CHO Cell Culture Process Intensification for Enhanced Production of IgG mAbs

Increased demand for higher product yield in biologics requires optimal use of existing manufacturing capacity. Process intensification is one way a facility can meet increased demand as it allows for fewer or smaller batches to provide the same amount of product in the same timeframe. Approaches for cell culture intensification may include addition of perfusion in seed train or production stages and modification of growth and production media to support cell densities not achievable in a standard fed-batch process. Perfusion requires specialised equipment such as tangential or alternating tangential flow (TF or ATF) controllers which require additional training and increase manufacturing costs.

Installation of this equipment may be limited by existing manufacturing site capabilities and could require costly expansion to accommodate their use. For facilities designed for fed-batch processes, the implementation of perfusion is too costly or impractical. Alternatively, significant gains in cell growth and productivity can be achieved by modification of media and feeds, capturing many of the benefits of perfusion without the additional costs related to equipment, facility upgrades, and training.

This poster shows a significant gain in growth and yield with similar product quality is achievable by modifying media and feeds. Intensification of the seed train was first assessed in these studies to allow for seeding a production vessel at a higher initial cell density. Multiple designs of experiments were executed. First, to intensify the seed train to deliver sufficient cell density for the production stage. Second, to evaluate the impact of production stage parameters on an intensified cell culture process in terms of growth, productivity, and product quality. Harvest studies were performed to ensure feasibility of processing the intensified cell culture at the manufacturing scale.

Objective

Create a process intensification workstream aimed at increasing the titer of CHO processes by 20–50%, adhering to the following constraints:

- No effect on the process timeline
- Maintains comparable product quality attributes
- No need for additional equipment purchases or training
- Compatible with all manufacturing facilities within Thermo Fisher's network.

Materials and methods

The intensification workstream was divided into two stages. The first stage defined the enriched media composition and operating parameters that enable achieving viable cell densities at passage sufficient to inoculate a production bioreactor at a seeding density ≥5.0 x 106 VC/mL. The media composition identified in this experiment would also function as the basal medium for the intensified production bioreactor. The second stage defined the operating parameters and feed strategy of intensified production bioreactor.

Cell Lines

Two CHO K1 cell lines were utilised to produce two recombinant human monoclonal antibodies, Rituximab and Herceptin. Intensification development was performed using the cell line expressing Rituximab. The intensified process developed using rituximab was then applied to the cell line expressing Herceptin to show response in an alternative cell line without optimisation.

Media and Feeds

Chemically defined catalog media and feeds were formulated per vendor instructions and then blended per experimental design to the specified formulations.

N-1 DOE

JMP statistical software was used to generate and analyse a custom DOE design evaluating the following factors: feed % in medium, seeding density, medium powder concentration, feed start day, and daily feed percentage. Conditions were run in parallel in 2 x 48 vessel Ambr™ 15 microbioreactor systems (Figure 1).

