CONVERSION OF AN INTENSIFIED FED-BATCH TO AN INTEGRATED CONTINUOUS BIOPROCESS

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INTRODUCTION

Despite advances in upstream and downstream Integrated Continuous Bioprocessing (ICB) technologies, reducing manufacturing costs and process development costs continue to be a concern.

- Conversion of a fed-batch (FB) or intensified fed-batch (IFB) process into a hybrid or end-to-end ICB process may be particularly challenging but manageable using key platform elements:
  - High specific productivity cell lines
  - Low-cost, concentrated perfusion media formulations
  - An optimized and scalable cell-retention device
- The impact to product quality remains as a potential issue stemming from higher culture cell densities, longer culture durations, and differences in product residence times.

This work involved the rapid conversion of an IFB mAb process to a hybrid ICB process using the Just-Evotec Biologics platform, resulting in several key project accomplishments:

- Mitigation of upstream IFB challenges: Filter fouling due to higher culture cell densities and durations was addressed by controlling cell density and implementing continuous harvest (CH).
- Significant productivity increase: 5-fold increase in product mass per working volume compared to original FB process at the 500L manufacturing scale.
- Short development time: < 6 months from project start to cGMP batch completion.
- Minimal risk from changes in product quality: most attributes (HMW and rCE) and process impurities (HCP, DNA, and Pro-A) comparable (minor differences in glycan distribution).

These results demonstrate that the rapid conversion of FB and IFB mAb processes to ICB can be met when implementing a robust ICB platform, thus supporting the biotherapeutics industry's need to quickly adapt to changing clinical and business circumstances.

PROCESS EVOLUTION STAGES

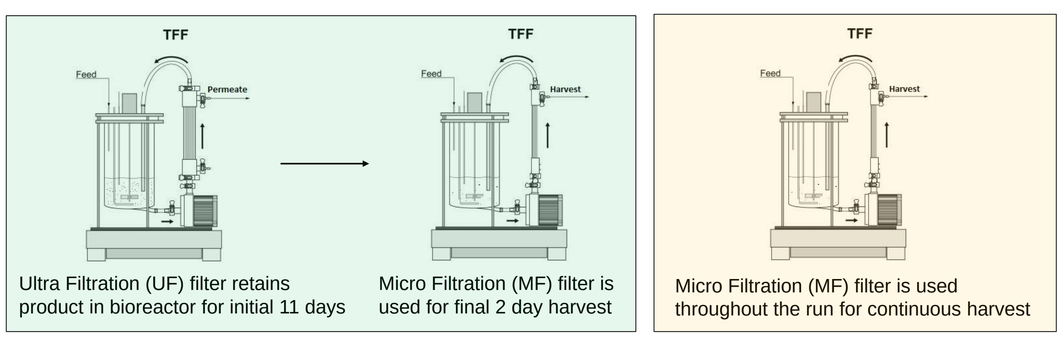
- Clinical mAb material generated from four distinct processes differing in bioreactor operation, harvest method, and capture step mode.
  - Need for high productivity and short project timeline: < 6 months from project start to cGMP DS.
  - Process transferred from original CDMO (versions 1 and 2) to Just-Evotec Biologics (versions 3 and 4).
- Intensified fed-batch process (IFB; version 3):
  - Short duration, high productivity and product concentration with low harvest volume.
  - Poor cell density reproducibility (high and uncontrolled) leading to filter fouling, frequent UF hollow fiber filter changes, and difficulty in restarting perfusion after filter changes.
- Continuous harvest process (CH; version 4) improved performance and productivity.
  - Controlled target cell density with continuous harvest removing and purifying product from the bioreactor on-line.
  - Maintaining consistent product quality across process versions was a concern due to changes in bioreactor residence time and capture column operation (batch vs. multi-column protein A affinity chromatography).

Figure 1. Process history and flow diagram for versions 3 and 4 (IFB – ICB (CH)).

CDMO and Process Version[1]		Production Bioreactor	Harvest	Capture Step	Viral Inactivation	Polishing Step 1	Polishing Step 2	Viral Inactivation	Final DS
Just-Evotec	3	Perfusion-IFB	UF MF	ProA Affinity	Low pH	Polish 1	Polish 2	Viral Filtration	UF/DF
Just-Evotec	4	Perfusion-CH	MF	ProA Affinity (continuous)	Low pH	Polish 1	Polish 2	Viral Filtration	UF/DF

[1] Process version 1 and 2 (fed-batch) were developed and performed at another CDMO and are not shown.

