DSM Biologics

Boosting Yield and Improving Quality by Process Intensification in Mammalian Cell Culture

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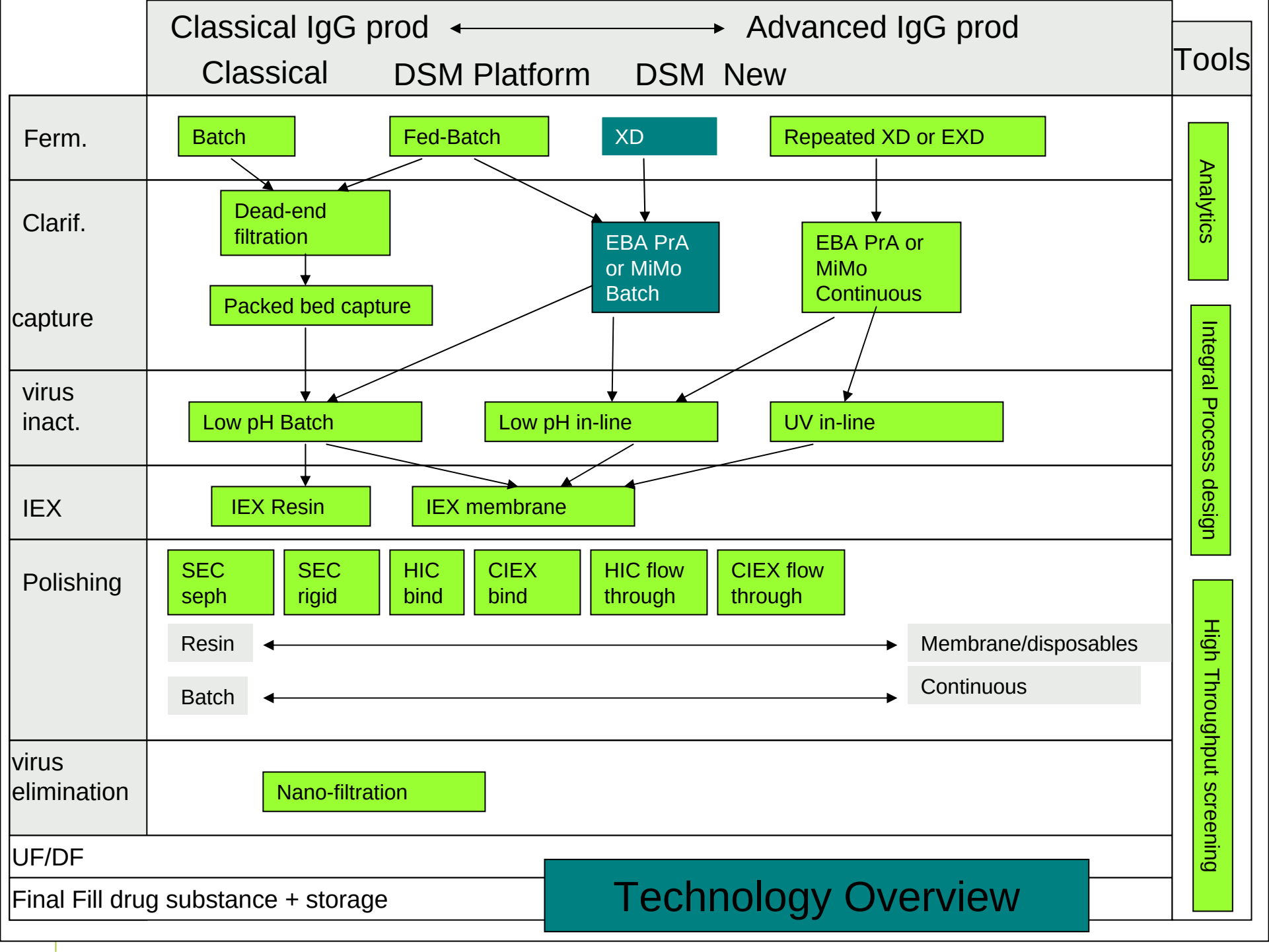
DSM Biologics.

BPN
October 2010


Classical IgG prod
Advanced IgG prod
Classical
DSM Platform
DSM New
Tools
Ferm.
Batch
Fed-Batch
XD
Repeated XD or EXD
Clarif.
Dead-end filtration
EBA PrA or MiMo Batch
EBA PrA or MiMo Continuous
capture
Packed bed capture
virus inact.
Low pH Batch
Low pH in-line
UV in-line
IEX
IEX Resin
IEX membrane
Polishing
SEC seph
SEC rigid
HIC bind
CIEX bind
HIC flow through
CIEX flow through
Resin
Membrane/disposables
Batch
Continuous
virus elimination
Nano-filtration
UF/DF
Final Fill drug substance + storage
Technology Overview
Analytics
Integral Process design
High Throughput screening

The XD® technology

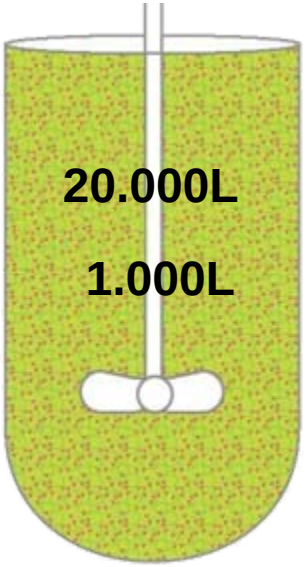
In more detail…

The Future of Protein Manufacturing

XX batches in 20,000L stainless steel bioreactors

Yesterday

Today



At 20g/L → 400Kg/batch

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What is XD® Process Technology?

- Extreme Density Cell-culture:

- DSM proprietary process technology
- Boosts titer/bioreactor productivity 5 -10 fold
- Boosts cell density 5 – 10 fold
- Maintains very high cell viability >90%
- Good Product Quality
- Applicable to many cell-lines (incl. CHO and PER.C6®)
- Batch time 14 – 20 days
- Single concentrated batch of product
- Also for existing processes !!

XD®: Process Intensification

Cell Culture Mode of Manufacturing

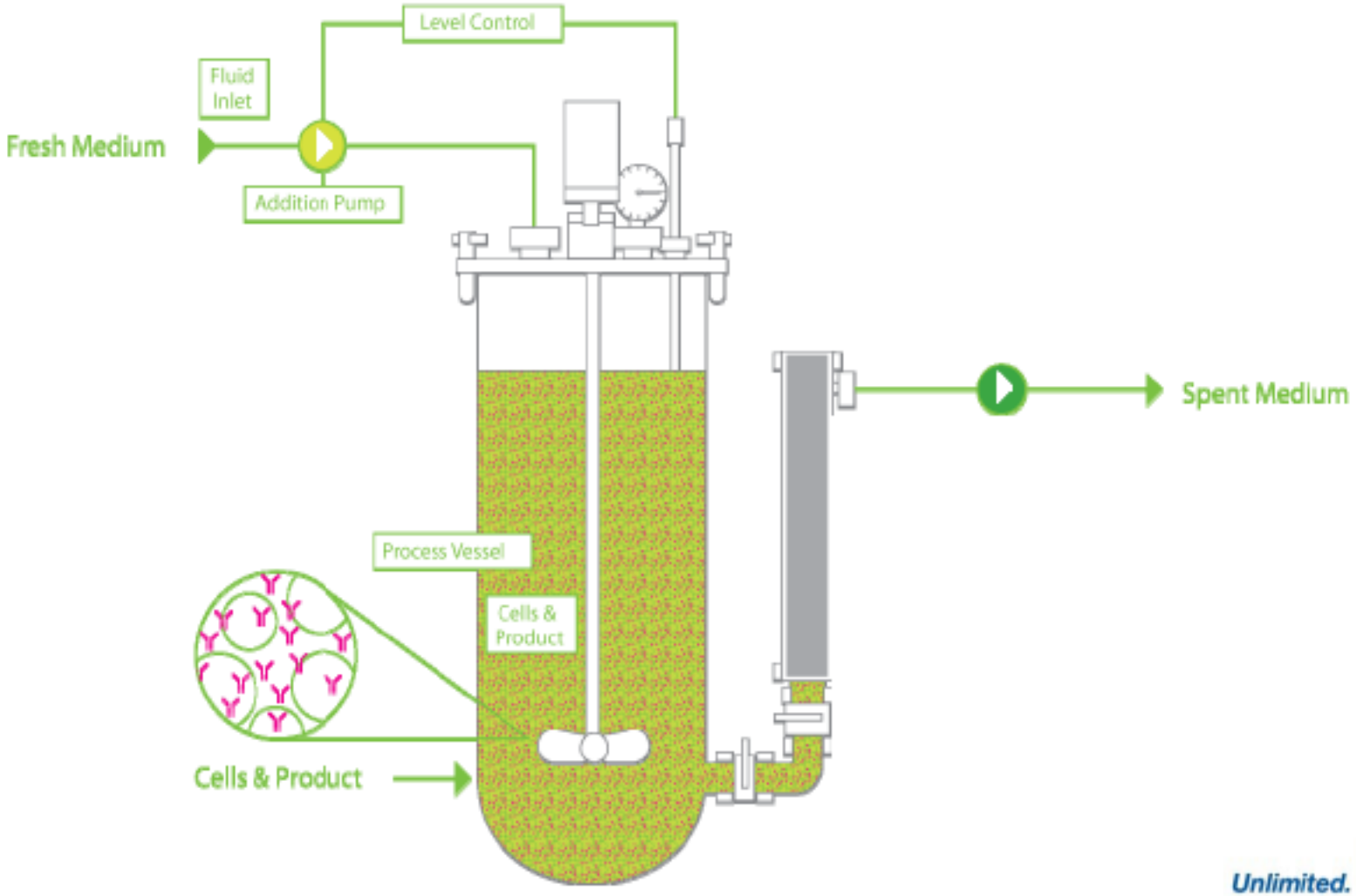
Fed Batch
Feed concentrate
Build up Metabolites
Osmo increase
Changing environment
Reducing cell viabilities
Concentrated Harvest
batch identification

