Biologics

Optimization of an intensified CHO cell culture process for enhanced production of IgG mAbs

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Introduction

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. Significant gains in cell growth and productivity have been achieved as previously presented by modification of media and feeds [1], capturing many of the benefits of perfusion without the added costs related to equipment, facility upgrades, and training. However, clones may respond to intensification with varying results. Optimization beyond medium enrichment and feed amount may be required for clones with higher specific productivity.

This poster shows an optimization of an intensified fed-batch process, assessing initial feed amount, feed tapering during production, and temperature shift. The inclusion of a feed taper was designed to limit the nutrients during the stationary phase to optimize productivity.

Materials and methods

Cell lines

Two CHO K1 cell lines were used to produce two recombinant human monoclonal antibodies, Rituximab and Herceptin. Intensification optimization was performed using the cell line expressing Herceptin with OFAT conditions for the cell line expression Rituximab.

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 analyze a custom DOE design evaluating the following factors: feed amount, feed taper amount, and temperature shift timing. Conditions were run in parallel in 1 x 24 vessel Ambr™ 250 microbioreactor system.

Experimental design

Table 1. This table delineates vessels and which clone they contained, as well as parameter values for initial feed amount, taper feed amount, and day of temperature shift per clone.

Vessel	Clone	Initial feed amount (ng/cell/day)	Taper feed amount (ng/cell/day)	Day of temperature shift
1	A	1.2X	X	X+1
2	A	X	X	X+2
3	A	X	1.2X	X+1
4	A	1.2X	1.2X	X
5	A	1.4X	1.6X	X+2
6	A	X	1.6X	X
7	A	1.2X	1.4X	X+1
8	A	1.4X	X	X
9	A	1.4X	X	X+2
10	A	1.4X	1.2X	X+1
11	A	1.2X	1.2X	X+2
12	A	X	X	X
13	A	1.2X	1.4X	X+1
14	A	X	1.6X	X+2
15	A	1.2X	1.6X	X+1
16	A	1.4X	1.6X	X
17	A	1.2X	1.2X	X+2
18	A	1.2X	1.2X	X+2
19	B	0.6X	2X	X+2
20	B	1.2X	1.2X	X+2
21	B	X	X	X+2
22	B	X	1.6X	X+2
23	B	1.4X	X	X+2
24	B	1.4X	1.6X	X+2

Results

Herceptin and Rituximab growth/VCD, viability, titer, and specific productivity.

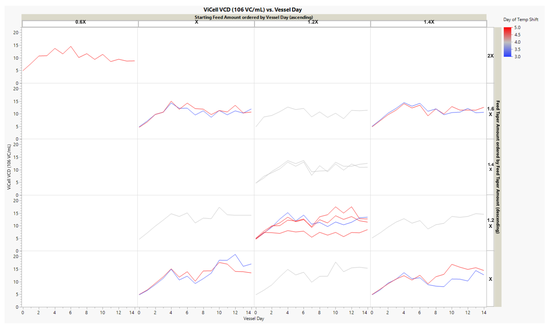

Figure 1. Herceptin growth is indicated by the VCD values, and show similar growth across all Herceptin conditions except for V17, which had a starting temperature of 34˚C.

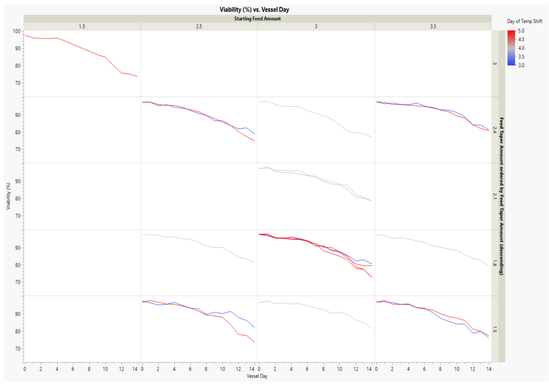

Figure 2. Herceptin final viability ranged from 70% to 80% on depending on vessel conditions. A temperature shift did show improvement of final viability for some conditions.

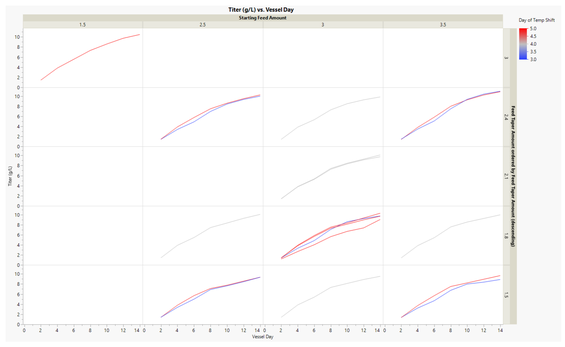

Figure 3. Highest observed titer for Herceptin clones was 11.01 g/L.