Figure 2. Production bioreactor: IFB vs. ICB (CH) perfusion and harvest details.

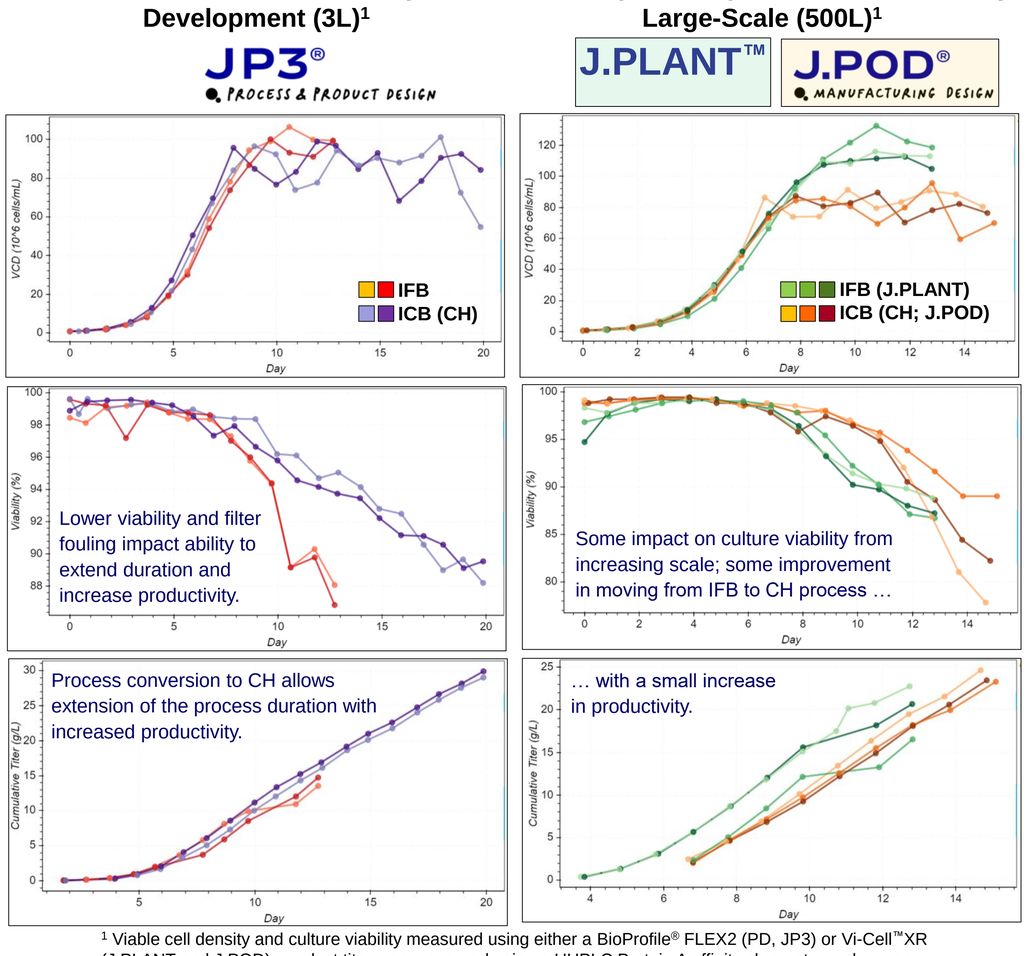


Bioreactor materials and methods: 3L glass bioreactor vessel (ChemGlass and Applikon) and controller (In-control, Applikon) or 500L Xcellerex XDR500 SUB (Cytiva) and controller (DeltaV) with automation system controlling TFF system flowrate as well as the perfusion feed, permeate/harvest, glucose, base, and antifoam flowrates via on-line scale measurements.

PRODUCTION BIOREACTOR RESULTS

- Development runs:
  - IFB process (12-13 days) had lower viability, increased filter fouling, and lower productivity.
  - ICB (CH) process extended from 15 to 20 days (assess product quality impact) resulting in an almost two-fold increase in productivity.
- Manufacturing runs: Similar trending yet smaller differences in viability and productivity for IFB vs. ICB (CH) processes; filter sizing experiments are in-progress to improve scalability.

Figure 3. Production bioreactor data (viable cell density, viability, and cumulative titer).



[1] Viable cell density and culture viability measured using either a BioProfile® FLEX2 (PD, JP3) or Vi-Cell™XR (J.PLANT and J.POD); product titer was measured using a UHPLC Protein A affinity chromatography assay.