Feed Start Day	Daily Feed Percentage	Feed Percentage in Medium	Seeding Density	Medium Concentration
2	2,5	0,10	0,6	0,8
2	2,5	0,05	0,6	1,0
2	2,5	0,00	0,6	1,2
2	2,5	0,10	1,2	0,8
2	2,5	0,05	1,2	1,0
2	2,5	0,00	1,2	1,2
2	2,5	0,10	1,8	0,8
2	2,5	0,05	1,8	1,2
2	2,5	0,00	1,8	1,0
2	3,5	0,10	0,6	1,0
2	3,5	0,05	0,6	1,2
2	3,5	0,00	0,6	0,8
2	3,5	0,10	1,2	1,2
2	3,5	0,05	1,2	0,8
2	3,5	0,00	1,2	1,0
2	3,5	0,10	1,8	1,2
2	3,5	0,05	1,8	0,8
2	3,5	0,00	1,8	1,0
2	4,5	0,10	0,6	0,8
2	4,5	0,05	0,6	1,0
2	4,5	0,00	0,6	1,2
2	4,5	0,10	1,2	0,8
2	4,5	0,05	1,2	1,0
2	4,5	0,00	1,2	1,2
2	4,5	0,10	1,8	0,8
2	4,5	0,05	1,8	1,2
2	4,5	0,00	1,8	1,0
3	2,5	0,10	0,6	0,8
3	2,5	0,05	0,6	1,0
3	2,5	0,00	0,6	1,2
3	2,5	0,10	1,2	0,8
3	2,5	0,05	1,2	1,0
3	2,5	0,00	1,2	1,2
3	2,5	0,10	1,8	0,8
3	2,5	0,05	1,8	1,2
3	2,5	0,00	1,8	1,0
3	3,5	0,10	0,6	1,0
3	3,5	0,05	0,6	1,2
3	3,5	0,00	0,6	0,8
3	3,5	0,10	1,2	1,2
3	3,5	0,05	1,2	0,8
3	3,5	0,00	1,2	1,0
3	3,5	0,10	1,8	1,2
3	3,5	0,05	1,8	0,8
3	3,5	0,00	1,8	1,0
3	4,5	0,10	0,6	0,8
3	4,5	0,05	0,6	1,0
3	4,5	0,00	0,6	1,2
3	4,5	0,10	1,2	0,8
3	4,5	0,05	1,2	1,0
3	4,5	0,00	1,2	1,2
3	4,5	0,10	1,8	0,8
3	4,5	0,05	1,8	1,2
3	4,5	0,00	1,8	1,0
4	2,5	0,10	0,6	0,8
4	2,5	0,05	0,6	1,0
4	2,5	0,00	0,6	1,2
4	2,5	0,10	1,2	0,8
4	2,5	0,05	1,2	1,0
4	2,5	0,00	1,2	1,2
4	2,5	0,10	1,8	0,8
4	2,5	0,05	1,8	1,2
4	2,5	0,00	1,8	1,0
4	3,5	0,10	0,6	1,0
4	3,5	0,05	0,6	1,2
4	3,5	0,00	0,6	0,8
4	3,5	0,10	1,2	1,2
4	3,5	0,05	1,2	0,8
4	3,5	0,00	1,2	1,0
4	3,5	0,10	1,8	1,2
4	3,5	0,05	1,8	0,8
4	3,5	0,00	1,8	1,0
4	4,5	0,10	0,6	0,8
4	4,5	0,05	0,6	1,0
4	4,5	0,00	0,6	1,2
4	4,5	0,10	1,2	0,8
4	4,5	0,05	1,2	1,0
4	4,5	0,00	1,2	1,2
4	4,5	0,10	1,8	0,8
4	4,5	0,05	1,8	1,2
4	4,5	0,00	1,8	1,0

Figure 1. The N-1 fed-batch design space on an Ambr 15 system

JMP software was used to construct a model of the intensified N-1 step showing the optimal parameters to maximise viable cell densities (VCD). This model provided a robust set of parameters to achieve a VCD >20 x 106 VC/mL in 4 days with no adverse impact to viability (Figure 2).

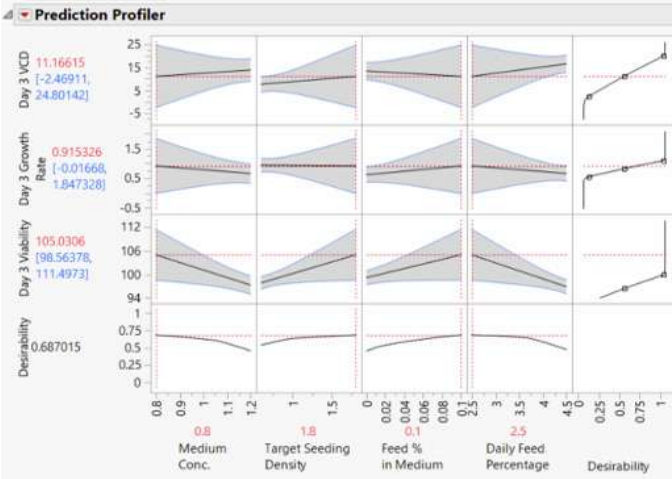


Figure 2. A model of the intensified N-1 step using JMP software

N-stage Production Vessel Intensification Development

JMP statistical software was used to generate and analyse a custom DOE design evaluating the following factors: seeding density, feed start day, feed amount per cell, and temperature shift target. Conditions were run in parallel in 1 x 24 vessel Ambr™ 250 bioreactor system (Figure 3).

		Feed Start Day									
		1			2			3			
		Feed Amount Per Cell			Feed Amount Per Cell			Feed Amount Per Cell			
		0,5	1,0	1,5	0,5	1,0	1,5	0,5	1,0	1,5	
Temperature Shift Target	37	X					X				2
	34							X			
	32			X	X				X		
	37							X			5
	34					XXX					
	32	X								X	
	37			X	X				X		8
	34	X								X	
	32		X				X	X			

Seeding Density

Figure 3. The N-stage production intensification design space on an Ambr 250 system

JMP software was used to construct a model of the intensified production step showing the optimal parameters for titer and specific productivity. This model provided a robust set of parameters to achieve 7 g/L in a standard 14 day fed batch (Figure 4).