XD®
Medium Feed
Wash out Metabolites
No Osmo increase
Constant environment
High cell viabilities
Concentrated Harvest
batch identification

Perfusion
Medium Feed
Wash out Metabolites
No Osmo increase
Constant environment
High cell viabilities
Dilute harvest
Large harvest

XD® Process

Bioreactor Set-up



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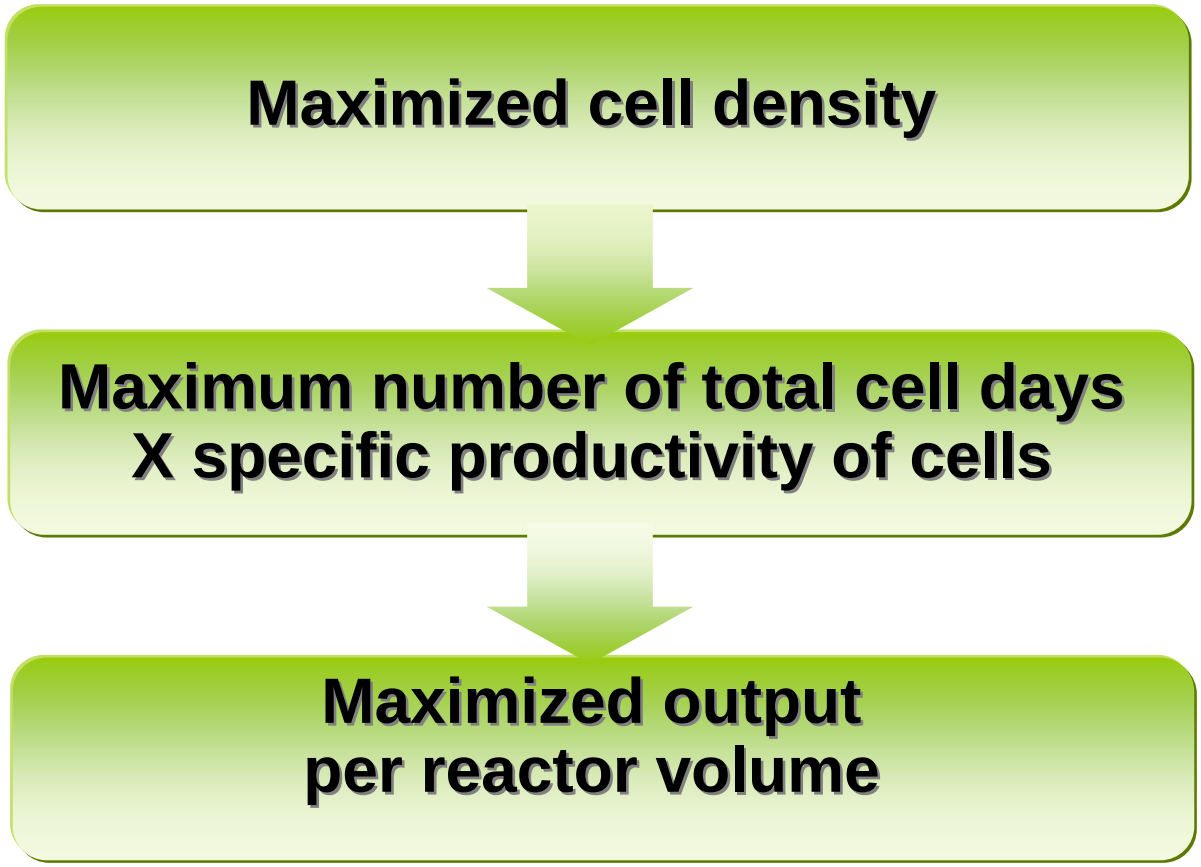
XD®: Process Intensification



XD® Process in cGMP

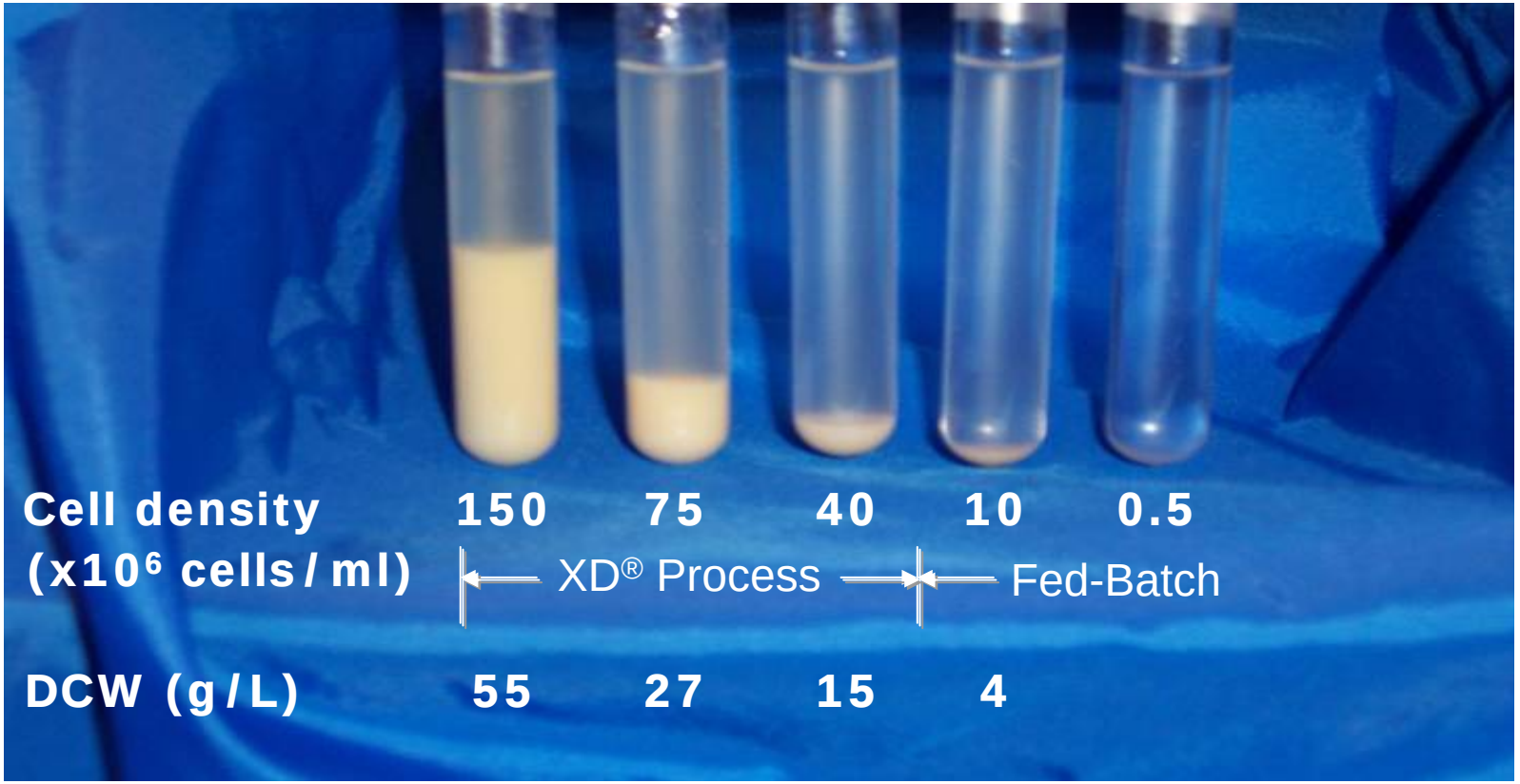
Controller

50L Single Use Bioreactor (SUB)