PRODUCT QUALITY RESULTS

- Largest impact on product quality attributes due to the switch from the IFB to ICB (CH) process on CEX and icIEF (acidic and main peaks), but the values remained within the specifications.
- Product residence time in the production bioreactor was reduced converting from process version 3 to 4 potentially impacting PQ results.

Table 2. Manufacturing-scale product quality data for process versions 3 and 4 (UF/DF pool).

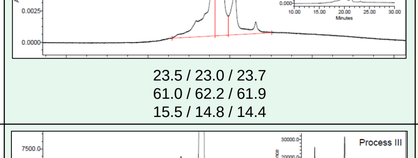
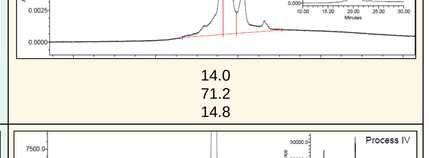
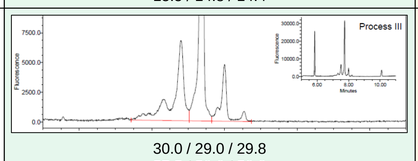
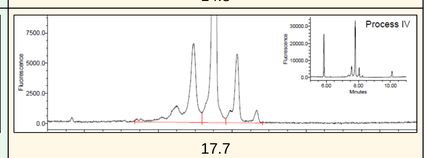
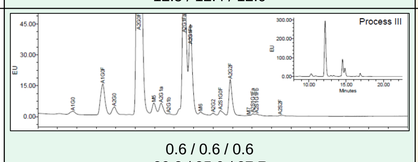
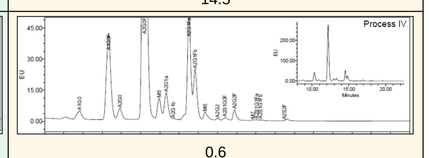
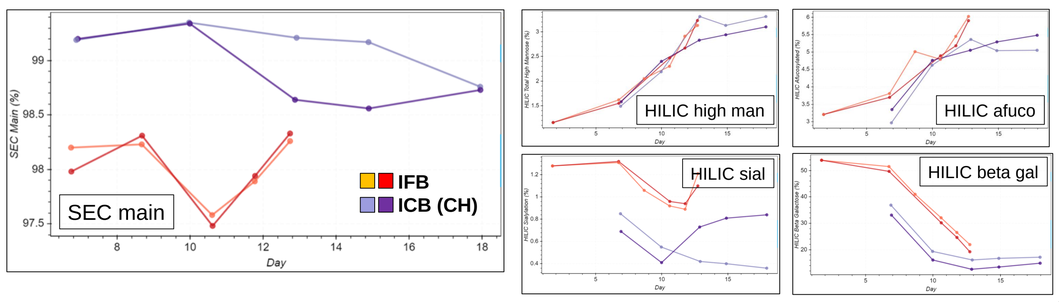
Method		J.PLANT IFB (3 Lots)	J.POD CH (2 CQV & 1 GMP Lot (shown))
SEC	% Main Peak (monomer)	98.0 / 98.8 / 98.8	99.2
rCE-SDS	LC + HC	96.9 / 97.2 / 97.4	97.4
CEX	Group or Main %		
	AcidicGrp Main BasicGrp	23.5 / 23.0 / 23.7 61.0 / 62.2 / 61.9 15.5 / 14.8 / 14.4	14.0 71.2 14.8
icIEF	Total % Area		
	Acidic Main Basic	30.0 / 29.0 / 29.8 57.5 / 58.6 / 58.2 12.5 / 12.4 / 12.0	17.7 67.8 14.5
HILIC	Result %		
	Sialylation, Beta-Galactose, Afucosylated, High Mannose	0.6 / 0.6 / 0.6 29.8 / 25.9 / 27.7 2.8 / 2.6 / 2.8 1.5 / 1.6 / 1.6	0.6 27.3 2.4 2.4

Figure 4. Bench-scale product quality data (Protein A column purified)



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CONCLUSIONS AND NEXT STEPS

- MAb process evolved due to increased mass demand and reduced timeline.
  - Fed-batch → intensified fed-batch → integrated continuous bioprocess (Continuous Harvest)
- Conversion to ICB (CH) process reduced filter fouling events while increasing process robustness and productivity.
- Productivity improved at large-scale but to a lesser degree than at bench-scale for ICB (CH).
  - Future work focused on improved scale-down model through filter sizing experiments.
- Product quality met specifications across process versions.
  - Differences likely due to product residence time differences in the production bioreactor.

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