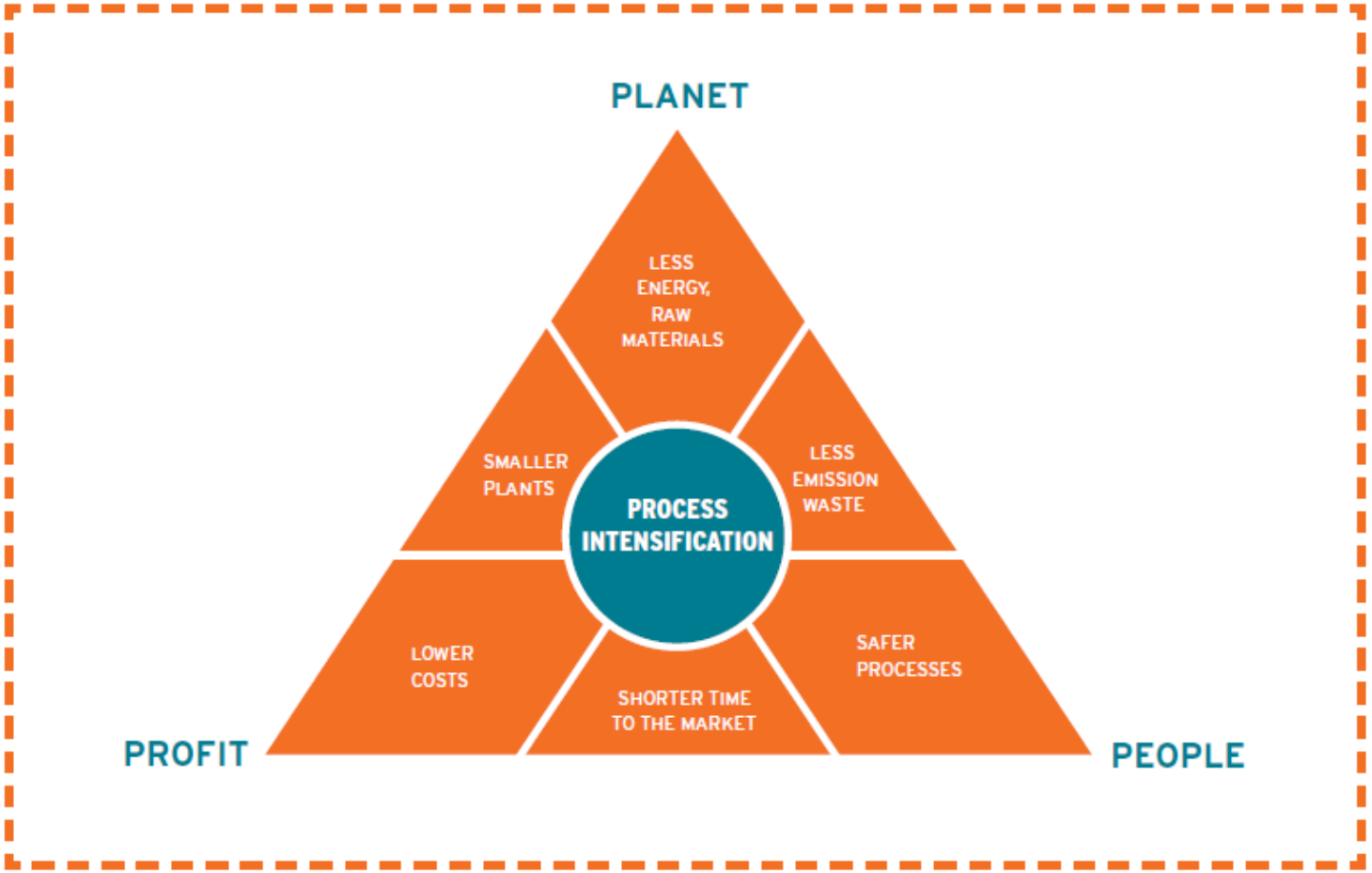

Figure C1 Process Intensification benefits align perfectly with the "Triple-P" philosophy of sustainable business

De Vries W., European roadmap to process intensification: https://www.rvo.nl/sites/default/files/bijlagen/European_Roadmap_Process_Intensification.pdf

Summary

- Process intensification can be implemented in most processes but should be evaluated already during development
- In USP the highest efficiency gain by improving the seed/inoculation train and a step elimination strategy
- Process intensification in DSP allows more options (resins, membranes, procedures, etc.)
- The biggest benefit in DSP by combining operations (precipitation, mixed mode resins, SPTFF and chromatography)
- Disposables have a huge impact on intensification by eliminating labor and preparation time
- Continuous processing is easiest established in the USP but new DSP approaches support end to end continuous processes
- Personalized medicine can significantly benefit from integrated intensified processes based on disposable elements

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Thanks - Questions?