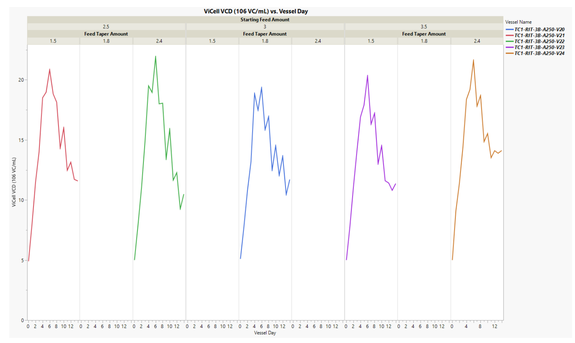

Figure 4. Rituximab growth is indicated by VCD values, and peaks ranged from 21.91 to 19.34 x 10^6 VC/mL, with the 3.0 to 1.8 feed strategy having the lowest VCD, and 2.5- to 2.4- feed strategy having the highest VCD.

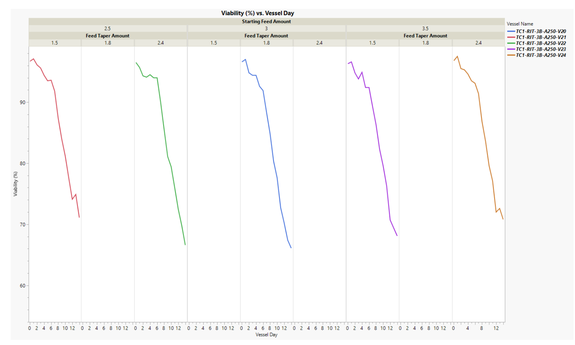

Figure 5. Rituximab final viability ranged from 66.1% to 71.1% for the 5x Rituximab vessels. Overall, feeding strategy did not have a large impact on the final viability for Rituximab.

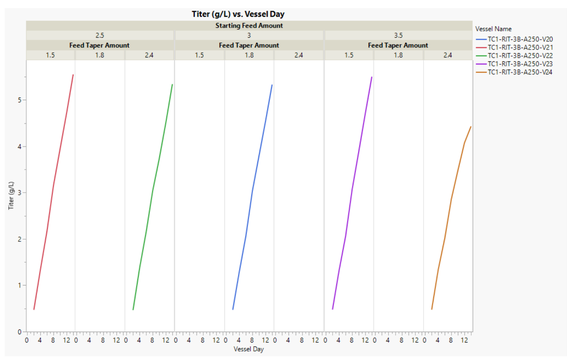

Figure 6. Highest observed titer for Rituximab was 5.6 g/L.

Herceptin prediction profiles for titer, desirability, and viability based on temperature and feed strategies.

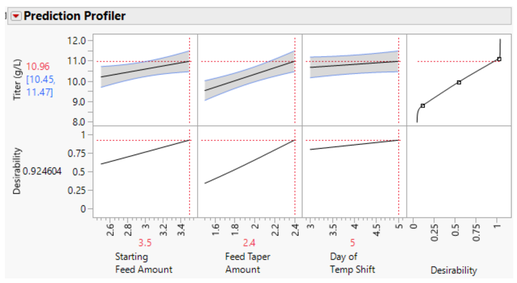

Figure 7. Prediction profiler of final titers observed using JMP software.

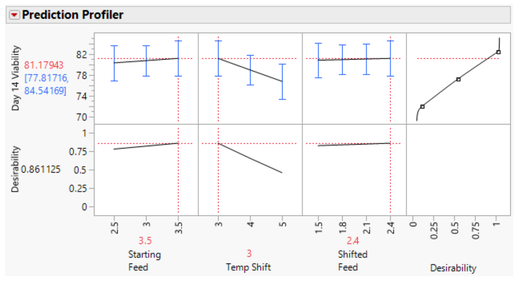

Figure 8. Prediction profiler of final viability on day 3 temperature shift observed using JMP software.

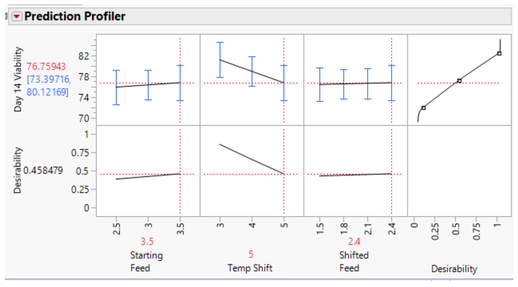

Figure 9. Prediction profiler of final viability on day 3 and day 5 temperature shift observed using JMP software. This shows that a day 5 shift increases titer with only a ~5% decrease in final viability.

Conclusions

Using the optimized intensified process, a consistent increase was observed for two clones producing different molecules (Table 2). This method can be applied successfully across multiple clones.

Table 2. Titer levels observed pre- and post-intensification.

Clone	Process	Day 14 titer (g/L)	Percent difference (%)
Clone A	Pre-intensification	3.4	–
Clone A	Intensified process	5.55	63.2
Clone B	Pre-intensification	6.7	–
Clone B	Intensified process	11.01	64.3

Further testing of additional cell lines and molecules is needed, particularly complex molecules such as Fc-fusion and bispecific molecules.

Reference

1. CHO cell culture process intensification for enhanced production of IgG mAbs – Kasparie, Bennett, Kuhne, Thermo Fisher Scientific

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