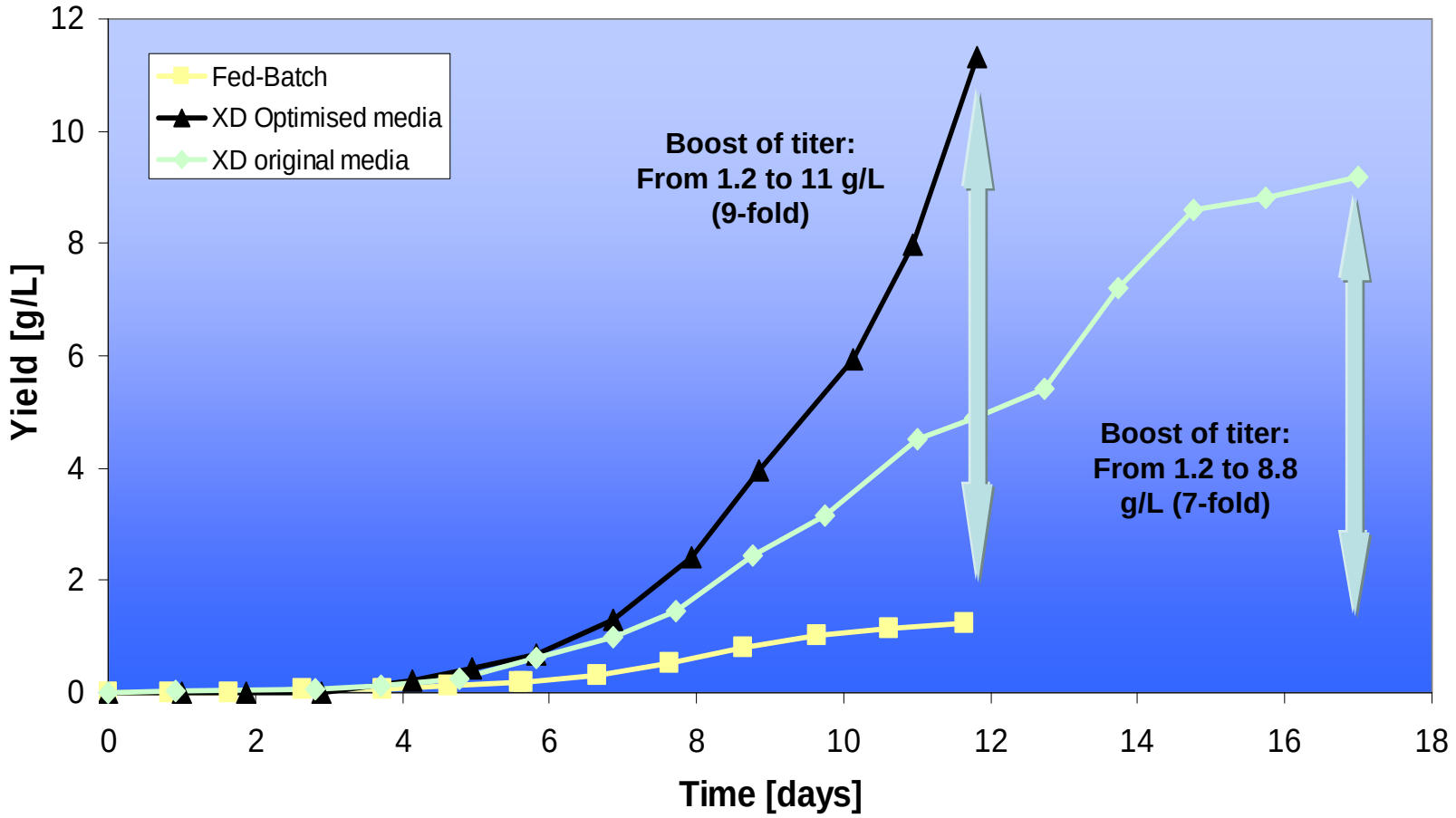
Hollow Fibre

XD®: Process Intensification

Unmatched biomass

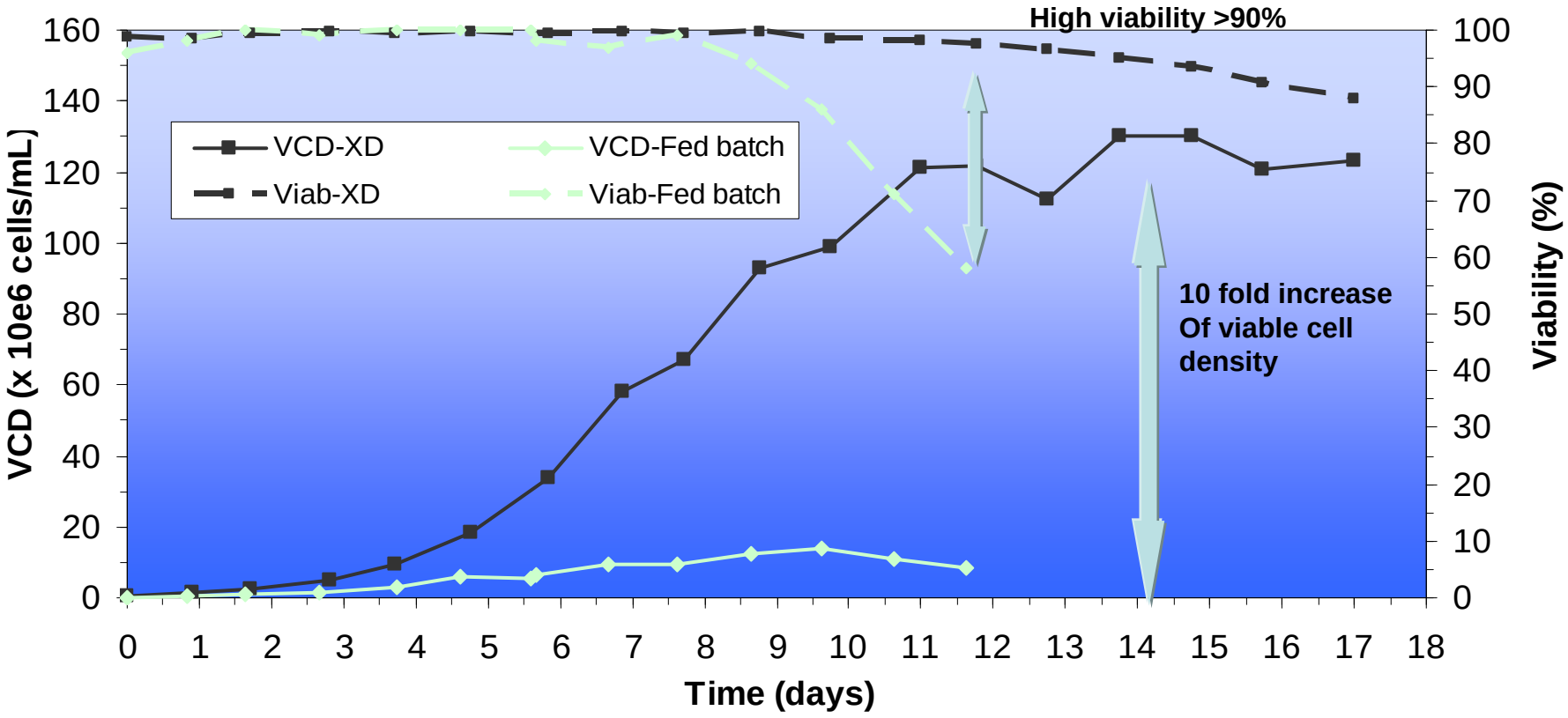


XD® - CHO Case Study 1
Fc - Fusion Protein



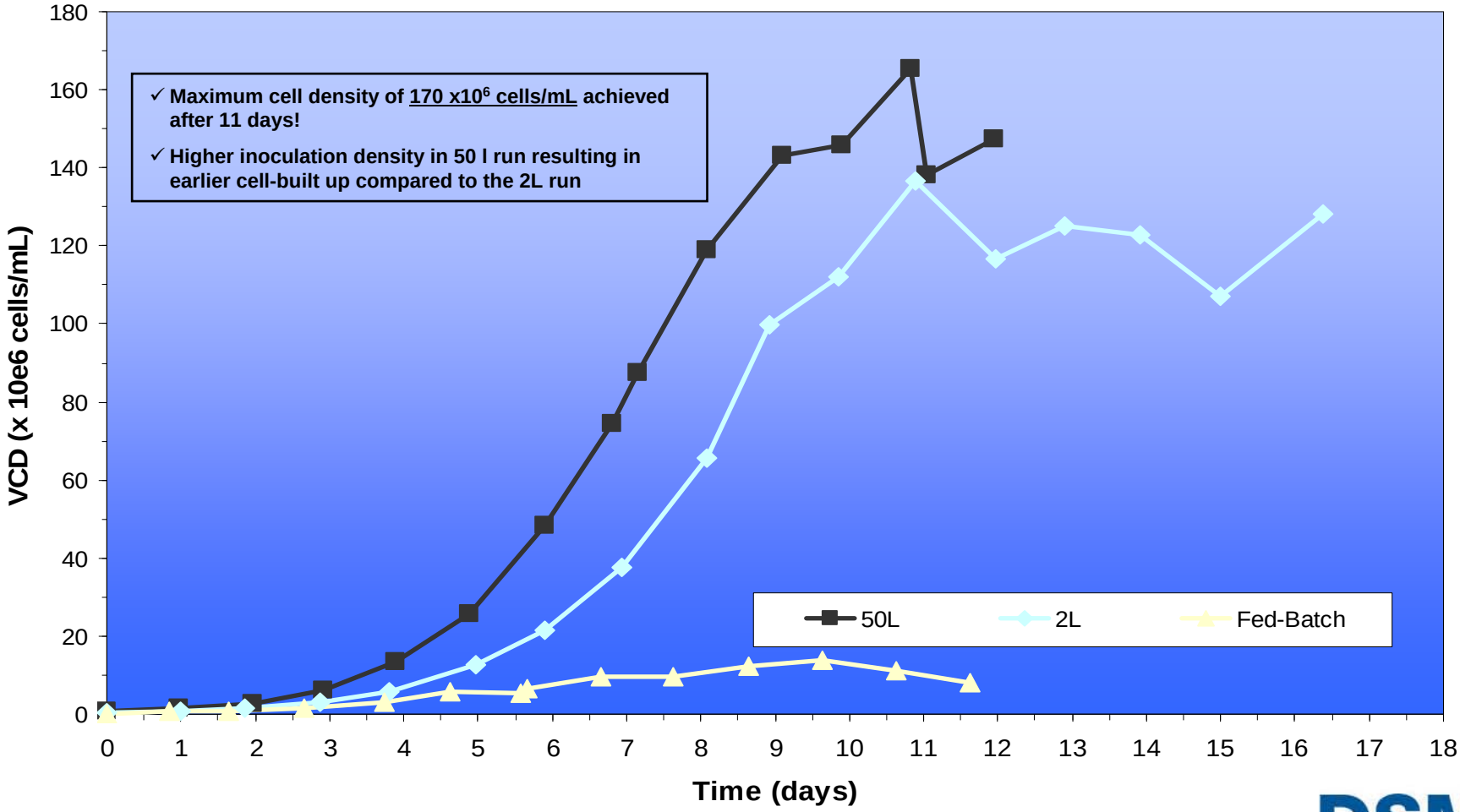
XD® - CHO Case Study 1
Fc-Fusion Protein