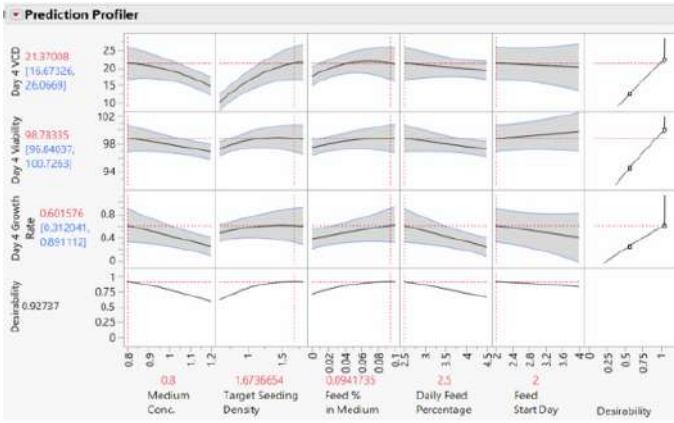


Figure 4. A model of the intensified production step using JMP software.

Results and Discussion

N-1 DOE

Figure 5 shows cell line expressing Rituximab that was scaled via the standard platform (blue) vs. the intensified process (red). Compared to the standard platform process, the intensified N-1 step process generated more than 4x as many cells to inoculate the production vessel.

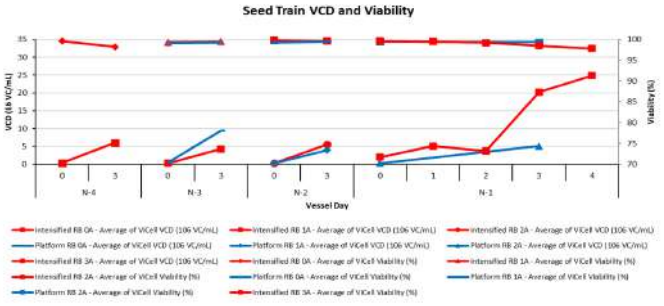


Figure 5. Effect of standard platform vs. the intensified process on VCD and viability from cell line expressing Rituximab

Figure 6 shows cell line expressing Herceptin that was scaled via the standard platform (blue) vs. the intensified process (red). Similar results were observed, with higher VCD and viability observed from cells produced using the intensified process.

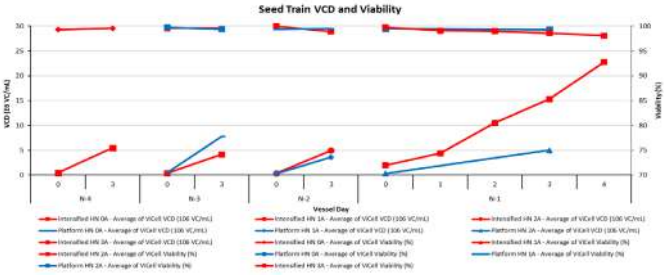
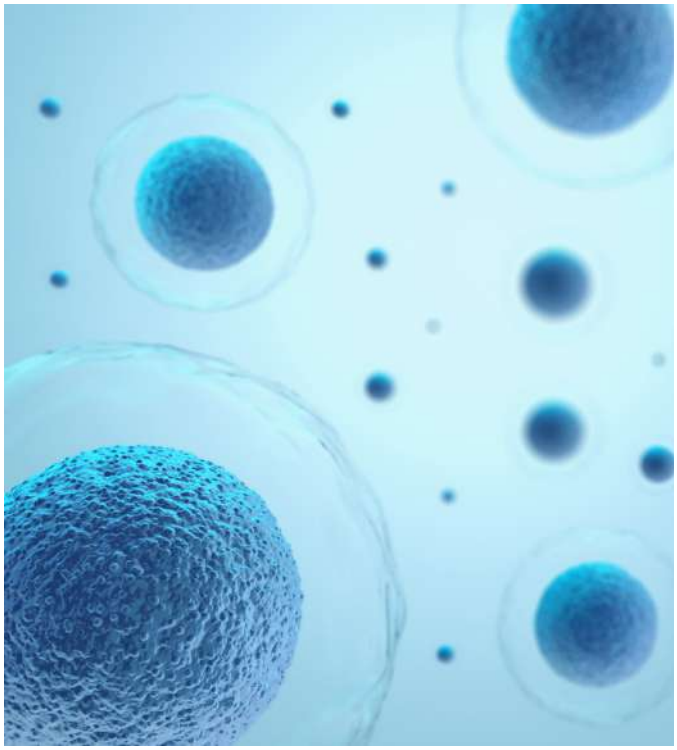


Figure 6. Effect of standard platform vs. the intensified process on VCD and viability from cell line expressing Herceptin



N-stage Production Vessel Intensification Development

Rituximab

Figure 7 shows the VCD, viability, titer, and specific productivity of cell line expressing Rituximab produced using the standard platform process (blue) vs. the intensified fed batch process (red).