Cell Density & Viability: XD® CHO vs Fed Batch



XD® - CHO Case Study 1
Fc-Fusion Protein

Comparison 2L, 50L XD® and fed-batch: Growth profile



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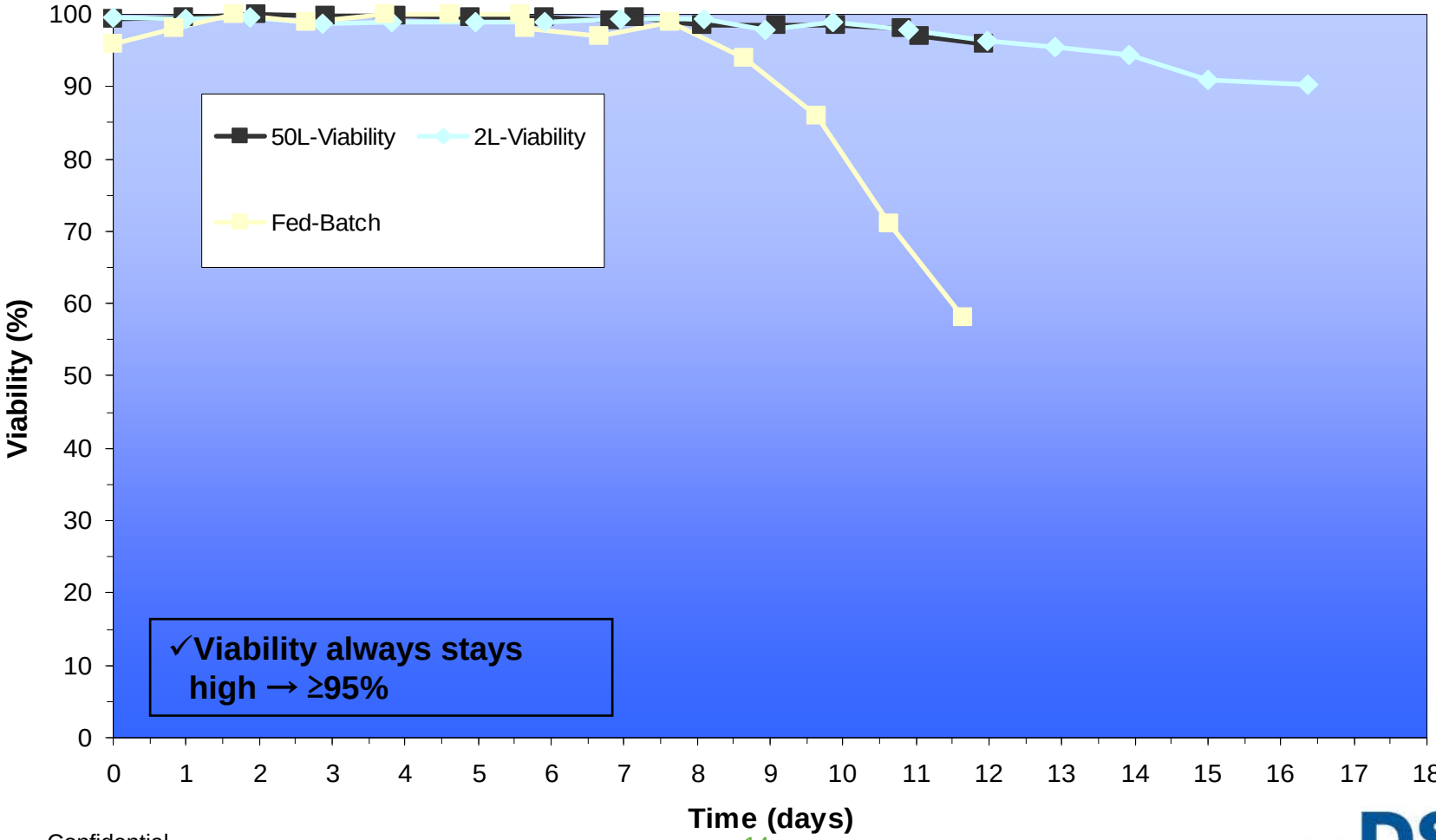
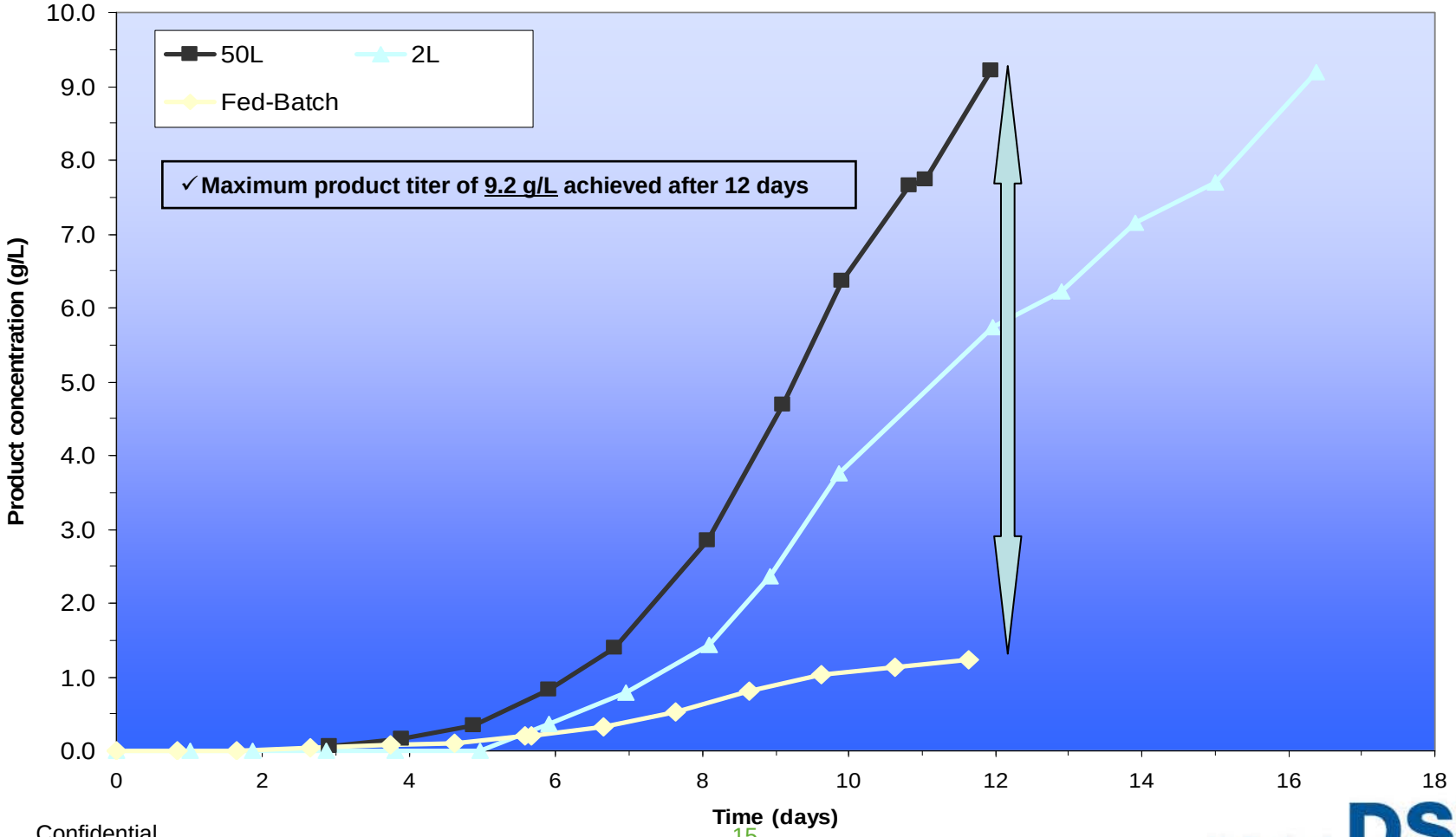
XD® - CHO Case Study 1
Fc-Fusion Protein