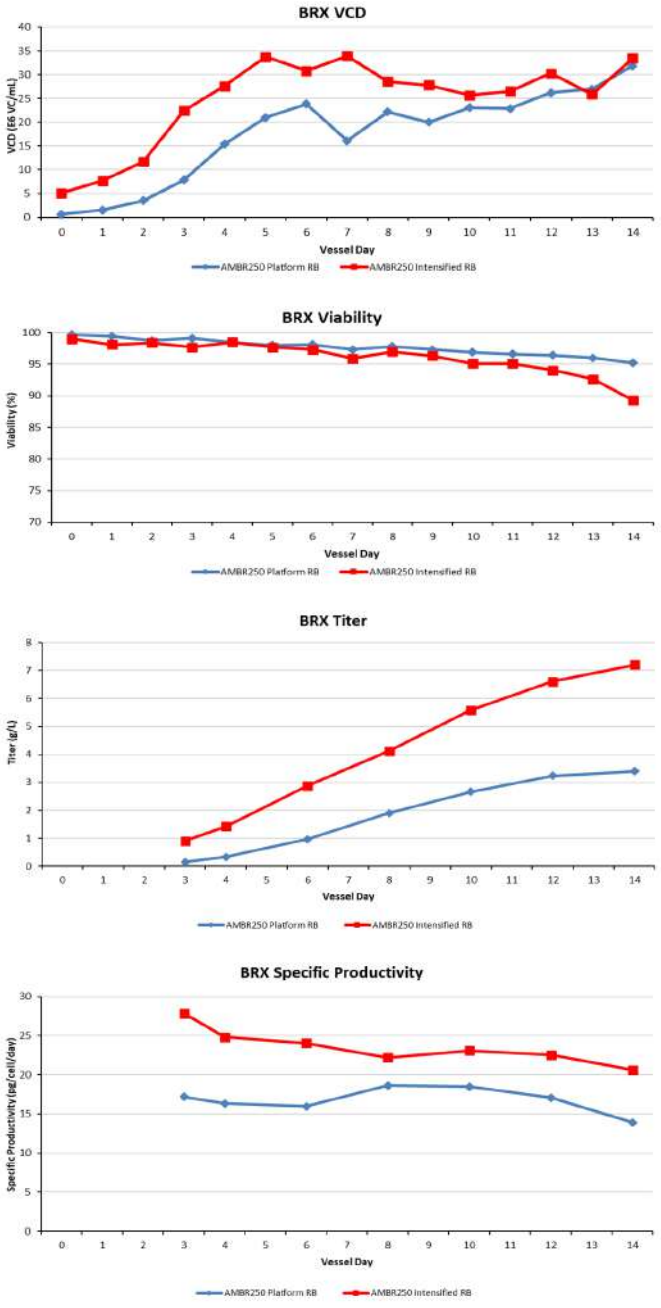


Figure 7. Effect of standard platform vs. the intensified process on cell line expressing Rituximab. (A) VCD, (B) viability, (C) titer, and (D) specific productivity

Herceptin

The same intensified process developed for the Rituximab-expressing cell line was applied to the Herceptin-expressing cell line. Higher titers were achieved while maintaining comparable product quality attributes (Figure 8).