Comparison 2L, 50L XD® and fed-batch: Viability



XD® - CHO Case Study 1
Fc-Fusion Protein

Comparison with 2L XD® and fed-batch: Product titer



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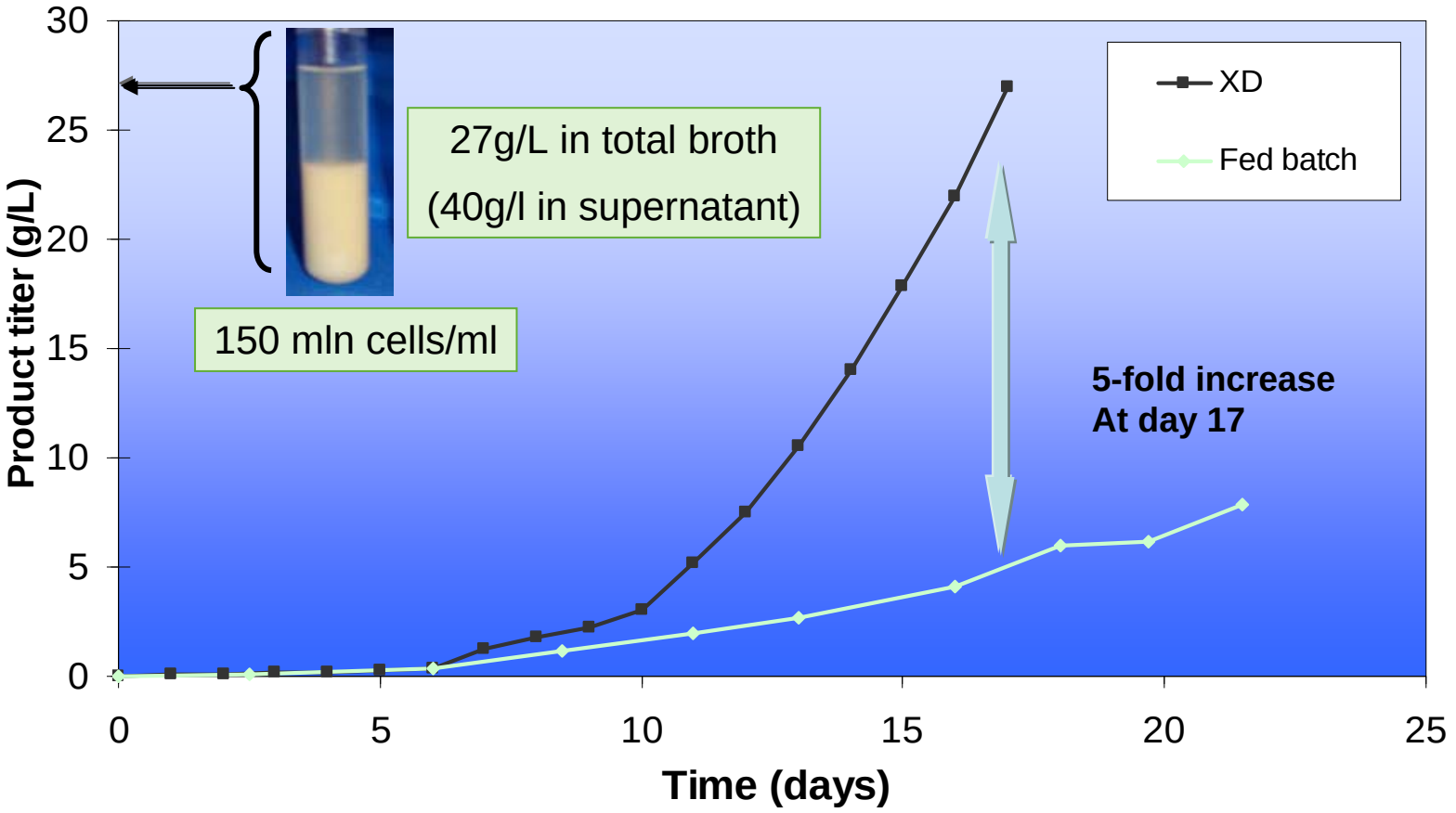
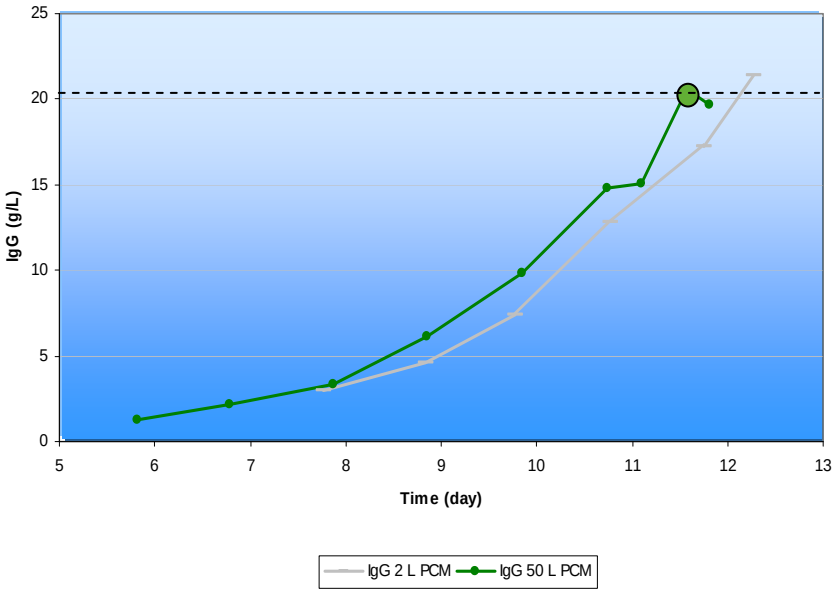
XD® - PER.C6® Results
IgG



XD® - Very good scalability !

Product Titer & Cell Density: XD® 2L & 50L Scale

20 g/l



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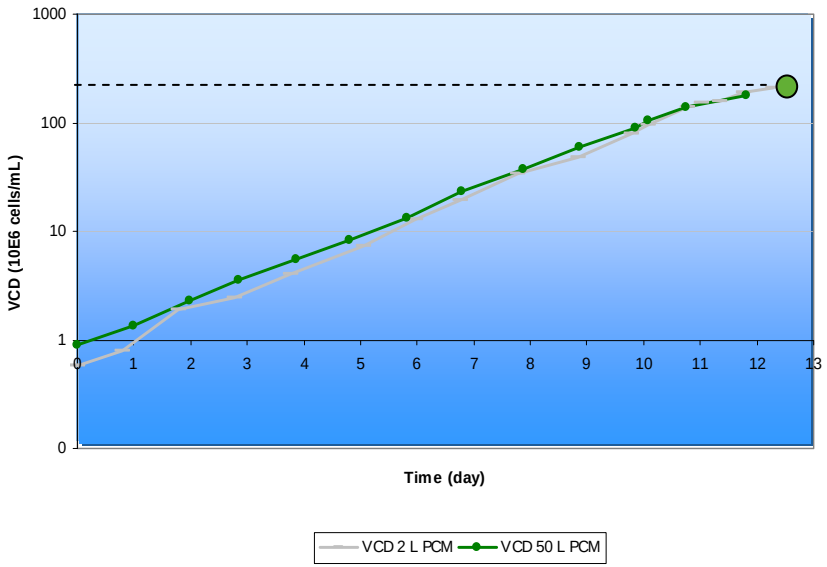
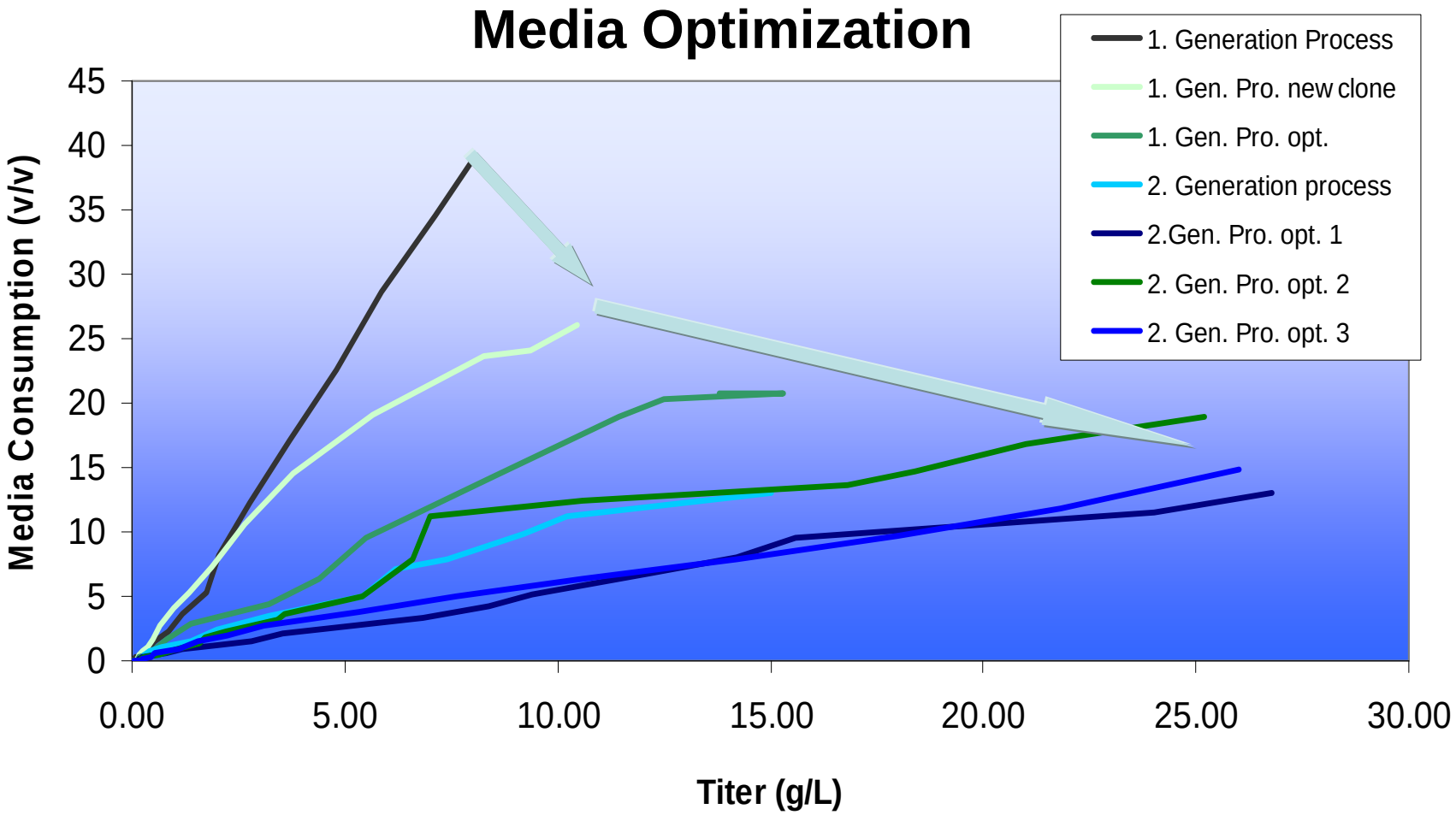