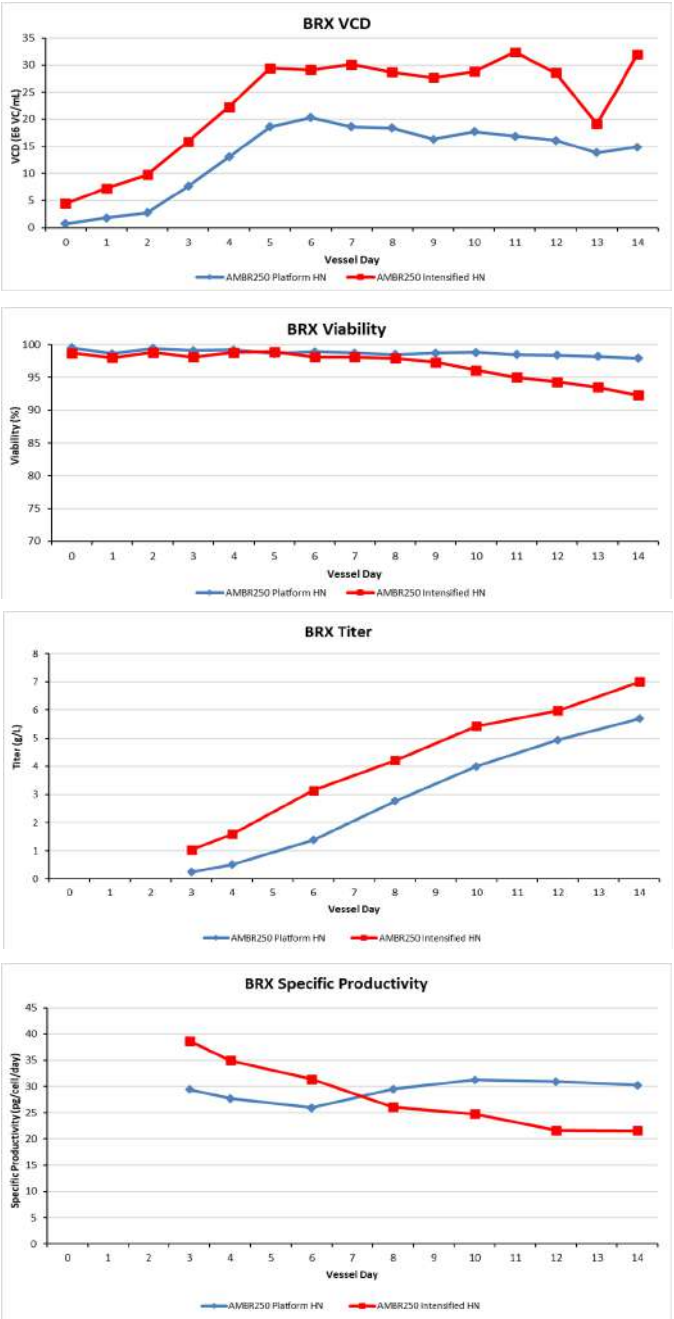


Figure 8. Effect of standard platform vs. the intensified process on cell line expressing Herceptin. (A) VCD, (B) viability, (C) titer, and (D) specific productivity

Table 1 shows that the intensified process delivered comparable product quality for cells expressing Rituximab compared to the standard platform process.

Process	SEC HMW (%)	SEC Monomer (%)	SEC LMW (%)	NR-CGE Peak (%)	NR-CGE Fragment (%)	iCE Acidic (%)	iCE Main (%)	iCE Basic (%)	Glycan-Sialylated (%)	Glycan – Mannose (%)	Glycan – Fucosylated (%)
Platform RB	2.5	97.5	0	97.1	2.9	53.3	43.4	3.3	0.3	0.6	94.2
Intensified RB	2.2	97.8	0	96.2	3.8	56.6	41.1	2.2	0.2	2.8	88.9

Table 1. Comparison of product quality attributes of Rituximab-expressing cells produced using the intensified vs. standard platform process

Table 2 shows that the intensified process delivered comparable product quality for cells expressing Herceptin compared to the standard platform process.

Process	SEC HMW (%)	SEC Monomer (%)	SEC LMW (%)	NR-CGE Peak (%)	NR-CGE Fragment (%)	iCE Acidic (%)	iCE Main (%)	iCE Basic (%)	Glycan-Sialylated (%)	Glycan – Mannose (%)	Glycan – Fucosylated (%)
Platform HN	2.2	96.5	1.4	97.8	2.2	55.0	39.8	5.2	0.2	1.6	89.3
Intensified HN	1.6	98.5	0	97.2	2.8	62.5	34.1	3.4	0.2	7.6	84.1

Table 2. Comparison of product quality attributes of Herceptin-expressing cells produced using the intensified vs. standard platform process

Conclusions

We successfully developed a workflow for intensifying CHO processes to increase titers from 20% to 100% while retaining comparable product quality attributes. The intensified process can be executed within our existing manufacturing platform and facilities. The intensified processes increased titers up to 100% when optimised for specifically for a molecule as shown in the case of Rituximab. An application of a general intensified process achieved a 20% increase in titer for a Herceptin-expressing cell line. No additional equipment, training, raw materials, or consumables were needed.

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