XD® Media Consumption



PER.C6®, IgG, 15 - 40 PCD

At harvest After 14 – 21 Days

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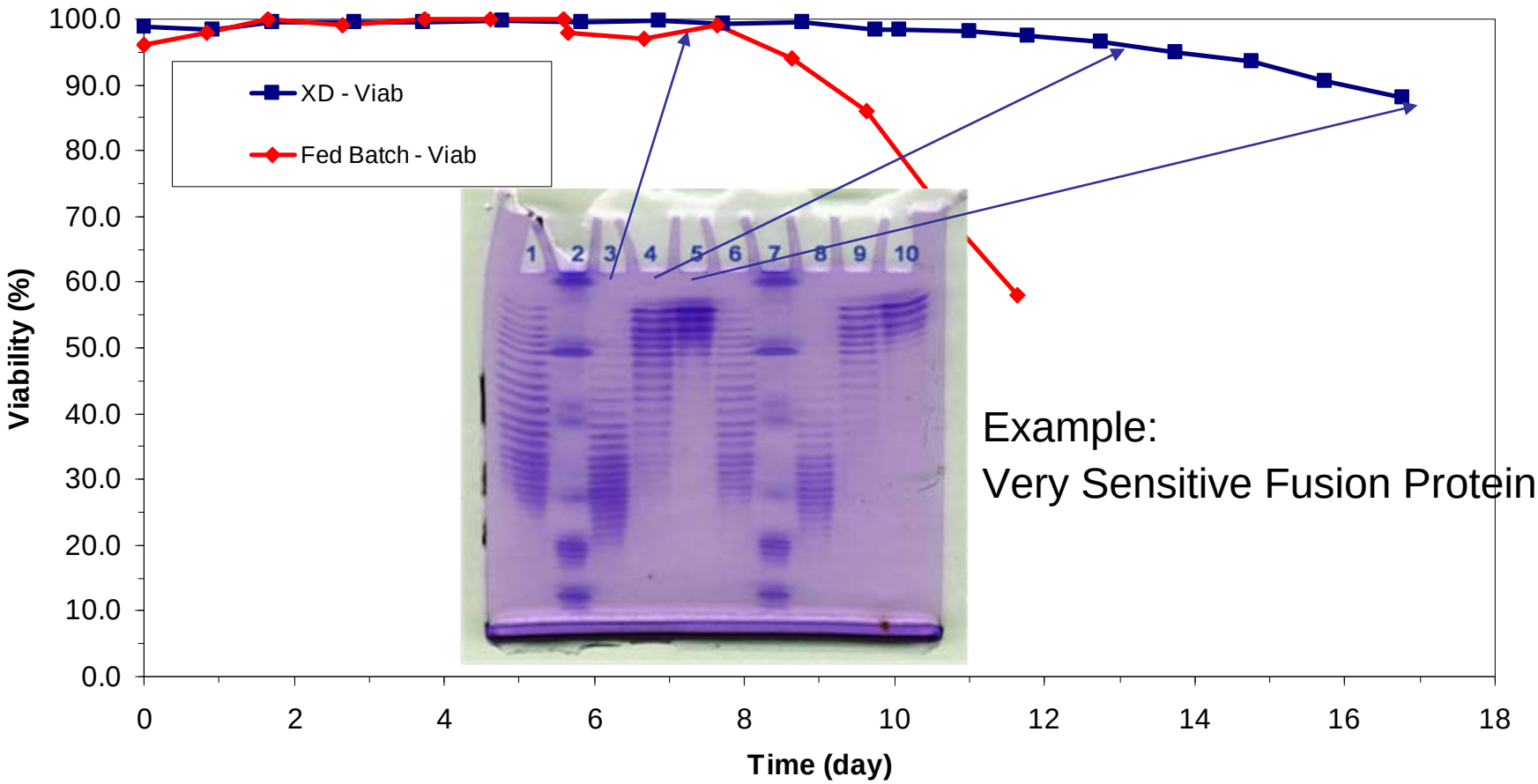
Titer boost results with XD®

- Cell line independent titer increase
  - Applicable to many cell lines:
    different CHO cell lines, Hybridoma,
    PER.C6® cell line, …
- Consistent titer boost by a factor 5x to 10x
  compared to fed batch

	XD®	
1 – 1.5 g/l	→	7 – 15 g/l
5 g/l	→	> 25 g/l

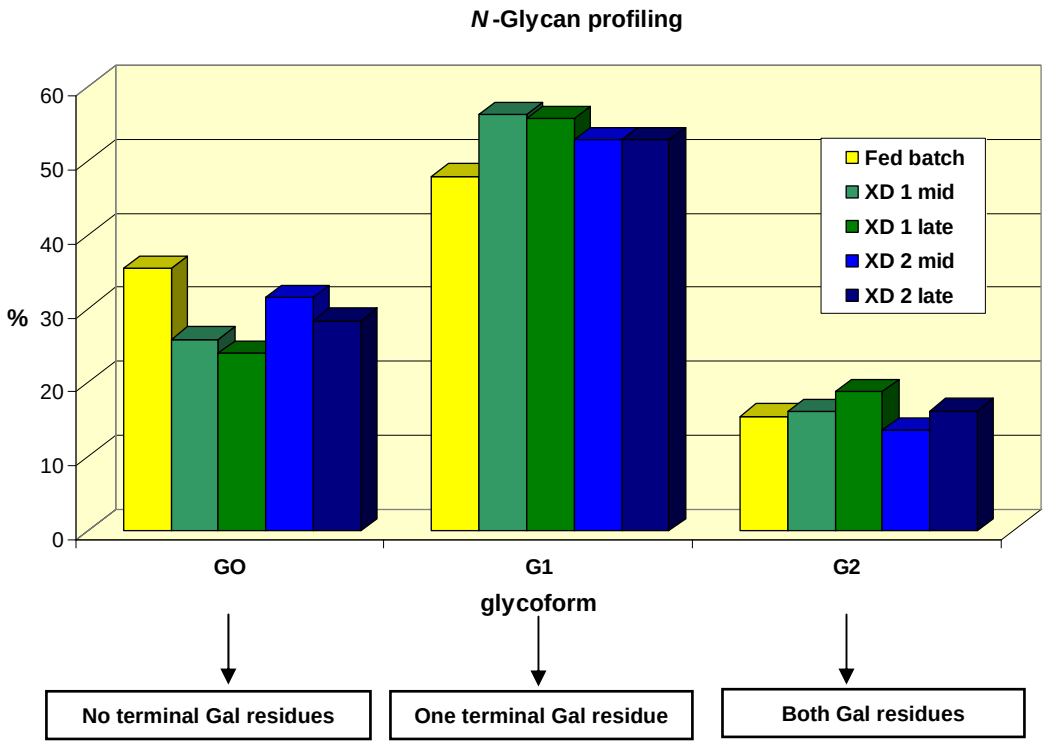
Product quality can be steered very well in XD®
because of “larger operation window and better batch control”

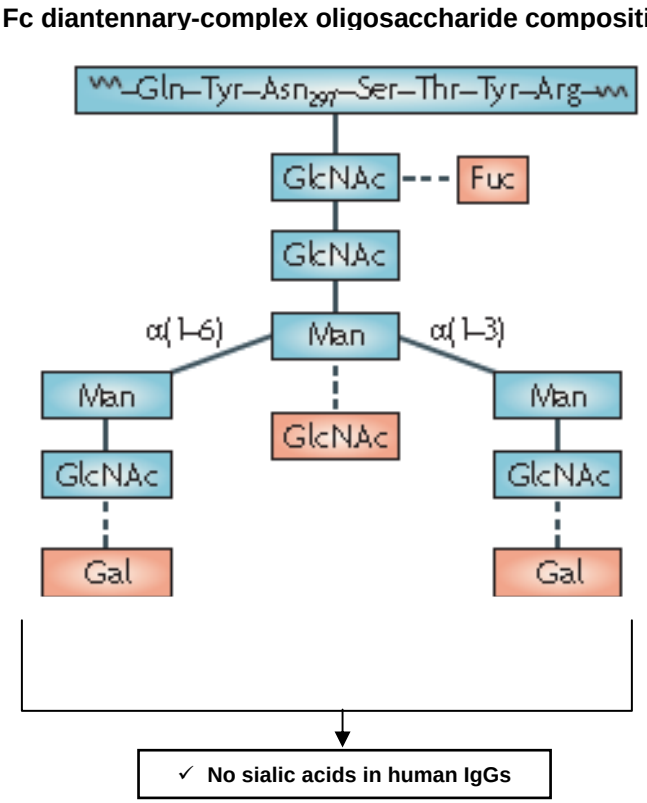


Many possible optimization parameters: media flow, max cell density, batch time, filter size, type of media, etc., while retaining high titers

Product quality - Glycosylation

- XD® samples are comparable to reference fed batch material in terms of *glycosylation* profile.





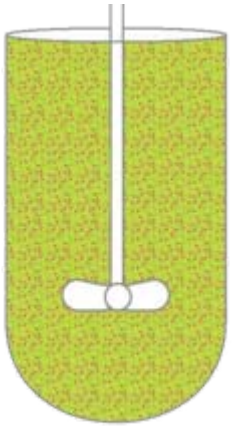
XD® Vision: Single Use Bioreactors

Clinical Devel → **Launch** → **Maturity**

50 L XD®

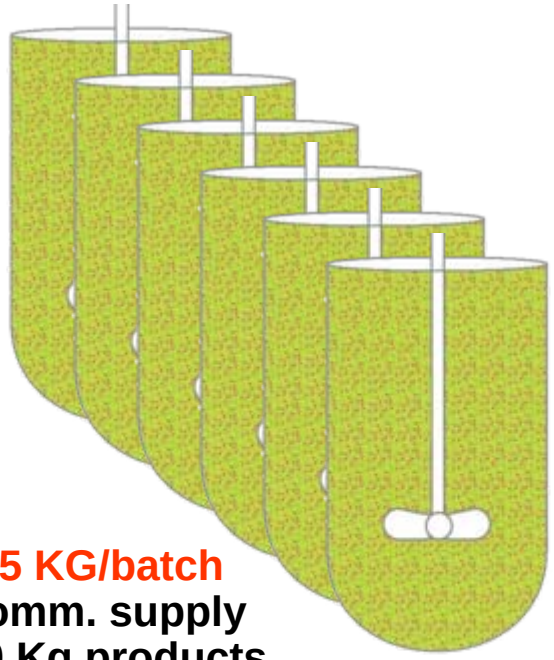
0.5 KG/batch
CT I/II supply

250 L XD®



2.5 KG/batch
CT III/comm. supply

Multiple 250 L XD®



2.5 KG/batch
Comm. supply
>20 Kg products

DSP technology

EBA – Expanded Bed Adsorption

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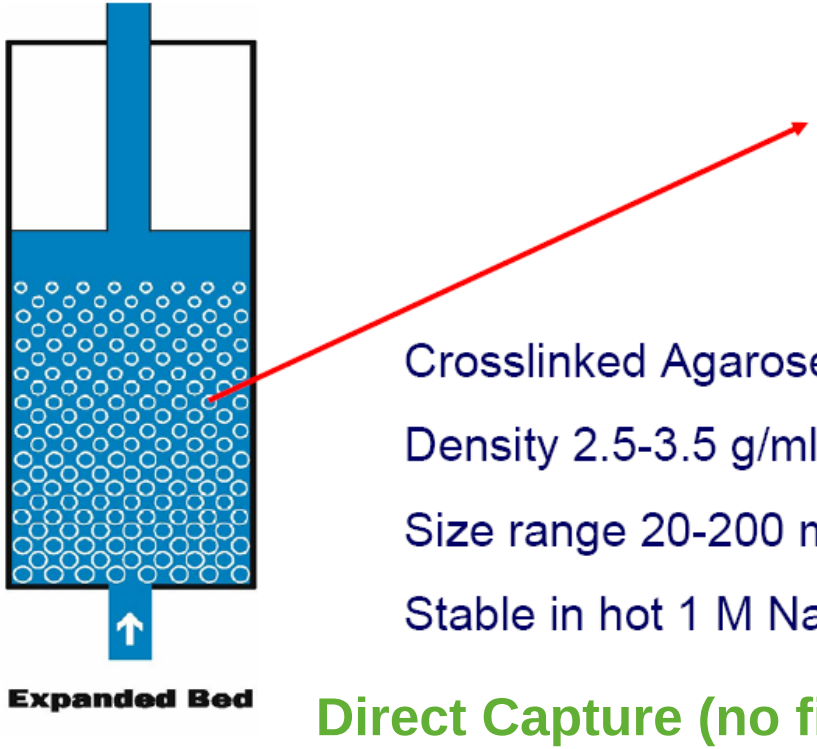
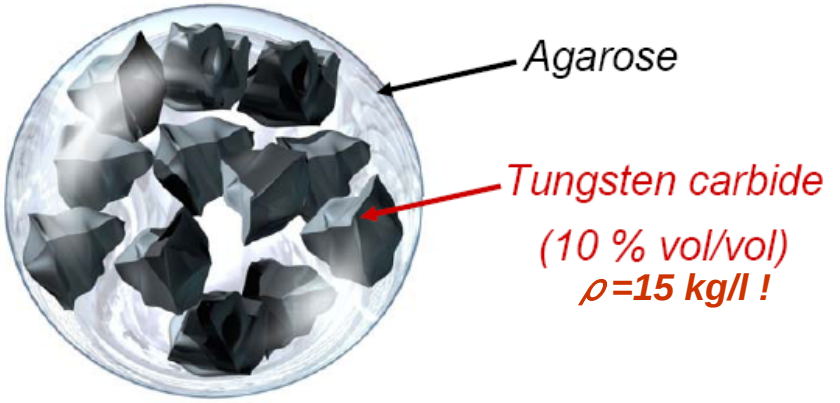
Rhobust™ Expanded Bed Adsorption





Crosslinked Agarose – tungsten carbide conglomerate

Density 2.5-3.5 g/ml

Size range 20-200 micrometer

Stable in hot 1 M NaOH

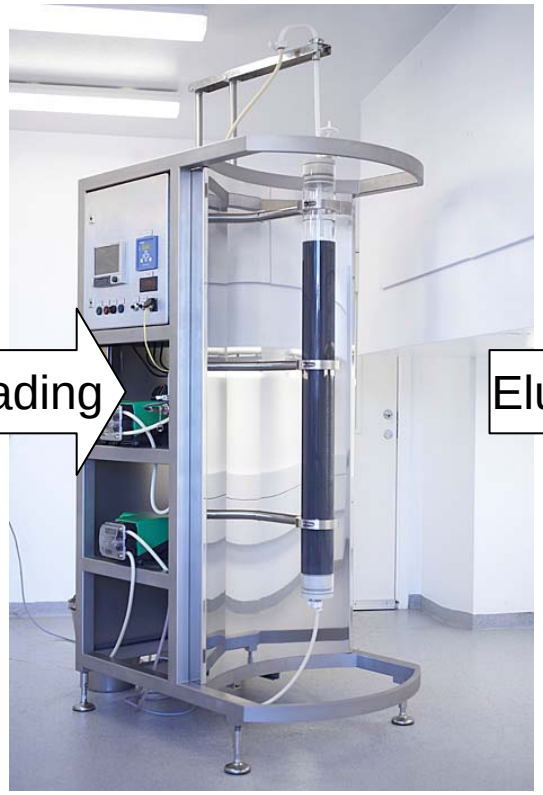
Direct Capture (no filter, no centrifuge)
Ideal Combination with XD®'s High Cell Densities

Rhobust™: Simplify Downstream Process

Single use Bioreactor in XD® mode

Rhobust™ : Integrated Clarification & Purification by Direct Capture

Loading



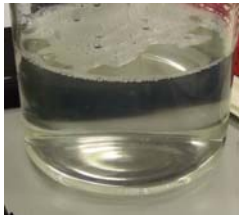
Elution

DSP

Rhobust™: in Action

XD® Harvest
~150x10^6 cells/mL

EBA: one step !



Post-Protein A intermediate

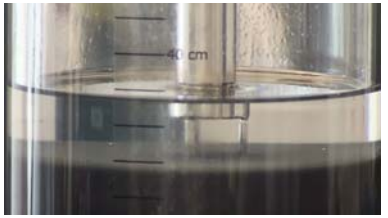
Equilibration

First cell breakthrough

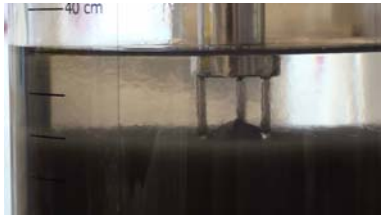
Complete cell breakthrough

Cell wash out

Wash



Elution



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EBA Experiments with XD®

Results Feb-batch, classical Protein-A packed bed versus XD®, EBA with Mixed Mode Ligand and Protein-A:

	Yield (%)	Purity (%)	HCP (ug/mg Mab)
Fed-batch, Filtration / Protein A Packed Bed	> 85	> 95	1 – 15 (n=15)
XD®, EBA - Mixed Mode Ligand	> 95	> 90	10 - 50 (n=5)
XD®, PrA EBA	> 95	> 90	4 - 23 (n=3)

With high cell viabilities consistent high yield

Purity still needs to be optimized (ligands/conditions)

HCP after column in normal range

Conclusion

- XD®
  - High yields
  - Increased flexibility
  - Targeted quality
  - Effective production cost

- EBA
  - Reduced Process steps
  - High yields
  - Effective production cost

DSM in Australia

Partner of:

The Commonwealth of Australia &

Queensland State Government

BPA
BioPharmaceuticals Australia

Open in 2013

Thank you for your kind attention!

Back-up slides

Rhobust™: EBA equipment

Systems	Diameter	Length	Material
Lab scale	1 cm	Variable	Glass columns
	2 cm	Variable	Glass columns
	2 cm	50 cm	Disposable
Pilot scale	2 cm	Variable	Disposable
	10 cm	Variable	Glass columns
	30 cm	Variable	Glass columns
	45 cm	Variable	Glass columns
	60 cm	Variable	Glass columns
Manufacturing	Custom made or Pilot scale in cGMP version		

Resin	Density	Ligands
Agarose + WC	2.5 - 3.5 g/mL	PrA, MiMo, IEX